Karin Fahl Wader

Hepatocyte growth factor, c-Met and syndecan-1 in multiple myeloma

Thesis for the degree of Philosophiae Doctor

Trondheim, October 2011

Norwegian University of Science and Technology
Faculty of Medicine
Department of Cancer Research and Molecular Medicine

NTNU – Trondheim
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Medicine
Department of Cancer Research and Molecular Medicine

ISBN 978-82-471-3100-8 (printed ver.)
ISBN 978-82-471-3101-5 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2011:267

Printed by NTNU-trykk

HEPATOCYTT-VEKSTFAKTOR, C-MET OG SYNDECAN-1 I MYELOMATOSE

Myelomgruppen ved IKM forsker på sykdommen myelomatose, en form for benmargskreft. Gruppens arbeid har vært fokusert på hvordan miljøet i benmargen, med løselige og cellebundne vekstfaktorer, påvirker kreftcellene/myelomcellene. En av flere vekstfaktor i benmargen er hepatocyttvekstfaktor (HGF). Myelomgruppen var de første til å vise at myelomceller produserer HGF og samtidig uttrykker dens receptor c-Met. Gruppen og andre har vist at høye nivåer av HGF i serum er et dårlig prognostisk tegn hos myelomatosepasienter, at HGF stimulerer vekst og overlevelse av myelomceller, og kan være viktig for den ødeleggelse av bein som sees ved myelomatose. Dette doktorgradsarbeidet fokuserer på HGF og c-Met og på syndecan-1, som er en viktig regulator av aktiviteten av vekstfaktorer i benmargen. I tillegg har vi undersøkt forekomsten av avvik i kromsomene i kreftcellene hos pasienter med myelomatose.

I den **første artikkelen** ønsket vi å undersøke hvorvidt HGF og c-Met er tilstede, og om systemet er aktivt, også i vev fra pasienter, og ikke bare i cellelinjer på laboratoriet. Vi undersøkte forekomsten av HGF og c-Met i benmargsprøver fra pasienter med myelomatose og beslektede sykdommer ved hjelp av immunfarging. 58 av 68 biopsier fra myelomatosepasienter, og 9 av 10 biopsier fra normal benmarg var positive for HGF. 25 av 63 biopsier fra myelomatosepasienter og ingen av 10 biopsier fra normal benmarg var positive for c-Met. Med fosfo-spesifikke antistoffer fant vi at c-Met var fosforylert (dvs aktivert) i 15 av 21 c-Met-positive pasienter. Dette viser at c-Met ikke bare er tilstede, men at det også går et aktivt signal gjennom denne. Studien indikerer at c-Met er en faktor som skiller maligne fra normale plasmaceller, og at c-Met er aktivert i myelomatosepasienter.

Omdanning av HGF fra inaktiv til aktiv form er avgjørende for biologisk funksjon. I den **andre artikkelen** undersøkte vi serumnivået av HGF aktivator (HGFA), som er en av de viktigste aktivatorer av HGF. Vi fant høyere serumnivåer av aktivert HGFA hos myelomatosepasienter enn hos friske kontrollpersoner. En mulig mekanisme for aktivering av HGF i myelomatose kan være økt nivå eller aktivitet av HGFA.

En ekstracellulær porsjon av c-Met kan kappes av til en løselig reseptor i serum. Den løselige reseptoren kan nedregulere effekten av HGF på flere måter, men dette har ikke vært undersøkt i myelomatose. I den **tredje artikkelen** undersøkte vi serumnivåer av løselig c-Met. Vi fant ingen forskjell i serumkonsentrasjon av løselig c-Met mellom myelomatosepasienter og friske kontrollpersoner. Likevel var det en negativ korrelasjon mellom serumkonsentrasjon av c-Met og sykdomsstadium, grad av plasmacelleinfiltrasjon i benmarg og nivå av M-komponent hos myelomatosepasienter. Studien indikerer at det kan være relevant å undersøke en mulig biologisk betydning av løselig c-Met i myelomatose.

Syndecan-1 er en viktig regulator av aktiviteten av flere vekstfaktorer. I den **fjerde artikkelen** undersøkte vi rollen til syndecan-1 som cofaktor i interaksjonen mellom HGF og c-Met. Det er kjent fra før at HGF kan binde til syndecan-1. Vi viser i denne studien at også c-Met kan binde til syndecan-1. Det er også tidligere vist at syndecan-1 lokaliseres til lipid rafts – kolesterolrike ”fett-flåter” – i cellemembranen. Slike lipid-flåter er viktige i cellesignalering, fordi viktige signalmolekylær konsentreres hit. Vi fant at HGF og c-Met lokaliseres sammen med syndecan-1 til lipid-flåter i myelomceller, og at intakte lipid-flåter er nødvendige for HGF-indusert signalering via PI3K-Akt, som er en viktig signalvei for overlevelse og tilvekst av myelomceller.

Det at myelomcellene er avhengig av faktorer i benmargen er én side av sykdomsutviklingen ved myelomatose. En annen side er genetiske forandringer i myelomcellene. I den **femte artikkelen** har vi undersøkt forekomsten av genetiske avvik i myelomcellene hos 250 norske myelomatosepasienter. Vi fant at 45% av pasientene hadde en translokasjon (overbytning av genmateriale) der immunglobulingenet, som er sentralt ved myelomatose, blir flyttet nært andre gen (onkogen) som ofte fremmer celleveksten. 35% hadde tap av deler (delesjon) av kromosom 13 og 19% hadde delesjon av kromosom 17. 10% hadde delesjon av korte armen av kromosom 1 og 34% hadde amplifikasjon av den lange armen av kromosom 1. Forekomsten av de genetiske avvikene hos norske myelomatosepasienter er lik den som er beskrevet i internasjonale materialer. Genetiske avvik kan komme til å få betydning for hvilken behandling som skal gis.

Metoder som er brukt i doktorgradsarbeidet er immunhistokjemi, ELISA, konfokalmikroskopi, flowcytometri, immunprecipitering, Western blot og interphase FISH.

Navn kandidat: Karin Inger Martina Fahl Wader
Institutt: Institutt for Kreftforskning og Molekylær Medisin
Veiledere: Anders Waage, Therese Standal, Magne Børset og Anna Bofin.
Finansieringskilde: Samarbeidsorganet Helse Midt-Norge – NTNU.

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i molekylær medisin.
Disputas finner sted i Auditoriet, Medisinsk Teknisk Forskningssenter, St Olavs Hospital, torsdag den 20 oktober 2011 kl 12.15.

ACKNOWLEDGEMENTS

This work has been carried out at the Department of Cancer Research and Molecular Medicine, NTNU, Trondheim. The work has been supported by grants from the Liaison Committee between the Central Norway Regional Health Authority and NTNU, the Norwegian Cancer Society, The Cancer Fund of St Olavs Hospital and the Research Council of Norway.

Finally finishing my thesis, I want to thank several persons. First, thanks to Anders Sundan, Anders Waage and Magne Børset for letting me start a research project in the myeloma group, and for your help along the way. Thanks to my main supervisor Anders Waage for all your advice, for your unreasonable optimism, and for your ability to see the big picture. Thanks to my co-supervisor Anna Bofin for guiding me through the bone marrow biopsies and introducing me to pathology, and to my co-supervisor Magne Børset for sharing your knowledge. Finally, thanks to my co-supervisor Therese Standal for being there all the way, from helping this hopeless novice in the lab bench and at the confocal microscope, through teaching basal skills of article writing and to reading the final thesis.

I am grateful to Borgny Ytterhus and Eli Johannesen for help and patience with the immunohistochemistry project. Thanks to Lill Anny Grøseth and Hanne Hella for teaching me flow cytometry, to Berit Størdal for teaching me all about ELISA, and to all three of you for all that help in the lab area, and for taking care of the cells. Thanks to Kjartan Egeberg for assistance with the confocal microscope. Thanks to Mari Sørensen for keeping everything at the lab in order. Thanks to all co-authors for collaboration, and thanks to former and present members of the myeloma group: Janne Bohnhorst, Gaute Brede, Anne-Tove Brenne, Torstein Baade Rø, Hong Yan Dai, Unn-Merete Fagerli, Berit Helen Grandaunet, Toril Holien, Randi Utne Holt, Håkon Hov, Sofia Jönsson, Siv Moen, Nikolai Nørgaard, Christoph Rampa, Magne Rekvig, Tobias Slørdahl, Olaf Strømme, Erming Tian and Thea Våtsveen. You have all been the lights of these years.

Thanks to Professor emeritus Jon Lamvik for your inspiring presence at the institute and for always being encouraging. Thanks to Thea Våtsveen for support. Thanks to Randi Utne Holt for making every room you enter a bit warmer. Thanks to my former room mate Øystein Grimstad for humoristic comments, to Asbjørn Nilsen for a nice day in the fridge, and to all members of the toll and Grill groups for friendly corridorship.

Special thanks to Unn-Merete Fagerli for collaboration in research and clinic, for all your support, and to you and Monika Eidem for your generosity, friendship and humor, making it a joy going to work. Thanks to my present and former boss at the Department of Oncology, Stein Kaasa and Stein Sundstrøm, for giving me the opportunity to finish this thesis.

Thanks to my husband Tony for being so open-minded and generous. And cool. Thanks to our four children for all the right-to-the-point comments. Finally, thanks to my parents for all your incredible help and support. I owe you.

LIST OF PAPERS

I. Wader KF, Fagerli UM, Børset M, Lydersen S, Hov H, Sundan A, Bofin A and Waage A. Immunohistochemical analysis of HGF and c-Met in plasma cell disease. Histopathology 2011; In Press.

II. Wader KF, Fagerli UM, Holt RU, Stordal B, Børset M, Sundan A and Waage A. Elevated serum concentrations of activated hepatocyte growth factor activator in patients with multiple myeloma. Eur J Haematol 2008; 81: 380-3

III. Wader KF, Fagerli UM, Holt RU, Børset M, Sundan A and Waage A. 2011. Soluble c-Met in serum of patients with multiple myeloma: Correlation with clinical parameters. Eur J Haematol 2011 Apr 5 [Epub ahead of print].

IV. Wader KF, Hov H, Holien T, Brede G, Majka M, Zebzda A, Børset M, Waage A, Sundan A and Standal T. HGF - c-Met interaction with syndecan-1 in lipid rafts promotes Akt signaling in myeloma cells. Manuscript.

V. Våtsveen TK, **Wader KF**, Grøseth LA, Dahl IM, Lindås R, Haukås E, Müller E, Gulbrandsen N, Gedde-Dahl T, Szatkowski D, Stenberg V, Loraas A, Sundan A, Aarset H, Dai HY, Børset M and Waage A. Genetic aberrations in Norwegian myeloma patients. Manuscript.

CONTENT

ABBREVIATIONS

ASCT – autologous stem cell transplant
ADAM – a disintegrin and metalloproteinase
APRIL – a proliferation-inducing ligand
BAD – Bcl-2 antagonist of cell death
BMP – bone morphogenetic protein
CR – complete remission
DKK-1 – dickkopf-related protein 1
EGF – epidermal growth factor
EMT – epithelial mesenchymal transition
ERK – extracellular signal-regulated kinase
FAK – focal adhesion kinase
FISH – fluorescence in situ hybridization
FGF – fibroblast growth factor
GAB1 – Grb2-associated binder 1
GAG – glycosaminoglycans
GDP – guanosine diphosphate
GEP – gene expression profiling
GTP – guanosine triphosphate
GRB2 – growth-factor-receptor-bound protein 2
GSK3β – glycogen synthase kinase 3β
HAI-1 and HAI-2 – hepatocyte growth factor activator inhibitor 1 and 2
HB-EGF – heparin-binding epidermal growth factor
HDT – high dose therapy
HMCL – human myeloma cell line
HS – heparan sulphate
HSPG – heparan sulphate proteoglycan
HGF – hepatocyte growth factor
HGFA – hepatocyte growth factor activator
ICAM-1 – intercellular adhesion molecule 1
Ig – immunoglobulin
IGF-1 – insulin-like growth factor 1
IGFBP – insulin-like growth factor-binding protein
IGF-1R – insulin-like growth factor 1 receptor
IκB – NF-κB inhibitor
IKK – IκB kinase
IL-6 – interleukin-6
IMWG – International Myeloma Working Group
ISS – international staging system
JAK – janus kinase
JNK – jun amino-terminal kinase
mAbs – monoclonal antibodies
MAPK – mitogen activated protein kinase
MAPKK – MAPK kinase
MEK – MAP-Erk kinase
MEKK – MEK kinase
MGUS – monoclonal gammopathy of undetermined significance
MIP-1α – macrophage inflammatory protein 1α
MM – multiple myeloma

MMP – matrix metalloproteinase
MSP – macrophage stimulating protein
mTOR – mammalian target of rapamycin
NF-κB – nuclear factor-κB
OPG – osteoprotegerin
PAK – p21-Activated kinase
PH – pleckstrin homology
PI3K – phosphatidylinositol 3-kinase
$PI(3,4,5)P_3$ – phosphatidylinositol triphosphate
PLCγ – phospholipase Cγ
PTEN – phosphatase and tensin homologue
RANK – receptor activator of NF-κB
RANKL – receptor activator of NF-κB ligand
SDF-1α – stromal derived factor-1α
SH2 domain – Src-homology-2 domain
SHC - Src homology domain containing
SHP2 – SH2-domain-containing protein tyrosine phosphatase 2
SOS – son of sevenless
SP – serine protease
STAT – signal transducer and activator of transcription
VEGF – vascular endothelial growth factor
VCAM-1 – vascular cell adhesion molecule 1
VLA-4 – very late antigen 4

INTRODUCTION

1.1. General aspects of multiple myeloma

1.1.1. Epidemiological and clinical aspects

Multiple myeloma (MM) is a malignant plasma cell disorder that accounts for approximately 1 % of cancer worldwide and 10-15% of all haematological malignancies (1, 2). Incidence rates vary from 0.4 to 6 per 100.000 (1, 3). In Norway, about 300 patients are diagnosed with MM per year (Cancer Registry of Norway). The median age at diagnosis is 65-70 years, but may be higher in unselected, population-based materials (3, 4). MM is more common in men than in women, and twice as common in African-Americans compared to Caucasians (2, 5). The incidence is higher in first-degree relatives of patients with MM (2). It is a disseminated disease which affects the bone marrow and frequently invades adjacent bone followed by bone destruction. Extramedullary expansions (plasmacytomas) of bone lesions, and true extramedullary plasmacytomas occur. The major clinical manifestations are symptoms from bone destruction with pain and/or fractures, anaemia, hypercalcaemia, renal failure and an increased risk of infections (6, 7). It has recently become clear that all, or almost all, cases of MM evolve from an asymptomatic premalignant state termed monoclonal gammopathy of undetermined significance (MGUS) (8, 9). MGUS is present in approximately 3% of the population above age 50, and about 1% per year progresses to MM or another B cell malignancy (10, 11). Other disorders of monoclonal plasma cells include MGUS, solitary plasmacytomas, systemic AL amyloidosis and POEMS (***p***olyneuropathy, ***o***rganomegaly, ***e***ndocrinopathy, ***M*** protein, ***s***kin changes) syndrome (12). Immunoglobulin M (IgM) MGUS and Waldenström's macroglobulinemia are related B-cell disorders that are not included in this work.

The current diagnostic classification distinguishes between smouldering/asymptomatic and symptomatic MM. The diagnostic criteria for symptomatic MM are [1] 10% or more clonal plasma cells on bone marrow examination, or a biopsy-proven plasmacytoma, [2] presence of serum and/or urine monoclonal protein and [3] signs of myeloma-related organ damage: Hypercalcaemia (C), renal insufficiency (R), anaemia (A), or bone destruction (B), with the acronym CRAB (6, 13). Exceptions from [2] are given in true non-secretory myeloma, which comprise approximately 2% of MM with no evidence of M protein on protein electrophoresis, serum immunofixation or serum-free light chain assay (12). The definition of smouldering myeloma requires 10% or more clonal bone marrow plasma cells and serum monoclonal protein (IgG or IgA) more than 30 g/L (12, 13).

MGUS	**Asymptomatic MM (Smouldering MM)**	**Symptomatic MM**
Clonal bone marrow plasma cells <10%	Clonal bone marrow plasma cells ≥ 10% *and/or*	Clonal bone marrow plasma cells ≥ 10%
Serum M protein < 30 g/L	Serum M protein ≥ 30 g/L	Serum and/or urine M protein present at any concentration
Absence of myeloma-related organ damage	Absence of myeloma-related organ damage	Myeloma-related organ damage (CRAB)

Table 1. *Diagnostic criteria for MGUS, asymptomatic and symptomatic MM (6, 12, 13)*

Routine primary diagnostic work-up includes bone marrow aspirate or biopsy, plain radiographs of the axial skeleton (skull, vertebral spine, pelvis and long bones) and analyses of M protein in serum and urine. The serum free light chain assay may be able to replace the need for urine electrophoresis in the situation of screening for MM, and can be used for monitoring of disease course and response to therapy in those without measurable M protein by serum electrophoresis or immunofixation. In addition, clinical and biochemical analyses are performed to detect signs of myeloma-related organ damage. Today, cytogenetic analyses

with conventional karyotyping and/or fluorescence in situ hybridization (FISH) are increasingly being considered as part of routine primary diagnostic work-up (6). Cytogenetic abnormalities in multiple myeloma will be further discussed in a later section. In some instances MRI of the skeleton and PET-CT may be of value.

1.1.2. Prognosis, staging, risk stratification and treatment

MM is still an incurable disease. After the introduction of new drugs during the last decade, median overall survival has improved and is now estimated to four to five years, compared to approximately three years earlier (14, 15). However, MM is a heterogeneous disease, and the individual variation is large with some patients experiencing an aggressive disease course with survival only of weeks or months, while other patients may live for 10 years and more with the disease. The staging systems by Durie Salmon (16, 17) and the International Staging System ISS (17, 18) both yield prognostic information. ISS also seem valid in the era of new drugs like thalidomide, lenalidomide and bortezomib (15), and is the current standard for staging of myeloma. Yet these systems are not useful for guiding therapeutic choices.

Stage I: Serum β2-microglobulin <3.5 mg/L and serum albumin ≥ 35 g/L
Stage II: Not fitting stage I or III
Stage III: Serum β2-microglobulin ≥ 5.5 mg/L

Table 2. *International Staging System*

There is increasing evidence that a risk stratification based on cytogenetics may be able to guide therapeutic decisions (19). Studies that have assessed various treatment options in patients with high risk cytogenetics include relatively small numbers of patients and limited

follow up, making it difficult to draw conclusions. While several studies suggest that use of bortezomib can improve the outcome of patients with t(4;14) or chromosome 13 abnormalities (20, 21), there is no conclusive evidence that currently available drugs can overcome the negative prognostic impact of 17p deletion (del17p)(21), and new therapeutic strategies will have to be further evaluated in these patients. High risk cytogenetic abnormalities will be detailed and further discussed in a later section. Plasma cell labelling index $\geq$ 3% was also shown to be associated with adverse prognosis (22), and the Mayo Clinic has implemented cytogenetics together with plasma cell labelling index in a risk adapted treatment algorithm (23). Gene expression profiling (GEP) can be of prognostic value and is used by some centres (24). However, general use of GEP is so far limited by the lack of availability and a uniform platform (6). The International Myeloma Working Group (IMWG) recently proposed a minimal testing panel for newly diagnosed MM patients which includes the established high risk cytogenetic factors t(4;14), t(14;16) and del17p (19, 23). Conventional metaphase cytogenetics is recommended in addition to FISH (25). However, tailored therapy based on risk factors and/or cytogenetic factors remains controversial.

Melphalan – Prednisone has been the cornerstone for MM treatment since its introduction in the early 1960s (26). High dose therapy (HDT) with autologous stem cell transplant (ASCT) was introduced in the late 1980s and prolongs overall survival for those eligible (27). Eligibility for HDT with ASCT has therefore been the first dividing point in all treatment algorithms. The introduction of new drugs which can produce very good partial remissions and complete remissions (CR) with less toxicity than HDT with ASCT may come to challenge this concept. Also, the improved outcome of MM patients during the last decade, in parallel to the introduction of new drugs, has lead to discussion of whether time has come for a paradigm shift, towards more aggressive therapy aiming at CR, rather than sequential use of

drugs aiming at disease control (28). This "cure versus control" debate is still ongoing. There are some data to support that high risk patients need aggressive therapy to achieve a CR for accomplishing long term survival, while achieving a CR may not affect survival in standard-risk patients (6, 29). Allogeneic stem cell transplantation has been associated with high mortality rates, and although new reduced conditioning regiments produce lower toxicity, the relapse rate increases, and allogeneic stem cell transplantation is still considered as investigational treatment (30, 31). A role for maintenance therapy with thalidomide, lenalidomide or bortezomib, after achieving optimal remission, is being evaluated in clinical trials (30). Further, the introduction of less toxic therapy and better risk stratification has brought up the topic of prophylactic treatment of smouldering myeloma (and even MGUS). However, until evidence for improved survival (or quality of life) by a prophylactic approach exist, observation until symptomatic disease remain the standard of care (6, 30). Several new drugs are currently being evaluated in trials (32). The IMWG uniform response and relapse criteria are detailed in (33).

1.2. Pathogenesis

1.2.1. Myeloma biology

Normal B-cell development. Early B-cell development starts in the bone marrow, where B-cell precursors go through rearrangement of the Ig heavy- and light-chain genes, resulting in a functional B-cell receptor. These cells then differentiate into mature**,** naïve B-cells which leave the bone marrow. After antigen activation, T helper cells stimulate the mature, antigen-activated B-cells to undergo proliferation, somatic hypermutation of Ig heavy (*IGH*) and light chain (*IGL*) sequences, and class-switch recombination of Ig. This takes place in the germinal centre of the lymph node, and results in memory B-cells and terminally differentiated, non-proliferating, long-lived plasma cells, capable of secreting antigen-specific antibodies (Figure

1). These cells home to the bone marrow where they receive survival signals as interleukin-6 (IL-6) from stromal cells, and live for months to years (34, 35).

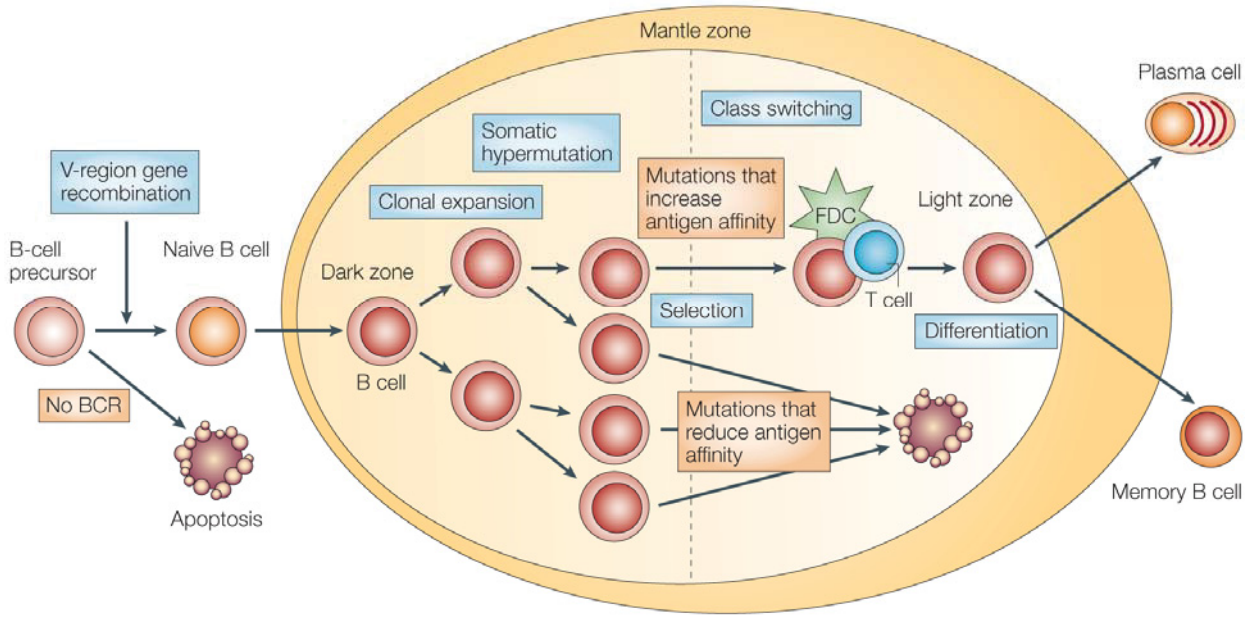

Figure 1. Normal B cell differentiation. Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Cancer (35), copyright 2005.

General aspects on myeloma biology. MM is caused by the expansion of a single clone of plasma cells derived from B cells in the bone marrow. They are long-lived, with a low labelling index, usually 1-2% (34, 36). GEP has shown that the mRNA profiles of MGUS and MM are similar, but different from that of normal plasma cells (37). MM pathogenesis is regarded as a multi-step process of genetic alterations of the myeloma cells (Figure 2) and changes in the bone marrow microenvironment, but it is still not clear at what state of B cell differentiation the primary oncogenic event occurs. As in other human cancers, involvement of cancer stem cells have been discussed also in MM, and there are indications of the existence of a small subpopulation of cells with clonogenic capacity, more resembling

memory B-cells, that would be responsible for initiation, relapse and progression of the disease (34, 36).

MM is a heterogeneous disease, both clinically and biologically. The basis for this heterogeneity is thought to involve both intrinsic disease biology and host factors. The IMWG molecular classification of multiple myeloma (19) is based on this intrinsic heterogeneity. It is a working classification based on today's knowledge and expected to be modified with growing knowledge in coming years.

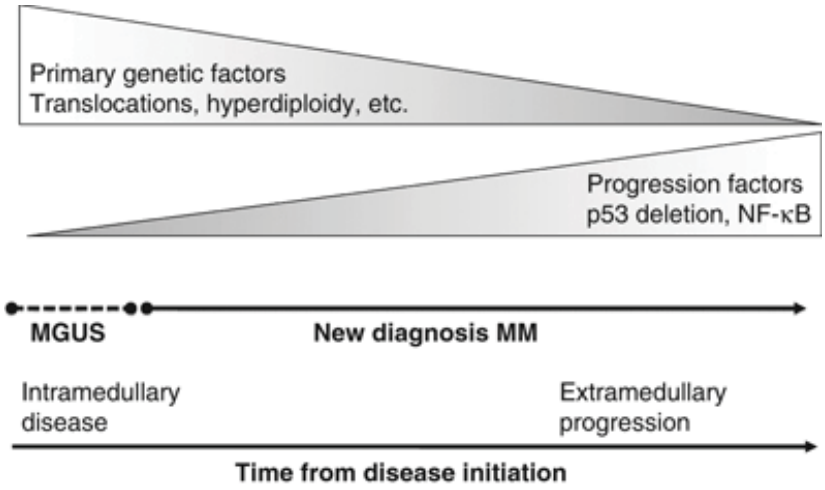

***Figure 2**. Schematic presentation of clonal evolution of malignant plasma cells. Reprinted by permission from Macmillan Publishers Ltd: Leukemia (19), copyright 2009.*

1.2.2. Genetics in multiple myeloma

MM can be divided into two categories based on chromosome numbers: hyperdiploid and non-hyperdiploid (19, 38, 39). This dichotomy has also been demonstrated in MGUS (40, 41), and the ploidy categories are valid over time (42). Patients in the hyperdiploid category have a better outcome (43, 44). The non-hyperdiploid group is characterized by a high frequency of

recurrent translocations involving the *IGH* locus on chromosome 14q32.3, of which the most common are t(11;14), t(4;14), t(14;16) and t(14;20) (19, 45, 46). These primary translocations cause various genes to be juxtaposed to a strong Ig enhancer that dysregulates their expression, and are looked upon as disease-defining events that remain during the course of the disease in a given patient (46). Translocations involving the *IGL* loci (2p12 or 22q11 for κ and λ, respectively) are less frequent (46).

MM pathogenesis is considered to be a multistep process with sequential accumulation of genetic aberrations, including chromosome 13q deletion (del13q) and monosomy, del17p and chromosome 1 abnormalities. Chromosome 13 abnormalities are detected in around 50% of myeloma patients, and are associated with shorter survival. However, this prognostic association is considered to be a surrogate of its association with non-hyperdiploid MM, especially t(4;14) (19).

Deletion of 17p13 (locus for the tumour suppressor gene *TP53*) is found in around 10% of MM patients (43), but is uncommon in MGUS. It is the cytogenetic factor with highest impact on prognosis. Patients with del17p have shorter overall survival, more aggressive disease and higher prevalence of extramedullary disease. In line with this, most myeloma cell lines have p53 abnormalities (19, 43). Chromosome 1 abnormalities, mainly 1p deletion (del1p) and 1q amplification, which are closely correlated, are also emerging as important prognostic factors (19, 47, 48), although contradictory data exist. Several authors have found a negative prognostic impact by chromosome 1 abnormalities (47-51), although the data is not yet considered sufficient to motivate routine use of chromosome 1 abnormalities to predict prognosis (25). Chromosome 1 abnormalities have also been implicated in transformation

from MGUS to smouldering myeloma and from smouldering myeloma to MM (50, 52). Several other candidates have been proposed as important progression factors (19).

GEP has also been used for prognostic classification of MM patients. The myeloma research group at University of Arkansas for Medial Sciences (UAMS) identified by GEP a set of 70 genes which predicted high-risk myeloma. They also identified 17 genes that were able to provide the same prognostic information (47). There was minimal overlap between the expressed signatures. Seven clusters of gene expression were identified, and this was the basis for the UAMS molecular classification of MM (24). The results have been validated by other groups, with identification of additional sub-groups (53). Some of the primary *IGH* translocations are associated with distinct gene expression profiles, like t(14;16) and t(14;20). However, some genetic events are not associated with any specific GEP pattern, and conversely, some GEP patterns are not associated with any known genetic events (19).

