Wnt signaling in the pathogenesis of multiple myeloma

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SUMMARY

Multiple myeloma (MM) is a clonal expansion of malignant plasma cells in the bone marrow (BM) that is typically accompanied by pancytopenia, renal failure, osteolytic bone lesions and hypercalcemia. MM tumor cells are highly dependent on a protective BM microenvironment for growth and survival. The Wnt signaling cascade is aberrantly activated in the majority of MMs and is involved in regulating tumor growth and drug resistance. Interestingly, cell-intrinsic Wnt pathway mutations are rare in MM. This suggests that aberrant Wnt signaling is driven by autocrine and/or paracrine Wnt ligands emanating from the BM niche.

Chapter 2 is a detailed review of the literature regarding the deregulation of Wnt signaling in MM cells. In particular, it focusses on genetic and epigenetic mechanisms that drive Wnt signaling in MM tumor cells, the effects of aberrant Wnt signaling on tumorigenesis and therapeutic strategies to target Wnt signaling in MM.

Chapter 3 describes the role of LGR4/R-spondin signaling in mediating aberrant Wnt activity in MM cells. The large majority of MMs expresses LGR4 on the cell surface, in contrast to normal plasma cells or B cells. LGR4 expression is transcriptionally driven by IL-6/STAT3 signaling, which plays a central role in MM pathogenesis. LGR4 expression allows MM cells to respond to R-spondins, which are secreted in the BM microenvironment by (pre)osteoblasts. Binding of R-spondin to its cognate receptor results in the stabilization of Wnt receptors on the cell membrane, which dramatically enhances the sensitivity of MM cells to both autocrine and paracrine Wnt ligands. Importantly, inhibition of Wnt secretion or silencing of LGR4 impairs the growth of MM cells that secrete autocrine Wnts. These results advocate targeting of the LGR4/R-spondin axis as a therapeutic strategy in MM.

Syndecan-1 (CD138) is a heparan sulfate proteoglycan (HSPG) capable of binding numerous growth factors and is used as the typical marker for the identification of both normal and malignant plasma cells. Chapter 4 describes the identification of syndecan-1 as an important coreceptor for both Wnt and LGR4/R-spondin signaling. Genetic or enzymatic removal of the heparan sulfate (HS) sidechains from syndecan-1 decreases Wnt signaling in MM cells. Mechanistically, syndecan-1 binds both Wnts and R-spondins and thereby facilitates signal transduction. Interestingly, removal of the HS side chains from syndecan-1 decreases growth of MM cells, which can be partially rescued by ectopic expression of genes that are a transcriptional target of Wnt signaling. These results indicate
that HSPGs function as important mediators of Wnt signaling in MM cells by binding an presenting both Wnt ligands and R-spondins.

Dickkopf-1 (DKK1) is a soluble Wnt antagonist that is secreted by MM cells. It plays an important role in the development of osteolytic bone lesions by attenuating osteoblast differentiation. **Chapter 5** describes the finding that DKK1 expression is lost in advanced MM as a result of hypermethylation of the DKK1 promotor. Treatment of MM cells with the demethylating agent decitabine results in the re-expression of DKK1. Importantly, DKK1 decreases Wnt signaling in MM tumor cells. Moreover, there is a positive correlation between hypermethylation of the DKK1 promotor and Wnt activation in MM, indicating that epigenetic silencing of the DKK1 promotor can unleash Wnt activation in tumor cells. Antibody-mediated inhibition of DKK1 was previously proposed as a therapeutic strategy targeting osteolytic bone lesions. However the findings in this chapter suggest that this strategy might activate Wnt signaling in MM cells and thereby enhance tumor growth.

In **chapter 6** the effect of genetic loss of the deubiquitinase cylindromatosis-1 (CYLD) on MM pathogenesis is investigated. Loss of CYLD is frequently observed in MMs and results from genetic deletion and/or mutation of the CYLD gene. CYLD is an enzyme that specifically removes lysine-63 linked polyubiquitin chains from proteins, including regulators of Wnt and NF kappa B signaling. This results in destabilization of these proteins and impairs signal transduction. Silencing of CYLD in MM cells indeed enhances activation of both Wnt and NF kappa B signaling. Conversely, reintroducing CYLD in deficient cell lines impairs signal transduction and attenuates tumor growth. Gene expression analysis in primary MMs reveals an inverse correlation between CYLD expression and both progression free and overall survival. Moreover, there is a strong negative correlation between CYLD expression and a Wnt transcriptional profile. These findings indicate that genetic loss of CYLD plays a role in MM pathogenesis, which involves facilitating Wnt activation in tumor cells.

