Regulation of cell growth in Multiple Myeloma: a role for the HGF/MET and WNT signaling pathways
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summary
Multiple myeloma (MM) growth and progression depends on various signals from the bone marrow (BM) microenvironment. These signals are supplied by growth factors and cytokines, and are initiated by binding of these molecules to receptors which can subsequently activate signaling cascades which guide and govern multiple processes like growth, survival, differentiation and migration. The studies described in this thesis have identified two potent oncogenic pathways, i.e. the HGF/MET and WNT signaling pathways, inducing growth and survival in MM, and substantiated a functional role for syndecan1 in MM pathobiology.

CHAPTER TWO presents data showing that MET is expressed on most myeloma cell lines and that MET expression is common on primary MMs as well as on extramedullary plasmacytomas. Furthermore, a functional and molecular characterization of the HGF/MET pathway in MM is presented. HGF-induced activation of MET on MM cells resulted in a rapid increase in the amount of GTP-bound RAS in both the MM cell lines as well as in primary BM-derived MM cells. Also, the downstream effector MAPKs ERK1 and -2 as well as PKB (aka AKT), were strongly activated in response to HGF stimulation. Subsequent functional studies showed that HGF stimulation of MM cells induced a strong dose-dependent increase of DNA synthesis, and a potent rescue from apoptosis. On a molecular level, we could show that the HGF-induced proliferation required activation of both MEK and PI3K. In contrast, the HGF controlled survival required activation of PI3K only, emphasizing the impact of the activation of PI3K/PKB signaling in MM growth and survival.

Expression of syndecan-1 (CD138) is characteristic of plasma cells and their malignant counterpart, multiple myeloma. Although widely used for diagnostic purposes, until recently, little was known about the functionality of syndecan1 on MM cells. Syndecan1 is a molecule which consists of proteoglycan core protein, to which a HS side chain is covalently linked. HS can bind hepan-binding glycoproteins and present them to their cognate receptors. CHAPTER THREE studies the distribution of HSPIGs on MM cells and describes the functional relationship between these molecules and the HGF/MET pathway. We showed that MM cells express a single HSPIG of approximately 90 kDa, which was also present in the lysates of syndecan1-transfected Namalwa Burkitt's lymphoma cells, but not in that of non-transfected or glypi- can1-transfected cells. The use of HSPIG-specific antibodies subsequently indicated that syndecan1 is the major, and most probably only HSPIG expressed on MM cells. We next investigated the ability of syndecan1 to interact with HGF. Experiments using HP1, a mutant form of HGF with a more than 50-fold decreased affinity for HS, and HS-specific enzymes, showed binding of HGF that was largely dependent on HS-moieties decorating syndecan1 on the MM cell surface. In order to explore the functional impact of the HGF-syndecan1 interaction in MM, we studied the impact of removal of the HS side chains on HGF-induced signaling. Not only the autophosphorylation of MET, but also the HGF-induced activation of downstream effector molecules, i.e. PKB and the MAPKs ERK1 and -2, were greatly inhibited upon removal of syndecan1-linked HS in MM cells. Non-specific effects of the heparitinase treatment on cell signaling were excluded, as their activation by insulin, which does not bind to HS, was unaffected. We therefore concluded that syndecan1 promotes HGF/MET signaling by either: (i) increasing the effective concentration of HGF at the plasma membrane, (ii) inducing an enhanced di- and oligomerization of MET, leading to enhanced receptor activation, or (iii) initiating a conformational change in HGF which may influence the affinity of HGF for MET. Our results indicate that the HGF/MET pathway and syndecan-1 form a complex controlling the growth and survival of MET positive MM cells.

CHAPTER FOUR describes the expression patterns of HGF and MET on normal tonsillar B cells, and on a large panel of several B cell malignancies. Expression of MET was detected in subsets of chronic lymphocytic lymphoma/leukemia (CLL), and follicular lymphoma (FL), and in the large number of Hodgkin's disease (HD), diffuse large B cell lymphoma (DLBCL) and MM. Furthermore, we detected expression of HGF within the tumor micro environment in all samples expressing MET, suggesting paracrine activation in B cell tumors. No indications of amplification of the MET locus (which is located on 7q) was found in any of the aforementioned B cell malignancies. However, we detected two missense germline mutations in four cases of B cell non-Hodgkin's lymphoma (B NHL). Mutations were located in a region juxtaposed to the PEST sequence in exon 14, a region implicated in the ubiquitin ligase-mediated degradation of proteins, and in exon 17, located adjacent to a tyrosine residue, necessary for activation of MET. The mutations described in the work presented here could therefore favor B cell tumor growth or progression, by inhibition of degradation or an increase in activation of MET. Finally, MET overexpression showed a correlation with a numerical increase of chromosome 7 in MM cells, suggesting a possible mechanism of expression regulation.

Even though WNT signaling is involved in early lymphocyte development and regulates the growth and progression of many tumors, a role for the WNT/β-catenin/TCF cascade in lymphoid malignancies has remained ambiguous. CHAPTER FIVE presents data showing the expression and functional impact of canonical WNT signaling

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in MM. Both primary BM-derived MM samples and the majority of the MM cell lines expressed large amounts of β-catenin, as well as detectable levels of the active, non-phosphorylated form of β-catenin, which represents the signaling-competent pool of cellular β-catenin. Compared to the β-catenin expression levels in MM, normal B cell subpopulations, plasma cells, and normal BM, showed very low or undetectable β-catenin levels, whereas the non-phosphorylated form of β-catenin was undetectable in these control samples. MM cells possess an intact WNT signaling pathway, illustrated by our finding that exogenous WNT stimuli, i.e., LiCl and Wnt3a, could induce accumulation and nuclear localization of β-catenin. We have furthermore suggested the possible contribution of WNT signaling to MM pathobiology, by showing that WNT stimuli can induce proliferation of MM cells. Moreover, repression of β-catenin/TCF-mediated transcription led to decreased proliferative responses, implying that constitutive, endogenous WNT signaling contributes to MM cell growth. Finally, we have detected WNT5a, WNT10b and WNT16 transcripts in malignant MM cells, whereas normal B cell populations and plasma cells did not show expression. This, combined with the absence of mutations in either APC or CCNTB1, suggested the possibility of an autocrine scenario, which we consider “illegitimate”, because it involves induction of proliferation within the most terminal compartment of B cell differentiation.