The role of epigenetic factors in MM pathogenesis is subject to rising interest. DNA methylation is altered in many cancers, and tumour suppressor genes, like p53, are found to be silenced by methylation in MM. Also, aberrant expression of histone deacetylases (HDACs) have been shown in MM, and HDAC inhibitors are promising therapeutic agents (54). Further, an important role of post-transcriptional gene regulation by microRNAs is increasingly acknowledged (55).

1.2.3. Cytokines and the bone marrow microenvironment

The clinical heterogeneity of MM is thought to mirror both differences in intrinsic disease biology and host factors. The term “host factors” generally refers to factors like age and comorbidity, affecting prognosis and treatment toxicity. However, also the micro milieu of the

bone marrow is crucial for survival and thriving of myeloma cells. It is well established that interaction between myeloma cells and the bone marrow microenvironment is essential in MM pathogenesis (34). In this interaction, two components are crucial: adhesion molecules and cytokines.

The bone marrow microenvironment consists of several cell types that secrete factors important for homing and adhesion of myeloma cells. These cell types include hematopoietic stem cells and progenitor cells, immune cells, stromal cells, endothelial cells, adipocytes, osteoclasts and osteoblasts (34).The chemokine stromal derived factor (SDF)-1α and its receptor CXCR4, which is expressed by myeloma cells, have crucial roles for homing of myeloma cells to the bone marrow (56). Binding of SDF-1α to CXCR4 induces motility and cytoskeletal rearrangements, i.e. migration, of myeloma cells. Several adhesion molecules then come to play, including CD44, very late antigen (VLA-4), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and syndecan-1 (34, 36). Adhesion of myeloma cells to the bone marrow affects gene expression both in the myeloma cells and in the bone marrow stromal cells, leading to up-regulation of several cytokines (36). Further, adhesion of myeloma cells to bone marrow endothelial cells (mediated by SDF-1α), up-regulates expression of many angiogenic cytokines, and leads to secretion of several myeloma growth factors, like insulin-like growth factor 1 (IGF-1), IL-6, vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), from the endothelial cells. At the same time, the myeloma cells may secrete factors that stimulate angiogenesis, like VEGF and basic fibroblast growth factor (bFGF) (34).

Osteoclasts produce myeloma growth factors, like IL-6. Conversely, myeloma cells activate osteoclasts, leading to bone destruction. Bone marrow stromal cells produce receptor activator

of nuclear factor-κB ligand (RANKL) and osteoblasts produce osteoprotegerin (OPG). RANKL, by interacting with its receptor RANK on osteoblasts, stimulates osteoclasts. OPG is a decoy receptor that binds to RANKL and prevents it from interacting with RANK, i.e. inhibits it from promoting osteoclastogenesis. By disturbing the OPG/RANKL ratio, myeloma cells promote bone destruction (57). Myeloma cells also inhibit osteoblast differentiation via dickkopf-related protein 1 (DKK1) (58) and HGF (59). Interactions between the myeloma cells and bone marrow cells thus trigger several signals which mediate growth, survival and migration of myeloma cells and, in addition, bone destruction and angiogenesis (34).

Several cytokines interact and synergize to promote myeloma cell survival and/or induce cell growth and proliferation, including IL-6, IGF-1, FGF, HGF, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), macrophage inflammatory protein 1α (MIP-1α), tumour necrosis factor (TNF), B cell activating factor (BAFF), a proliferation-inducing ligand (APRIL), IL-15, IL-21 and more (60-70). Some of them are produced by the myeloma cells, and some by bone marrow stromal cells, thus acting in an autocrine or paracrine manner. Among these cytokines, IL-6 is since long time regarded as the major growth and survival factor of myeloma cells (60, 71). IL-6 and one of the most important other cytokines, IGF-1, will be described in more detail, but the focus of this work is on HGF, its receptor c-Met and related factors.

1.2.4. Signalling common pathways and redundancy

Several cytokines converge to activate common intracellular pathways, like the Ras/mitogen activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, nuclear factor-κB (NF-κB) and janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathways (72). As an example consistent with this notion, it was shown that in the

presence of bone marrow stromal cells, myeloma cells survive independently of IL-6/STAT3 signalling (73), indicating that additional factors from the bone marrow microenvironment might substitute for its actions. This is thought to explain the often disappointing results of inhibiting single cytokines, like IL-6, as these signalling cascades will be redundantly activated by other cytokines (72). The intracellular pathways most relevant for this work are further described in the c-Met signalling section.

1.2.5. IL-6

IL-6 was early recognized as the main cytokine inducing terminal differentiation of B cells into plasma cells (74). IL-6 has proved to be a major myeloma growth factor, and many myeloma cell lines are dependent of IL-6 for thriving (60, 71, 75). IL-6 knock out mice do not develop plasma cell malignancies (76). IL-6 promotes growth, protects myeloma cells from apoptosis and, in addition, may induce resistance to dexamethason (77). Serum levels of IL-6 correspond with disease severity (78). When myeloma cells adhere to bone marrow stromal cells or osteoblasts, these are triggered to produce IL-6 (79-82). IL-6 may also be produced by myeloma cells (60, 83). IL-6-production by bone marrow stromal cells and by myeloma cells is stimulated by several cytokines, like TNF-α and IL-1 (84, 85). IL-6 thus acts in an autocrine or paracrine upon the IL-6 receptor complex which is expressed by the malignant cells of most MM patients, and by almost all myeloma cell lines (86, 87). The IL-6 receptor complex is composed of a ligand binding domain, IL-6 receptor-α (IL-6Rα), and a signal transducing domain (GP130/CD130). When bound to ligand, IL-6Rα dimerizes GP130 followed by activation of intracellular signalling pathways. The intracellular portion of the IL-6R complex lacks tyrosine kinase activity, but recruits other intra-cytoplasmic tyrosine kinases for downstream signalling mainly through the JAK/STAT3, Ras/MAPK and PI3K/Akt pathways (88-90).

1.2.6. IGF-1

IGF-1 has a physiologic role in normal regulation of metabolism, development and growth, but is also thought to play crucial roles in many types of cancer (91). It is mainly produced by the liver under the influence of growth hormone and insulin, and circulates in a complex bound to IGF binding protein 3 (IGFBP-3). When IGFBP-3 binds to matrix proteins or cell surfaces, or is cleaved by proteases, it releases IGF-1. In addition, IGF-1 can be secreted locally in tissues, like the bone marrow, where it is produced by bone marrow stromal cells and osteoblasts, and by cancer cells. Thus IGF-1 may act in an endocrine, paracrine or autocrine manner (91). Numerous studies in cell lines and mouse models have established an important role of IGF-1 in myeloma pathogenesis (61, 92, 93). Klein and co-workers found that IL-6 and IGF-1 were equally important for myeloma cell growth and survival (92). Expression of the IGF-1 receptor (IGF-1R) on myeloma cells was found to be a negative prognostic factor (93-95), and serum concentration of IGF-1 was associated with shorter survival in MM patients (96). Upon binding of IGF-1 the IGF-1R goes through a conformational change, inducing kinase activity, leading to activation of the PI3K/Akt and MEK/ERK pathways (91). IGF-1 mediates proliferation, survival, homing and adhesion of myeloma cells (92, 97, 98). In addition, the growth factor activity of IL-6, heparin-binding EGF (HB-EGF) and HGF was found to be partly dependent on IGF-1/IGF-1R signalling (93).

1.2.7. The HGF/c-Met system.

HGF was first discovered in 1984 as a mitogen for rat hepatocytes (99). In 1989, cDNA for human HGF was cloned, and the structure was clarified (100, 101). In 1991, it was discovered that scatter factor (102), a motogen for epithelial cells, was identical to HGF (103, 104). HGF is now known as a mitogenic, motogenic and morphogenic factor, and has essential roles in human embryogenesis/development (105, 106). It is mainly secreted by mesenchymal cells,

and acts in a paracrine manner on epithelial cells. Knocking out HGF, or its receptor c-Met, is embryonically lethal due to impaired development of placenta and liver (105, 106).

HGF is synthesized as a single chain pre-pro-form of 728 amino acids. The first 31 amino acids are cleaved to form pro-HGF. Pro-HGF is then further cleaved between Arg494 and Val495 to form the biologically active form. Active HGF is a heterodimer, composed of a 69 kDa alpha-chain containing a hairpin domain and four kringle domains, and a 34 kDa serine protease (SP)-like beta-chain, linked by a disulphide bond (100, 101, 106) (Figure 3). Although the SP domain has lost its protease activity due to replacement of two amino acids in the active site, HGF is structurally similar to plasminogen, and its activation follows a similar pattern as the activation steps of the serine proteases of the coagulation cascade. HGF activator (HGFA), urokinase-type plasminogen activator (uPA), tissue plasminogen activator (tPA), coagulation factor XI and XII, plasma kallikrein and the membrane bound proteases matriptase and hepsin have all been shown to activate single chain HGF (107-113). HGFA, matriptase and hepsin are the most potent processors, the HGF-converting potency of HGFA being more than 1000 times that of uPA (108). HGFA is further described below.

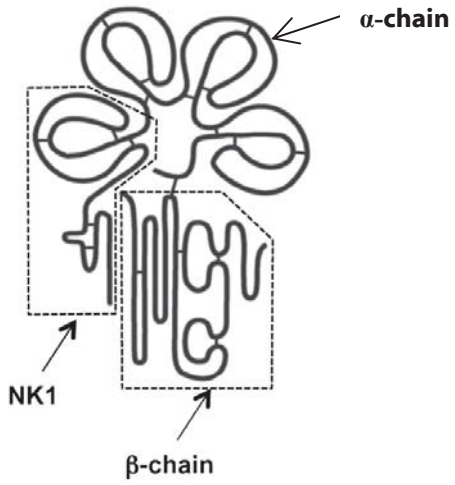

Figure 3. Schematic illustration of HGF. Modified by permission from Blackwell Publishing: J Gastroenterol Hepatol (106), c*opyright 2011.*

The naturally occurring splice variants of HGF named NK1 and NK2 consist of the N-terminal hairpin loop and one or two of the kringle domains, respectively (Figure 3). Both NK1 and NK2 can act either as agonists or as competitive antagonists (114). The synthetic splice variant NK4 is composed of the hairpin loop and four kringle domains and has antagonistic activity (115).

HGF is a heparan sulphate (HS)-binding protein, and numerous studies have confirmed that HGF binds to sulphated glycosaminoglycans (GAG), or GAG covalently bound to proteins, termed heparan sulphate proteoglycans (HSPG) (116, 117). In studies of the interactions between HS and growth factors, heparin has commonly been used as a substitute for HSPG, mainly due to structural and chemical similarity (the main difference is that heparin has a higher amount of sulphation) and greater availability. The main heparin binding site is located in the N-terminal hairpin loop of HGF (116, 118). By binding to HSPG, HGF is sequestered in the extracellular matrix of most tissues, mainly in its inactive form (119). HSPG can potentiate HGF activity (116, 120, 121), but may also have opposite effects on HGF activity depending on their concentration (122). The interaction between HGF and the most abundant HSPG on plasma cells, syndecan-1, is further described below.

HGFA. Activation of HGF in the bone marrow microenvironment is critical for HGF/c-Met signalling. One of the most potent activators is the factor XII-related serine protease HGFA (123). HGFA is a member of the kringle-containing serine protease super-family, and is mainly secreted by the liver, although extrahepatic expression has been reported in a number of normal and tumour tissues (124). It circulates in plasma as a single-chain 96-kDa pro-form, which is activated by thrombin, in the presence of negatively charged molecules, such as heparin, HS and chondroitin sulphate, in injured tissues and in tumours. The activated form of

HGFA consists of a heterodimer with a 66 kDa heavy chain and a 32 kDa light chain (125). Plasma kallikrein may further splice the HGFA heavy chain, resulting in a 34 kDa two-chain short form, which retains its enzymatic activity (125). An alternative mechanism for activation of pro-HGFA is by the kallikrein related peptidases 4 and 5 (126). Activation of HGFA leads to an increased heparin-binding capacity, possibly concentrating activated HGFA to cell surface HSPG (127). Since the major activator of HGFA is thrombin, it follows that the conversion of prothrombin to thrombin, i.e. activation of the coagulation cascade in injured tissues, is an important step in activation of the HGF/c-Met system.

The system is regulated by the HGF activator inhibitors (HAI) -1 and -2 and plasma protein C inhibitor (124, 128). HAI-1 and HAI-2 are both membrane bound serine protease inhibitors. The activity of HGFA is suppressed by reversible binding to cell surface HAI-1, but the HGFA-HAI-1 complex can also be released by metalloproteinase-mediated shedding of the HAI-1 ectodomain. So, while HAI-2 is a strong inhibitor, it is possible that HAI-1 not only works as an inhibitor, but also as a reservoir of HGFA at the cell surface (113, 124, 129, 130).

The role of HGFA in physiological and pathological conditions is not fully elucidated. Serum from HGFA knockout mice was unable to process pro-HGF, suggesting that HGFA is indeed the major activator of HGF in serum (131). Still, while knocking out HGF is embryonically lethal, no obvious developmental abnormalities were shown in $HGFA^{-/-}$ mice, clearly indicating that HGFA is redundant during tissue development, and can be compensated for by other proteases like matriptase and hepsin, acting in the local tissue environment (113, 131). On the other hand, HGFA has important roles in regeneration of injured tissue (113, 127). Several studies also indicate a role in tumour progression, and expression of HGFA has been observed in several tumour types, reviewed in (113). It has also been shown that myeloma

cells can express HGFA (132). HGFA may also activate pro-macrophage stimulating protein (MSP), a protein with structural homology to HGF (113).

c-Met.

The HGF receptor, c-Met, was cloned in 1984 (133). It is member of the scatter factor receptor family, together with the tyrosine kinase Ron, which is the receptor for MSP (134). c-Met is produced as a single chain precursor, which is cleaved by furin to form a 50 kDa extracellular alpha-chain and a 145 kDa transmembrane beta-chain, linked by a disulfide bond (133, 135). The extracellular portion of c-Met contains a Sema domain, to which semaphorin-type proteins can bind (134), a cysteine-rich Met-related-sequence (MRS) domain, and four Ig-like structures (136, 137) (Figure 4). High affinity binding between HGF and c-Met occurs via the α-chain of HGF and the Ig-like region of c-Met (138), independently of HGF activation. Low affinity binding occurs only after activation of HGF, by binding of the HGF β-chain to the Sema domain of c-Met (139, 140). While the alpha-chain of HGF is necessary for high affinity binding to the receptor (but does not activate the receptor), the low-affinity binding of the β-chain to the c-Met Sema domain is necessary for receptor dimerization and activation (135, 139). It is believed that HGF-induced c-Met activation is mediated by formation of a 2:2 complex where c-Met dimerization is primarily mediated by dimer formation of HGF (141).

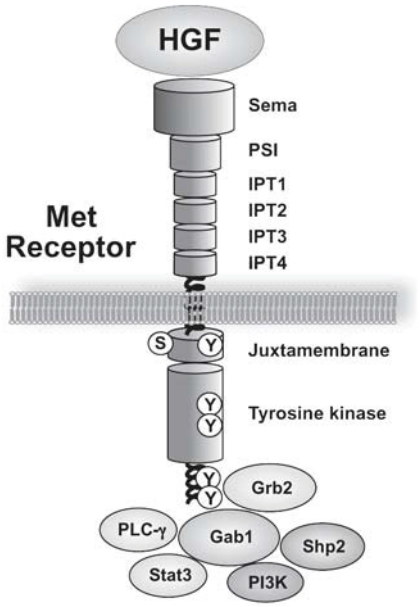

Figure 4. Schematic illustration of c-Met. Reprinted by permission from Blackwell Publishing: J Gastroenterol Hepatol (106), copyright 2011.

The intracellular portion of c-Met is composed of a juxtamembranous domain, a tyrosine kinase domain, and a carboxy-terminal (C-terminal) regulatory tail. The juxtamembranous domain with tyrosine 1003 is essential in downregulation of the receptor (142, 143). In the tyrosine kinase domain, two tyrosine residues (Tyr 1234 and Tyr 1235) regulate the kinase activity, while two other tyrosine residues (Tyr 1349 and Tyr 1356), located in the C-terminal regulatory tail, are essential for recruitment of downstream adapter molecules as described in the following section (105, 144).

HGF - c-Met signalling. Upon HGF binding c-Met is dimerized, and autophosphorylation occurs on tyrosine residues Y1234 and Y1235 in the activation loop of the tyrosine kinase domain, which induces kinase activity, while phosphorylation on Y1349 and Y1356 form a C-terminal multifunctional docking site. This phosphorylation of C-terminal tyrosine residues in

the docking site recruit scaffolding adaptor proteins, including growth-factor-receptor-bound protein 2 (GRB2)-associated binder 1 (GAB1) and α6β4-integrin. These adaptors act as supplementary docking platforms for further binding of intracellular signalling molecules, including GRB2, PI3K, p120 Ras-GTPase-activating protein (p120), phospholipase Cγ 1 (PLCγ1), SH2-domain-containing protein tyrosine phosphatase 2 (SHP2), Src, Src-homology-2 domain-containing transforming protein (SHC) and STAT3, summarized in (135). The scaffolding adaptor protein GAB1 is the most crucial substrate for HGF/c-Met signalling, and is responsible for most of the cellular responses to c-Met activation. Knocking out GAB1 is, like knocking out HGF or c-Met, embryonically lethal (145).

While the c-Met receptor with its docking site and scaffolding adaptor proteins is unique, its downstream signalling pathways are the same as those evoked by several other tyrosine kinase receptors. Downstream effectors of c-Met include the MAPK cascades, the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway, the Src/focal adhesion kinase (FAK) pathway, the NF-κB inhibitor-α (IκBα) – NF-κB complex (105, 135) and the STAT3 pathway. These are distinct but interacting cascades. As earlier described, several cytokines converge to activate common intracellular pathways.

The PI3K-Akt pathway. c-Met can activate PI3K either indirectly via activation of Ras, or directly. Direct activation occurs when the regulatory subunit p85 of PI3K binds to the docking site of c-Met, leading to recruitment of the catalytic subunit p110, whereby PI3K is activated. The main function of active PI3K is to generate phosphatidylinositol (3,4,5)-triphosphate ($PI(3,4,5)P_3$), which in turn recruits pleckstrin homology (PH)-domain containing molecules to the plasma membrane. One of these is the serine/threonine kinase Akt (Figure 5). Activated Akt may inactivate the pro-apoptotic protein BCL-2 antagonist of cell

death (BAD), and promote degradation of the pro-apoptotic protein p53. By these two mechanisms, Akt acts anti-apoptotic. Akt also inhibits apoptosis indirectly via the NF-κB pathway. Further, Akt inactivates glycogen synthase kinase 3β (GSK3β), which normally suppresses expression of the positive cell cycle regulators Myc and cyclin D1, and activates mTOR. By these mechanisms, Akt stimulates proliferation and cell growth. The PI3K – Akt pathway is one of the major oncogenic pathways, and aberrations involving components of this pathway are commonly seen in cancer. One example is loss of phosphatase and tensin homologue (PTEN), the $PI(3,4,5)P_3$ -phosphatase which converts $PI(3,4,5)P_3$ back to $PI(4,5)P_2$. PTEN is a tumour suppressor, and is absent in many tumours (146).

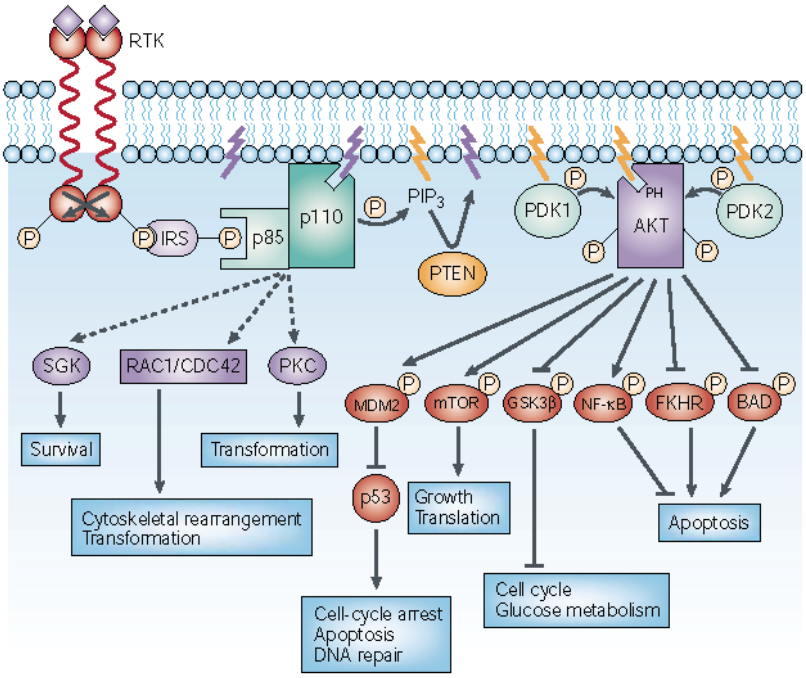

Figure 5. *Schematic illustration of the PI3K/Akt pathway. See text for details. Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Cancer (146), copyright 2002.*

The MAPK pathways. The MAPK subfamilies are characteristically composed of a series of phosphokinases, where the MAPK kinase kinase (MAPKKK) activates the MAPK kinase (MAPKK), which in turn activates the MAPK (Figure 6).

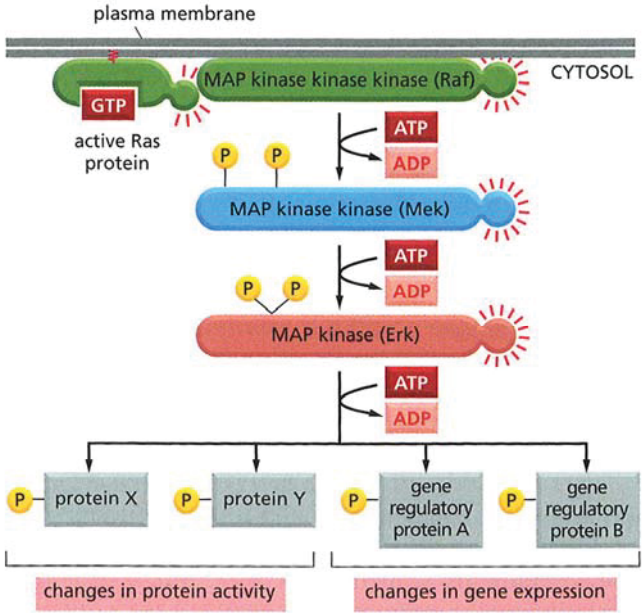

***Figure 6**. Schematic illustration of the MAPK pathway. See text for details. Copyright © 2008 From MolecularBiology of The Cell by Bruce Alberts, et al. Reproduced by permission of Garland Science/Taylor & Francis Books, Inc.*

The MAPK family includes the extracellular signal-regulated kinase 1 (ERK1) and ERK2, jun amino-terminal kinases (JNKs) and p38 pathways. Activation of the ERK pathway is triggered by Ras. Ras is activated by transition from the guanosine diphosphate (GDP) to guanosine triphosphate (GTP) state, and c-Met activates Ras through the GRB2 – son of sevenless (SOS) complex. This activation may occur directly or via an SHC adaptor. It may also occur via SHP2, which dephosphorylates the p120-binding site on GAB1, which normally deactivates Ras. Activated Ras recruits the serine/threonine kinase Raf, leading to a conformational change of Raf, which is thereby phosphorylated, and in turn activates MAPK-

ERK kinase (MEK)1 and MEK2, which in turn phosphorylates ERK1 and ERK2. The JNK pathway starts with Rac mediating phosphorylation of MEK kinase (MEKK)1, which leads to phosphorylation of MEK4, which leads to activation of JNK 1,2 and 3. The p38 pathway also starts with Rac mediating phosphorylation of MEKK, leading to phosphorylation of MEK3 and 6, leading to phosphorylation of p38α, p38β, p38γ and p38δ. The MAPK pathways are involved in cell proliferation, differentiation, transformation and apoptosis.

Other important pathways include the p120/STAT3 pathway, implicated in cell proliferation and differentiation. The Ras-Rac1-p21 activated kinase (PAK)-pathway is important for cytoskeletal rearrangement and cell adhesion, and the Src/focal adhesion kinase (FAK) pathway regulates cell adhesion and migration. The NF-κB system is a family of transcription factors that are kept inactive in the cytoplasm by the inhibitory IκBs. In response to tyrosine kinase activation, the PI3K- and the Src pathways mediate activation of the IκB kinase (IKK). IKK mediates degradation of the IκBs, leading to release of NF-κB, which translocates to the nucleus and stimulates transcription of mitogenic and anti-apoptotic regulators (105, 135).

Regulation of c-Met activity. HGF – c-Met activity may be enhanced by co-receptors, including HSPGs as described in the HGF and syndecan-1 sections (pages 22 and 36). CD44 is another transmembrane glycoprotein, which works as a linker between the extracellular matrix and the intracellular actin cytoskeleton (147). CD44 can collaborate with c-Met, and is under some conditions necessary for c-Met signalling (148). c-Met may also interact with semaphorin receptors (plexins), and when oligomerized with plexins, c-Met can be activated by semaphorins independently of HGF (134, 135).

Several protein-tyrosine phosphatases can hamper c-Met signalling by dephosphorylation of tyrosines in the catalytic or docking domain (135). c-Met signalling is also negatively regulated by the ubiquitin ligase Cbl, which binds to phosphorylated Y1003 in the juxtamembranous region of c-Met, resulting in c-Met ubiquitination, endocytosis and degradation, providing a mechanism for terminating c-Met signalling (143). Another mechanism for downregulation of c-Met is by activation of protein kinase C, leading to phosphorylation of Ser985 in the juxtamembranous domain of c-Met, which in turn leads to suppression of HGF-induced activation of c-Met (142). Finally, c-Met can be downregulated by ectodomain shedding.

c-Met shedding. Extracellular fragments of c-Met can be shed from the cell surface under physiologic conditions and in cancer (149-153). The membrane-bound metalloproteinases of the A Disintegrin And Metalloproteinase (ADAM) family, ADAM-10 (154) and ADAM-17 (155), have been proposed as mediators of cell surface shedding of c-Met, and several splice variants of c-Met have been described (149, 153, 156). There is compelling evidence from cancer cell lines and mouse models that a soluble extracellular fragment of c-Met can act as a decoy receptor and downregulate HGF/c-Met activity (138, 152, 156-158). Ectodomain shedding may downregulate c-Met by three different mechanisms. First, the soluble ectodomain can compete for HGF, and prevent HGF from interacting with c-Met at the cell surface. Second, the soluble ectodomain can interact with full size c-Met, preventing it from dimerization and activation. By these mechanisms, c-Met decoys may inhibit both HGF-dependent and HGF-independent c-Met activation (157). Third, shedding of the ectodomain may leave a surface-associated cytoplasmic remnant, which is subsequently detached from the membrane and degraded (152, 155). Contradictory data exist, though, since others have found that deletion of the c-Met ectodomain can lead to activation of the remaining tyrosine kinase

domain (159). Also, c-Met ectodomain fragments found in culture supernatant of mammary carcinoma cells could not compete efficiently with intact cellular c-Met for HGF binding (153). In conclusion, the biological effects of c-Met shedding may be different in different tumours and under different circumstances, but existing data suggest an important effect of c-Met shedding in down-regulation of its activity.

Effects of HGF/c-Met activity. The characteristic cellular responses to HGF - c-Met signalling in epithelial cells include "scattering" and invasion, induced by dissociation of cells and increased motility. By promoting cell survival, proliferation, morphogenesis, migration and angiogenesis, HGF/c-met signalling has essential roles in human embryogenesis, wound healing and tissue repair (105, 106). Efforts are made to utilize the positive effects of HGF in tissue regeneration, and HGF-treatment have also shown effectiveness in prohibiting development of fibrosis in various disease models including liver cirrhosis, lung fibrosis and dilated cardiomyopathy, reviewed in (106). Beyond the physiological roles, there is growing interest in the role of HGF/c-Met activity in tumour development. HGF/c-Met signalling is crucial in the event termed epithelial mesenchymal transition (EMT), a biological program characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility (160). EMT is considered a central mechanism for metastasizing of epithelial tumours, in the biological program termed "invasive growth" (161, 162). In this way mechanisms that were meant for embryonic development and tissue repair are adopted by cancer cells for invasion and metastasis.

HGF/c-Met activity is normally tightly regulated, by paracrine ligand delivery, ligand activation at the cell surface, and ligand-activated receptor internalisation and degradation (135). Deregulation of the HGF/c-Met pathway is common in cancer, and HGF and c-Met

expression and signalling have been demonstrated in several cancer types, including breast, colorectal, gastric, head & neck, liver, lung, pancreas, ovarian, renal, prostate, sarcoma and lymphoma (www.vai.org/met/) and (105). c-Met activation in cancer can be ligand-dependent or ligand-independent. Ligand-dependent deregulation may occur by paracrine activation – interactions between HGF, secreted by stromal cells, and c-Met, expressed by tumour cells, are central in the tumour-stromal interactions of several malignancies. It may also occur by or autocrine activation, as some tumours concomitantly secrete HGF and express c-Met (105, 106). An additional mechanism is by acceleration of the conversion step of HGF to its activated form. It was recently shown that upregulation of matriptase had oncogenic effects via the Akt - mTOR pathway, mediated by conversion of pro-HGF to active HGF and signalling through c-Met (163). Ligand-independent activation may occur by mutation in the kinase or juxtamembranous domains of the *MET* gene (105), which is the causative genetic disorder in hereditary renal papillary carcinoma (164). *MET* mutations have been shown also in other cancer types including lung cancer, hepatocellular carcinoma and gastric carcinoma (105, 165). Although *MET* mutations occur at a relatively low frequency, they provide evidence of the oncogenic potential of the HGF/c-Met pathway (164). Also, HGF-independent activation may occur through trans-activation of c-Met by other membrane receptors, including CD44, integrins, plexins, Ron and Fas. c-Met may also cooperate with other tyrosine kinase receptors in oncogenesis. It has been shown that the gene encoding c-Met is amplified in a subset of patients with non-small cell lung cancer resistant to the EGFR tyrosine kinase inhibitor gefitinib, and that c-Met can take over the activation of PI3K and ErbB3 from EGFR. In these instances, it would be necessary to inhibit both EGFR and c-Met to induce cell death (165, 166).