The MM BM microenvironment is hypoxic, which requires tumor cells to adapt to the low oxygen levels. During disease progression, there is an increase in micro vessel density, which was proposed to be driven by hypoxia in the tumor environment. **Chapter 7** investigates the transcriptional response of MM cells to hypoxia. Using gene expression analysis, the pro-angiogenic protein adenomedullin (ADM) was identified as the most upregulated gene in hypoxic conditions. In addition, ADM is secreted by several MMs in normoxic conditions, indicating that other mechanisms can also drive its expression.
Functionally, ADM enhances the angiogenic properties of endothelial cells, which can be efficiently blocked by specific small molecule ADM inhibitors. These results indicate that MM cells secrete ADM in response to hypoxia, which enhances angiogenesis in advanced stages of MM.

DISCUSSION

Canonical Wnt signaling in multiple myeloma

Oncogenic Wnt signaling is typically caused by cell-intrinsic mutations that drive constitutive, ligand-independent pathway activation. Whereas most MMs display hallmarks of active canonical Wnt signaling, activating Wnt pathway mutations are rare. This suggests that aberrant Wnt signaling is driven by autocrine and/or paracrine Wnts originating from the BM niche.\(^1\) In MM, activation of canonical Wnt signaling has been implicated in proliferation and drug resistance of tumor cells. In this thesis, we describe the identification of various genetic and epigenetic alterations that facilitate aberrant Wnt activation in MM cells. I) Overexpression of the LGR4 receptor, which sensitizes MM cells to Wnt ligands in response to preosteoblast-derived R-spondins (Chapter 3) II) expression of the heparan sulfate proteoglycan syndecan-1, which mediates Wnt signaling by binding both Wnts and R-spondins (Chapter 4) III) epigenetic silencing of the secreted Wnt antagonist DKK1, in particular in advanced stages of MM (Chapter 5) IV) genetic loss of the deubiquitinase CYLD, resulting in enhanced sensitivity to Wnt ligands (Chapter 6).

Collectively, these findings indicate that Wnt activation in MM is the results of ‘releasing the breaks’ rather than ‘hitting the gas’. Intriguingly, MM cells also secrete the soluble Wnt antagonist DKK1, which plays an important role in the development of osteolytic bone lesions.\(^2\) DKK1 expression is largely restricted to early disease stages. In advanced disease stages, DKK1 is epigenetically silenced, which suggests that it exerts stage specific functions. As discussed in the review of chapter 2, we propose a model in which DKK1-mediated suppression of osteoblast differentiation indirectly enhances tumorigenesis in early disease stages by creating a tumor permissive environment. Consistent with this scenario, osteoblast precursors secrete high levels of cytokines and growth factors, such as IL-6 and R-spondin.\(^3-5\) During disease progression, MM cells lose their dependence on the BM niche and DKK1 is silenced. In cooperation with the above described genetic and epigenetic abnormalities, this facilitates aberrant Wnt activation in MM cells. Importantly, the Wnt signaling pathway in MM is essentially intact and Wnt activation is largely ligand dependent. This implies that Wnt signaling can be targeted by drugs that interfere with
Wnt secretion, prevent binding of Wnt ligands to the receptor or block LGR4/R-spondin signaling.

**Regulation of angiogenesis by adrenomedullin**

At the transition from the pre-malignant MGUS to a full-blown MM there is a marked increase in bone marrow angiogenesis, which has been designated as the ‘angiogenic switch’. Importantly, the increased microvessel density in the BM of MM patients is correlated to disease progression and adverse outcome. Because typical angiogenic factors such as VEGF and bFGF were previously found to be equally expressed between MGUS and MM patients, the angiogenic switch was proposed to be driven by an increase in tumor burden or the loss of anti-angiogenic factors. However, in functional essays, MM cells display increased pro-angiogenic properties compared to normal plasma cells. Moreover, hypoxia induces HIF1α stabilization in MM cells, which is accompanied by increased production of pro-angiogenic factors such as VEGF. This suggests that the production of pro-angiogenic factors might be an important driving force behind the ‘angiogenic switch’. Hypoxia in the BM might not just be an epiphenomenon, but also play an instrumental role in tumorigenesis. This is supported by the observation that highly tumorigenic, quiescent MM cells typically reside in the hypoxic endosteal niche, in close proximity to osteoblasts. Moreover, hypoxia has been shown to regulate drug resistance, tumor dissemination and stem cell-like properties of MM cells.

In chapter 6 we studied the transcriptional response of MM cells to hypoxia by gene expression analysis. The pro-angiogenic factor adrenomedullin (ADM) was identified as the most upregulated gene in hypoxic conditions. Functionally, adrenomedullin enhances endothelial cell proliferation and mesh formation *in vitro*, which can be efficiently blocked by small molecule ADM inhibitors. Intriguingly, certain cell lines also express ADM in normoxic condition, even in the absence of apparent HIF1α stabilization. This indicates that alternative mechanisms can also drive ADM expression. In support of this notion, there was no significant correlation between ADM mRNA expression and the expression of other HIF1α target genes. Collectively, these results suggest that ADM contributes to MM-induced angiogenesis and is driven by both hypoxia/HIF1α-dependent and -independent mechanisms. Specific ADM inhibitors are available and might be tested as an anti-angiogenic therapy in MM.
REFERENCES


