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

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General Introduction

Chapter 1
Introduction

This chapter introduces the molecular and cellular aspects of B cell development, differentiation and function, as well as the pathogenesis of B cell malignancies. The first section briefly describes the early development of B cells and their subsequent differentiation into memory B cells or plasma cells, the malignant transformation of B cells, and the basic principles of lymphocyte homing and lymphoma dissemination. The second section of this chapter introduces the B cell antigen receptor-, chemokine-, and HGF/Met-signaling pathways, which, because of their role in the control of differentiation and function of normal B cells and the pathogenesis of multiple myeloma, were the focus of the studies described in this thesis.

1. B cell development, differentiation and malignant transformation

1.1. B cell development and differentiation

Antibodies (Ab) are immunoglobulin (Ig) proteins secreted on exposure to an antigen by terminally differentiated B cells (plasma cells). Antibodies function as mediators of specific humoral immunity by binding to pathogens and promote their elimination by other components of the immune system. During their development and differentiation, each B cell becomes genetically programmed to produce antibodies of a single specificity, and all the B cells combined can make an almost unlimited repertoire of antibodies. B cell development occurs in the bone marrow (BM), where committed lymphoid progenitors develop in an antigen-independent manner into immature B cells (Figure 1). The BM microenvironment provides the B cells with signals crucial for progression through the sequential stages of their development. During the initial stages of development, by Recombination-Activating Gene (RAG)-mediated step-wise gene-rearrangement, B cells recombine the variable (V), diversity (D) and joining (J) gene segments of the immunoglobulin heavy-chain (IgH) \( \mu \) gene locus. Expression of the \( \mu \) IgH, which associates with the surrogate light chain (SLC) and with Ig\( \alpha \) and Ig\( \beta \) results in formation of the pre-B cell antigen receptor (pre-BCR). Signaling by the pre-BCR is essential for survival of these cells and induces rearrangement of the V and J segments of Ig light chain. Subsequently, the cells develop into immature B cells expressing a mature BCR of the IgM isotype and enter the circulation. In the BM and spleen, signaling by the B cell antigen receptor (BCR) in immature B cells plays a role in depletion of B cells producing auto-reactive immunoglobulins, a process that leads to self-tolerance.

Next, naïve B cells will migrate to the secondary lymphoid organs like the spleen, peripheral lymph nodes and mucosa associated lymphoid tissue (tonsils and Peyer’s Patches in the gut) (Figure 1). Here, B cells can undergo antigen-specific B cell differentiation, which requires multiple interactions of B cells with T cells, follicular dendritic cells (FDCs), and with the extracellular matrix (ECM). These interactions take place in the germinal center (GC), a specialized microenvironment within the B cell area or follicle of secondary lymphoid organs [1,2]. The B cells first migrate into the GC dark zone where they undergo rapid clonal
expansion and somatic hypermutation of their immunoglobulin genes. Mutated B cells then progress to centrocytes and move to the basal light zone of the GC. Here they re-encounter antigen, presented as immune complexes by FDCs, and undergo affinity selection. Based on the affinity of their BCR, the B cells are selected to undergo clonal expansion and Ig-isotype switching (high affinity BCR) or apoptosis (low affinity and autoreactive BCR). Both somatic hypermutation and isotype switching involve the enzymatic activity of activation-induced deaminase (AID) [3]. As a consequence of isotype switching of the IgH genes, the antibodies produced are better adapted to their specific effector functions. Subsequently, B cells will differentiate in antibody secreting plasma cells which control the humoral immune response, or in memory B cells, which upon antigen recall cause a rapid, more specific secondary immune response [1].