The HGF/c-Met signalling pathway as a target in cancer therapy. As one of the most frequently activated oncogenic proteins in human cancer (www.vai.org/met/) and (105), the HGF/c-Met pathway has become an increasingly attractive target in cancer treatment. Silencing the overexpressed MET gene in tumour cells was shown to suppress tumour growth and metastasis, and induced regression of established metastases in mouse models (167). Several inhibitors of the HGF/c-Met pathway are under preclinical and clinical development. Small synthetic molecules that inhibit c-Met tyrosine kinase activity comprise the largest group of agents currently under evaluation. Other approaches to inhibit HGF-c-Met activity include siRNA, ribozymes, neutralizing monoclonal antibodies (mAbs) directed against HGF or c-Met, soluble c-Met receptors, HGF-forms that resist proteolytic activation or its conformational consequences, and antagonists composed of selected domains of HGF (168, 169). Among the latter group, NK4 was the first described inhibitor. NK4 consists of the HGF N-terminal and the four kringle domains and is a competitive inhibitor of HGF-induced c-Met activation. In addition, anti-angiogenic properties of NK4 has been demonstrated, and NK4 have been shown to inhibit invasion and metastasis in several cancer types (170). NK4 also inhibits growth of myeloma cells (115). Other advanced drug candidates are mAbs against either HGF or c-Met. Most of them work by blocking HGF/c-Met binding, while the mAb DN-30 works by a different mechanism, by inducing ectodomain shedding and receptor degradation (152).

HGF and c-Met in multiple myeloma. HGF is secreted by bone marrow stromal cells as well as by hematopoietic cells of the myeloid lineage and mature neutrophils in the bone marrow microenvironment (171-173), but neither HGF nor c-Met are expressed by normal plasma cells (93, 174, 175). In 1996, Børset et al showed that malignant plasma cells can produce HGF, and also express c-Met (176). HGF may therefore interact with c-Met in a

paracrine or autocrine manner. Serum HGF levels are elevated in MM patients as compared to healthy individuals, and high levels are associated with poor prognosis (177-179). The levels of HGF are higher in the bone marrow than in the circulation of MM patients (180), suggesting that the bone marrow is the main source of the elevated serum HGF. Also, levels of HGF mRNA in crude bone marrow biopsies of MM patients are significantly higher than in healthy individuals (Tian et al, manuscript in preparation). HGF was the only growth factor among the 70 most upregulated genes in malignant compared to normal plasma cells, as assessed by gene array analysis (174). Others have confirmed upregulation of the genes encoding HGF (175) and c-Met (93, 175) in myeloma cells as compared to normal plasma cells, and in another study, HGF and c-Met were among the transcripted genes distinguishing MM from the related B-cell lymphoproliferative disorders chronic lymphocytic leukaemia and Waldenströms macroglobulinemia (181).

In vitro, HGF stimulates survival, proliferation, adhesion and migration of malignant plasma cells (63, 182-184), stimulate angiogenesis (185) and inhibit osteoblastogenesis (59). Besides its own actions as a growth factor, HGF may also potentiate the actions of IL-6 in proliferation and migration of myeloma cells (186). HGF and c-Met may thereby in many ways contribute to MM pathogenesis, and are considered as potential therapeutic targets also in MM (115) . However, the functional data on the actions of HGF and c-Met in MM are mainly derived from *in vitro* studies, and further studies in mouse models are warranted.

1.2.8. Syndecan-1

The syndecan family of HSPGs comprises four members, named syndecan-1 to -4 (187). Almost all cells express at least one of the syndecans, and they are the major source of cell surface HS. Syndecan-1 (CD138) is a type-I membrane protein with HS (and sometimes

chondroitin or dermatan sulphate) attached to its extracellular domain. The HS chains mediate attachment of heparin binding growth factors, and their structure is modified by heparanase and sulphatases. The short cytoplasmic tail of the core protein consists of a variable region (unique for syndecan-1), flanked by two conserved regions (that are identical between all syndecans). The C-terminal conserved region has a binding site for PDZ-domain containing proteins. These proteins are thought to connect syndecan-1 to signalling and cytoskeletal components (188, 189).

Syndecan-1 is expressed in epithelial cells, and in some lymphoid cells: It is the predominating HSPG on normal and malignant plasma cells, and is also present on pre-B cells (190). Its relative specificity for plasma cells has made it useful for immunomagnetic separation of plasma cells from bone marrow (191).

The syndecans bind a wide variety of molecules via their HS chains, thereby promoting adhesion between cells, and between cells and extracellular matrix (192, 193). They synergize with integrins to regulate cell adhesion (194). An important characteristic is also the ability to bind growth factors, thereby concentrating them at the cell surface (195). Syndecan-1 may also be shed from the cell surface, and by binding to soluble syndecan-1, growth factors may be sequestered/concentrated in the extracellular matrix (195). Several cytokines are known to bind to HS chains, such as FGF (196, 197), HGF (120, 122, 198), APRIL (199), VEGF (200), HB-EGF (64) and members of the bone morphogenetic protein (BMP) family (201). IGF-1 does not bind HS, but it has been shown that binding of IGFBP-3 to HS chains weakens its affinity for IGF-1, leading to local release of IGF-1, indicating that both cell surface-bound and soluble syndecan-1 also can contribute to local concentration of IGF-1 (202).

By presenting a growth factor for its receptor, HSPGs can facilitate receptor dimerization. This phenomenon is best characterized for the FGF receptor, which is dependent on HS for signalling (Figure 7) (196, 203). Further, upon binding to a ligand, syndecans on the cell surface may translocate to lipid rafts (204), which are detergent insoluble, cholesterol-rich microdomains of the plasma membrane that function as platforms for cellular signalling (205). Possible consequences/implications of this latter phenomenon for growth factor signalling in cells abundant of syndecan-1 are largely unknown.

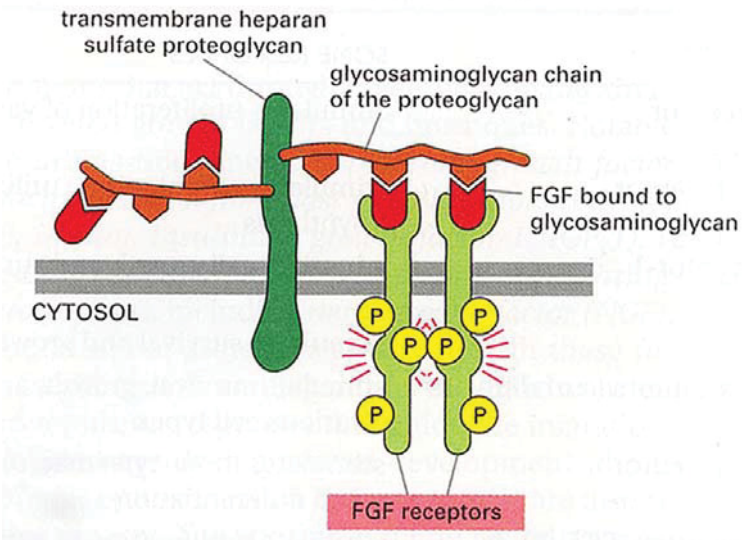

Figure 7. *By presenting a growth factor for its receptor, HSPGs can facilitate receptor dimerization. Copyright © 2002 From MolecularBiology of The Cell by Bruce Alberts, et al. Reproduced by permission of Garland Science/Taylor & Francis Books, Inc.*

Several mediators of syndecan-1 shedding have been described, including matrix metalloproteinase (MMP)1, MMP7 (matrilysin), MMP9, and uPA (206-209). Soluble syndecan-1 remains biologically active, and accumulate in the extracellular matrix of the bone marrow, where it can act as a reservoir for growth factors (195). These reservoirs are thought to play a role in promoting myeloma pathogenesis, and possibly contribute to regeneration of

tumour after chemotherapy. There is a gradient of syndecan-1 concentration between bone marrow and peripheral blood, levels being higher in bone marrow than in serum of MM (180). High serum levels of soluble syndecan-1 in MM patients are associated with a poor prognosis (210, 211).

We have taken particular interest in the interaction of syndecan-1 with HGF and c-Met in MM. It has previously been shown that soluble syndecan-1 can be measured at high levels in the bone marrow of MM patients (122, 180), and that HGF and syndecan-1 can exist as a complex (122). Syndecan-1 is targeted to the uropod of polarized myeloma cells where it can sequester HS-binding proteins (198), and it has been shown that syndecan-1 promotes HGF/c-Met signalling (120). This phenomenon has mainly been explained by a syndecan-1-mediated concentration of HGF at the cell surface, presentation of HGF to c-Met and facilitation of receptor dimerization, in a mechanism parallel to the way in which FGFR dimerization is facilitated by HSPG (Figure 7) (196, 203). FGF signalling requires not only FGF-HS binding, but also FGF receptor – HS interaction (212), and a similar mechanism has been suggested in the case of HGF signalling, where HS may interact with both HGF and c-Met (118, 137, 213). In contrast, others have demonstrated little or no heparin binding capacity of c-Met (117). Recently, it was shown that heparanase, an enzyme that cleaves HS chains but also enhances syndecan-1 synthesis and shedding (206, 214, 215), in addition may stimulate HGF expression, the activity of which is further enhanced by interaction with shed syndecan-1 (216). Syndecan-1-dependent growth of myeloma cells stimulated by other heparin-binding growth factors, such as EGF and APRIL, has also been demonstrated (64, 199).

Thus, syndecan-1 regulates and promotes the activity of several myeloma-relevant growth factors, acting both on the cell surface and within the extracellular matrix of the bone marrow

environment. Since, within the bone marrow, syndecan-1 is almost exclusively expressed by plasma cells, it would theoretically be an ideal therapeutic target in MM (217). Antibodies against syndecan-1 can be used for targeting cytotoxic agents to myeloma cells, exemplified by murine/human syndecan-1-specific mAbs conjugated with cytotoxic maytansinoid derivates which have shown efficacy *in vitro* and *in vivo* (218). Antibodies against syndecan-1 could also be used to stimulate immune-mediated myeloma cell killing (219, 220), or the syndecan-1 molecule itself could be the target. Knocking down syndecan-1 expression by RNA interference, as well as altering the HS structure, inhibited growth of primary myeloma tumours in severe combined immunodeficiency (SCID) mice (217, 221). Syndecan-1 knockdown cells formed fewer focal subcutaneous tumour lesions when injected intravenously into mice, and syndecan-1 knockdown tumours exhibited lower levels of VEGF and reduced angiogenesis compared with tumours expressing normal syndecan-1 levels (221). Inhibition of heparanase, which enhances syndecan-1 synthesis and shedding, also inhibited myeloma growth *in vivo* (217).

2. AIMS

The first aim of this work. The results of several *in vitro* studies point to HGF and its tyrosine kinase receptor c-Met as important contributors in MM pathogenesis. Most studies have been carried out in myeloma cell lines, and it is important to gain more knowledge about the expression of these factors in patients with MM. Thus, the first aim of this work was to contribute to knowledge about expression of c-Met, HGF and HGFA in patients with malignant plasma cell disease. More specifically, we wanted to investigate:

1) whether HGF and c-Met are present in bone marrow and extramedullary tumour biopsies from patients with monoclonal plasma cell disease and whether c-Met is activated, using a phospho-specific anti-c-Met antibody.

2) whether the soluble form of c-Met can be detected in serum and bone marrow plasma of MM patients, and whether the concentration is different than in healthy individuals.

3) whether HGFA can be detected in serum and bone marrow plasma of MM patients, and whether the concentration is different than in healthy individuals.

The second aim of this work was to expand the knowledge about HGF/c-Met/syndecan-1 interaction. It has previously been shown that syndecan-1 promote c-Met signalling. This has mainly been assigned to syndecan-1-mediated concentration of HGF at the cell surface, and to presentation of HGF to c-Met with facilitation of receptor dimerization. The syndecans have previously also been shown to localize in lipid rafts, and to mediate raft dependent endocytosis. We wanted to investigate whether HGF and c-Met are localized in lipid rafts together with syndecan-1, and whether lipid raft localization, and possibly raft dependent endocytosis, affects c-Met signalling.

The third aim of this work. Dependence on the bone marrow microenvironment and cytokines is one side of MM pathogenesis. Cytogenetic aberrations in the myeloma cells are another side, and the third aim of this work was to examine the prevalence of cytogenetic aberrations in Norwegian MM patients. We wanted to investigate whether there was a correlation between the defined cytogenetic abnormalities and clinical parameters at diagnosis. The final aim is to examine a possible prognostic impact of the defined genetic aberrations in terms of progression free and overall survival. The survival analysis will start by the end of 2011, and in this work we therefore report only findings at diagnosis.

3. MATERIAL AND METHODS

3.1. Statement of approval

All the studies were approved by the Regional Ethics Review Board, and were performed according to the declaration of Helsinki.

3.2. Patient samples

Paper I – III includes serum and biopsies from patients diagnosed with MM, MGUS or solitary plasmacytoma in Central Norway from January 1996 to December 2005.

For paper I, biopsies from patients with MM, MGUS and solitary plasmacytoma were identified from registered diagnostic codes at the Department of Pathology, St Olav's Hospital, Trondheim. The biopsy material includes bone marrow biopsies, bone marrow clots from aspirates, and biopsies from plasmacytomas. During this time period, bone marrow biopsies were not routinely performed in all MM patients in this region. Thus, the study does not include all MM patients diagnosed in Central Norway in 1996-2005, but a selection based on the availability of adequate amount and quality of paraffin-embedded biopsy material.

Paper II and III include serum and bone marrow plasma samples from MM patients diagnosed at St Olav's Hospital during the same time period. The samples represent a selection of patients based on availability of serum and bone marrow plasma that had been frozen at the time of diagnosis, before initiation of treatment.

Paper IV presents in vitro studies, mainly in human myeloma cell lines. The study also includes primary myeloma cells from freshly separated CD138-positive cells from four patients with MM.

The study described in Paper V is performed in another cohort of patients, and includes bone marrow samples from Norwegian MM patients examined by FISH from January 2006 to December 2010. The study includes all Norwegian MM patients from whom a bone marrow sample was sent for FISH analysis during this time period, provided that adequate amount and quality of bone marrow was available and that the FISH analysis was technically successful. Patients from 23 Norwegian hospitals were included. Plasma cells were purified by 3 different methods: In 2006 – 2008 the analyses were performed on bone marrow smears or smears of mononuclear cells, and the plasma cells were identified by antibodies against Ig kappa and lambda. This is a time consuming method, and from 2008 the analyses were performed on plasma cells after automated CD138 separation, which has been the standard since.

3.3. Experimental procedures

Immunohistochemistry for CD138 (syndecan-1), HGF, c-Met and phospho-c-Met on sections of paraffin-embedded biopsies was performed as detailed in Paper I. As very few earlier publications present immunohistochemical studies of HGF in bone marrow, we first validated the method in a pilot study as described. ***Enzyme-linked immunosorbent assays (ELISAs)*** were used for the measurement of activated HGFA, HGF and soluble c-Met in serum and bone marrow plasma, as detailed in Papers II and III. As the c-Met ELISA had been validated only for use in cell culture supernatants by the manufacturer, we first performed a pilot study in order to validate the ELISA kit for analyses in serum, and to confirm that it was able to detect the extracellular portion of c-Met. ***Confocal microscopy*** was used for studies on cellular distribution and colocalization of HGF, syndecan-1 and c-Met, as detailed in Paper IV. ***Flow cytometry*** was used for cell surface detection of HGF, c-Met and syndecan-1 as described in Paper IV. ***Immunoblotting*** was used for detection of non-phosphorylated and

phosphorylated proteins after stimulation with cytokines with or without pharmacological inhibitors, as described in Paper IV. Complexes between c-Met and syndecan-1 were detected by ***immunoprecipitation*** followed by Western Blot, as described in Paper IV. Chromosomal abnormalities (Paper V) were detected by ***interphase FISH***.

3.4. Statistics

Pearson's χ^2 or Fisher's exact tests were used for between-group comparisons of discrete variables. Comparisons between groups for continuous variables were performed using Student's T-test or Mann Whitney U test. Correlations between two parameters were estimated by Spearman's rank correlation analysis. Survival between groups was compared by the log rank test. For analysis of immunohistochemical staining, the inter-observer agreement was estimated by Cohen's kappa statistics. Kappa between 0.4 and 0.6 was considered as a moderate agreement, kappa between 0.6 and 0.8 as a substantial agreement, and kappa >0.8 as an excellent agreement (222). In 2 x 2 tables with small or zero values (Paper I), exact p-values and exact confidence intervals for OS were computed using StatXact 8 (Cytel Inc.Cambride, MA, USA). All other statistical calculations were performed by SPSS 14.0 - 16.0 (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at $p = 0.05$. All p-values were 2-tailed.

4. MAIN RESULTS/SUMMARY OF THE WORK

Paper I

In this study we aimed to examine whether HGF and c-Met are present in bone marrow and extramedullary tumour biopsies from patients with monoclonal plasma cell disease and whether c-Met is activated, using a phospho-specific anti-c-Met antibody. Expression of HGF, c-Met and phospho-c-Met was studied by immunohistochemistry in biopsies from 80 patients with MM, MGUS and solitary plasmacytomas. We found cytoplasmic staining for HGF in the plasma cells in 58 of 68 biopsies from MM patients (85%), but also in biopsies from nine of ten healthy individuals. We found membranous staining for c-Met in 25 of 63 MM patients (40%), and in none of ten healthy individuals. Membranous staining for phospho-c-Met was found in biopsies from 15 of 21 c-Met-positive MM patients. Thus, this study indicates that c-Met is a factor that discriminates normal from malignant plasma cells, and that the HGF/c-Met system is activated in MM patients.

Paper II.

Conversion of pro-HGF to its active form is a critical limiting step for its biological effects. In this study we aimed to examine the levels of one of its most potent activators, HGFA, in serum and bone marrow plasma of patients with MM. The activated form of HGFA was measured by ELISA in serum (n = 49) and bone marrow plasma (n = 16) from MM patients, and in serum from healthy controls (n = 24). The median concentration of activated HGFA in MM and control sera was 39.7 ng/mL (range 6.2 - 450.0) and 17.6 ng/mL (range 4.8 - 280.6), respectively. The difference was statistically significant (p=0.037). The median concentration of activated HGFA in bone marrow plasma was 6.1 ng/mL (range 3.5 - 30.0). In conclusion, we found that the concentration of the activated form of HGFA was elevated in serum from

patients with MM compared to healthy individuals, providing a possible mechanism for increased activation of HGF in MM.

Paper III

A soluble extracellular fragment of c-Met may function as a decoy receptor and downregulate the biological effects of HGF and c-Met. In this paper, we aimed to examine serum levels of soluble c-Met in MM patients and healthy individuals, and to investigate a possible relationship with clinical disease parameters and survival. The concentration of c-Met and HGF were measured by ELISA in serum (n = 49) and bone marrow plasma (n = 16) from MM patients, and in serum from healthy controls (n = 26). The median serum concentration of soluble c-Met was 186 ng/mL (range 22-562) in MM patients and 189 ng/mL (range 124-397) in healthy individuals. There was a significant negative correlation between serum c-Met and disease stage, bone marrow plasma cell percentage, and serum concentration of M-protein. In conclusion, we found equal median concentration of soluble c-Met in MM patients and healthy individuals, but still there was a negative correlation between serum soluble c-Met and parameters of disease burden in MM patients.

Paper IV

In this paper we aimed to study the interactions between HGF, c-Met and syndecan-1 in MM. It has previously been shown that syndecan-1 promote c-Met signalling. The syndecans have also been shown to localize in lipid rafts, and to mediate raft dependent endocytosis. We wanted to investigate whether HGF and c-Met are localized in lipid rafts together with syndecan-1, and whether lipid raft localization, and possibly raft dependent endocytosis, affects c-Met signalling. We studied cell lines and primary myeloma cells by confocal

microscopy, flow cytometry, immunoprecipitation and Western Blot. We found that c-Met can exist as a complex with syndecan-1 in myeloma cells, and that c-Met is concentrated to the uropod of myeloma cells together with syndecan-1. We also found that HGF, c-Met and syndecan-1 are located to lipid rafts in the plasma membrane of myeloma cells. Disruption of lipid rafts by methyl-β-cyclodextrin inhibited HGF-mediated phosphorylation of Akt, but not phosphorylation of c-Met and ERK 1/2. Inhibition of dynamin by Dynasore inhibited endocytosis and reduced HGF-induced phosphorylation of Akt, whereas phosphorylation of c-Met and ERK1/2 was unaffected. This study indicates that binding of HGF and c-Met to syndecan-1 and localization in lipid rafts, followed by raft dependent endocytosis, is important for HGF-induced Akt signalling in myeloma cells.

Paper V

Detection of cytogenetic abnormalities by fluorescence *in situ* hybridization (FISH) yields prognostic information in MM. In this study we examined the prevalence of the most common primary translocations and deletions/amplifications in 250 Norwegian MM patients, of whom 214 were previously untreated. The final aim of the study is to examine a possible prognostic impact of the defined genetic aberrations in terms of progression free and overall survival. The survival analysis will start by the end of 2011, and in this work we therefore report only findings at diagnosis. FISH was performed on CD138 separated cells or with cytoplasmic-immunoglobulin-FISH on mononuclear cells to detect *IGH* split, del13q, del17p, del1p and 1q amplification. When an *IGH* split was found, FISH was performed for t(4;14), t(11;14), t(6;14) and t(14;16). The results are summarized in table 3. There was no correlation between any of the *IGH* translocations and del13q, del17p or chromosome 1 abnormalities, but there was a strong correlation between del13q and del17p ($p = 0.001$), and between del1p and amp1q ($p <$

0.001). Clinical information was available in 135 patients. In these patients there were no significant correlations between genetic and immunological or clinical features.

***IGH* split**	**t(4;14)**	**t(11;14)**	**t(6;14)**	**t(14;16)**	**t(?;14)** [1]	**Del13q**	**Del17p**	**Del1p**	**Amp1q**
45%	14%	16%	1%	2%	12%	35%	19%	10%	34%

Table 3. *Frequency of cytogenetic aberrations in Norwegian MM patients.*

[1] *Patients in whom an IGH split was found, but the translocation partner was not identified. In a majority of these patients there were not enough material to perform analysis of t(6;14) and t(14;16).*

5. DISCUSSION

5.1. Methodological considerations

Results and interpretation are critically dependent on the quality of the methods used, and this chapter will focus on some important methodological considerations of this work.

Patient samples and myeloma cell lines. As described in the Methods section, the immunohistochemistry (Paper I) and the serum studies (Paper II and III) do not include all MM, MGUS and plasmacytoma patients diagnosed in Central Norway in 1996-2005, but a selection based on availability of biopsy material, serum and/or bone marrow plasma. During this time period, bone marrow biopsy was not routinely performed in all MM patients. There is therefore a possibility that the immunohistochemistry study comprises a selection of patients in whom there were particular differential diagnostic considerations, leading the physician to perform a bone marrow biopsy in addition to a bone marrow smear. Therefore, we cannot state that this population is representative of the general Norwegian MM population. Similar considerations apply to the MGUS patients of the immunohistochemistry study: the samples include only a small selection of patients in whom bone marrow biopsies were performed. This might have selected patients in whom the distinction between MGUS and MM was difficult. Finally, we cannot exclude sampling bias caused by selection of patients who where diagnosed and treated at a University Hospital.

A strength and weakness of the studies presented in Paper I – III lies in the long follow up time for the patients. Including patients diagnosed as far as 15 years ago gives opportunity for long time follow up. However, as introduction of new drugs has improved the overall prognosis during the last decade, a patient diagnosed in 1996 is not necessarily comparable with one diagnosed in 2005. This may affect survival analysis, as further discussed below. β2-

microglobulin was not routinely analysed in the MM patients during the first years, making retrospective ISS staging incomplete. Further, the retrospective nature of these studies leaves us without knowledge about cytogenetic changes in the patients.

Paper V includes a different cohort of MM patients from 23 Norwegian Hospitals from whom bone marrow samples were sent for FISH analysis from January 2006 to December 2010. All Norwegian MM patients who were analysed by FISH during this time period were included, provided a successful FISH analysis was performed. Although this cohort includes patients from several local hospitals there is an over-representation of patients from University Hospitals, which might lead to a selection, particularly of younger patients.

For paper IV we worked with the human myeloma cell lines (HMCL) INA-6 and CAG. HMCLs grown in monoculture in the laboratory are central research tools in most preclinical studies in MM, as isolated primary myeloma cells only rarely survive outside the bone marrow microenvironment. HMCLs differ significantly from primary myeloma cells. Most HMCLs are established from extramedullary manifestations in relapsed patients, often from pleural effusions, or from blood in plasma cell leukaemia, representing cells that are independent of the bone marrow microenvironment (223, 224). During cell line establishment, a clonal selection of rapidly proliferating cells may also occur (225). Thus, these cells are typically more proliferative, and exhibit genetic features of highly aggressive disease, with secondary genetic changes rarely encountered in newly diagnosed MM patients. The limitations with HMCLs as a tool for studies of factors thought to be important in the bone marrow microenvironment are obvious. Still, HMCLs have proven useful in providing simplified systems for studies on biological and molecular mechanisms for central functions

of myeloma cells (223, 226), and have provided essential knowledge that have during the years also contributed to development of new therapy (227).

We also worked with variants of the EBV-transformed B-lymphoblastoid cell line ARH-77, which was established from a patient with plasma cell leukaemia, and which does not express syndecan-1 (192). ARH-77^{syn-1} and ARH-77^{A5P3} stably express syndecan-1, while ARH-77^{neo} expresses only control vector (193, 228). Although these cell lines are useful tools that have earlier been used in several studies on the function of cell-bound syndecan-1, they are not true myeloma cell lines, making it even more obvious that observations made in these cells are not necessarily valid for the *in vivo* situation in MM, and have to be interpreted with caution.

Because of these considerations, we also studied freshly isolated primary myeloma cells when this was possible. Primary myeloma cells were isolated from bone marrow aspirates by immunomagnetic CD138 separation (191). This was done "by hand" using immunomagnetic beads before our laboratory in 2008 had access to automated CD138 separation (RoboSep©). The high specificity of CD138 (syndecan-1) for plasma cells in the bone marrow has made it to the most common tool for separation of plasma cells (191). CD138 has also become the most common way of identifying plasma cells on histological examination (229). However, it should be noted that a CD138-negative subpopulation of myeloma cells with more immature features and higher proliferative potential have been described, which will be excluded in all research performed on CD138-selected cells (230). Kappa/lambda staining is an alternative way of identifying plasma cells in immunocytochemical/-histochemical studies, that would also include a possible CD138-negative population, and could be considered for future studies.

Immunohistochemistry is a semi-quantitative method. It is common in research publications to present a rather detailed analysis of immunohistochemical staining by grading the number of stained cells and/or the staining intensity, and sometimes to combine these parameters to a "staining index". After testing of the antibodies and experience with their staining characteristics, we decided to modify this approach for the study presented in Paper I. The immunohistochemical staining was performed by the same, very experienced, technician in the same laboratory. The scoring was performed independently by two researchers. The specificity of the staining was tested by three types of controls: Omitting the primary antibody, replacing it with non-immune serum, and pre-adsorbing the antibody with corresponding antigen peptide. For c-Met and phospho-c-Met, we encountered problems with non-specific staining in some sections, and the non-specific staining was difficult to discriminate from a weak cytoplasmic staining of the cells. We therefore chose to define a *cell* as positive for c-Met or phospho-c-Met only if there was a clear membranous staining. We also decided to define a *biopsy section* as positive if 10% or more of the cells were positive, with no further grading/quantification than negative or positive. The strict but rather coarse definitions used may underestimate the percentage of positive cells and the number of positive biopsies. However, we found it to be the most robust way to categorize and interpret the results.

Confocal microscopy. Some methodological issues, like non-specific antibody staining, are common to immunohistochemistry and confocal microscopy, making rigorous control measures necessary. Even with adequate controls for non-specific staining, there will remain a risk for cross-reactivity of the primary antibody with similar epitopes on other proteins, especially when using short peptide antibodies. The interpretation of results will always comprise some degree of subjectivity, and there is a possibility for bias when capturing

images. By examining the same phenomenon by more than one method, exemplified by the combination of confocal studies with immunoprecipitation and flow cytometry for the studies on c-Met – syndecan-1 interaction in Paper IV, we have efforted to make the results more robust.

Serum analyses. Only serum from peripheral blood, and plasma from bone marrow, were available for analyses in the studies presented in Paper II and III. Optimally, we would have analyzed either serum or plasma from both localizations. Although the difference between measurements in serum and plasma in many cases are negligible, there can be significant differences. In control experiments, we found lower concentrations of activated HGFA in plasma than in serum from blood samples drawn at the same time from the same patient. Because this phenomenon was also confirmed by the ELISA manufacturer (IBL, Japan), we did not perform a full control series to quantify the serum/plasma difference, but settled with the fact that we could not compare bone marrow plasma samples with serum samples. Results by this ELISA were otherwise reproducible with variation coefficients <10%.

