Signaling pathways involved in B cell differentiation and disease: the role of Ral, Btk and Met

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Summary

Signaling by the B cell antigen receptor, integrin-mediated adhesion and chemokine-controlled migration are processes critically involved in early B cell development in the bone marrow, recirculation and homing of B cells via the bloodstream, and antigen-specific B cell differentiation and function underlying a proper immune response in the secondary lymphoid organs. The ability of lymphocytes to home into specialized microenvironments that control their differentiation and survival and to migrate to sites of antigenic insult, together with the related process of malignant B and T cell dissemination is discussed in chapter 2.

The B cell antigen receptor (BCR) plays an essential role in B cell development, differentiation, and function by controlling adhesion, survival, and proliferation. Signaling by the BCR involves activation of several members of the Ras superfamily of small GTPases, among which is Ras itself. Ras can control the activity of multiple effectors, including Raf, phosphatidylinositol3-kinase (PI3K), and guanine nucleotide exchange factors for the small GTPase Ral. Ras, Raf, and PI3K have been implicated in a variety of processes underlying B cell development, differentiation, and function; however, the role of Ral in B lymphocytes remained to be established. In chapter 3, we show that Ral is activated upon BCR stimulation in human tonsillar and mouse splenic B lymphocytes and in B cell lines. Using signaling molecule-deficient B cells, we demonstrate that this activation is mediated by Lyn and Syk, Bruton’s tyrosine kinase (Btk), Phospholipase C-γ2 (PLCγ2), and inositol-1,4,5-trisphosphate receptor-mediated Ca^{2+} release. In addition, although Ral can be activated by Ras-independent mechanisms, we demonstrate that BCR-controlled activation of Ral is dependent on Ras. By means of expression of the dominant-negative mutants RasN17 and RalN28, or of RalBPΔGAP, a Ral effector mutant which sequesters active Ral, we show that Ras and Ral mediate BCR-controlled transcription of c-fos. Furthermore, while not involved in NF-κB activation, Ras and Ral mediate BCR-controlled activation of JUN/ATF2 and NFAT transcription factors. Taken together, our data show that Ral is activated upon BCR stimulation and mediates BCR-controlled activation of AP-1 and NFAT transcription factors.

Next, we demonstrated that several of these mediators of BCR signaling are also involved in chemokine-induced adhesion and migration. In chapter 4 we show that the chemokine SDF-1 induces activation of Bruton’s tyrosine kinase (Btk) and that integrin-mediated adhesion and migration in response to SDF-1 or CXCL13, as well as in vivo homing to lymphoid organs, is impaired in Btk-deficient (pre-)B cells. Furthermore, SDF-1 induced tyrosine phosphorylation of PLCγ2, which, unlike activation of the migration regulatory GTPases Rac or Rap1, is mediated by Btk. PLCγ2-deficient B cells also exhibited impaired SDF-1-controlled migration. In addition, a role for Lyn and/or Syk in B cell migration was found. These results reveal that Btk, PLCγ2 and Lyn/Syk mediate chemokine-controlled migration, thereby providing insights into the control of B cell homeostasis, trafficking, and function, as well as into the pathogenesis of the immunodeficiency disease X-linked agammaglobulinemia (XLA).

As discussed in chapter 2, chemokine-controlled migration, besides in B cell development, differentiation and function, also plays a critical role in the pathogenesis of
B cell malignancies, including the plasma cell neoplasm multiple myeloma (MM). In chapter 5 we demonstrate that stimulation of B cells and MM cells with the chemokine SDF-1 induces strong migration and activation of Ral. Inhibition of Ral, by expression of the dominant negative RalN28 mutant or of RalBPΔGAP, results in impaired SDF-1-induced migration of B cells and MM cells. Of the two Ral isoforms, RalA and RalB, RalB was found to mediate SDF-1-induced migration. SDF-1-induced Ral activation is not affected in B cells deficient in Btk, PLCγ2 and Lyn/Syk. Also treatment with pharmacological inhibitors of PI3K and PLC, or expression of a dominant negative Ras mutant did not impair SDF-1-induced Ral activation. Taken together, these results reveal a novel function for Ral, i.e. regulation of SDF-1-induced migration of B cells and MM cells. This provides new insights into the pathogenesis of MM, and, together with its role in BCR signaling, suggests that Ral plays an important role in B cell development, trafficking and function.

The evolution of MM depends on complex signals from the bone marrow (BM) microenvironment, supporting the proliferation and survival of MM cells. In chapter 6 we show that hepatocyte growth factor/scatter factor (HGF), a growth factor produced by BM stromal cells and frequently by MM cells themselves, controls growth and survival of MM cells. Its receptor Met is expressed by the majority of MM cell lines and by approximately half of the primary plasma cell neoplasms tested. Stimulation of MM cells with HGF led to the activation of the Ras/mitogen-activated protein kinase (Ras/MAPK) and PI3K/protein kinase B (PI3K/PKB) pathways, signaling routes that have been implicated in the regulation of cell proliferation and survival. Indeed, functional studies demonstrated that HGF has strong proliferative and anti-apoptotic effects on MM cell lines and primary MM cells. Furthermore, by applying pharmacological inhibitors, we demonstrated that MEK is required for HGF-induced proliferation, whereas activation of PI3K is required for both HGF-induced proliferation and for rescue of MM cells from apoptosis. Taken together, our data indicate that HGF is a potent myeloma growth and survival factor.