**Figure 1. B cell development and differentiation and the corresponding B cell neoplasia.**
Schematic representation of B cell development and differentiation. The malignant counterparts are indicated in parenthesis. See text for more detail. BM, bone marrow; LN, lymph node; MALT; mucosa associated lymphoid tissue; ALL, acute lymphoblastic leukemia; MCL, mantle cell lymphoma; IC, immunocytoma; DLBCL, diffuse large B cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphatic leukemia/lymphoma; FL, follicular lymphoma; BL, Burkitt’s lymphoma; MM, multiple myeloma.
1.2. Malignant transformation of B cells

The DNA-modifying processes, which B cells undergo during their development and differentiation, involve introduction of double stranded DNA-breaks and induction of mutations. In combination with rapid clonal expansion these processes predispose to malignant transformation (Figure 1). Indeed, the chromosomal translocations involving one of the immunoglobulin loci and a proto-oncogene, which are common oncogenic events in non-Hodgkin lymphomas (NHLs), result from errors during gene rearrangement, somatic hypermutagenesis and isotype switching [4,5]. Besides the genetic transformation events, interaction with the tumor microenvironment also plays a critical role in the pathogenesis of B cell malignancies [5]. In addition, similar to non-malignant B cells, selection for expression of a functional BCR also occurs in certain B cell lymphomas, including chronic lymphocytic leukemias (CLLs), follicular, Burkitt’s and MALT lymphomas [4-6].

Multiple myeloma (MM) is a neoplasm of terminally differentiated B cells expanding in the BM. MM is an incurable malignancy for which the median survival has remained fixed at about 3 years for the past decade. This disease accounts for approximately 2% of all cancer deaths and nearly 20% of deaths caused by hematological malignancies [7]. In MM cells, chromosomal translocations frequently place oncogenes under transcriptional control of enhancers regions at the IgH gene locus (14q32) [7,8]. Translocation partners include CCND1 on 11q13 (15-20%) and CCND3 on 6p21 (5%) resulting in overexpression of cyclin D1 and D3 respectively, 4p16 (12%) which leads to dysregulation of FGFR3 and MMSET expression, and of c-MAF on 16q23 (5-10%) with as a consequence overexpression of this basic-leucine zipper transcription factor [7,8]. In addition, complex genomic rearrangements that occur during late stages of MM cause dysregulation of c-myc [9]. MM is preceded by a pre-malignant expansion of plasma cells called monoclonal gammopathy of undetermined significance (MGUS).

MM is located at several sites in the BM compartment. Normal long-lived plasma cells and all stages of intramedullary MM are dependent on the BM microenvironment, where cytokines produced by BM stromal cells, like IL-6, VEGF and IGF, induce survival and growth of these cells [7,8]. Furthermore, direct physical contact of MM cells with BM stromal cells via integrins, can mediate growth and survival and triggers cytokine production [10,11]. A hallmark of MM is osteolytic bone disease, which is caused by increased osteoclast-mediated bone resorption and suppressed osteoblast-mediated bone formation, causing 90% of the MM patients to develop bone lesions [12].

1.3. Lymphocyte homing and lymphoma dissemination

The orchestration of systemic immune responses is critically dependent on coordinated lymphocyte migration and recirculation. This ‘homing’ guides lymphocyte sub-sets to the specialized microenvironments that control their differentiation and survival, disperses the immunologic repertoire, and targets effector lymphocytes to sites of antigenic insult. Lymphocyte homing is a multi-step process that requires chemotaxis and cell adhesion coupled with strategies to overcome physical barriers. At the molecular level, it is regulated by adhesion molecules in concert with chemokines and facilitated by intrinsic molecular programs that allow ‘amoeboid’ shape change of lymphocytes. These properties allow highly effective lymphocyte traffic between different tissue compartments. In case of malignant transformation, however, the fact that lymphocytes are ‘licensed to move’ forms a serious threat to the organism, since it allows rapid tumor dissemination irrespective of the conventional anatomic boundaries limiting early spread in most other types of cancer. The
dissemination patterns often reflect basic rules of lymphocyte homing, explaining the strikingly tissue-specific dissemination of, for example, malignant lymphomas of the mucosa-associated lymphoid tissues, cutaneous lymphomas, and MM. A better understanding of the molecular mechanisms underlying this behavior may provide novel targets for treatment of lymphoma patients. This will be discussed in more detail in chapter 2 of this thesis.

2. Signaling pathways underlying differentiation and function of B cells and pathogenesis of MM

This section introduces several of the kinases, small GTPases and transcription factors, involved in the regulation of proliferation, differentiation, motility and tumorigenesis in general, or in B cells in particular. Subsequently, the specific function of several of these signaling mediators will be discussed in more detail in the context of BCR-, chemokine receptor-, and HGF/Met-signaling.