This chapter has focused only on some important methodological considerations of this work. However, the methodological problems mentioned are universal and shared by all investigators in the field. There are possible uncertainties with all laboratory methods, underscoring the importance of adequate controls. By relevant control measures and, when possible, by studying the same phenomenon by more than one method, we have tried to partly overcome these uncertainties.

5.2. General discussion, conclusion and future directions

MM pathogenesis is multifaceted and involves intrinsic properties of the myeloma cells, including the disease-defining primary translocations and later occurring genetic events, but also complex interactions with several cell types and cytokines in the bone marrow microenvironment. While Paper V focuses on cytogenetic aberrations, the main focus of this work lies on the cytokine HGF and its receptor tyrosine kinase c-Met.

5.2.1. Expression of HGF and c-Met in the bone marrow of MM patients.

HGF and its receptor c-Met are established as mediators of growth, survival, adhesion and migration of myeloma cells *in vitro* (63, 182-184). HGF/c-Met signalling can also contribute to angiogenesis (175, 180) and inhibit osteoblastogenesis (59), and may therefore by multiple means contribute to MM pathogenesis. Most studies have been carried out in myeloma cell lines, and this work aimed at gaining more knowledge about the expression of these and related factors in patients with MM. c-Met is upregulated in MM patients compared to healthy individuals at the mRNA level (93, 175). In this work, we have shown that c-Met also at the protein level is a factor that distinguishes malignant from normal plasma cells. We also show that c-Met exist in its phosphorylated state in a proportion of MM patients, supporting that the HGF/system is active in MM (Figure 8).

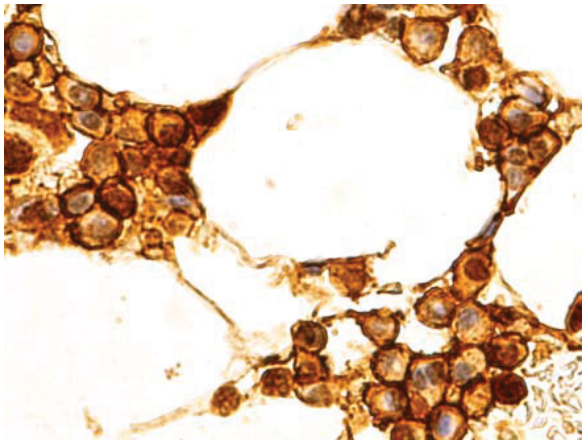

Figure 8. Bone marrow section showing membranous staining for phosphorylated c-Met in the plasma cells of a patient with MM. Enlarged section from photography with original magnification x 600. Photography by A Bofin, Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, NTNU, Trondheim.

These findings are significant both from a biological and a clinical point of view: First, they add substance to earlier *in vitro* data on the effects of HGF/c-Met signalling in myeloma cell lines, and in this way add to knowledge about myeloma biology. Second, they support that the HGF/c-Met axis should be evaluated as a therapeutic target in MM, and that immunohistochemistry could be a method for identifying patients who are candidates for HGF/c-Met targeted therapy. However, before immunohistochemical analyses of c-Met and phospho-c-Met could be introduced as methods for the clinical setting, the methods will have to be further validated, and one should search for antibodies with a better signal-to-noise ratio than the ones used here, to make evaluation feasible.

We found positive staining for c-Met and phospho-c-Met in the nucleus of the myeloma cells in some samples (Figure 9). Nuclear localization of c-Met, or a carboxy-terminal fragment of c-Met, has previously been described in other cancer cell types (231, 232). Because the pre-

defined criterion for a c-Met- or phospho-c-Met-positive case in this study was the presence of a clear membranous staining, we did not include nuclear staining in the analysis, but a possible translocation of c-Met to the nucleus in MM is a new finding that should be subject to future studies.

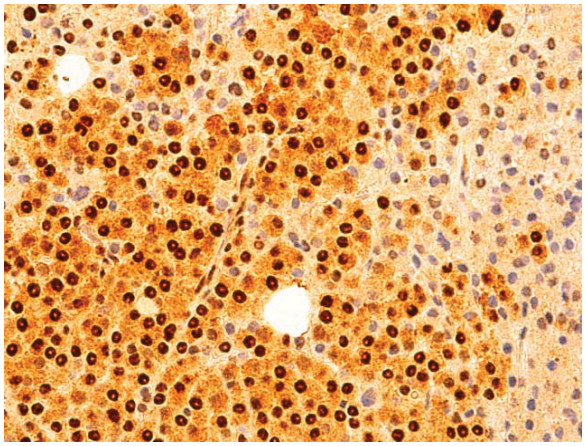

Figure 9. Bone marrow section showing nuclear staining for c-Met in the plasma cells of a patient with MM. Original magnification x 400. Photography by KF Wader.

Malignant plasma cells can produce HGF, but HGF is also secreted by many other cell types in the bone marrow, including stromal cells and cells of the myeloid lineage. Immunostaining for HGF could therefore be expected be found in several cell types and in the extracellular matrix. In contrast, we found HGF staining concentrated to the myeloma cells, with a comparatively very week staining of the background and other cell types (Figure 10).

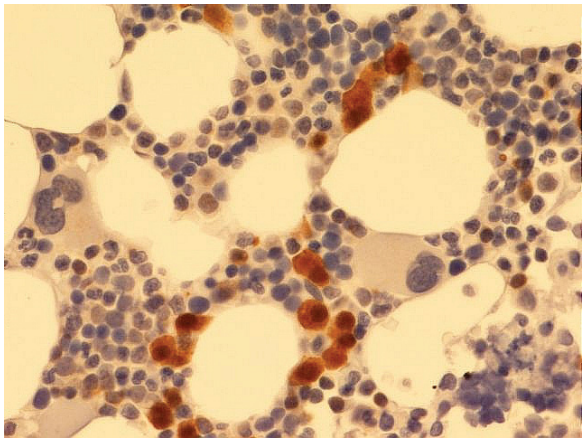

Figure 10. Bone marrow section showing HGF staining concentrated to myeloma cells, with a comparatively week staining of the background and other cell types. Original magnification x 600. Photography by KF Wader.

We also found HGF immunoreactivity of normal plasma cells, seemingly in contrast to earlier gene expression data which showed that HGF is not expressed by normal plasma cells (174, 175). A reasonable explanation of this contradictory finding is that HGF is concentrated to the plasma cells through binding to syndecan-1. A general impression from the microscopy part of this study was that the HGF-staining everywhere appeared as a mirror of the syndecan-1 (CD138) staining. In this scenario, HGF would bind both to normal and malignant plasma cells, although it would have biological consequences only in malignant cells that express the c-Met receptor. In conclusion, this study supports that bone marrow plasma cells are richly supplied with HGF, but immunohistochemistry will probably not prove useful as a method for discriminating myeloma patients with HGF-producing plasma cells from those who are soaked in HGF from paracrine sources.

5.2.2. Syndecan-1, lipid rafts and Akt signalling

Previous studies have shown that HGF can exist in a complex with syndecan-1, and bind to syndecan-1 at the surface of myeloma cells (120, 122, 198). In the present work (Paper IV)

we have shown that also c-Met can exist in a complex with syndecan-1, which would lead to a tightly bound ternary complex between HGF, c-Met and syndecan-1 in MM cells (Figure 11). Syndecan-1 has previously been shown to localize in lipid rafts, which are cholesterol-rich microdomains of the plasma membrane that function as platforms for cellular signalling (204, 205, 233). We found that HGF and c-Met colocalize with syndecan-1 in lipid rafts, and further, this raft localization (followed by raft dependent endocytosis) was important for HGF/c-Met signalling through the PI3K/Akt pathway. Thus, a mechanism by which syndecan-1 promotes Akt signalling in MM cells may be recruitment of c-Met to these signalling microdomains (Figure 11). In this way c-Met signal transduction seem to be assisted not only by functional interaction with signalling amplifiers, but also by structural and topographical regulation at the plasma membrane.

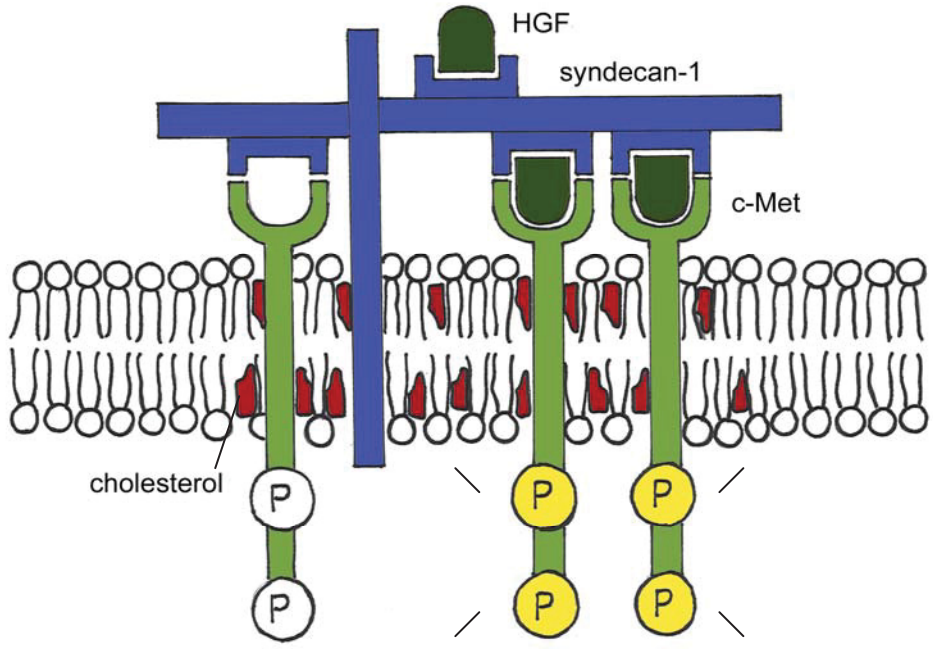

Figure 11. Schematic illustration of syndecan-1, HGF and c-Met in a lipid raft domain of the plasma membrane. See text for details. Illustrated by KF Wader.

So, while recent years' research on syndecan-1 in MM mainly has focused on the shed, soluble form of syndecan-1 (195, 234), our data suggest an important role also for cell surface-bound syndecan-1. Akt-signalling is an important pro-survival factor in MM cells (90, 235), and the relevance of our findings is mainly a contribution to insights into the mechanisms by which HGF, c-Met and syndecan-1 interact to promote HGF/c-Met signalling through Akt in myeloma cells. Hopefully improved knowledge about such basic mechanisms in the future can contribute to identification of ways to inhibit the HGF/c-Met axis. An equally attractive target for MM therapy could be syndecan-1, as a promoter of the activity of several myeloma-relevant growth factors.

5.2.3. Activation of HGF

For biological activity, HGF has to be activated from its pro-form by proteolytic cleavage. HGFA is one of the main activators of HGF (113), and we have shown in this work that serum levels of activated HGFA are higher in myeloma patients than in healthy individuals of the same age. This finding points to another mechanism by which HGF/c-Met activity may be enhanced in multiple myeloma - by increased level and/or activity of one of its main activators. The fact that HGFA is mainly activated by thrombin (113, 125) poses an interesting connection between activation of the coagulation system and activation of a tumour-promoting cytokine. Further, it is interesting that HGFA earlier has been shown to bind to HSPG (127) and that its activation requires the presence of negatively charged molecules, such as heparin, HS or chondroitin sulphate (125). In that way, not only HGF, but also activated HGFA, could possibly be sequestered in the bone marrow and concentrated to myeloma cells mainly via syndecan-1. It has also earlier been shown that myeloma cells may produce HGFA (132). We do not know whether the malignant plasma cells are the source of the elevated serum concentration of activated HGFA in myeloma patients, or if other factors,

like an activated coagulation system, may contribute. It is a draw back of our study, that only serum from peripheral blood, and bone marrow plasma, were available for analysis. The assay we used measures lower HGFA concentrations in plasma than in serum, and thus we could not reliably examine whether there was a difference between the levels of HGFA in the bone marrow and in the peripheral blood. Several other activators of HGF exist, and the role of the most potent ones, matriptase and hepsin, would be relevant to study in MM. It would also be relevant to examine the expression and function of the HGFA inhibitors, especially HAI-1, in MM.

5.2.4. Down-regulation of c-Met activity by decoy receptors

HGF/c-Met activity may be regulated in several ways. One way of down-regulating its activity is by decoy c-Met receptors. Compelling evidence support that shedding of the c-Met ectodomain can hamper HGF/c-Met signalling, by different mechanisms as described in the introduction (page 33). Thus, the soluble ectodomain can compete for HGF, and prevent HGF from interacting with c-Met at the cell surface. The soluble ectodomain can also interact with full size c-Met, preventing it from dimerization and activation. Shedding of the ectodomain also leave a surface-associated cytoplasmic remnant, which is subsequently degraded. Serum levels of c-Met may therefore be of relevance in MM patients. In our study the median serum concentration was not different in MM patients than in healthy individuals, but serum levels of soluble c-Met still showed a consistent negative correlation with several parameters of disease burden: bone marrow plasma cell percentage, ISS stage and concentration of serum M protein. Although these correlations should be regarded only as observations that give no information about causality, they may indicate a biological relevance of c-Met shedding in multiple myeloma, which hopefully will be elucidated in the future.

5.2.5. General remarks

Papers I, II and III are the first ones to comprehensively study the concentration in serum and the expression in biopsies, respectively, of the examined factors in MM patients. In none of these studies we found an impact on survival by the factors studied. The studies are however retrospectively performed in relatively small patient cohorts, and in a heterogeneous population with regards to therapy. For example, few of the earliest patients had access to new drugs like thalidomide, lenalidomide and bortezomib. To proper answer the question of a possible prognostic impact of serum levels of HGFA and c-Met, and of c-Met expression by immunohistochemistry, new studies should be performed based on the knowledge gained in these first studies, and in a more homogeneous population with regards to therapy.

However, it should be noted that factors that may be biologically important in the pathogenesis of a disease, do not necessarily need to have prognostic associations. For example, if a given subtype of MM was dependent on a specific factor, the presence of this factor would not necessarily have any *prognostic* impact in MM patients (as a group), but could still be an important *predictive* factor, as it would sort out patients who could be considered for treatment directed against this specific factor. This way of thinking has lead to the concepts “prognostic classification” versus “predictive classification” (19).

5.2.6. Conclusive remarks on HGF and c-Met in multiple myeloma

This work has made contributions to a growing mass of knowledge about HGF, c-Met and related factors in MM. Do the accumulated data to date mean that the HGF/c-Met axis is important in MM? Still, there are no really good *in vivo* data to firmly establish HGF and c-Met as major players in myeloma pathogenesis. Serum levels of HGF are elevated and associated with poor prognosis in MM patients, and there are correlations between serum

levels of HGF and bone marrow angiogenesis (180) and between serum levels of HGF and bone disease (59). These correlations, however, do not prove causality, and there is a possibility that HGF in these studies is merely a marker for tumour burden. Another possibility is that serum HGF is a marker for syndecan-1, which might be a more important actor, given its role as a multifunctional regulator of several cytokines in the bone marrow. Still, the accumulating mass of data on the effects of HGF on crucial functions in myeloma pathogenesis, as cell survival, proliferation, adhesion, migration, angiogenesis and impaired osteoblastogenesis, its prognostic impact in serum, and the fact that both HGF and c-Met are upregulated in malignant compared to normal plasma cells (93, 174, 175), supports that the HGF/c-Met axis should be further evaluated *in vivo*. The future will hopefully bring valid data from mouse models, followed by studies on targeting the HGF/c-Met axis in MM patients.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108.
2. Alexander DD, Mink PJ, Adami HO, Cole P, Mandel JS, Oken MM, et al. Multiple myeloma: a review of the epidemiologic literature. Int J Cancer. 2007;120 Suppl 12:40-61.
3. Wisloff F, Andersen P, Andersson TR, Brandt E, Eika C, Fjaestad K, et al. Has the incidence of multiple myeloma in old age been underestimated? The myeloma project of health region I in Norway. I. Eur J Haematol. 1991 Nov;47(5):333-7.
4. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003 Jan;78(1):21-33.
5. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/racial groups: support for genetic factors in pathogenesis. Leukemia. 2009 Oct;23(10):1691-7.
6. Rajkumar SV. Multiple myeloma: 2011 update on diagnosis, risk-stratification, and management. Am J Hematol. 2011 Jan;86(1):57-65.
7. Kyle RA, Rajkumar SV. Multiple myeloma. N Engl J Med. 2004 Oct 28;351(18):1860-73.
8. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood. 2009 May 28;113(22):5412-7.
9. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. Blood. 2009 May 28;113(22):5418-22.
10. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. N Engl J Med. 2002 Feb 21;346(8):564-9.
11. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med. 2006 Mar 30;354(13):1362-9.
12. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia. 2009 Jan;23(1):3-9.
13. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol. 2003 Jun;121(5):749-57.
14. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008 Mar 1;111(5):2516-20.
15. Kastritis E, Zervas K, Symeonidis A, Terpos E, Delimbassi S, Anagnostopoulos N, et al. Improved survival of patients with multiple myeloma after the introduction of novel agents and the applicability of the International Staging System (ISS): an analysis of the Greek Myeloma Study Group (GMSG). Leukemia. 2009 Jun;23(6):1152-7.
16. Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer. 1975 Sep;36(3):842-54.
17. Hari PN, Zhang MJ, Roy V, Perez WS, Bashey A, To LB, et al. Is the International Staging System superior to the Durie-Salmon staging system? A comparison in multiple myeloma patients undergoing autologous transplant. Leukemia. 2009 Aug;23(8):1528-34.

18. Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Blade J, et al. International staging system for multiple myeloma. J Clin Oncol. 2005 May 20;23(15):3412-20.
19. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia. 2009 Dec;23(12):2210-21.
20. Jagannath S, Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, et al. Bortezomib appears to overcome the poor prognosis conferred by chromosome 13 deletion in phase 2 and 3 trials. Leukemia. 2007 Jan;21(1):151-7.
21. Avet-Loiseau H, Leleu X, Roussel M, Moreau P, Guerin-Charbonnel C, Caillot D, et al. Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p). J Clin Oncol. 2010 Oct 20;28(30):4630-4.
22. Greipp PR, Lust JA, O'Fallon WM, Katzmann JA, Witzig TE, Kyle RA. Plasma cell labeling index and beta 2-microglobulin predict survival independent of thymidine kinase and C-reactive protein in multiple myeloma. Blood. 1993 Jun 15;81(12):3382-7.
23. Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood. 2003 Jun 1;101(11):4569-75.
24. Zhou Y, Barlogie B, Shaughnessy JD, Jr. The molecular characterization and clinical management of multiple myeloma in the post-genome era. Leukemia. 2009 Nov;23(11):1941-56.
25. Munshi NC, Anderson KC, Bergsagel PL, Shaughnessy J, Palumbo A, Durie B, et al. Consensus recommendations for risk stratification in multiple myeloma: report of the International Myeloma Workshop Consensus Panel 2. Blood. 2011 May 5;117(18):4696-700.
26. Bergsagel DE, Sprague CC, Ross SW. Evaluation of new chemotherapeutic agents in the treatment of multiple myeloma. I. Plan of study. Cancer Chemother Rep. 1962 Aug;21:69-74.
27. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med. 1996 Jul 11;335(2):91-7.
28. Rajkumar SV. Treatment of myeloma: cure vs control. Mayo Clin Proc. 2008 Oct;83(10):1142-5.
29. Haessler J, Shaughnessy JD, Jr., Zhan F, Crowley J, Epstein J, van Rhee F, et al. Benefit of complete response in multiple myeloma limited to high-risk subgroup identified by gene expression profiling. Clin Cancer Res. 2007 Dec 1;13(23):7073-9.
30. Kumar S. Multiple myeloma - current issues and controversies. Cancer Treat Rev. 2010 May;36 Suppl 2:S3-11.
31. Lokhorst H, Einsele H, Vesole D, Bruno B, San Miguel J, Perez-Simon JA, et al. International Myeloma Working Group consensus statement regarding the current status of allogeneic stem-cell transplantation for multiple myeloma. J Clin Oncol. 2010 Oct 10;28(29):4521-30.
32. Kastritis E, Charidimou A, Varkaris A, Dimopoulos MA. Targeted therapies in multiple myeloma. Target Oncol. 2009 Jan;4(1):23-36.
33. Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006 Sep;20(9):1467-73.
34. Anderson KC, Carrasco RD. Pathogenesis of myeloma. Annu Rev Pathol. 2011 Feb 28;6:249-74.

35. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005 Apr;5(4):251-62.
36. Mahindra A, Hideshima T, Anderson KC. Multiple myeloma: biology of the disease. Blood Rev. 2010 Nov;24 Suppl 1:S5-11.
37. Davies FE, Dring AM, Li C, Rawstron AC, Shammas MA, O'Connor SM, et al. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. Blood. 2003 Dec 15;102(13):4504-11.
38. Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, Winkler JM, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood. 2003 Oct 1;102(7):2562-7.
39. Smadja NV, Fruchart C, Isnard F, Louvet C, Dutel JL, Cheron N, et al. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. Leukemia. 1998 Jun;12(6):960-9.
40. Chng WJ, Van Wier SA, Ahmann GJ, Winkler JM, Jalal SM, Bergsagel PL, et al. A validated FISH trisomy index demonstrates the hyperdiploid and nonhyperdiploid dichotomy in MGUS. Blood. 2005 Sep 15;106(6):2156-61.
41. Brousseau M, Leleu X, Gerard J, Gastinne T, Godon A, Genevieve F, et al. Hyperdiploidy is a common finding in monoclonal gammopathy of undetermined significance and monosomy 13 is restricted to these hyperdiploid patients. Clin Cancer Res. 2007 Oct 15;13(20):6026-31.
42. Chng WJ, Winkler JM, Greipp PR, Jalal SM, Bergsagel PL, Chesi M, et al. Ploidy status rarely changes in myeloma patients at disease progression. Leuk Res. 2006 Mar;30(3):266-71.
43. Avet-Loiseau H, Attal M, Moreau P, Charbonnel C, Garban F, Hulin C, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. Blood. 2007 Apr 15;109(8):3489-95.
44. Fassas AB, Spencer T, Sawyer J, Zangari M, Lee CK, Anaissie E, et al. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. Br J Haematol. 2002 Sep;118(4):1041-7.
45. Bergsagel PL, Chesi M, Nardini E, Brents LA, Kirby SL, Kuehl WM. Promiscuous translocations into immunoglobulin heavy chain switch regions in multiple myeloma. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):13931-6.
46. Bergsagel PL, Kuehl WM. Chromosome translocations in multiple myeloma. Oncogene. 2001 Sep 10;20(40):5611-22.
47. Shaughnessy JD, Jr., Zhan F, Burington BE, Huang Y, Colla S, Hanamura I, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007 Mar 15;109(6):2276-84.
48. Chang H, Qi X, Jiang A, Xu W, Young T, Reece D. 1p21 deletions are strongly associated with 1q21 gains and are an independent adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma. Bone Marrow Transplant. 2010 Jan;45(1):117-21.
49. Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B. Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. Blood. 1998 Mar 1;91(5):1732-41.
50. Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, Sawyer JR, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood. 2006 Sep 1;108(5):1724-32.

51. Chng WJ, Gertz MA, Chung TH, Van Wier S, Keats JJ, Baker A, et al. Correlation between array-comparative genomic hybridization-defined genomic gains and losses and survival: identification of 1p31-32 deletion as a prognostic factor in myeloma. Leukemia. 2010 Apr;24(4):833-42.
52. Chiecchio L, Dagrada GP, Protheroe RK, Stockley DM, Smith AG, Orchard KH, et al. Loss of 1p and rearrangement of MYC are associated with progression of smouldering myeloma to myeloma: sequential analysis of a single case. Haematologica. 2009 Jul;94(7):1024-8.
53. Broyl A, Hose D, Lokhorst H, de Knegt Y, Peeters J, Jauch A, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood. 2010 Oct 7;116(14):2543-53.
54. Smith EM, Boyd K, Davies FE. The potential role of epigenetic therapy in multiple myeloma. Br J Haematol. 2010 Mar;148(5):702-13.
55. Calvo KR, Landgren O, Roccaro AM, Ghobrial IM. Role of microRNAs from monoclonal gammopathy of undetermined significance to multiple myeloma. Semin Hematol. 2011 Jan;48(1):39-45.
56. Hideshima T, Chauhan D, Hayashi T, Podar K, Akiyama M, Gupta D, et al. The biological sequelae of stromal cell-derived factor-1alpha in multiple myeloma. Mol Cancer Ther. 2002 May;1(7):539-44.
57. Terpos E, Efstathiou E, Christoulas D, Roussou M, Katodritou E, Dimopoulos MA. RANKL inhibition: clinical implications for the management of patients with multiple myeloma and solid tumors with bone metastases. Expert Opin Biol Ther. 2009 Apr;9(4):465-79.
58. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003 Dec 25;349(26):2483-94.
59. Standal T, Abildgaard N, Fagerli UM, Stordal B, Hjertner O, Borset M, et al. HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. Blood. 2007 Apr 1;109(7):3024-30.
60. Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, et al. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature. 1988 Mar 3;332(6159):83-5.
61. Ge NL, Rudikoff S. Insulin-like growth factor I is a dual effector of multiple myeloma cell growth. Blood. 2000 Oct 15;96(8):2856-61.
62. Otsuki T, Yamada O, Yata K, Sakaguchi H, Kurebayashi J, Nakazawa N, et al. Expression of fibroblast growth factor and FGF-receptor family genes in human myeloma cells, including lines possessing t(4;14)(q16.3;q32. 3) and FGFR3 translocation. Int J Oncol. 1999 Dec;15(6):1205-12.
63. Derksen PW, de Gorter DJ, Meijer HP, Bende RJ, van Dijk M, Lokhorst HM, et al. The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. Leukemia. 2003 Apr;17(4):764-74.
64. Mahtouk K, Cremer FW, Reme T, Jourdan M, Baudard M, Moreaux J, et al. Heparan sulphate proteoglycans are essential for the myeloma cell growth activity of EGF-family ligands in multiple myeloma. Oncogene. 2006 Nov 16;25(54):7180-91.
65. Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, et al. Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. Blood. 2001 Jul 15;98(2):428-35.
66. Lentzsch S, Gries M, Janz M, Bargou R, Dorken B, Mapara MY. Macrophage inflammatory protein 1-alpha (MIP-1 alpha) triggers migration and signaling cascades

mediating survival and proliferation in multiple myeloma (MM) cells. Blood. 2003 May 1;101(9):3568-73.
67. Tai YT, Li XF, Breitkreutz I, Song W, Neri P, Catley L, et al. Role of B-cell-activating factor in adhesion and growth of human multiple myeloma cells in the bone marrow microenvironment. Cancer Res. 2006 Jul 1;66(13):6675-82.
68. Moreaux J, Legouffe E, Jourdan E, Quittet P, Reme T, Lugagne C, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. Blood. 2004 Apr 15;103(8):3148-57.
69. Hjorth-Hansen H, Waage A, Borset M. Interleukin-15 blocks apoptosis and induces proliferation of the human myeloma cell line OH-2 and freshly isolated myeloma cells. Br J Haematol. 1999 Jul;106(1):28-34.
70. Brenne AT, Ro TB, Waage A, Sundan A, Borset M, Hjorth-Hansen H. Interleukin-21 is a growth and survival factor for human myeloma cells. Blood. 2002 May 15;99(10):3756-62.
71. Chauhan D, Uchiyama H, Urashima M, Yamamoto K, Anderson KC. Regulation of interleukin 6 in multiple myeloma and bone marrow stromal cells. Stem Cells. 1995 Aug;13 Suppl 2:35-9.
72. Lentzsch S, Chatterjee M, Gries M, Bommert K, Gollasch H, Dorken B, et al. PI3-K/AKT/FKHR and MAPK signaling cascades are redundantly stimulated by a variety of cytokines and contribute independently to proliferation and survival of multiple myeloma cells. Leukemia. 2004 Nov;18(11):1883-90.
73. Chatterjee M, Honemann D, Lentzsch S, Bommert K, Sers C, Herrmann P, et al. In the presence of bone marrow stromal cells human multiple myeloma cells become independent of the IL-6/gp130/STAT3 pathway. Blood. 2002 Nov 1;100(9):3311-8.
74. Lue C, Kiyono H, McGhee JR, Fujihashi K, Kishimoto T, Hirano T, et al. Recombinant human interleukin 6 (rhIL-6) promotes the terminal differentiation of in vivo-activated human B cells into antibody-secreting cells. Cell Immunol. 1991 Feb;132(2):423-32.
75. Zhang XG, Gaillard JP, Robillard N, Lu ZY, Gu ZJ, Jourdan M, et al. Reproducible obtaining of human myeloma cell lines as a model for tumor stem cell study in human multiple myeloma. Blood. 1994 Jun 15;83(12):3654-63.
76. Hilbert DM, Kopf M, Mock BA, Kohler G, Rudikoff S. Interleukin 6 is essential for in vivo development of B lineage neoplasms. J Exp Med. 1995 Jul 1;182(1):243-8.
77. Juge-Morineau N, Francois S, Puthier D, Godard A, Bataille R, Amiot M. The gp 130 family cytokines IL-6, LIF and OSM but not IL-11 can reverse the anti-proliferative effect of dexamethasone on human myeloma cells. Br J Haematol. 1995 Jul;90(3):707-10.
78. Bataille R, Jourdan M, Zhang XG, Klein B. Serum levels of interleukin 6, a potent myeloma cell growth factor, as a reflect of disease severity in plasma cell dyscrasias. J Clin Invest. 1989 Dec;84(6):2008-11.
79. Uchiyama H, Barut BA, Mohrbacher AF, Chauhan D, Anderson KC. Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. Blood. 1993 Dec 15;82(12):3712-20.
80. Klein B, Zhang XG, Jourdan M, Content J, Houssiau F, Aarden L, et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood. 1989 Feb;73(2):517-26.
81. Lokhorst HM, Lamme T, de Smet M, Klein S, de Weger RA, van Oers R, et al. Primary tumor cells of myeloma patients induce interleukin-6 secretion in long-term bone marrow cultures. Blood. 1994 Oct 1;84(7):2269-77.
82. Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, et al. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol. 1990 Nov 15;145(10):3297-303.

