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CHAPTER

6

Summary

Summary

Multiple myeloma (MM) is a largely incurable hematological malignancy characterized by a clonal expansion of malignant plasma cell in the bone marrow (BM). The survival and proliferation of MM cells in the BM is highly dependent on signals emanating from the BM microenvironment.

In most MMs, aberrant activation of the Wnt/ β -catenin signaling drives proliferation and is associated with disease progression, however, canonical Wnt-pathway activation mutations are rare, indicating that Wnt pathway activation in MM is driven by autocrine and/or paracrine Wnt ligands. **In chapter 2**, we uncover a previously unknown role for the R-spondin/ LGR4 axis in regulating Wnt/ β -catenin activation in MM. We showed that LGR4 is expressed in primary MM cells and human MM cell lines, but not in normal plasma cells or other early B cells. This aberrant expression of LGR4 in MM is transcriptionally driven by IL-6/STAT3 signaling. The expression of LGR4 allows MM cells to hijack R-spondins secreted by (pre)osteoblasts, thereby deregulating Wnt receptors stabilization and resulting in a dramatically enhanced sensitivity to autocrine and paracrine Wnts. Furthermore, we demonstrate that inhibition of Wnt/ β -catenin signaling by means of blocking β -catenin-mediated transcription, inhibition of autocrine Wnt secretion, or knockdown of LGR4 impairs MM cell expansion. In conclusion, we demonstrate that R-spondin/LGR4 signaling plays an important role in the aberrant activation of Wnt signaling in MM. These results advocate LGR4 as a therapeutic target in MM.

High expression of syndecan-1 is a hallmark of plasma cell and its malignant counterpart MM. **In chapter 3**, we first confirm that human myeloma cell lines display constitutive Wnt/ β -catenin signaling and that inhibition of Wnt signaling by disruption β -catenin mediated transcription or by blocking of autocrine Wnt secretion impairs MM cell growth. We show that both human MM cell lines and primary MM highly express cell surface HS. Next, we show that CRISPR/Cas9-mediated knockout EXT1 results in complete loss of cell surface syndecan-1 HS. HS-deficient MM cells display strongly decreased autocrine Wnt/ β -catenin pathway activity and Wnt-target gene expression. In addition, inducible knockdown of EXT1 significantly inhibits Wnt signaling-dependent MM cell growth. Importantly, loss of HS induced growth inhibition in MM could not be overcome by co-culture with BMSCs. Next, we demonstrate that loss of HS also impairs Wnt pathway activation by paracrine Wnts. In line with chapter 2, we show that co-stimulation with R-spondin is required for optimal activation of Wnt-signaling in many MM cell lines and primary MMs. Loss of syndecan-1 HS mitigates the potentiating effect of R-spondin on Wnt signaling. Mechanistically, we demonstrated that regulation of Wnt signaling by syndecan-1 mainly takes place at the cell surface by binding Wnt ligands and R-spondins via its HS side chains. Furthermore, we demonstrate that thrombospondin protein domain of R-spondin is critical for its potentiating effect in Wnt signaling and plays an important role in R-spondin interaction

with HS. Taken together, this chapter shows that HS chains decorating syndecan-1 bind Wnts and R-spondins to promote aberrant Wnt signaling activation and cell growth in MM.

BMSCs secreted CXCL12 α plays an important role in MM cell homing and adhesion in the BM niche. However, the role of its alternative splicing isoform CXCL12 γ in MM BM microenvironment is still unknown. **In chapter 4**, we show that CXCL12 γ is expressed in situ by stromal cells in both the normal and MM bone marrow microenvironment and is highly expressed by isolated BMSCs as well as by BMSC cell lines. Importantly, upon secretion, CXCL12 γ is retained on the surface of BMSCs by HSPGs. Functionally, recombinant CXCL12 γ induces a strong adhesion of MM cells to VCAM-1 and fibronectin. Interestingly, deletion of the CXCL12 γ isoform or EXT1 in BMSCs strongly reduced MM cell adhesion to BMSCs and impaired BMSC-mediated protection of MM cells from bortezomib-induced cell death, indicating that HSPG-CXCL12 γ on the BMSC surface plays an important role in cell adhesion-mediated drug-resistance. The data presented in this chapter suggest that CXCL12 γ functions as a 'niche chemokine' that, in conjunction with cell surface HSPGs, plays a unique role in controlling MM cell adhesion, BM retention, and drug resistance.

In chapter 5, we reviewed the critical role of HSPGs in the interaction between normal and malignant plasma cells and the BM microenvironment. After B cells differentiation to plasma cells, the BCR is no longer expressed on the cell surface. The survival of plasma cells in the BM mainly dependent on the BM microenvironment derived survival signals. Plasma cell acquire strong expression of the HSPG syndecan-1. In this review, we first briefly introduce the B cell development and HSPGs structure, synthesis, and their general functions. Then we discuss the literature showing a critical role of syndecan-1 in the metamorphosis of B cells to plasma cells and in the pathogenesis of MM. Next, we reviewed the mechanism of the function of syndecan-1 in normal plasma and MM cell survival. Syndecan-1 promote normal plasma and MM cell survival by interacting with many growth factors and cytokines such as IL6, APRIL, HGF, Wnts, R-spondins, and HB-EGF, which are produced in the BM microenvironment. Then we turn to the BM niche-derived HSPGs, in particular, we discuss the important roles of BMSC derived-HSPGs on adhesion and BMSC-induced drug resistance in MM. Finally, we review the current therapeutic strategies and clinical trials, targeting HSPGs and/or their modifying enzymes.