83. Frassanito MA, Cusmai A, Iodice G, Dammacco F. Autocrine interleukin-6 production and highly malignant multiple myeloma: relation with resistance to drug-induced apoptosis. Blood. 2001 Jan 15;97(2):483-9.
84. Kawano M, Tanaka H, Ishikawa H, Nobuyoshi M, Iwato K, Asaoku H, et al. Interleukin-1 accelerates autocrine growth of myeloma cells through interleukin-6 in human myeloma. Blood. 1989 Jun;73(8):2145-8.
85. Carter A, Merchav S, Silvian-Draxler I, Tatarsky I. The role of interleukin-1 and tumour necrosis factor-alpha in human multiple myeloma. Br J Haematol. 1990 Apr;74(4):424-31.
86. Barille S, Thabard W, Robillard N, Moreau P, Pineau D, Harousseau JL, et al. CD130 rather than CD126 expression is associated with disease activity in multiple myeloma. Br J Haematol. 1999 Aug;106(2):532-5.
87. Rawstron AC, Fenton JA, Ashcroft J, English A, Jones RA, Richards SJ, et al. The interleukin-6 receptor alpha-chain (CD126) is expressed by neoplastic but not normal plasma cells. Blood. 2000 Dec 1;96(12):3880-6.
88. Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity. 1999 Jan;10(1):105-15.
89. Daeipour M, Kumar G, Amaral MC, Nel AE. Recombinant IL-6 activates p42 and p44 mitogen-activated protein kinases in the IL-6 responsive B cell line, AF-10. J Immunol. 1993 Jun 1;150(11):4743-53.
90. Hsu JH, Shi Y, Hu L, Fisher M, Franke TF, Lichtenstein A. Role of the AKT kinase in expansion of multiple myeloma clones: effects on cytokine-dependent proliferative and survival responses. Oncogene. 2002 Feb 21;21(9):1391-400.
91. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer. 2008 Dec;8(12):915-28.
92. Ferlin M, Noraz N, Hertogh C, Brochier J, Taylor N, Klein B. Insulin-like growth factor induces the survival and proliferation of myeloma cells through an interleukin-6-independent transduction pathway. Br J Haematol. 2000 Nov;111(2):626-34.
93. Sprynski AC, Hose D, Caillot L, Reme T, Shaughnessy JD, Jr., Barlogie B, et al. The role of IGF-1 as a major growth factor for myeloma cell lines and the prognostic relevance of the expression of its receptor. Blood. 2009 May 7;113(19):4614-26.
94. Bataille R, Robillard N, Avet-Loiseau H, Harousseau JL, Moreau P. CD221 (IGF-1R) is aberrantly expressed in multiple myeloma, in relation to disease severity. Haematologica. 2005 May;90(5):706-7.
95. Chng WJ, Gualberto A, Fonseca R. IGF-1R is overexpressed in poor-prognostic subtypes of multiple myeloma. Leukemia. 2006 Jan;20(1):174-6.
96. Standal T, Borset M, Lenhoff S, Wisloff F, Stordal B, Sundan A, et al. Serum insulinlike growth factor is not elevated in patients with multiple myeloma but is still a prognostic factor. Blood. 2002 Dec 1;100(12):3925-9.
97. Vanderkerken K, Asosingh K, Braet F, Van Riet I, Van Camp B. Insulin-like growth factor-1 acts as a chemoattractant factor for 5T2 multiple myeloma cells. Blood. 1999 Jan 1;93(1):235-41.
98. Tai YT, Podar K, Catley L, Tseng YH, Akiyama M, Shringarpure R, et al. Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of beta1-integrin and phosphatidylinositol 3'-kinase/AKT signaling. Cancer Res. 2003 Sep 15;63(18):5850-8.
99. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem Biophys Res Commun. 1984 Aug 16;122(3):1450-9.

100. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, et al. Molecular cloning and expression of human hepatocyte growth factor. Nature. 1989 Nov 23;342(6248):440-3.
101. Miyazawa K, Tsubouchi H, Naka D, Takahashi K, Okigaki M, Arakaki N, et al. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. Biochem Biophys Res Commun. 1989 Sep 15;163(2):967-73.
102. Stoker M, Gherardi E, Perryman M, Gray J. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. Nature. 1987 May 21-27;327(6119):239-42.
103. Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, et al. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. EMBO J. 1991 Oct;10(10):2867-78.
104. Gherardi E, Stoker M. Hepatocytes and scatter factor. Nature. 1990 Jul 19;346(6281):228.
105. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol. 2003 Dec;4(12):915-25.
106. Nakamura T, Sakai K, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. J Gastroenterol Hepatol. 2011 Jan;26 Suppl 1:188-202.
107. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. Am J Pathol. 1993 Sep;143(3):949-58.
108. Shimomura T, Miyazawa K, Komiyama Y, Hiraoka H, Naka D, Morimoto Y, et al. Activation of hepatocyte growth factor by two homologous proteases, blood-coagulation factor XIIa and hepatocyte growth factor activator. Eur J Biochem. 1995 Apr 1;229(1):257-61.
109. Lee SL, Dickson RB, Lin CY. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. J Biol Chem. 2000 Nov 24;275(47):36720-5.
110. Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, et al. Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. EMBO J. 1992 Dec;11(13):4825-33.
111. Herter S, Piper DE, Aaron W, Gabriele T, Cutler G, Cao P, et al. Hepatocyte growth factor is a preferred in vitro substrate for human hepsin, a membrane-anchored serine protease implicated in prostate and ovarian cancers. Biochem J. 2005 Aug 15;390(Pt 1):125-36.
112. Peek M, Moran P, Mendoza N, Wickramasinghe D, Kirchhofer D. Unusual proteolytic activation of pro-hepatocyte growth factor by plasma kallikrein and coagulation factor XIa. J Biol Chem. 2002 Dec 6;277(49):47804-9.
113. Kataoka H, Kawaguchi M. Hepatocyte growth factor activator (HGFA): pathophysiological functions in vivo. FEBS J. 2010 May;277(10):2230-7.
114. Tolbert WD, Daugherty-Holtrop J, Gherardi E, Vande Woude G, Xu HE. Structural basis for agonism and antagonism of hepatocyte growth factor. Proc Natl Acad Sci U S A. 2010 Jul 27;107(30):13264-9.
115. Du W, Hattori Y, Yamada T, Matsumoto K, Nakamura T, Sagawa M, et al. NK4, an antagonist of hepatocyte growth factor (HGF), inhibits growth of multiple myeloma cells: molecular targeting of angiogenic growth factor. Blood. 2007 Apr 1;109(7):3042-9.
116. Kemp LE, Mulloy B, Gherardi E. Signalling by HGF/SF and Met: the role of heparan sulphate co-receptors. Biochem Soc Trans. 2006 Jun;34(Pt 3):414-7.
117. Lyon M, Deakin JA, Gallagher JT. The mode of action of heparan and dermatan sulfates in the regulation of hepatocyte growth factor/scatter factor. J Biol Chem. 2002 Jan 11;277(2):1040-6.

118. Lietha D, Chirgadze DY, Mulloy B, Blundell TL, Gherardi E. Crystal structures of NK1-heparin complexes reveal the basis for NK1 activity and enable engineering of potent agonists of the MET receptor. EMBO J. 2001 Oct 15;20(20):5543-55.
119. Lyon M, Deakin JA, Mizuno K, Nakamura T, Gallagher JT. Interaction of hepatocyte growth factor with heparan sulfate. Elucidation of the major heparan sulfate structural determinants. J Biol Chem. 1994 Apr 15;269(15):11216-23.
120. Derksen PW, Keehnen RM, Evers LM, van Oers MH, Spaargaren M, Pals ST. Cell surface proteoglycan syndecan-1 mediates hepatocyte growth factor binding and promotes Met signaling in multiple myeloma. Blood. 2002 Feb 15;99(4):1405-10.
121. Zioncheck TF, Richardson L, Liu J, Chang L, King KL, Bennett GL, et al. Sulfated oligosaccharides promote hepatocyte growth factor association and govern its mitogenic activity. J Biol Chem. 1995 Jul 14;270(28):16871-8.
122. Seidel C, Borset M, Hjertner O, Cao D, Abildgaard N, Hjorth-Hansen H, et al. High levels of soluble syndecan-1 in myeloma-derived bone marrow: modulation of hepatocyte growth factor activity. Blood. 2000 Nov 1;96(9):3139-46.
123. Miyazawa K, Shimomura T, Kitamura A, Kondo J, Morimoto Y, Kitamura N. Molecular cloning and sequence analysis of the cDNA for a human serine protease reponsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation factor XII. J Biol Chem. 1993 May 15;268(14):10024-8.
124. Kataoka H, Miyata S, Uchinokura S, Itoh H. Roles of hepatocyte growth factor (HGF) activator and HGF activator inhibitor in the pericellular activation of HGF/scatter factor. Cancer Metastasis Rev. 2003 Jun-Sep;22(2-3):223-36.
125. Shimomura T, Kondo J, Ochiai M, Naka D, Miyazawa K, Morimoto Y, et al. Activation of the zymogen of hepatocyte growth factor activator by thrombin. J Biol Chem. 1993 Oct 25;268(30):22927-32.
126. Mukai S, Fukushima T, Naka D, Tanaka H, Osada Y, Kataoka H. Activation of hepatocyte growth factor activator zymogen (pro-HGFA) by human kallikrein 1-related peptidases. FEBS J. 2008 Mar;275(5):1003-17.
127. Miyazawa K, Shimomura T, Kitamura N. Activation of hepatocyte growth factor in the injured tissues is mediated by hepatocyte growth factor activator. J Biol Chem. 1996 Feb 16;271(7):3615-8.
128. Suzuki K. Hepatocyte growth factor activator (HGFA): its regulation by protein C inhibitor. FEBS J. 2010 May;277(10):2223-9.
129. Kataoka H, Shimomura T, Kawaguchi T, Hamasuna R, Itoh H, Kitamura N, et al. Hepatocyte growth factor activator inhibitor type 1 is a specific cell surface binding protein of hepatocyte growth factor activator (HGFA) and regulates HGFA activity in the pericellular microenvironment. J Biol Chem. 2000 Dec 22;275(51):40453-62.
130. Kawaguchi T, Qin L, Shimomura T, Kondo J, Matsumoto K, Denda K, et al. Purification and cloning of hepatocyte growth factor activator inhibitor type 2, a Kunitz-type serine protease inhibitor. J Biol Chem. 1997 Oct 31;272(44):27558-64.
131. Itoh H, Naganuma S, Takeda N, Miyata S, Uchinokura S, Fukushima T, et al. Regeneration of injured intestinal mucosa is impaired in hepatocyte growth factor activator-deficient mice. Gastroenterology. 2004 Nov;127(5):1423-35.
132. Tjin EP, Derksen PW, Kataoka H, Spaargaren M, Pals ST. Multiple myeloma cells catalyze hepatocyte growth factor (HGF) activation by secreting the serine protease HGF-activator. Blood. 2004 Oct 1;104(7):2172-5.
133. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature. 1984 Sep 6-11;311(5981):29-33.

134. Trusolino L, Comoglio PM. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat Rev Cancer. 2002 Apr;2(4):289-300.
135. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol. 2010 Dec;11(12):834-48.
136. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science. 1991 Feb 15;251(4995):802-4.
137. Gherardi E, Youles ME, Miguel RN, Blundell TL, Iamele L, Gough J, et al. Functional map and domain structure of MET, the product of the c-met protooncogene and receptor for hepatocyte growth factor/scatter factor. Proc Natl Acad Sci U S A. 2003 Oct 14;100(21):12039-44.
138. Basilico C, Arnesano A, Galluzzo M, Comoglio PM, Michieli P. A high affinity hepatocyte growth factor-binding site in the immunoglobulin-like region of Met. J Biol Chem. 2008 Jul 25;283(30):21267-77.
139. Kong-Beltran M, Stamos J, Wickramasinghe D. The Sema domain of Met is necessary for receptor dimerization and activation. Cancer Cell. 2004 Jul;6(1):75-84.
140. Stamos J, Lazarus RA, Yao X, Kirchhofer D, Wiesmann C. Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor. EMBO J. 2004 Jun 16;23(12):2325-35.
141. Gherardi E, Sandin S, Petoukhov MV, Finch J, Youles ME, Ofverstedt LG, et al. Structural basis of hepatocyte growth factor/scatter factor and MET signalling. Proc Natl Acad Sci U S A. 2006 Mar 14;103(11):4046-51.
142. Gandino L, Longati P, Medico E, Prat M, Comoglio PM. Phosphorylation of serine 985 negatively regulates the hepatocyte growth factor receptor kinase. J Biol Chem. 1994 Jan 21;269(3):1815-20.
143. Peschard P, Fournier TM, Lamorte L, Naujokas MA, Band H, Langdon WY, et al. Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. Mol Cell. 2001 Nov;8(5):995-1004.
144. Ponzetto C, Bardelli A, Zhen Z, Maina F, dalla Zonca P, Giordano S, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. Cell. 1994 Apr 22;77(2):261-71.
145. Sachs M, Brohmann H, Zechner D, Muller T, Hulsken J, Walther I, et al. Essential role of Gab1 for signaling by the c-Met receptor in vivo. J Cell Biol. 2000 Sep 18;150(6):1375-84.
146. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002 Jul;2(7):489-501.
147. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol. 2003 Jan;4(1):33-45.
148. Orian-Rousseau V, Chen L, Sleeman JP, Herrlich P, Ponta H. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev. 2002 Dec 1;16(23):3074-86.
149. Prat M, Crepaldi T, Gandino L, Giordano S, Longati P, Comoglio P. C-terminal truncated forms of Met, the hepatocyte growth factor receptor. Mol Cell Biol. 1991 Dec;11(12):5954-62.
150. Galvani AP, Cristiani C, Carpinelli P, Landonio A, Bertolero F. Suramin modulates cellular levels of hepatocyte growth factor receptor by inducing shedding of a soluble form. Biochem Pharmacol. 1995 Sep 28;50(7):959-66.
151. Wajih N, Walter J, Sane DC. Vascular origin of a soluble truncated form of the hepatocyte growth factor receptor (c-met). Circ Res. 2002 Jan 11;90(1):46-52.

152. Petrelli A, Circosta P, Granziero L, Mazzone M, Pisacane A, Fenoglio S, et al. Ab-induced ectodomain shedding mediates hepatocyte growth factor receptor down-regulation and hampers biological activity. Proc Natl Acad Sci U S A. 2006 Mar 28;103(13):5090-5.
153. Athauda G, Giubellino A, Coleman JA, Horak C, Steeg PS, Lee MJ, et al. c-Met ectodomain shedding rate correlates with malignant potential. Clin Cancer Res. 2006 Jul 15;12(14 Pt 1):4154-62.
154. Kopitz C, Gerg M, Bandapalli OR, Ister D, Pennington CJ, Hauser S, et al. Tissue inhibitor of metalloproteinases-1 promotes liver metastasis by induction of hepatocyte growth factor signaling. Cancer Res. 2007 Sep 15;67(18):8615-23.
155. Foveau B, Ancot F, Leroy C, Petrelli A, Reiss K, Vingtdeux V, et al. Down-regulation of the met receptor tyrosine kinase by presenilin-dependent regulated intramembrane proteolysis. Mol Biol Cell. 2009 May;20(9):2495-507.
156. Tiran Z, Oren A, Hermesh C, Rotman G, Levine Z, Amitai H, et al. A novel recombinant soluble splice variant of Met is a potent antagonist of the hepatocyte growth factor/scatter factor-Met pathway. Clin Cancer Res. 2008 Jul 15;14(14):4612-21.
157. Michieli P, Mazzone M, Basilico C, Cavassa S, Sottile A, Naldini L, et al. Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. Cancer Cell. 2004 Jul;6(1):61-73.
158. Coxon A, Rex K, Meyer S, Sun J, Chen Q, Radinsky R, et al. Soluble c-Met receptors inhibit phosphorylation of c-Met and growth of hepatocyte growth factor: c-Met-dependent tumors in animal models. Mol Cancer Ther. 2009 May;8(5):1119-25.
159. Merlin S, Pietronave S, Locarno D, Valente G, Follenzi A, Prat M. Deletion of the ectodomain unleashes the transforming, invasive, and tumorigenic potential of the MET oncogene. Cancer Sci. 2009 Apr;100(4):633-8.
160. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell. 2008 Jun;14(6):818-29.
161. Christofori G. New signals from the invasive front. Nature. 2006 May 25;441(7092):444-50.
162. Gentile A, Comoglio PM. Invasive growth: a genetic program. Int J Dev Biol. 2004;48(5-6):451-6.
163. Szabo R, Rasmussen AL, Moyer AB, Kosa P, Schafer JM, Molinolo AA, et al. c-Met-induced epithelial carcinogenesis is initiated by the serine protease matriptase. Oncogene. 2011 Apr 28;30(17):2003-16.
164. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997 May;16(1):68-73.
165. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res. 2008 May 15;14(10):2895-9.
166. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007 May 18;316(5827):1039-43.
167. Corso S, Migliore C, Ghiso E, De Rosa G, Comoglio PM, Giordano S. Silencing the MET oncogene leads to regression of experimental tumors and metastases. Oncogene. 2008 Jan 24;27(5):684-93.
168. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov. 2008 Jun;7(6):504-16.
169. Cecchi F, Rabe DC, Bottaro DP. Targeting the HGF/Met signalling pathway in cancer. Eur J Cancer. 2010 May;46(7):1260-70.

170. Nakamura T, Sakai K, Matsumoto K. Anti-cancer approach with NK4: Bivalent action and mechanisms. Anticancer Agents Med Chem. 2010 Jan;10(1):36-46.
171. Takai K, Hara J, Matsumoto K, Hosoi G, Osugi Y, Tawa A, et al. Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. Blood. 1997 Mar 1;89(5):1560-5.
172. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem. 1996 Apr;119(4):591-600.
173. Toyama T, Ido A, Sasak H, Maeda K, Yamashita K, Kubuki Y, et al. Possible involvement of neutrophils in a serum level increase of hepatocyte growth factor in non-Hodgkin's lymphoma. Oncol Rep. 2005 Mar;13(3):439-44.
174. Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood. 2002 Mar 1;99(5):1745-57.
175. Hose D, Moreaux J, Meissner T, Seckinger A, Goldschmidt H, Benner A, et al. Induction of angiogenesis by normal and malignant plasma cells. Blood. 2009 Jul 2;114(1):128-43.
176. Borset M, Lien E, Espevik T, Helseth E, Waage A, Sundan A. Concomitant expression of hepatocyte growth factor/scatter factor and the receptor c-MET in human myeloma cell lines. J Biol Chem. 1996 Oct 4;271(40):24655-61.
177. Seidel C, Borset M, Turesson I, Abildgaard N, Sundan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. The Nordic Myeloma Study Group. Blood. 1998 Feb 1;91(3):806-12.
178. Seidel C, Lenhoff S, Brabrand S, Anderson G, Standal T, Lanng-Nielsen J, et al. Hepatocyte growth factor in myeloma patients treated with high-dose chemotherapy. Br J Haematol. 2002 Dec;119(3):672-6.
179. Iwasaki T, Hamano T, Ogata A, Hashimoto N, Kitano M, Kakishita E. Clinical significance of vascular endothelial growth factor and hepatocyte growth factor in multiple myeloma. Br J Haematol. 2002 Mar;116(4):796-802.
180. Andersen NF, Standal T, Nielsen JL, Heickendorff L, Borset M, Sorensen FB, et al. Syndecan-1 and angiogenic cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival. Br J Haematol. 2005 Jan;128(2):210-7.
181. Chng WJ, Schop RF, Price-Troska T, Ghobrial I, Kay N, Jelinek DF, et al. Gene-expression profiling of Waldenstrom macroglobulinemia reveals a phenotype more similar to chronic lymphocytic leukemia than multiple myeloma. Blood. 2006 Oct 15;108(8):2755-63.
182. Holt RU, Baykov V, Ro TB, Brabrand S, Waage A, Sundan A, et al. Human myeloma cells adhere to fibronectin in response to hepatocyte growth factor. Haematologica. 2005 Apr;90(4):479-88.
183. Holt RU, Fagerli UM, Baykov V, Ro TB, Hov H, Waage A, et al. Hepatocyte growth factor promotes migration of human myeloma cells. Haematologica. 2008 Apr;93(4):619-22.
184. Hov H, Holt RU, Ro TB, Fagerli UM, Hjorth-Hansen H, Baykov V, et al. A selective c-met inhibitor blocks an autocrine hepatocyte growth factor growth loop in ANBL-6 cells and prevents migration and adhesion of myeloma cells. Clin Cancer Res. 2004 Oct 1;10(19):6686-94.
185. You WK, McDonald DM. The hepatocyte growth factor/c-Met signaling pathway as a therapeutic target to inhibit angiogenesis. BMB Rep. 2008 Dec 31;41(12):833-9.
186. Hov H, Tian E, Holien T, Holt RU, Vatsveen TK, Fagerli UM, et al. c-Met signaling promotes IL-6-induced myeloma cell proliferation. Eur J Haematol. 2009 Apr;82(4):277-87.
187. Tumova S, Woods A, Couchman JR. Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. Int J Biochem Cell Biol. 2000 Mar;32(3):269-88.

188. Rapraeger AC. Molecular interactions of syndecans during development. Semin Cell Dev Biol. 2001 Apr;12(2):107-16.
189. Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? Nat Rev Mol Cell Biol. 2003 Dec;4(12):926-37.
190. Sanderson RD, Lalor P, Bernfield M. B lymphocytes express and lose syndecan at specific stages of differentiation. Cell Regul. 1989 Nov;1(1):27-35.
191. Borset M, Helseth E, Naume B, Waage A. Lack of IL-1 secretion from human myeloma cells highly purified by immunomagnetic separation. Br J Haematol. 1993 Nov;85(3):446-51.
192. Ridley RC, Xiao H, Hata H, Woodliff J, Epstein J, Sanderson RD. Expression of syndecan regulates human myeloma plasma cell adhesion to type I collagen. Blood. 1993 Feb 1;81(3):767-74.
193. Liebersbach BF, Sanderson RD. Expression of syndecan-1 inhibits cell invasion into type I collagen. J Biol Chem. 1994 Aug 5;269(31):20013-9.
194. Morgan MR, Humphries MJ, Bass MD. Synergistic control of cell adhesion by integrins and syndecans. Nat Rev Mol Cell Biol. 2007 Dec;8(12):957-69.
195. Sanderson RD, Yang Y. Syndecan-1: a dynamic regulator of the myeloma microenvironment. Clin Exp Metastasis. 2008;25(2):149-59.
196. Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science. 1991 Jun 21;252(5013):1705-8.
197. Schlessinger J, Plotnikov AN, Ibrahimi OA, Eliseenkova AV, Yeh BK, Yayon A, et al. Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. Mol Cell. 2000 Sep;6(3):743-50.
198. Borset M, Hjertner O, Yaccoby S, Epstein J, Sanderson RD. Syndecan-1 is targeted to the uropods of polarized myeloma cells where it promotes adhesion and sequesters heparin-binding proteins. Blood. 2000 Oct 1;96(7):2528-36.
199. Moreaux J, Sprynski AC, Dillon SR, Mahtouk K, Jourdan M, Ythier A, et al. APRIL and TACI interact with syndecan-1 on the surface of multiple myeloma cells to form an essential survival loop. Eur J Haematol. 2009 Aug;83(2):119-29.
200. Jakobsson L, Kreuger J, Holmborn K, Lundin L, Eriksson I, Kjellen L, et al. Heparan sulfate in trans potentiates VEGFR-mediated angiogenesis. Dev Cell. 2006 May;10(5):625-34.
201. Jiao X, Billings PC, O'Connell MP, Kaplan FS, Shore EM, Glaser DL. Heparan sulfate proteoglycans (HSPGs) modulate BMP2 osteogenic bioactivity in C2C12 cells. J Biol Chem. 2007 Jan 12;282(2):1080-6.
202. Clemmons DR. Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. Nat Rev Drug Discov. 2007 Oct;6(10):821-33.
203. Tkachenko E, Rhodes JM, Simons M. Syndecans: new kids on the signaling block. Circ Res. 2005 Mar 18;96(5):488-500.
204. Fuki IV, Meyer ME, Williams KJ. Transmembrane and cytoplasmic domains of syndecan mediate a multi-step endocytic pathway involving detergent-insoluble membrane rafts. Biochem J. 2000 Nov 1;351 Pt 3:607-12.
205. Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. Nat Rev Mol Cell Biol. 2010 Oct;11(10):688-99.
206. Purushothaman A, Chen L, Yang Y, Sanderson RD. Heparanase stimulation of protease expression implicates it as a master regulator of the aggressive tumor phenotype in myeloma. J Biol Chem. 2008 Nov 21;283(47):32628-36.
207. Brule S, Charnaux N, Sutton A, Ledoux D, Chaigneau T, Saffar L, et al. The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is

accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. Glycobiology. 2006 Jun;16(6):488-501.
208. Li Q, Park PW, Wilson CL, Parks WC. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell. 2002 Nov 27;111(5):635-46.
209. Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, et al. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. J Biol Chem. 2003 Oct 17;278(42):40764-70.
210. Seidel C, Sundan A, Hjorth M, Turesson I, Dahl IM, Abildgaard N, et al. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood. 2000 Jan 15;95(2):388-92.
211. Lovell R, Dunn JA, Begum G, Barth NJ, Plant T, Moss PA, et al. Soluble syndecan-1 level at diagnosis is an independent prognostic factor in multiple myeloma and the extent of fall from diagnosis to plateau predicts for overall survival. Br J Haematol. 2005 Aug;130(4):542-8.
212. Pellegrini L, Burke DF, von Delft F, Mulloy B, Blundell TL. Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. Nature. 2000 Oct 26;407(6807):1029-34.
213. Rubin JS, Day RM, Breckenridge D, Atabey N, Taylor WG, Stahl SJ, et al. Dissociation of heparan sulfate and receptor binding domains of hepatocyte growth factor reveals that heparan sulfate-c-met interaction facilitates signaling. J Biol Chem. 2001 Aug 31;276(35):32977-83.
214. Mahtouk K, Hose D, Raynaud P, Hundemer M, Jourdan M, Jourdan E, et al. Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma. Blood. 2007 Jun 1;109(11):4914-23.
215. Yang Y, Macleod V, Miao HQ, Theus A, Zhan F, Shaughnessy JD, Jr., et al. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. J Biol Chem. 2007 May 4;282(18):13326-33.
216. Ramani VC, Yang Y, Ren Y, Nan L, Sanderson RD. Heparanase plays a dual role in driving hepatocyte growth factor (HGF) signaling by enhancing HGF expression and activity. J Biol Chem. 2011 Feb 25;286(8):6490-9.
217. Yang Y, MacLeod V, Dai Y, Khotskaya-Sample Y, Shriver Z, Venkataraman G, et al. The syndecan-1 heparan sulfate proteoglycan is a viable target for myeloma therapy. Blood. 2007 Sep 15;110(6):2041-8.
218. Ikeda H, Hideshima T, Fulciniti M, Lutz RJ, Yasui H, Okawa Y, et al. The monoclonal antibody nBT062 conjugated to cytotoxic Maytansinoids has selective cytotoxicity against CD138-positive multiple myeloma cells in vitro and in vivo. Clin Cancer Res. 2009 Jun 15;15(12):4028-37.
219. Dhodapkar KM, Krasovsky J, Williamson B, Dhodapkar MV. Antitumor monoclonal antibodies enhance cross-presentation ofcCellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. J Exp Med. 2002 Jan 7;195(1):125-33.
220. Ragnarsson L, Stromberg T, Wijdenes J, Totterman TH, Weigelt C. Multiple myeloma cells are killed by syndecan-1-directed superantigen-activated T cells. Cancer Immunol Immunother. 2001 Sep;50(7):382-90.
221. Khotskaya YB, Dai Y, Ritchie JP, MacLeod V, Yang Y, Zinn K, et al. Syndecan-1 is required for robust growth, vascularization, and metastasis of myeloma tumors in vivo. J Biol Chem. 2009 Sep 18;284(38):26085-95.
222. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977 Mar;33(1):159-74.

223. Drexler HG, Matsuo Y. Malignant hematopoietic cell lines: in vitro models for the study of multiple myeloma and plasma cell leukemia. Leuk Res. 2000 Aug;24(8):681-703.
224. Duperray C, Klein B, Durie BG, Zhang X, Jourdan M, Poncelet P, et al. Phenotypic analysis of human myeloma cell lines. Blood. 1989 Feb;73(2):566-72.
225. Verdelli D, Mattioli M, Fabris S, Nobili L, Intini D, Guerneri S, et al. Molecular and biological characterization of three novel interleukin-6-dependent human myeloma cell lines. Haematologica. 2005 Nov;90(11):1541-8.
226. Lombardi L, Poretti G, Mattioli M, Fabris S, Agnelli L, Bicciato S, et al. Molecular characterization of human multiple myeloma cell lines by integrative genomics: insights into the biology of the disease. Genes Chromosomes Cancer. 2007 Mar;46(3):226-38.
227. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res. 2001 Apr 1;61(7):3071-6.
228. Liu W, Litwack ED, Stanley MJ, Langford JK, Lander AD, Sanderson RD. Heparan sulfate proteoglycans as adhesive and anti-invasive molecules. Syndecans and glypican have distinct functions. J Biol Chem. 1998 Aug 28;273(35):22825-32.
229. Chilosi M, Adami F, Lestani M, Montagna L, Cimarosto L, Semenzato G, et al. CD138/syndecan-1: a useful immunohistochemical marker of normal and neoplastic plasma cells on routine trephine bone marrow biopsies. Mod Pathol. 1999 Dec;12(12):1101-6.
230. Reid S, Yang S, Brown R, Kabani K, Aklilu E, Ho PJ, et al. Characterisation and relevance of CD138-negative plasma cells in plasma cell myeloma. Int J Lab Hematol. 2010 Dec;32(6 Pt 1):e190-6.
231. Matteucci E, Bendinelli P, Desiderio MA. Nuclear localization of active HGF receptor Met in aggressive MDA-MB231 breast carcinoma cells. Carcinogenesis. 2009 Jun;30(6):937-45.
232. Gomes DA, Rodrigues MA, Leite MF, Gomez MV, Varnai P, Balla T, et al. c-Met must translocate to the nucleus to initiate calcium signals. J Biol Chem. 2008 Feb 15;283(7):4344-51.
233. Patra SK. Dissecting lipid raft facilitated cell signaling pathways in cancer. Biochim Biophys Acta. 2008 Apr;1785(2):182-206.
234. Yang Y, Yaccoby S, Liu W, Langford JK, Pumphrey CY, Theus A, et al. Soluble syndecan-1 promotes growth of myeloma tumors in vivo. Blood. 2002 Jul 15;100(2):610-7.
235. Younes H, Leleu X, Hatjiharissi E, Moreau AS, Hideshima T, Richardson P, et al. Targeting the phosphatidylinositol 3-kinase pathway in multiple myeloma. Clin Cancer Res. 2007 Jul 1;13(13):3771-5.

Paper I

Immunohistochemical analysis of HGF and c-Met in plasma cell disease

Wader KF[1,2], Fagerli UM[1,2], Børset M[1,3], Lydersen S[1], Hov H[4], Sundan A[1], Bofin A[5] and Waage A[1,6]

[1]Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, [2]Department of Oncology, St Olav's Hospital, Trondheim, [3]Department of Immunology and Transfusion Medicine, St Olav's Hospital, Trondheim, [4]Department of Pathology, St Olav's Hospital, Trondheim, [5]Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, NTNU, Trondheim and [6]Department of Hematology, St Olav's Hospital, Trondheim, Norway.

Running title: HGF and c-Met in plasma cell disease

Key words: multiple myeloma; hepatocyte growth factor; c-Met; phospho-c-Met; immunohistochemistry

Corresponding author:

Karin Fahl Wader

Norwegian University of Science and Technology, Faculty of Medicine, Department of Cancer Research and Molecular Medicine, Postboks 8905, Medisinsk teknisk forskningssenter, N-7491 Trondheim, Norway

Telephone: +47 72825263
Fax: +47 72571463

E-mail: karin.f.wader@ntnu.no

Abstract

Aims

Interaction with the bone marrow microenvironment plays an important role for homing and survival of myeloma cells. One cytokine involved in this process is hepatocyte growth factor (HGF). HGF, by binding to the receptor tyrosine kinase c-Met, mediates a broad range of tumor progression activities. Our aims were to investigate whether HGF and c-Met are present in bone marrow and extramedullary tumors from patients with monoclonal plasma cell disease, and whether c-Met is activated.

Methods and Results

Expression of HGF, c-Met and phospho-c-Met was studied by immunohistochemistry in biopsies from 80 patients with monoclonal plasma cell disease. Cytoplasmic staining for HGF in the plasma cells was demonstrated in 58 out of 68 biopsies from multiple myeloma patients (85%), but also in biopsies from nine of ten healthy individuals. Membranous staining for c-Met was found in 25 of 63 multiple myeloma patients (40%), and in none of ten healthy individuals. Membranous staining for phospho-c-Met was found in biopsies from 15 of 21 c-Met-positive myeloma patients (71%).

Conclusions

Our data point to c-Met as one of the factors that distinguish normal from malignant plasma cells, and indicate that the HGF/c-Met system is activated in multiple myeloma patients.

Introduction

Multiple myeloma is an incurable hematological malignancy, caused by clonal expansion of malignant plasma cells in the bone marrow. The bone marrow microenvironment plays an important role for homing and survival of myeloma cells. Several cytokines are involved in the interaction between malignant plasma cells and the bone marrow microenvironment. One of them is hepatocyte growth factor (HGF). HGF is produced by bone marrow stromal cells,[1,2] and may also be produced by myeloma cells.[1,3,4] Serum HGF levels are elevated in myeloma patients as compared to healthy individuals, and high levels are associated with poor prognosis.[5,6]

When binding to HGF, the receptor tyrosine kinase c-Met becomes phosphorylated at specific tyrosine residues in the cytoplasmic domain, creating docking sites for intracellular signal transducers. Important signaling pathways downstream of c-Met are the Ras – mitogen activated protein kinase (MAPK), the phosphatidylinositol 3-kinase (PI3K) – Akt, and the signal transducer and activator of transcription (STAT) signaling pathways.[7] *In vitro*, HGF stimulates survival, proliferation, adhesion and migration of malignant plasma cells[8-10] and inhibit osteoblastogenesis,[11] suggesting that the HGF/c-Met system by several mechanisms may contribute to multiple myeloma pathogenesis. Besides its own actions as a growth factor, HGF may also potentiate the actions of interleukin-6 in proliferation and migration of myeloma cells.[12] Growing evidence points to HGF as an important factor in the development of multiple myeloma, and the HGF/c-Met system is therefore a promising target for multiple myeloma therapy.[13,14]

We aimed to investigate whether HGF and c-Met are present in bone marrow and extramedullary tumors from patients with monoclonal plasma cell disease and whether c-Met is activated, using a phospho-specific anti-c-Met antibody. Secondly, we aimed to investigate a possible relationship between HGF/c-Met staining and patient outcome in terms of survival.

Material and methods

Patient samples

We analyzed bone marrow samples from 80 patients diagnosed with monoclonal plasma cell disease in central Norway between 1996 and 2005. 68 patients had multiple myeloma, six patients had monoclonal gammopathy of undetermined significance (MGUS), and six patients had a solitary plasmacytoma (table 1A). The material consisted of bone marrow trephine biopsies, clots from bone marrow aspirates and biopsies from plasmacytomas localized extramedullary or in bone. For ten of the myeloma patients, bone marrow plasma and serum were also available. All samples were obtained at diagnosis, and before initiation of treatment. We included all biopsies that were taken from previously untreated patients with monoclonal plasma cell disease during this time period, provided that sufficient material was available. We also examined ten bone marrow biopsies that were taken during the same time period from individuals in whom a bone marrow disease had been suspected, but was not confirmed. These persons are in the following sections termed as healthy individuals, referring to their morphologically normal bone marrow, although their complete health status is not known.

Clinical information about the myeloma patients was obtained retrospectively from the patient records, without knowledge of the HGF/c-Met results. Registered information was stage according to Durie Salmon and International Scoring System (ISS), type and concentration of serum and urin M-protein, plasma cell percentage in bone marrow aspirate, serum β_2-microglobulin and overall survival. Bone disease was assessed by X-ray, and scored semiquantitatively. Five regions were defined: The cranium, vertebral spine, pelvis, long bones and other areas. Each region was scored according to the following system: 0 (no osteolytic lesions), 1 (less than five osteolytic lesions) or 2 (five or more lesions). The total score was calculated as the sum of all five regions. The serum M-protein was of IgG type in 66%, IgA type in 7%, other Ig isotypes in 4% and more than one isotype in 3% of the patients. 16% of the patients had only light chain secretion and 3% had non-secretory myeloma. 23% of the patients were in International Scoring System (ISS) stage 1, 31% were in stage 2 and 23% in stage 3; for 22% no ISS information was available (Table 1B). The study protocol was approved by the Regional Ethics Committee. The study was carried out in accordance with the declaration of Helsinki.

Antibodies and other reagents

A polyclonal antibody against the HGF β chain (H495) from IBL (Hamburg, Germany) was used at dilution 1:50. A polyclonal antibody against the HGF alpha chain (H-145) from Santa Cruz Biotechnology (SCBT) (Santa Cruz, CA, USA) was used at dilution 1:100. The monoclonal HGF antibody 2D7 was made in our laboratory as previously described[5] and used at dilution 1:100. The anti-c-Met C-28 antibody from SCBT was used at dilution 1:200. The anti-phospho-c-Met pYpYpY1230/1234/1235 antibody from Biosource/Invitrogen (Carlsbad, CA,USA) was used at dilution 1:100. The anti-CD138 antibody (clone MI15) from Dako

(Glostrup, Denmark) was used at dilution 1:100. Peptides for control experiments were purchased from IBL (HGF), SCBT (c-Met) and Biosource (phospho-c-Met).

Immunohistochemistry

The biopsies were fixed in formalin, decalcified in EDTA and embedded in paraffin. Sections cut at 4 μm were mounted on Cryostat glass, deparaffinised in xylene and rehydrated through a graded series of ethanol solutions to distilled water. Heat induced antigen retrieval was done in a steamer for 12 min. For the HGF staining, the sections were incubated with primary antibody in Tris-buffered saline, 0.25% BSA, 0.25% Tween 20, pH 7.6 for 1 hour at room temperature. For the c-Met and phospho-c-Met staining, blocking of non-specific staining in Tris-buffered saline, 2.5% BSA, pH 7.6 was done for 1 hour, before incubation with the primary antibodies in Tris-buffered saline, 0.25% BSA, 0.25% Tween 20, pH 7.6 for 24 hours at 4 ºC. Endogenous peroxidase activity was quenched with H_2O_2, and immunohistochemical reactions were visualized with DakoCytomation EnVisionDAB, K5007. Sections were counterstained with hematoxylin. All the slides were processed by the same technician in the same laboratory.

We validated the HGF immunohistochemistry method in a pilot study, using the three antibodies described above on 12 bone marrow biopsies. These biopsies were not part of the main study population. The HGF beta antibody from IBL had a minimum of background/non-specific staining and was chosen for the main study. The C-28 anti-c-Met antibody has been widely used for immunohistochemistry studies,[15,16] and was therefore not included in the pilot study. Control for specificity was carried out by 1) omitting the primary antibody, 2)

replacing it with non-immune rabbit immunoglobulins, and 3) preincubating the primary antibody over night at 4 ºC with a molar excess of the antigen by which it was raised. To test the specificity of the immunoreaction of phosphorylated c-Met, a blocking experiment was performed, where the primary antibody was pre-incubated over night at 4 ºC with a molar excess of the phosphorylated or non-phosphorylated antigen peptide. An immunohistochemical staining that was blocked by the phosphorylated, but not by the non-phosphorylated peptide, was considered a phospho-specific reaction.

The plasma cells were identified by CD 138 staining of sections adjacent to the HGF and c-Met sections. Only cases with positive immunoreaction for CD138 in the plasma cells were included. For each antigen, we evaluated the percentage of immunoreactive cells per case and the cellular location of the staining. The percentage of positively stained cells was determined by counting at least 200 plasma cells from two different areas. For patients with MGUS, and for healthy individuals, a lower number of plasma cells were counted. We scored the expression as positive when the proportion of immunoreactive plasma cells was 10% or higher. The scoring was performed independently by two researchers (KFW and UMF) without knowledge of diagnosis or clinical information. Discrepancies were resolved by joint review together with a senior pathologist (AB) on a multihead microscope. The inter-observer agreement by kappa statistics was estimated to 0.58, defined as moderate agreement.[17] The main source of disagreement was coexistence of background staining and faint cell staining in the same slide. Images were captured using a Nikon Eclipse 80i microscope and Nikon dig SIGHT DS5-M camera, and processed by Nikon Imaging Software NIS-Elements (Nikon Instruments Europe B.V., Badhoevedorp, Netherlands).

ELISA

HGF was measured with an ELISA from R&D systems (Minneapolis, MN, USA). The assay was performed according to the manufacturer's instructions. All samples were run in duplicates. The standard curve was linear between 0.5 and 8 ng/mL. Due to limited quantities of sample material the measurements could not be repeated and therefore samples with HGF concentrations above 8 ng/mL were given the value 8 ng/mL. Variation coefficients for the measurements were <10%.

Statistics

Inter-observer agreement was estimated by Cohen's kappa statistics. Kappa between 0.4 and 0.6 was considered as a moderate agreement, kappa between 0.6 and 0.8 as a substantial agreement, and kappa >0.8 as an excellent agreement.[17] Pearson's χ^2 or Fisher's exact tests were used for between-group comparison of discrete variables. Comparisons between groups for continuous variables were performed using Mann Whitney U test. Survival between groups was compared by the log rank test. In 2 x 2 tables with small or zero values (Table 2A,B), exact p-values and exact confidence intervals for OS was computed using StatXact 8 (Cytel Inc.Cambride, MA, USA). All other statistical calculations were performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at $p = 0.05$. All p-values were 2-tailed.

Results

HGF accumulates in normal and malignant plasma cells

First, we examined the expression of HGF as assessed by immunohistochemistry in biopsies from 80 previously untreated patients with plasma cell disease and ten healthy individuals. Cytoplasmic staining for HGF in the plasma cells was demonstrated in 58 of 68 biopsies from multiple myeloma patients (85%), six of six MGUS biopsies (100%) and six of six plasmacytoma biopsies (100%). We also found positive reaction for HGF in plasma cells in nine of ten of the normal bone marrow samples (90%), and in the plasma cells of a reactive gingival lesion (Table 2A) (Figure 1). The specificity of the staining was verified by control experiments as described in Methods (Figure 1). There was no significant difference in percentage of positive cases, or staining intensity, between biopsies from healthy individuals and biopsies from patients with multiple myeloma, MGUS or solitary plasmacytoma (Table 2A). In the bone marrow of normal individuals, MGUS and multiple myeloma, other hematopoietic cells, mainly megakaryocytes, myeloid precursors at all stages and mature neutrophils were faintly positive for HGF, but by far the strongest staining was seen in the plasma cells (Figure 1).

The HGF-negative patients had a lower bone marrow plasma cell percentage as assessed by bone marrow aspirate (median plasma cell infiltration 10%) than the HGF-positive patients (median plasma cell infiltration 34%). The difference was statistically significant ($p = 0.016$). Information of ISS stage was available for six of the ten patients with a HGF-negative biopsy. None of the six HGF-negative patients were in ISS stage 3, while 16 of 47 (34%) of the HGF-positive patients were in ISS stage 3. The difference was not statistically significant ($p =$

0.10). There was no significant difference in the severity of bone disease, concentration of serum M-protein, serum β_2-microglobulin or overall survival between patients with a HGF-negative or positive biopsy (data not shown).

We then examined the concentration of HGF in serum and bone marrow plasma of ten of the myeloma patients. The concentration of HGF was higher in the bone marrow than in serum in eight of ten patients. In one patient the levels were higher in serum, and one patient was not evaluable due to levels above the dynamic range (Figure 3).

c-Met is expressed in malignant plasma cells.

Sections from the same 68 biopsies were then stained for c-Met. Biopsies from five of the 68 patients were not evaluable because of high background staining, and were therefore excluded from analysis. Positive staining for c-Met in the plasma cells was demonstrated in biopsies from 25 of 63 evaluable multiple myeloma patients (40%) (Table 2B). The staining pattern was membranous and/or cytoplasmic. In some cases there was also a faint nuclear staining. The staining of the plasma cells was specific, as shown by the control experiments described in Methods. However, there was some background staining in several cases, and due to difficulties in discriminating a weak cytoplasmic/nuclear staining from the background, we decided only to regard cells with a clear membranous staining as truly positive. None of the ten biopsies from healthy individuals had positive c-Met staining in plasma cells. The difference between myeloma patients and healthy individuals was statistically significant, $p = 0.021$. Positive staining for c-Met was also seen in four of six MGUS patients (67%), but in only one of six patients with a solitary plasmacytoma (17%) (Table 2B).

There was concomitant positive staining for both HGF and c-Met in the plasma cells of 23 of 63 myeloma patients (36%), four of six MGUS patients (67%) and one of six plasmacytoma patients (17%). There was no significant correlation between positive HGF and c-Met staining. There was no significant difference in disease stage according to ISS, severity of bone disease, concentration of serum M-protein, serum β_2-microglobulin, or overall survival between patients with a c-Met-negative or positive biopsy (data not shown).

c-Met is phosphorylated in malignant plasma cells.

We then examined whether c-Met is phosphorylated in patients with malignant plasma cell disease and in healthy individuals. For this purpose, we performed immunohistochemical staining with a phospho-specific antibody, in a subset of 21 biopsies from myeloma patients, four biopsies from MGUS patients, and seven biopsies from healthy individuals. The selection criteria were: 1) Biopsy positive for total c-Met and 2) Biopsy of optimal technical quality. The phospho-c-Met staining pattern of the plasma cells was membranous and/or cytoplasmic. In some cases there was also a faint nuclear staining. Following the same evaluation criteria as for total c-Met, we decided only to regard cells with a clear membranous staining as truly positive (Figure 2). Specificity was shown by a peptide competition experiment as described in Methods (data not shown). Positive staining for phospho-c-Met in the plasma cells was demonstrated in biopsies from 15 of the 21 myeloma patients (71%), and one of the four MGUS patients. All of the biopsies from healthy individuals were negative.

Discussion

In this study we examined expression of HGF and c-Met by immunohistochemistry in biopsies from bone marrow or extramedullary plasmacytomas in patients with plasma cell disease. We show that HGF and c-Met are concomitantly expressed in the plasma cells, and that c-Met exists in its phosphorylated state, in a substantial proportion of myeloma patients, suggesting that the HGF/c-Met system is active in myeloma patients *in vivo*. Expression of c-Met and phospho-c-Met was strictly confined to malignant plasma cells, as opposed to plasma cells of healthy subjects. Thus, this study points to c-Met and its activation as one of the factors that discriminate malignant from normal plasma cells.

Serum levels of HGF are elevated in multiple myeloma patients as compared to healthy individuals,[5,6] and levels of HGF are higher in the bone marrow than in the circulation.[5,18] Levels of HGF mRNA in crude bone marrow biopsies of myeloma patients are significantly higher than in healthy individuals (Tian et al, manuscript in preparation). HGF can be produced by myeloma cells,[3,4] and by stromal cells as well as by hematopoietic cells of the myeloid lineage and mature neutrophils in the bone marrow microenvironment.[1,2,19] In this study, we found the strongest staining for HGF in the plasma cells, and a comparatively weak staining of other cells of the bone marrow. We found negative immunostaining for HGF in only a small subset of ten myeloma patients (15%). In accordance with other studies, our study demonstrates that the bone marrow is richly supplied with HGF, and that HGF accumulates in the plasma cells.

We found positive immunostaining for HGF also in normal plasma cells, seemingly in conflict with gene expression data from Zhan et al[20] and Hose et al,[21] who showed that HGF is not expressed by normal plasma cells. However, there are several possible explanations for the accumulation of HGF in plasma cells. HGF is a heparin binding growth factor, which is able to interact with heparan sulfate proteoglycans (HSPG). The main HSPG on myeloma cells is CD138 (syndecan-1). We have earlier shown that HGF can exist as a complex with syndecan-1,[22] and bind to syndecan-1 on the surface of myeloma cells.[23] It is therefore possible that the strong immunostaining for HGF in both normal, MGUS and myeloma plasma cells found in this study, reflects HGF that is secreted by other cells and thereafter bound to syndecan-1 on the plasma cell surface. Alternatively, the detected HGF originates from the plasma cells. It is also possible that both these mechanisms are operative.

In contrast to HGF, c-Met staining was exclusively confined to MGUS and myeloma patients as there was negative staining of the bone marrow plasma cells of ten healthy individuals. Expression of c-Met has earlier been detected by various methods in multiple myeloma cell lines.[3,13] Upregulation of the gene coding for c-Met has previously been shown by gene expression profiling in plasma cells of myeloma patients as compared to healthy donors.[21,24] Using immunohistochemistry, we found expression of c-Met also at the protein level in 40% of myeloma patients. Our results agree well with Derksen et al, who found expression of c-Met by Western blot in cell lysates of frozen-stored bone marrow aspirates in seven of 13 multiple myeloma patients, but in none of seven normal bone marrow samples.[8] We and others have earlier by *in vitro* studies shown that the HGF/c-Met system may promote proliferation, survival, adhesion and migration of myeloma cells[8-10] and contribute to the myeloma bone disease.[11] Importantly, in this study we found positive staining for

phosphorylated c-Met in a subset of biopsies, supporting that the HGF/c-Met system is active not only in cell lines, but also in myeloma patients.

HGF and c-Met expression and signaling have been demonstrated in several cancer types, including breast, colorectal, gastric, head & neck, liver, lung, pancreas, ovarian, renal, prostate, sarcoma and lymphoma.[7,25] The HGF/c-Met system, mediating a broad range of tumor progression features like proliferation, invasion, survival, metastasis and angiogenesis,[26] has been proposed as a promising target for therapy in different types of cancer,[25] including multiple myeloma.[13] Several studies on targeting c-Met in cancer have included competitors of HGF or c-Met, monoclonal antibodies directed against HGF or c-Met, and small-molecule tyrosine kinase inhibitors directed against c-Met.[13,25,27-29] Our data support that the HGF/c-Met system is a potential target also in multiple myeloma. In this context, immunohistochemistry might be a useful method to select patients who could be candidates for such therapy.

In conclusion, our study points to c-Met as one of the factors that discriminate normal plasma cells from myeloma cells. It exists in its phosphorylated state in biopsies from myeloma patients, indicating that the HGF/c-Met system is activated, and thus may be a target for therapeutic intervention in multiple myeloma.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgements

We are grateful to Borgny Ytterhus and Eli Johannesen for excellent technical assistance. This work was supported by grants from the Norwegian Cancer Society, The Cancer Fund of St Olav's Hospital, The Research Council of Norway and The Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU).

References

1. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem.* 1996; **119:** 591-600.

2. Takai K, Hara J, Matsumoto K, *et al.* Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. *Blood.* 1997; **89:** 1560-1565.

3. Borset M, Lien E, Espevik T, Helseth E, Waage A, Sundan A. Concomitant expression of hepatocyte growth factor/scatter factor and the receptor c-MET in human myeloma cell lines. *J Biol Chem.* 1996; **271:** 24655-24661.

4. Hov H, Holt RU, Ro TB*, et al.* A selective c-met inhibitor blocks an autocrine hepatocyte growth factor growth loop in ANBL-6 cells and prevents migration and adhesion of myeloma cells. *Clin Cancer Res.* 2004; **10:** 6686-6694.

5. Seidel C, Borset M, Turesson I, Abildgaard N, Sundan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. The Nordic Myeloma Study Group. *Blood.* 1998; **91:** 806-812.

6. Iwasaki T, Hamano T, Ogata A, Hashimoto N, Kitano M, Kakishita E. Clinical significance of vascular endothelial growth factor and hepatocyte growth factor in multiple myeloma. *Br J Haematol.* 2002; **116:** 796-802.

7. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol.* 2003 Dec; **4**(12)**:** 915-925.

8. Derksen PW, de Gorter DJ, Meijer HP, *et al.* The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. *Leukemia.* 2003; **17:** 764-774.

9. Holt RU, Baykov V, Ro TB*, et al.* Human myeloma cells adhere to fibronectin in response to hepatocyte growth factor. *Haematologica.* 2005; **90:** 479-488.

10. Holt RU, Fagerli UM, Baykov V*, et al.* Hepatocyte growth factor promotes migration of human myeloma cells. *Haematologica.* 2008; **93:** 619-622.

11. Standal T, Abildgaard N, Fagerli UM, *et al.* HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. *Blood.* 2007; **109:** 3024-3030.

12. Hov H,Tian E, Holien T, *et al.* c-Met signaling promotes IL-6-induced myeloma cell proliferation. *Eur J Haematol.* 2009; **82:** 277-287.

13. Du W, Hattori Y, Yamada T*, et al.* NK4, an antagonist of hepatocyte growth factor (HGF), inhibits growth of multiple myeloma cells: molecular targeting of angiogenic growth factor. *Blood.* 2007; **109:** 3042-3049.

14. Stellrecht CM, Phillip CJ, Cervantes-Gomez F, Gandhi V. Multiple myeloma cell killing by depletion of the MET receptor tyrosine kinase. *Cancer Res.* 2007; **67:** 9913-9920.

15. Teofili L, Di Febo AL, Pierconti F, *et al.* Expression of the c-met proto-oncogene and its ligand, hepatocyte growth factor, in Hodgkin disease. *Blood.* 2001; **97:** 1063-1069.

16. Ayhan A, Ertunc D, Tok EC. Expression of the c-Met in advanced epithelial ovarian cancer and its prognostic significance. *Int J Gynecol Cancer.* 2005; **15:** 618-623.

17. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* 1977; **33:** 159-174.

18. Andersen NF, Standal T, Nielsen JL*, et al.* Syndecan-1 and angiogenic cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival. *Br J Haematol.* 2005; **128:** 210-217.

19. Toyama T, Ido A, Sasak H, *et al.* Possible involvement of neutrophils in a serum level increase of hepatocyte growth factor in non-Hodgkin's lymphoma. *Oncol Rep.* 2005; **13:** 439-444.

20. Zhan F, Hardin J, Kordsmeier B, *et al.* Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood.* 2002; **99:** 1745-1757.

21. Hose D, Moreaux J, Meissner T*, et al.* Induction of angiogenesis by normal and malignant plasma cells. *Blood.* 2009; **114:** 128-143.

22. Seidel C, Borset M, Hjertner O*, et al.* High levels of soluble syndecan-1 in myeloma-derived bone marrow: modulation of hepatocyte growth factor activity. *Blood.* 2000; **96:** 3139-3146.

23. Borset M, Hjertner O, Yaccoby S, Epstein J, Sanderson RD. Syndecan-1 is targeted to the uropods of polarized myeloma cells where it promotes adhesion and sequesters heparin-binding proteins. *Blood.* 2000; **96:** 2528-2536.

24. Sprynski AC, Hose D, Caillot L, *et al*. The role of IGF-1 as a major growth factor for myeloma cell lines and the prognostic relevance of the expression of its receptor. Blood. 2009; **113**: 4614-4626.

25. Eder JP, Vande Woude GF, Boerner SA, LoRusso PM. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. *Clin Cancer Res.* 2009; **15:** 2207-2214.

26. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. *Nat Rev Cancer.* 2006 Aug; **6**(8)**:** 637-645.

27. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov.* 2008; **7:** 504-516.

28. Matsumoto K, Nakamura T. NK4 (HGF-antagonist/angiogenesis inhibitor) in cancer biology and therapeutics. *Cancer Sci.* 2003; **94:** 321-327.

29. Burgess T, Coxon A, Meyer S*, et al.* Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. *Cancer Res.* 2006; **66:** 1721-1729.

Table 1A. Overview over cases and biopsy material.

Diagnosis	No of cases	No of biopsies stained for HGF	No of biopsies stained for c-Met	No of biopsies stained for p-Met
Healthy	10	10	10	7
MGUS	6	6	6	4
Myeloma	68	68	68	21
Solitary plasmacytoma of bone	2	2	2	0
Solitary extramedullary plasmacytoma	4	4	4	0

Table 1B. Patient characteristics

Age	Median (range)	69 (30 – 88)
Sex	Male/female	47/21
ISS	1	16 (23%)
	2	21 (31%)
	3	16 (23%)
	Information not available	15 (22%)
DS	1	9 (13%)
	2	25 (37%)
	3	32 (47%)
	Information not available	2 (3%)
DS A or B	A	54 (79%)
	B	11 (16%)
	Information not available	3 (4%)
M-protein isotype	IgG	45 (66%)
	IgA	5 (7%)
	IgG + IgA	2 (3%)
	Other Ig subtype	3 (4%)
	Light chain only	11 (16%)
	Non-secretory	2 (3%)
Serum M-protein, g/L	Median (range)	25 (0 – 76)

Table 2A. Number and percentage of HGF-positive plasma cells in biopsies from patients with MGUS, multiple myeloma and solitary plasmacytoma, as compared with healthy individuals.

HGF					
	No of cases	No of positive cases	% positive cases	95% CI of OR*	p-value
Healthy	10	9	90	reference	reference
MGUS	6	6	100	0.015, infinite*	1.000
Myeloma	68	58	85	0.013, 5.68	1.000
Solitary plasmacytoma	6	6	100	0.015, infinite*	1.000

*The odds ratio (OR) and the upper limit of the 95% confidence interval (CI) is infinite due to zero values in one or more cells.

Table 2B. Number and percentage of c-Met-positive plasma cells in biopsies from patients with MGUS, multiple myeloma and solitary plasmacytoma, as compared with healthy individuals.

c-Met					
	No of cases	No of positive cases	% positive cases	95% CI for OR*	p-value
Healthy	10	0	0	reference	reference
MGUS	6	4	67	1.61, infinite*	0.016
Myeloma	63	25	40	1.32, infinite*	0.021
Solitary plasmacytoma	6	1	17	0.043, infinite*	0.750

*The odds ratio (OR) and the upper limit of the 95% confidence interval (CI) is infinite due to zero values in one or more cells.

Figure legends

Figure 1. Immunohistochemical staining of biopsies with malignant or non-malignant plasma cells. Panel (A-C) show 4 μm sections with cytoplasmic staining for HGF in (A) multiple myeloma plasma cells, (B) plasma cells of a reactive lesion and (C) plasma cells of normal bone marrow. Panel (E-G) show membranous and cytoplasmic staining for c-Met in (E) multiple myeloma plasma cells. Only cells with a clear membranous staining were defined as positive. No membranous staining was seen in (F) plasma cells of a reactive lesion and (G) plasma cells of normal bone marrow. (D+H) Negative control. Controls for specificity were carried out as described in the Methods section. (I-K) CD138 and (L) Hematoxylin-Eosin-Saffron (HES) staining for identification of plasma cells. Images were captured using a Nikon Eclipse 80i microscope and Nikon dig SIGHT DS5-M camera, and processed by Nikon Imaging Software NIS-elements (Nikon Instruments Europe B.V., Badhoevedorp, Netherlands). Original magnification x 1000.

Figure 2. Immunohistochemical staining for phospho-c-Met in bone marrow biopsies from patients with monoclonal plasma cell disease. Only cases that were positive for c-Met were included. Panel (A) shows membranous staining for phospho-c-Met. Only cells with a clear membranous staining were defined as positive. (B) Negative control section, showing non-specific staining of cytoplasm, but no membranous staining. Images were captured using a Nikon Eclipse 80i microscope and Nikon dig SIGHT DS5-M camera, and processed by Nikon Imaging Software NIS-elements (Nikon Instruments Europe B.V., Badhoevedorp, Netherlands). Original magnification x 1000.

Figure 3. Concentration of HGF as measured by ELISA in bone marrow plasma and serum of a subset of ten multiple myeloma patients. All samples were run in duplicates. The standard curve was linear between 0.5 and 8 ng/mL. Due to limited quantities of sample material the measurements could not be repeated and therefore samples with HGF concentrations above 8 ng/mL were given the value 8 ng/mL.

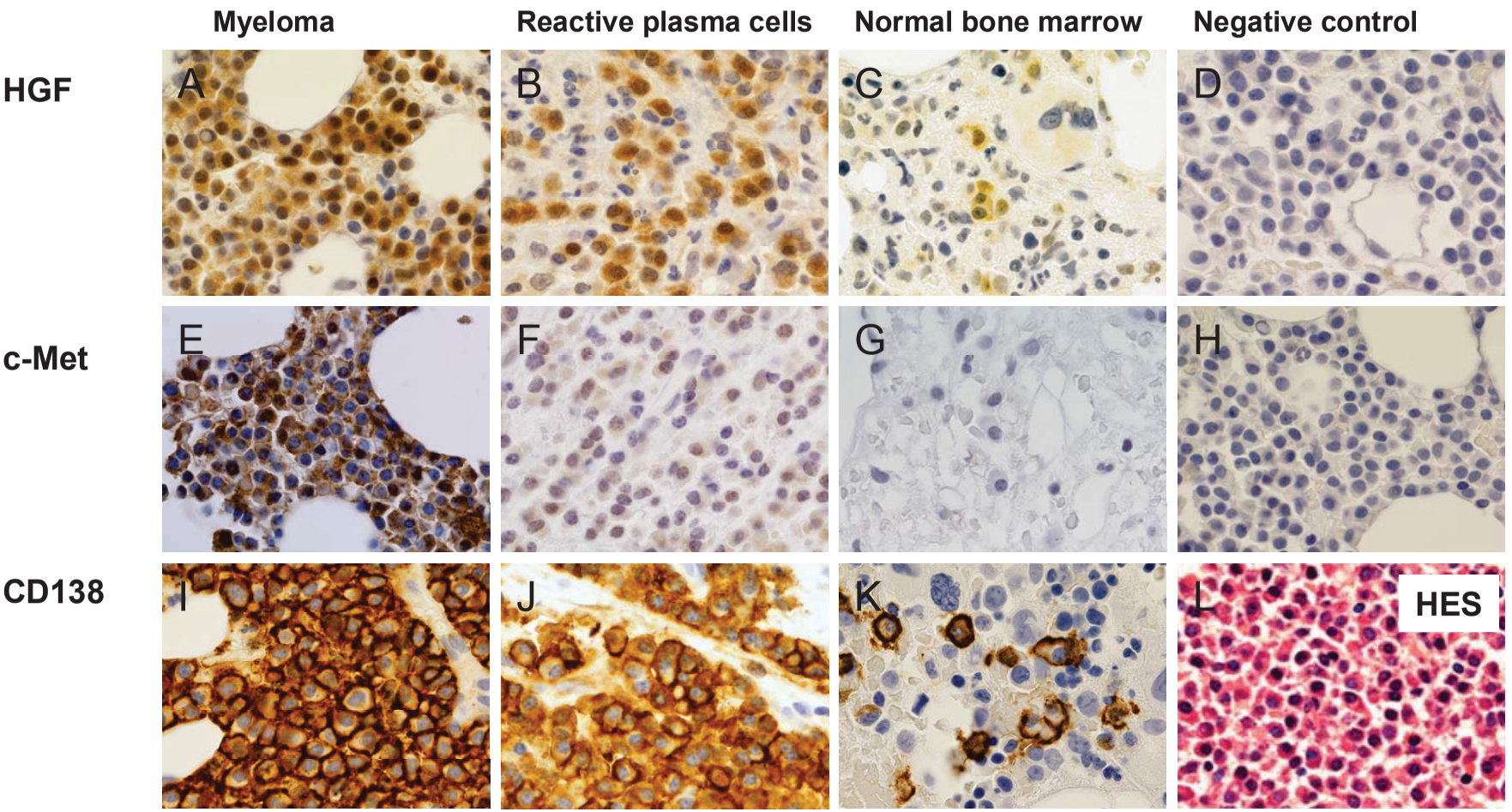
Myeloma
Reactive plasma cells
Normal bone marrow
Negative control
HGF
c-Met
CD138
A
B
G
D
E
F
G
H
I
J
K
L
HES

Figure 1

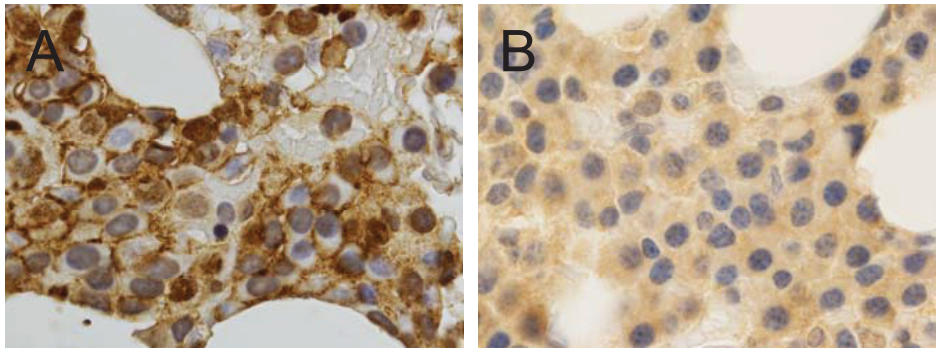

Figure 2

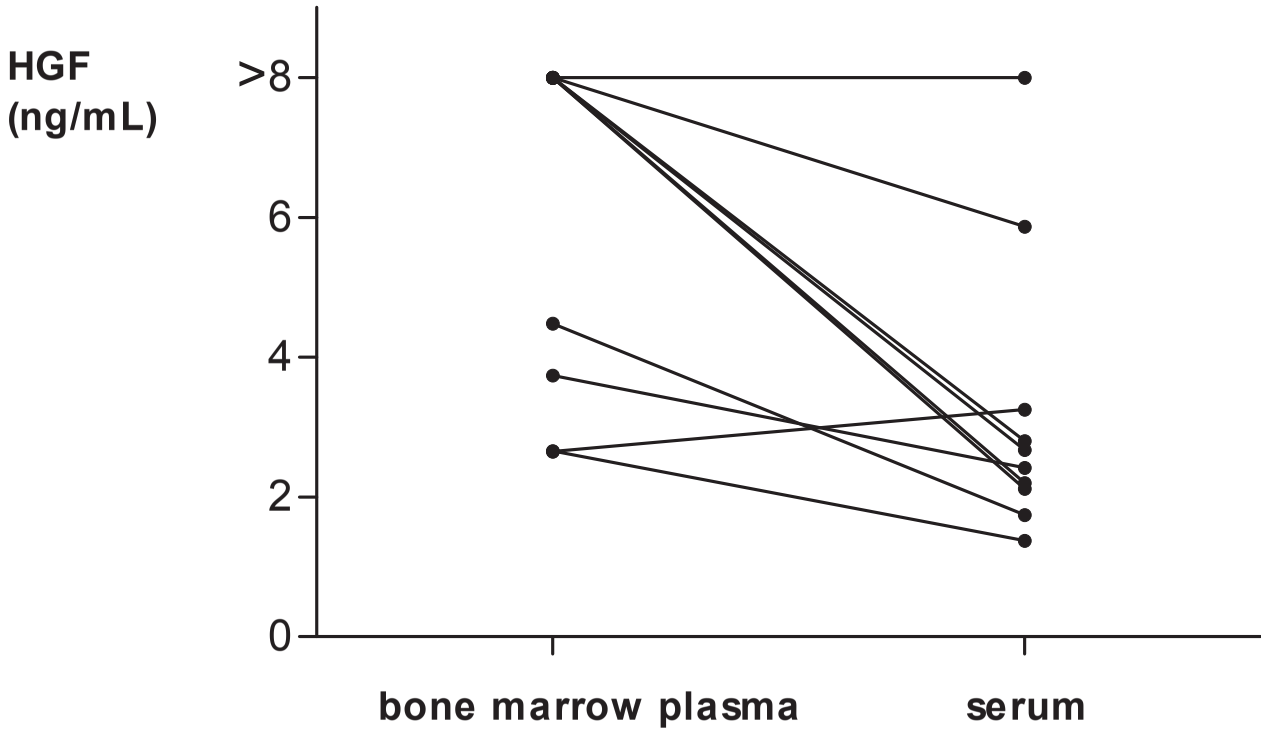

Figure 3

Paper II

European Journal of Haematology ISSN 0902-4441

ORIGINAL ARTICLE

Elevated serum concentrations of activated hepatocyte growth factor activator in patients with multiple myeloma

K. F. Wader[1,2], U. M. Fagerli[1,2], R. U. Holt[1,3], B. Stordal[1], M. Børset[1,4], A. Sundan[1], A. Waage[1,5]

[1]Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim; [2]Department of Oncology, St Olavs Hospital, Trondheim; [3]Department of Food and Medical Technology, Sør-Trøndelag University College, Trondheim; [4]Department of Immunology and Transfusion Medicine, St Olavs Hospital, Trondheim; [5]Department of Hematology, St Olavs Hospital, Trondheim, Norway

Abstract

Objectives: Hepatocyte growth factor (HGF) is a potential key factor in multiple myeloma. Conversion of pro-HGF to its active form is a critical limiting step for its biological effects. We aimed to examine the levels of the most potent activator, the hepatocyte growth factor activator (HGFA), in serum and bone marrow plasma of patients with multiple myeloma. *Methods:* The activated form of HGFA was measured by an enzyme-linked immunosorbent assay in serum (n = 49) and bone marrow plasma (n = 16) from multiple myeloma patients, and in serum from healthy controls (n = 24). *Results:* The median concentrations of activated HGFA in myeloma and control sera were 39.7 (range 6.2–450.0) and 17.6 ng/mL (range 4.8–280.6), respectively. The difference was statistically significant (P = 0.037). The median concentration of activated HGFA in bone marrow plasma was 6.1 ng/mL (range 3.5–30.0). *Conclusion:* We here show for the first time that the activated form of HGFA is present at high levels in serum and bone marrow of myeloma patients, thus providing a necessary prerequisite for the activation of HGF.

Key words multiple myeloma; hepatocyte growth factor; scatter factor; hepatocyte growth factor activator

Correspondence Karin Fahl Wader, Department of Cancer Research and Molecular Medicine, Medical Technical Research Center, Faculty of Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway. Tel: +47 73550451; Fax: +47 73598801; e-mail: karin.f.wader@ntnu.no

Accepted for publication 29 July 2008 doi:10.1111/j.1600-0609.2008.01130.x

Hepatocyte growth factor (HGF) stimulates survival, proliferation (1), adhesion (2) and migration (3) of malignant plasma cells and is a potential contributor to the bone disease of multiple myeloma (4). HGF is produced by myeloma cells and by stromal cells in the bone marrow microenvironment, and thereby acts in an autocrine or paracrine manner through its receptor c-Met (5–7). We and others have previously shown that serum HGF levels are elevated in myeloma patients compared with normal controls, and associated with poor prognosis (8, 9).

HGF is secreted as a single chain precursor which is proteolytically converted to its biologically active heterodimeric form. The most potent activator is the factor XII-related serine protease hepatocyte growth factor activator (HGFA) (10, 11). HGFA is mainly secreted by the liver, although extrahepatic expression has been reported in a number of normal and tumour tissues (12). It circulates in plasma as a single-chain 96-kDa pro-form, which is activated by thrombin in the presence of negatively charged molecules to its 34-kDa active two-chain heterodimeric form (13). The HGFA activity is regulated by the HGF activator inhibitors (HAI)-1 and -2, reviewed in (12).

Tjin *et al.* (14) showed that myeloma cells express HGFA and thereby proteolytically convert single chain HGF into its active form. We aimed to examine the levels of the activated form of HGFA in serum and bone marrow plasma from myeloma patients, and to correlate the serum levels with clinical stage, parameters of disease

activity and survival. Secondly, we aimed to investigate a possible relationship between the concentrations of HGFA and HGF.

Patients and methods

We examined serum samples drawn at diagnosis from 49 patients diagnosed with multiple myeloma in mid-Norway between 1996 and 2005. We also examined bone marrow plasma from the same patients when available ($n = 16$). Serum and bone marrow plasma samples were drawn before initiation of treatment and frozen at −80°C until they were analyzed. In six patients, we also examined serum drawn at time of first response, defined according to the EBMT/IBMTR/ABMTR criteria (15) and at first relapse, defined as the time point where treatment was re-introduced. Control samples were obtained from 24 healthy volunteers. Because of limited quantities of sample material, HGF was analyzed in only 20 of the 24 controls. Clinical information about the myeloma patients was obtained retrospectively from the patient records. Registered information was stage according to Durie Salmon and International Scoring System (ISS), type and concentrations of serum and urine M-component, plasma cell percentage in bone marrow aspirate, serum β_2-microglobulin and overall survival. The study protocol was approved by the Regional Medical Ethics Committee and the study was performed according to the declaration of Helsinki.

The median age of the myeloma patients (33 men and 16 women) was 65 yr (range 30–87 yr), and of the controls (15 men and 9 women) was 68 yr (range 44–81 yr). The patients were representative of the general myeloma population with serum M-component of IgG type in 29 patients (59%), IgA in seven patients (14%), other Ig isotypes in three patients (6%), only light chain secretion in nine patients (18%) and non-secretory myeloma in one patient (2%). Twenty patients (41%) were in stage 1 according to ISS, 13 patients (26%) in stage 2 and 11 patients (22%) in stage 3; for five patients (10%), no information was available.

We used a commercially available enzyme-linked immunosorbent assay (ELISA) for the measurement of activated HGFA (IBL, Gunma, Japan) in serum and bone marrow plasma. The assay was performed according to the manufacturer's instructions. All samples were run in duplicates. The standard curve was linear between 0.9 and 15 ng/mL, and samples were diluted to concentrations within this range. The intra-assay and interassay variation coefficients for this assay are 5.5% and 5.5% at 6.5 ng/mL according to the manufacturer. Variation coefficients for our measurements were < 10%.

HGF was measured with an ELISA from R&D systems (Minneapolis, MN, USA). The assay was performed according to the manufacturer's instructions. All samples were run in duplicates. The standard curve was linear between 0.5 and 8 ng/mL. Because of limited quantities of sample material, the measurements could not be repeated and therefore samples with HGF concentrations lower than 0.5 ng/mL and above 8 ng/mL were given the values 0.5 and 8 ng/mL. Variation coefficients for our measurements were < 10%. Up to two freeze–thaw cycles of serum did not affect the measured levels of HGF or HGFA.

SPSS Statistical Software version 14.0 was used for statistic calculations (SPSS Inc, Chicago, IL, USA). Comparisons between groups were performed by the Mann–Whitney *U*-test. Correlations between two parameters were estimated by Spearman's rank correlation analysis. Survival analysis was conducted by the Kaplan–Meier method, using median values as cut off. The level of statistical significance was set at $P < 0.05$. *P*-values were two-tailed.

Results

Serum levels of activated HGFA in patients at the time of diagnosis and in controls are shown in Fig. 1. The median HGFA concentrations in myeloma and control sera were 39.7 (range 6.2–450.0) and 17.6 ng/mL (range 4.8–280.6), respectively. The difference was statistically significant ($P = 0.037$). The median level of activated HGFA in bone marrow plasma of myeloma patients was 6.1 ng/mL (range 3.5–30.0) (data not shown). Thus, HGFA levels were lower in bone marrow plasma than in serum. However, serum and plasma HGFA levels cannot be directly compared, as measurement of levels in serum will be 2–3 times higher than in plasma in this assay according to the manufacturer and own validation experiments (data not shown).

There was no correlation between the serum levels of HGFA and disease stage according to ISS or Durie

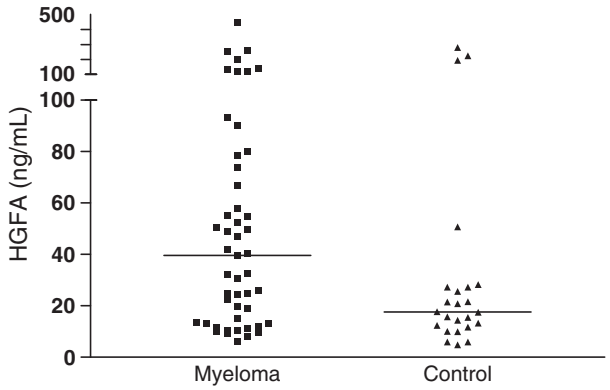

Figure 1 Serum concentrations of activated HGFA measured by ELISA in 49 multiple myeloma patients and 24 age- and gender-matched controls. Bars indicate median concentration.

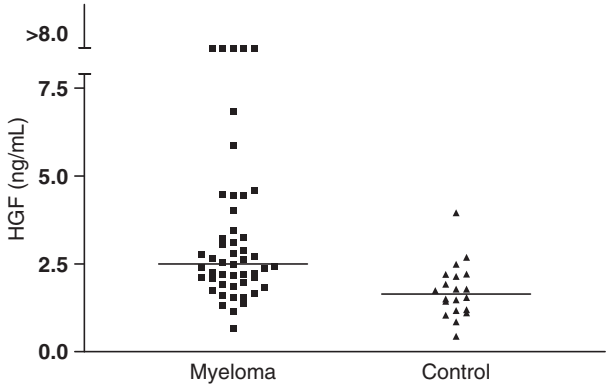

Figure 2 Serum concentrations of HGF measured by ELISA in 49 myeloma patients and 20 age- and gender-matched controls. Bars indicate median concentration.

Salmon, concentration of serum M-component, serum β_2-microglobulin, percentage of plasma cells in the bone marrow or overall survival (data not shown). The HGFA levels did not covariate with disease activity in serial measurements of serum drawn at diagnosis, remission and relapse in six myeloma patients (data not shown).

The median HGF concentrations in myeloma and control sera were 2.5 (range 0.7–8.0) and 1.6 ng/mL (range 0.5–4.0), respectively (Fig. 2). The difference was statistically significant ($P < 0.001$). The median HGF concentration in bone marrow plasma was 8.0 ng/mL. There was no correlation between the levels of HGFA and HGF in serum ($r_s = 0.14$, $P = 0.26$) or bone marrow plasma ($r_s = 0.31$, $P = 0.38$).

Discussion

HGF has a number of myeloma-relevant activities; however, it has to be converted to its heterodimeric form to be biologically active. Urokinase-type plasminogen activator (uPA), tissue plasminogen activator, factor XIIa and matriptase have all been shown to activate single chain HGF at low rates (11, 16–18). The most potent activator is, however, the factor XII-related serine protease HGFA, with an HGF-converting potency of more than 1000 times that of uPA (11). Tjin *et al.* (14) showed that myeloma cells express HGFA, thereby activating HGF. We here demonstrate for the first time that HGFA exists in its activated form in serum from myeloma patients, and that serum concentrations are higher than in healthy controls. We also found detectable activated HGFA in 16 of 16 samples of bone marrow plasma from myeloma patients.

The role of HGFA in regulating HGF activity in injured tissue is well established (12). Recent data support an important function of HGFA also in solid tumours such as colorectal cancer (19) and glioblastoma (20). Among lymphomas, the HGF receptor is predominantly expressed in diffuse large B-cell lymphoma (DLBCL), and interestingly, DLBCL cells also express HGFA, possibly activating HGF produced by macrophages in the tumour microenvironment (21).

The activity of HGFA is tightly regulated. Secreted as an inactive single chain pro-form, cleavage by thrombin is essential for its function. In a recent publication, the kallikrein-related peptidases 4 and 5 were shown to have HGFA-activating properties similar to thrombin (22). The activity of HGFA is also controlled by the Kunitz type serine protease inhibitors HAI-1 and HAI-2 (12).

It is possible that the myeloma cells directly contribute to the elevated HGFA levels in serum of myeloma patients. However, we found no correlation between the serum HGFA concentration and disease stage or traditional markers of tumour burden. As we have measured only the activated form of HGFA, the elevated levels in myeloma patients might also mirror a higher degree of activation of pro-HGFA in patients compared with controls. The complex mechanisms regulating activation of HGF in multiple myeloma, including a potential role for the HGFA inhibitors HAI-1 and HAI-2, should be addressed in further studies.

We found no correlation between serum levels of HGFA and HGF. This is partly in disagreement with Nagakawa *et al.* (23), who found a positive correlation between serum levels of HGF and HGFA in patients with untreated and advanced stage prostate cancer. However, the fact that we have measured total HGF, which is both single chain HGF and the active heterodimer, may obscure a positive correlation between HGFA and active HGF.

In conclusion, activated HGFA is present at high levels in serum and bone marrow of myeloma patients. Although this study has obvious limitations because of the relatively small number of study subjects, it clearly demonstrates the presence of a necessary prerequisite for activation of the HGF system in multiple myeloma. It also points to the activation step of HGF as a possible target for therapeutic intervention.

Acknowledgements

This work was supported by grants from the Norwegian Cancer Society, The Cancer Fund of St Olavs Hospital, The Research Council of Norway and The Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU).

References

1. Derksen PW, de Gorter DJ, Meijer HP, Bende RJ, van Dijk M, Lokhorst HM, Bloem AC, Spaargaren M, Pals

ST. The hepatocyte growth factor/met pathway controls proliferation and apoptosis in multiple myeloma. *Leukemia* 2003;**17**:764–74.
2. Holt RU, Baykov V, Ro TB, Brabrand S, Waage A, Sundan A, Borset M. Human myeloma cells adhere to fibronectin in response to hepatocyte growth factor. *Haematologica* 2005;**90**:479–88.
3. Holt RU, Fagerli UM, Baykov V, Ro TB, Hov H, Waage A, Sundan A, Borset M. Hepatocyte growth factor promotes migration of human myeloma cells. *Haematologica* 2008;**93**:619–22.
4. Standal T, Abildgaard N, Fagerli UM, Stordal B, Hjertner O, Borset M, Sundan A. Hgf inhibits bmp-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. *Blood* 2007;**109**:3024–30.
5. Hov H, Holt RU, Ro TB, Fagerli UM, Hjorth-Hansen H, Baykov V, Christensen JG, Waage A, Sundan A, Borset M. A selective c-met inhibitor blocks an autocrine hepatocyte growth factor growth loop in anbl-6 cells and prevents migration and adhesion of myeloma cells. *Clin Cancer Res* 2004;**10**:6686–94.
6. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem* 1996;**119**:591–600.
7. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;**4**:915–25.
8. Seidel C, Borset M, Turesson I, Abildgaard N, Sundan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. The nordic myeloma study group. *Blood* 1998;**91**:806–12.
9. Iwasaki T, Hamano T, Ogata A, Hashimoto N, Kitano M, Kakishita E. Clinical significance of vascular endothelial growth factor and hepatocyte growth factor in multiple myeloma. *Br J Haematol* 2002;**116**:796–802.
10. Miyazawa K, Shimomura T, Kitamura A, Kondo J, Morimoto Y, Kitamura N. Molecular cloning and sequence analysis of the cDNA for a human serine protease reponsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation factor XII. *J Biol Chem* 1993;**268**:10024–8.
11. Shimomura T, Miyazawa K, Komiyama Y, Hiraoka H, Naka D, Morimoto Y, Kitamura N. Activation of hepatocyte growth factor by two homologous proteases, blood-coagulation factor XIIA and hepatocyte growth factor activator. *Eur J Biochem* 1995;**229**:257–61.
12. Kataoka H, Miyata S, Uchinokura S, Itoh H. Roles of hepatocyte growth factor (HGF) activator and HGF activator inhibitor in the pericellular activation of HGF/scatter factor. *Cancer Metastasis Rev* 2003;**22**:223–36.
13. Shimomura T, Kondo J, Ochiai M, Naka D, Miyazawa K, Morimoto Y, Kitamura N. Activation of the zymogen of hepatocyte growth factor activator by thrombin. *J Biol Chem* 1993;**268**:22927–32.
14. Tjin EP, Derksen PW, Kataoka H, Spaargaren M, Pals ST. Multiple myeloma cells catalyze hepatocyte growth factor (HGF) activation by secreting the serine protease HGF-activator. *Blood* 2004;**104**:2172–5.
15. Smith A, Wisloff F, Samson D. Guidelines on the diagnosis and management of multiple myeloma 2005. *Br J Haematol* 2006;**132**:410–51.
16. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993;**143**:949–58.
17. Lee SL, Dickson RB, Lin CY. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. *J Biol Chem* 2000;**275**:36720–5.
18. Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F, Comoglio PM. Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. *EMBO J* 1992;**11**:4825–33.
19. Kataoka H, Hamasuna R, Itoh H, Kitamura N, Koono M. Activation of hepatocyte growth factor/scatter factor in colorectal carcinoma. *Cancer Res* 2000;**60**:6148–59.
20. Uchinokura S, Miyata S, Fukushima T, Itoh H, Nakano S, Wakisaka S, Kataoka H. Role of hepatocyte growth factor activator (HGF activator) in invasive growth of human glioblastoma cells *in vivo*. *Int J Cancer* 2006;**118**:583–92.
21. Tjin EP, Groen RW, Vogelzang I, Derksen PW, Klok MD, Meijer HP, van Eeden S, Pals ST, Spaargaren M. Functional analysis of HGF/met signaling and aberrant hgf-activator expression in diffuse large b-cell lymphoma. *Blood* 2006;**107**:760–8.
22. Mukai S, Fukushima T, Naka D, Tanaka H, Osada Y, Kataoka H. Activation of hepatocyte growth factor activator zymogen (pro-HGFA) by human kallikrein 1-related peptidases. *FEBS J* 2008;**275**:1003–17.
23. Nagakawa O, Yamagishi T, Fujiuchi Y, Junicho A, Akashi T, Nagaike K, Fuse H. Serum hepatocyte growth factor activator (hgfa) in benign prostatic hyperplasia and prostate cancer. *Eur Urol* 2005;**48**:686–90.

Paper III

Is not included due to copyright

Paper IV

Is not included due to copyright

Paper V

Is not included due to copyright

Dissertations at the Faculty of Medicine, NTNU

1977

1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978

3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979

5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980

6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981

8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983

9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984

11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AND STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
17. Trond Haug: FACTORS REGULATING BEHAVIORAL EFFECTS OG DRUGS.

1985

18. Sven Erik Gisvold: RESUSCITATION AFTER COMPLETE GLOBAL BRAIN ISCHEMIA.
19. Terje Espevik: THE CYTOSKELETON OF HUMAN MONOCYTES.
20. Lars Bevanger: STUDIES OF THE Ibc (c) PROTEIN ANTIGENS OF GROUP B STREPTOCOCCI.
21. Ole-Jan Iversen: RETROVIRUS-LIKE PARTICLES IN THE PATHOGENESIS OF PSORIASIS.
22. Lasse Eriksen: EVALUATION AND TREATMENT OF ALCOHOL DEPENDENT BEHAVIOUR.
23. Per I. Lundmo: ANDROGEN METABOLISM IN THE PROSTATE.

1986

24. Dagfinn Berntzen: ANALYSIS AND MANAGEMENT OF EXPERIMENTAL AND CLINICAL PAIN.
25. Odd Arnold Kildahl-Andersen: PRODUCTION AND CHARACTERIZATION OF MONOCYTE-DERIVED CYTOTOXIN AND ITS ROLE IN MONOCYTE-MEDIATED CYTOTOXICITY.
26. Ola Dale: VOLATILE ANAESTHETICS.

1987

27. Per Martin Kleveland: STUDIES ON GASTRIN.
28. Audun N. Øksendal: THE CALCIUM PARADOX AND THE HEART.
29. Vilhjalmur R. Finsen: HIP FRACTURES

1988

30. Rigmor Austgulen: TUMOR NECROSIS FACTOR: A MONOCYTE-DERIVED REGULATOR OF CELLULAR GROWTH.

31. Tom-Harald Edna: HEAD INJURIES ADMITTED TO HOSPITAL.
32. Joseph D. Borsi: NEW ASPECTS OF THE CLINICAL PHARMACOKINETICS OF METHOTREXATE.
33. Olav F. M. Sellevold: GLUCOCORTICOIDS IN MYOCARDIAL PROTECTION.
34. Terje Skjærpe: NONINVASIVE QUANTITATION OF GLOBAL PARAMETERS ON LEFT VENTRICULAR FUNCTION: THE SYSTOLIC PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT.
35. Eyvind Rødahl: STUDIES OF IMMUNE COMPLEXES AND RETROVIRUS-LIKE ANTIGENS IN PATIENTS WITH ANKYLOSING SPONDYLITIS.
36. Ketil Thorstensen: STUDIES ON THE MECHANISMS OF CELLULAR UPTAKE OF IRON FROM TRANSFERRIN.
37. Anna Midelfart: STUDIES OF THE MECHANISMS OF ION AND FLUID TRANSPORT IN THE BOVINE CORNEA.
38. Eirik Helseth: GROWTH AND PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN GLIOMAS AND BRAIN METASTASES - WITH SPECIAL REFERENCE TO TRANSFORMING GROWTH FACTOR BETA AND THE EPIDERMAL GROWTH FACTOR RECEPTOR.
39. Petter C. Borchgrevink: MAGNESIUM AND THE ISCHEMIC HEART.
40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

1989

43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
44. Rolf A. Walstad: CEFTAZIDIME.
45. Rolf Salvesen: THE PUPIL IN CLUSTER HEADACHE.
46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
51. Tore B. Halvorsen: PROGNOSTIC FACTORS IN COLORECTAL CANCER.

1990

52. Asbjørn Nordby: CELLULAR TOXICITY OF ROENTGEN CONTRAST MEDIA.
53. Kåre E. Tvedt: X-RAY MICROANALYSIS OF BIOLOGICAL MATERIAL.
54. Tore C. Stiles: COGNITIVE VULNERABILITY FACTORS IN THE DEVELOPMENT AND MAINTENANCE OF DEPRESSION.
55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.

67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.

108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

1996

110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

1997

124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs.

1998

132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.
133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.

138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

1999

141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

2000

158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
159. xxxxxxxxx (blind number)
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.

168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM

198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES

223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS

251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT
272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS

280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN
297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS
304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM

306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS
324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING

335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT
350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY
354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS
358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL-LIKE RECEPTOR 2 –EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.

363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE AND STRENGTH TRAINING IN CORONARY ARTERY DISEASE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS
369. Anja Bye: GENE EXPRESSION PROFILING OF *INHERITED* AND *ACQUIRED* MAXIMAL OXYGEN UPTAKE – RELATIONS TO THE METABOLIC SYNDROME.
370. Oluf Dimitri Røe: MALIGNANT MESOTHELIOMA: VIRUS, BIOMARKERS AND GENES. A TRANSLATIONAL APPROACH
371. Ane Cecilie Dale: DIABETES MELLITUS AND FATAL ISCHEMIC HEART DISEASE. ANALYSES FROM THE HUNT1 AND 2 STUDIES
372. Jacob Christian Hølen: PAIN ASSESSMENT IN PALLIATIVE CARE: VALIDATION OF METHODS FOR SELF-REPORT AND BEHAVIOURAL ASSESSMENT
373. Erming Tian: THE GENETIC IMPACTS IN THE ONCOGENESIS OF MULTIPLE MYELOMA
374. Ole Bosnes: KLINISK UTPRØVING AV NORSKE VERSJONER AV NOEN SENTRALE TESTER PÅ KOGNITIV FUNKSJON
375. Ola M. Rygh: 3D ULTRASOUND BASED NEURONAVIGATION IN NEUROSURGERY. A CLINICAL EVALUATION
376. Astrid Kamilla Stunes: ADIPOKINES, PEROXISOME PROFILERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS AND SEROTONIN. COMMON REGULATORS OF BONE AND FAT METABOLISM
377. Silje Engdal: HERBAL REMEDIES USED BY NORWEGIAN CANCER PATIENTS AND THEIR ROLE IN HERB-DRUG INTERACTIONS
378. Kristin Offerdal: IMPROVED ULTRASOUND IMAGING OF THE FETUS AND ITS CONSEQUENCES FOR SEVERE AND LESS SEVERE ANOMALIES
379. Øivind Rognmo: HIGH-INTENSITY AEROBIC EXERCISE AND CARDIOVASCULAR HEALTH
380. Jo-Åsmund Lund: RADIOTHERAPY IN ANAL CARCINOMA AND PROSTATE CANCER

2009

381. Tore Grüner Bjåstad: HIGH FRAME RATE ULTRASOUND IMAGING USING PARALLEL BEAMFORMING
382. Erik Søndenaa: INTELLECTUAL DISABILITIES IN THE CRIMINAL JUSTICE SYSTEM
383. Berit Rostad: SOCIAL INEQUALITIES IN WOMEN'S HEALTH, HUNT 1984-86 AND 1995-97, THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
384. Jonas Crosby: ULTRASOUND-BASED QUANTIFICATION OF MYOCARDIAL DEFORMATION AND ROTATION
385. Erling Tronvik: MIGRAINE, BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM
386. Tom Christensen: BRINGING THE GP TO THE FOREFRONT OF EPR DEVELOPMENT
387. Håkon Bergseng: ASPECTS OF GROUP B STREPTOCOCCUS (GBS) DISEASE IN THE NEWBORN. EPIDEMIOLOGY, CHARACTERISATION OF INVASIVE STRAINS AND EVALUATION OF INTRAPARTUM SCREENING
388. Ronny Myhre: GENETIC STUDIES OF CANDIDATE TENE3S IN PARKINSON'S DISEASE
389. Torbjørn Moe Eggebø: ULTRASOUND AND LABOUR
390. Eivind Wang: TRAINING IS MEDICINE FOR PATIENTS WITH PERIPHERAL ARTERIAL DISEASE
391. Thea Kristin Våtsveen: GENETIC ABERRATIONS IN MYELOMA CELLS
392. Thomas Jozefiak: QUALITY OF LIFE AND MENTAL HEALTH IN CHILDREN AND ADOLESCENTS: CHILD AND PARENT PERSPECTIVES
393. Jens Erik Slagsvold: N-3 POLYUNSATURATED FATTY ACIDS IN HEALTH AND DISEASE – CLINICAL AND MOLECULAR ASPECTS

394. Kristine Misund: A STUDY OF THE TRANSCRIPTIONAL REPRESSOR ICER. REGULATORY NETWORKS IN GASTRIN-INDUCED GENE EXPRESSION
395. Franco M. Impellizzeri: HIGH-INTENSITY TRAINING IN FOOTBALL PLAYERS. EFFECTS ON PHYSICAL AND TECHNICAL PERFORMANCE
396. Kari Hanne Gjeilo: HEALTH-RELATED QUALITY OF LIFE AND CHRONIC PAIN IN PATIENTS UNDERGOING CARDIAC SURGERY
397. Øyvind Hauso: NEUROENDOCRINE ASPECTS OF PHYSIOLOGY AND DISEASE
398. Ingvild Bjellmo Johnsen: INTRACELLULAR SIGNALING MECHANISMS IN THE INNATE IMMUNE RESPONSE TO VIRAL INFECTIONS
399. Linda Tømmerdal Roten: GENETIC PREDISPOSITION FOR DEVELOPMENT OF PREEMCLAMPSIA – CANDIDATE GENE STUDIES IN THE HUNT (NORD-TRØNDELAG HEALTH STUDY) POPULATION
400. Trude Teoline Nausthaug Rakvåg: PHARMACOGENETICS OF MORPHINE IN CANCER PAIN
401. Hanne Lehn: MEMORY FUNCTIONS OF THE HUMAN MEDIAL TEMPORAL LOBE STUDIED WITH fMRI
402. Randi Utne Holt: ADHESION AND MIGRATION OF MYELOMA CELLS – IN VITRO STUDIES –
403. Trygve Solstad: NEURAL REPRESENTATIONS OF EUCLIDEAN SPACE
404. Unn-Merete Fagerli: MULTIPLE MYELOMA CELLS AND CYTOKINES FROM THE BONE MARROW ENVIRONMENT; ASPECTS OF GROWTH REGULATION AND MIGRATION
405. Sigrid Bjørnelv: EATING– AND WEIGHT PROBLEMS IN ADOLESCENTS, THE YOUNG HUNT-STUDY
406. Mari Hoff: CORTICAL HAND BONE LOSS IN RHEUMATOID ARTHRITIS. EVALUATING DIGITAL X-RAY RADIOGRAMMETRY AS OUTCOME MEASURE OF DISEASE ACTIVITY, RESPONSE VARIABLE TO TREATMENT AND PREDICTOR OF BONE DAMAGE
407. Siri Bjørgen: AEROBIC HIGH INTENSITY INTERVAL TRAINING IS AN EFFECTIVE TREATMENT FOR PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE
408. Susanne Lindqvist: VISION AND BRAIN IN ADOLESCENTS WITH LOW BIRTH WEIGHT
409. Torbjørn Hergum: 3D ULTRASOUND FOR QUANTITATIVE ECHOCARDIOGRAPHY
410. Jørgen Urnes: PATIENT EDUCATION IN GASTRO-OESOPHAGEAL REFLUX DISEASE. VALIDATION OF A DIGESTIVE SYMPTOMS AND IMPACT QUESTIONNAIRE AND A RANDOMISED CONTROLLED TRIAL OF PATIENT EDUCATION
411. Elvar Eyjolfsson: 13C NMRS OF ANIMAL MODELS OF SCHIZOPHRENIA
412. Marius Steiro Fimland: CHRONIC AND ACUTE NEURAL ADAPTATIONS TO STRENGTH TRAINING
413. Øyvind Støren: RUNNING AND CYCLING ECONOMY IN ATHLETES; DETERMINING FACTORS, TRAINING INTERVENTIONS AND TESTING
414. Håkon Hov: HEPATOCYTE GROWTH FACTOR AND ITS RECEPTOR C-MET. AUTOCRINE GROWTH AND SIGNALING IN MULTIPLE MYELOMA CELLS
415. Maria Radtke: ROLE OF AUTOIMMUNITY AND OVERSTIMULATION FOR BETA-CELL DEFICIENCY. EPIDEMIOLOGICAL AND THERAPEUTIC PERSPECTIVES
416. Liv Bente Romundstad: ASSISTED FERTILIZATION IN NORWAY: SAFETY OF THE REPRODUCTIVE TECHNOLOGY
417. Erik Magnus Berntsen: PREOPERATIV PLANNING AND FUNCTIONAL NEURONAVIGATION – WITH FUNCTIONAL MRI AND DIFFUSION TENSOR TRACTOGRAPHY IN PATIENTS WITH BRAIN LESIONS
418. Tonje Strømmen Steigedal: MOLECULAR MECHANISMS OF THE PROLIFERATIVE RESPONSE TO THE HORMONE GASTRIN
419. Vidar Rao: EXTRACORPOREAL PHOTOCHEMOTHERAPY IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMA OR GRAFT-vs-HOST DISEASE
420. Torkild Visnes: DNA EXCISION REPAIR OF URACIL AND 5-FLUOROURACIL IN HUMAN CANCER CELL LINES

2010

421. John Munkhaugen: BLOOD PRESSURE, BODY WEIGHT, AND KIDNEY FUNCTION IN THE NEAR-NORMAL RANGE: NORMALITY, RISK FACTOR OR MORBIDITY ?
422. Ingrid Castberg: PHARMACOKINETICS, DRUG INTERACTIONS AND ADHERENCE TO TREATMENT WITH ANTIPSYCHOTICS: STUDIES IN A NATURALISTIC SETTING

423. Jian Xu: BLOOD-OXYGEN-LEVEL-DEPENDENT-FUNCTIONAL MAGNETIC RESONANCE IMAGING AND DIFFUSION TENSOR IMAGING IN TRAUMATIC BRAIN INJURY RESEARCH
424. Sigmund Simonsen: ACCEPTABLE RISK AND THE REQUIREMENT OF PROPORTIONALITY IN EUROPEAN BIOMEDICAL RESEARCH LAW. WHAT DOES THE REQUIREMENT THAT BIOMEDICAL RESEARCH SHALL NOT INVOLVE RISKS AND BURDENS DISPROPORTIONATE TO ITS POTENTIAL BENEFITS MEAN?
425. Astrid Woodhouse: MOTOR CONTROL IN WHIPLASH AND CHRONIC NON-TRAUMATIC NECK PAIN
426. Line Rørstad Jensen: EVALUATION OF TREATMENT EFFECTS IN CANCER BY MR IMAGING AND SPECTROSCOPY
427. Trine Moholdt: AEROBIC EXERCISE IN CORONARY HEART DISEASE
428. Øystein Olsen: ANALYSIS OF MANGANESE ENHANCED MRI OF THE NORMAL AND INJURED RAT CENTRAL NERVOUS SYSTEM
429. Bjørn H. Grønberg: PEMETREXED IN THE TREATMENT OF ADVANCED LUNG CANCER
430. Vigdis Schnell Husby: REHABILITATION OF PATIENTS UNDERGOING TOTAL HIP ARTHROPLASTY WITH FOCUS ON MUSCLE STRENGTH, WALKING AND AEROBIC ENDURANCE PERFORMANCE
431. Torbjørn Øien: CHALLENGES IN PRIMARY PREVENTION OF ALLERGY. THE PREVENTION OF ALLERGY AMONG CHILDREN IN TRONDHEIM (PACT) STUDY.
432. Kari Anne Indredavik Evensen: BORN TOO SOON OR TOO SMALL: MOTOR PROBLEMS IN ADOLESCENCE
433. Lars Adde: PREDICTION OF CEREBRAL PALSY IN YOUNG INFANTS. COMPUTER BASED ASSESSMENT OF GENERAL MOVEMENTS
434. Magnus Fasting: PRE- AND POSTNATAL RISK FACTORS FOR CHILDHOOD ADIPOSITY
435. Vivi Talstad Monsen: MECHANISMS OF ALKYLATION DAMAGE REPAIR BY HUMAN AlkB HOMOLOGUES
436. Toril Skandsen: MODERATE AND SEVERE TRAUMATIC BRAIN INJURY. MAGNETIC RESONANCE IMAGING FINDINGS, COGNITION AND RISK FACTORS FOR DISABILITY
437. Ingeborg Smidesang: ALLERGY RELATED DISORDERS AMONG 2-YEAR OLDS AND ADOLESCENTS IN MID-NORWAY – PREVALENCE, SEVERITY AND IMPACT. THE PACT STUDY 2005, THE YOUNG HUNT STUDY 1995-97
438. Vidar Halsteinli: MEASURING EFFICIENCY IN MENTAL HEALTH SERVICE DELIVERY: A STUDY OF OUTPATIENT UNITS IN NORWAY
439. Karen Lehrmann Ægidius: THE PREVALENCE OF HEADACHE AND MIGRAINE IN RELATION TO SEX HORMONE STATUS IN WOMEN. THE HUNT 2 STUDY
440. Madelene Ericsson: EXERCISE TRAINING IN GENETIC MODELS OF HEART FAILURE
441. Marianne Klokk: THE ASSOCIATION BETWEEN SELF-REPORTED ECZEMA AND COMMON MENTAL DISORDERS IN THE GENERAL POPULATION. THE HORDALAND HEALTH STUDY (HUSK)
442. Tomas Ottemo Stølen: IMPAIRED CALCIUM HANDLING IN ANIMAL AND HUMAN CARDIOMYOCYTES REDUCE CONTRACTILITY AND INCREASE ARRHYTHMIA POTENTIAL – EFFECTS OF AEROBIC EXERCISE TRAINING
443. Bjarne Hansen: ENHANCING TREATMENT OUTCOME IN COGNITIVE BEHAVIOURAL THERAPY FOR OBSESSIVE COMPULSIVE DISORDER: THE IMPORTANCE OF COGNITIVE FACTORS
444. Mona Løvlien: WHEN EVERY MINUTE COUNTS. FROM SYMPTOMS TO ADMISSION FOR ACUTE MYOCARDIAL INFARCTION WITH SPECIAL EMPHASIS ON GENDER DIFFERECES
445. Karin Margaretha Gilljam: DNA REPAIR PROTEIN COMPLEXES, FUNCTIONALITY AND SIGNIFICANCE FOR REPAIR EFFICIENCY AND CELL SURVIVAL
446. Anne Byriel Walls: NEURONAL GLIAL INTERACTIONS IN CEREBRAL ENERGY – AND AMINO ACID HOMEOSTASIS – IMPLICATIONS OF GLUTAMATE AND GABA
447. Cathrine Fallang Knetter: MECHANISMS OF TOLL-LIKE RECEPTOR 9 ACTIVATION
448. Marit Følsvik Svindseth: A STUDY OF HUMILIATION, NARCISSISM AND TREATMENT OUTCOME IN PATIENTS ADMITTED TO PSYCHIATRIC EMERGENCY UNITS
449. Karin Elvenes Bakkelund: GASTRIC NEUROENDOCRINE CELLS – ROLE IN GASTRIC NEOPLASIA IN MAN AND RODENTS

450. Kirsten Brun Kjelstrup: DORSOVENTRAL DIFFERENCES IN THE SPATIAL REPRESENTATION AREAS OF THE RAT BRAIN
451. Roar Johansen: MR EVALUATION OF BREAST CANCER PATIENTS WITH POOR PROGNOSIS
452. Rigmor Myran: POST TRAUMATIC NECK PAIN. EPIDEMIOLOGICAL, NEURORADIOLOGICAL AND CLINICAL ASPECTS
453. Krisztina Kunszt Johansen: GENEALOGICAL, CLINICAL AND BIOCHEMICAL STUDIES IN *LRRK2* – ASSOCIATED PARKINSON'S DISEASE
454. Pål Gjerden: THE USE OF ANTICHOLINERGIC ANTIPARKINSON AGENTS IN NORWAY. EPIDEMIOLOGY, TOXICOLOGY AND CLINICAL IMPLICATIONS
455. Else Marie Huuse: ASSESSMENT OF TUMOR MICROENVIRONMENT AND TREATMENT EFFECTS IN HUMAN BREAST CANCER XENOGRAFTS USING MR IMAGING AND SPECTROSCOPY
456. Khalid S. Ibrahim: INTRAOPERATIVE ULTRASOUND ASSESSMENT IN CORONARY ARTERY BYPASS SURGERY – WITH SPECIAL REFERENCE TO CORONARY ANASTOMOSES AND THE ASCENDING AORTA
457. Bjørn Øglænd: ANTHROPOMETRY, BLOOD PRESSURE AND REPRODUCTIVE DEVELOPMENT IN ADOLESCENCE OF OFFSPRING OF MOTHERS WHO HAD PREECLAMPSIA IN PREGNANCY
458. John Olav Roaldset: RISK ASSESSMENT OF VIOLENT, SUICIDAL AND SELF-INJURIOUS BEHAVIOUR IN ACUTE PSYCHIATRY – A BIO-PSYCHO-SOCIAL APPROACH
459. Håvard Dalen: ECHOCARDIOGRAPHIC INDICES OF CARDIAC FUNCTION – NORMAL VALUES AND ASSOCIATIONS WITH CARDIAC RISK FACTORS IN A POPULATION FREE FROM CARDIOVASCULAR DISEASE, HYPERTENSION AND DIABETES: THE HUNT 3 STUDY
460. Beate André: CHANGE CAN BE CHALLENGING. INTRODUCTION TO CHANGES AND IMPLEMENTATION OF COMPUTERIZED TECHNOLOGY IN HEALTH CARE
461. Latha Nrugham: ASSOCIATES AND PREDICTORS OF ATTEMPTED SUICIDE AMONG DEPRESSED ADOLESCENTS – A 6-YEAR PROSPECTIVE STUDY
462. Håvard Bersås Nordgaard: TRANSIT-TIME FLOWMETRY AND WALL SHEAR STRESS ANALYSIS OF CORONARY ARTERY BYPASS GRAFTS – A CLINICAL AND EXPERIMENTAL STUDY

Cotutelle with University of Ghent: Abigail Emily Swillens: A MULTIPHYSICS MODEL FOR IMPROVING THE ULTRASONIC ASSESSMENT OF LARGE ARTERIES

2011

463. Marte Helene Bjørk: DO BRAIN RHYTHMS CHANGE BEFORE THE MIGRAINE ATTACK? A LONGITUDINAL CONTROLLED EEG STUDY
464. Carl-Jørgen Arum: A STUDY OF UROTHELIAL CARCINOMA: GENE EXPRESSION PROFILING, TUMORIGENESIS AND THERAPIES IN ORTHOTOPIC ANIMAL MODELS
465. Ingunn Harstad: TUBERCULOSIS INFECTION AND DISEASE AMONG ASYLUM SEEKERS IN NORWAY. SCREENING AND FOLLOW-UP IN PUBLIC HEALTH CARE
466. Leif Åge Strand: EPIDEMIOLOGICAL STUDIES AMONG ROYAL NORWEGIAN NAVY SERVICEMEN. COHORT ESTABLISHMENT, CANCER INCIDENCE AND CAUSE-SPECIFIC MORTALITY
467. Katrine Høyer Holgersen: SURVIVORS IN THEIR THIRD DECADE AFTER THE NORTH SEA OIL RIG DISASTER OF 1980. LONG-TERM PERSPECTIVES ON MENTAL HEALTH
468. MarianneWallenius: PREGNANCY RELATED ASPECTS OF CHRONIC INFLAMMATORY ARTHRITIDES: DISEASE ONSET POSTPARTUM, PREGNANCY OUTCOMES AND FERTILITY. DATA FROM A NORWEGIAN PATIENT REGISTRY LINKED TO THE MEDICAL BIRTH REGISTRY OF NORWAY
469. Ole Vegard Solberg: 3D ULTRASOUND AND NAVIGATION – APPLICATIONS IN LAPAROSCOPIC SURGERY
470. Inga Ekeberg Schjerve: EXERCISE-INDUCED IMPROVEMENT OF MAXIMAL OXYGEN UPTAKE AND ENDOTHELIAL FUNCTION IN OBESE AND OVERWEIGHT INDIVIDUALS ARE DEPENDENT ON EXERCISE-INTENSITY
471. Eva Veslemøy Tyldum: CARDIOVASCULAR FUNCTION IN PREECLAMPSIA – WITH REFERENCE TO ENDOTHELIAL FUNCTION, LEFT VENTRICULAR FUNCTION AND PRE-PREGNANCY PHYSICAL ACTIVITY
472. Benjamin Garzón Jiménez de Cisneros: CLINICAL APPLICATIONS OF MULTIMODAL MAGNETIC RESONANCE IMAGING

473. Halvard Knut Nilsen: ASSESSING CODEINE TREATMENT TO PATIENTS WITH CHRONIC NON-MALIGNANT PAIN: NEUROPSYCHOLOGICAL FUNCTIONING, DRIVING ABILITY AND WEANING
474. Eiliv Brenner: GLUTAMATE RELATED METABOLISM IN ANIMAL MODELS OF SCHIZOPHRENIA
475. Egil Jonsbu: CHEST PAIN AND PALPITATIONS IN A CARDIAC SETTING; PSYCHOLOGICAL FACTORS, OUTCOME AND TREATMENT
476. Mona Høysæter Fenstad: GENETIC SUSCEPTIBILITY TO PREECLAMPSIA : STUDIES ON THE NORD-TRØNDELAG HEALTH STUDY (HUNT) COHORT, AN AUSTRALIAN/NEW ZEALAND FAMILY COHORT AND DECIDUA BASALIS TISSUE
477. Svein Erik Gaustad: CARDIOVASCULAR CHANGES IN DIVING: FROM HUMAN RESPONSE TO CELL FUNCTION
478. Karin Torvik: PAIN AND QUALITY OF LIFE IN PATIENTS LIVING IN NURSING HOMES
479. Arne Solberg: OUTCOME ASSESSMENTS IN NON-METASTATIC PROSTATE CANCER
480. Henrik Sahlin Pettersen: CYTOTOXICITY AND REPAIR OF URACIL AND 5-FLUOROURACIL IN DNA
481. Pui-Lam Wong: PHYSICAL AND PHYSIOLOGICAL CAPACITY OF SOCCER PLAYERS: EFFECTS OF STRENGTH AND CONDITIONING
482. Ole Solheim: ULTRASOUND GUIDED SURGERY IN PATIENTS WITH INTRACRANIAL TUMOURS
483. Sten Roar Snare: QUANTITATIVE CARDIAC ANALYSIS ALGORITHMS FOR POCKET-SIZED ULTRASOUND DEVICES
484. Marit Skyrud Bratlie: LARGE-SCALE ANALYSIS OF ORTHOLOGS AND PARALOGS IN VIRUSES AND PROKARYOTES

485. Anne Elisabeth F. Isern: BREAST RECONSTRUCTION AFTER MASTECTOMY – RISK OF RECURRENCE AFTER DELAYED LARGE FLAP RECONSTRUCTION – AESTHETIC OUTCOME, PATIENT SATISFACTION, QUALITY OF LIFE AND SURGICAL RESULTS; HISTOPATHOLOGICAL FINDINGS AND FOLLOW-UP AFTER PROPHYLACTIC MASTECTOMY IN HEREDITARY BREAST CANCER
486. Guro L. Andersen: CEREBRAL PALSY IN NORWAY – SUBTYPES, SEVERITY AND RISK FACTORS
487. Frode Kolstad: CERVICAL DISC DISEASE – BIOMECHANICAL ASPECTS
488. Bente Nordtug: CARING BURDEN OF COHABITANTS LIVING WITH PARTNERS SUFFERING FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE OR DEMENTIA
489. Mariann Gjervik Heldahl: EVALUATION OF NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER BASED ON MR METHODOLOGY
490. Lise Tevik Løvseth: THE SUBJECTIVE BURDEN OF CONFIDENTIALITY
491. Marie Hjelmseth Aune: INFLAMMATORY RESPONSES AGAINST GRAM NEGATIVE BACTERIA INDUCED BY TLR4 AND NLRP12
492. Tina Strømdal Wik: EXPERIMENTAL EVALUATION OF NEW CONCEPTS IN HIP ARTHROPLASTY
493. Solveig Sigurdardottir: CLINICAL ASPECTS OF CEREBRAL PALSY IN ICELAND. A POPULATION-BASED STUDY OF PRESCHOOL CHILDREN
494. Arne Reimers: CLINICAL PHARMACOKINETICS OF LAMOTRIGINE
495. Monica Wegling: KULTURMENNESKETS BYRDE OG SYKDOMMENS VELSIGNELSE. KAN MEDISINSK UTREDNING OG INTERVENSJON HA EN SELVSTENDIG FUNKSJON UAVHENGIG AV DET KURATIVE?
496. Silje Alvestad: ASTROCYTE-NEURON INTERACTIONS IN EXPERIMENTAL MESIAL TEMPORAL LOBE EPILEPSY – A STUDY OF UNDERLYING MECHANISMS AND POSSIBLE BIOMARKERS OF EPILEPTOGENESIS
497. Javaid Nauman: RESTING HEART RATE: A MATTER OF LIFE OR DEATH – PROSPECTIVE STUDIES OF RESTING HEART RATE AND CARDIOVASCULAR RISK (THE HUNT STUDY, NORWAY)
498. Thuy Nguyen: THE ROLE OF C-SRC TYROSINE KINASE IN ANTIVIRAL IMMUNE RESPONSES
499. Trine Naalsund Andreassen: PHARMACOKINETIC, PHARMACODYNAMIC AND PHARMACOGENETIC ASPECTS OF OXYCODONE TREATMENT IN CANCER PAIN
500. Eivor Alette Laugsand: SYMPTOMS IN PATIENTS RECEIVING OPIOIDS FOR CANCER PAIN – CLINICAL AND PHARMACOGENETIC ASPECTS

501. Dorthe Stensvold: PHYSICAL ACTIVITY, CARDIOVASCULAR HEALTH AND LONGEVITY IN PATIENTS WITH METABOLIC SYNDROME
502. Stian Thoresen Aspenes: PEAK OXYGEN UPTAKE AMONG HEALTHY ADULTS – CROSS-SECTIONAL DESCRIPTIONS AND PROSPECTIVE ANALYSES OF PEAK OXYGEN UPTAKE, PHYSICAL ACTIVITY AND CARDIOVASCULAR RISK FACTORS IN HEALTHY ADULTS (20-90 YEARS)
503. Reidar Alexander Vigen: PATHOBIOLOGY OF GASTRIC CARCINOIDS AND ADENOCARCINOMAS IN RODENT MODELS AND PATIENTS. STUDIES OF GASTROCYSTOPLASTY, GENDER-RELATED FACTORS, AND AUTOPHAGY
504. Halvard Høilund-Kaupang: MODELS AND METHODS FOR INVESTIGATION OF REVERBERATIONS IN NONLINEAR ULTRASOUND IMAGING
505. Audhild Løhre: WELLBEING AMONG SCHOOL CHILDREN IN GRADES 1-10: PROMOTING AND ADVERSE FACTORS
506. Torgrim Tandstad: VOX POPULI. POPULATION-BASED OUTCOME STUDIES IN TESTICULAR CANCER
507. Anna Brenne Grønskag: THE EPIDEMIOLOGY OF HIP FRACTURES AMONG ELDERLY WOMEN IN NORD-TRØNDELAG. HUNT 1995-97, THE NORD-TRØNDELAG HEALTH STUDY
508. Kari Ravndal Risnes: BIRTH SIZE AND ADULT MORTALITY: A SYSTEMATIC REVIEW AND A LONG-TERM FOLLOW-UP OF NEARLY 40 000 INDIVIDUALS BORN AT ST. OLAV UNIVERSITY HOSPITAL IN TRONDHEIM 1920-1960
509. Hans Jakob Bøe: LONG-TERM POSTTRAUMATIC STRESS AFTER DISASTER – A CONTROLLED STUDY OF SURVIVORS' HEALTH 27 YEARS AFTER THE CAPSIZED NORTH SEA OIL RIG
510. Cathrin Barbara Canto, Cotutelle with University of Amsterdam: LAYER SPECIFIC INTEGRATIVE PROPERTIES OF ENTORHINAL PRINCIPAL NEURONS
511. Ioanna Sandvig: THE ROLE OF OLFACTORY ENSHEATHING CELLS, MRI, AND BIOMATERIALS IN TRANSPLANT-MEDIATED CNS REPAIR
512. Karin Fahl Wader: HEPATOCYTE GROWTH FACTOR, C-MET AND SYNDECAN-1 IN MULTIPLE MYELOMA