2.1. Kinases

Kinases are enzymes that transfer a phosphate-group from ATP to its specific protein- or lipid-substrate, and play an essential role in signal transduction processes. Protein kinases comprise the largest group of kinases and can phosphorylate their targets at specific tyrosine-, serine-, or threonine-residues. The covalent linking of the phosphate group causes a change in charge and conformation of the phosphorylated substrate, thereby affecting its enzymatic activity and/or ability to interact with other molecules. Kinases can be subdivided in receptor kinases, of which Met discussed in section 3.6 is an example, and in cytoplasmic kinases, which will be discussed here.

Phosphoinositide 3-kinase

Phosphoinositide 3-kinases (PI3Ks) are activated in response to a wide variety of stimuli and mediate numerous biological responses, including cell growth, differentiation, survival, proliferation, cell metabolism and migration. On basis of structural similarities, the PI3K family is subdivided into three classes, class I, class II and class III. In turn the class I can be subdivided in class IA PI3Ks, which are activated by receptor tyrosine kinases, antigen receptors, co-stimulatory and cytokine receptors, and the class IB PI3K, PI3K\gamma, which is activated by G protein-coupled receptors (GPCRs), a large family of receptor proteins that includes the chemokine receptors [13]. Class IA PI3Ks are heterodimeric enzymes consisting of a regulatory subunit (p85α, p85β or p55γ) and a catalytic subunit (p110α, p110β or p110δ) [13]. All Class I PI3Ks phosphorylate phosphatidylinositol-(4,5)-bisphosphate (PIP2) resulting in the formation of phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). This causes membrane recruitment of proteins that contain PIP3-binding Plekstrin homology domains (PH-domains). These include the serine/threonine kinase protein kinase B (PKB, also known as AKT), which has an important role in cell proliferation, growth, survival and metabolism, and tyrosine kinases of the TEC family, such as Bruton’s tyrosine kinase (Btk), which will be discussed
later. PI3K signaling is negatively regulated by the lipid phosphatase PTEN (phosphatase with tensin homology, which is deleted on chromosome 10), which dephosphorylates PIP$_3$ to generate PIP$_2$ [14]. Notably, constitutive activation of PI3K signaling as a consequence of mutation, deletion or deregulation of PTEN promotes cell survival, proliferation and chemoresistance in many tumours.

**Protein kinase B**

The serine/threonine kinase PKB is an important effector of PI3K [15,16]. PIP$_3$ generated by active PI3K allows binding and thus membrane recruitment of PKB and PDK1 via their PH-domains [15,16]. The constitutively active PDK1 phosphorylates PKB at threonine 309, which results in activation of this kinase. Subsequently PKB is phosphorylated at serine 474 leading to maximal PKB activation. The identity of the kinase phosphorylating PKB at ser-474 is still unknown. Once activated PKB phosphorylates several substrates and thereby modulates their activity. Downstream effector molecules for PKB include the Bcl-2 family member Bad, which can exert pro-apoptotic activity by interacting with Bcl-2 [17], and Glycogen Synthase Kinase-3 (GSK3), which is involved in glycogen metabolism [18]. Both are inhibited by phosphorylation by PKB. Furthermore, PKB is implicated in regulation of gene transcription. Phosphorylation of IKK by PKB has shown to activate the pro-survival Nuclear Factor-κB (NF-κB) pathway [19], whereas phosphorylation of the FOXO transcription factors, which control cell cycle arrest and apoptosis, inhibits their activity [20] (see below).

PKB appeared to be essential in PI3K-mediated survival of various B cell malignancies. In diffuse large B cell lymphomas (DLBCLs), inhibition of PI3K by LY294002 induced apoptosis, and high phospho-PKB levels was found to associate with short survival [21]. Also in MM cells PI3K-PKB signaling results in proliferation and rescue from apoptosis [22].

**Bruton’s tyrosine kinase**

Bruton’s tyrosine kinase (Btk) belongs to the Tec family of tyrosine kinases, which also include Tec, Itk, Rlk, and Bmx, a family of non-receptor tyrosine kinases that contain a PH-domain [23]. Tec-family kinases are mediating signaling by cytokine receptors, lymphocyte antigen receptors, GPCRs and integrins. Btk is a 659 amino acid, 77 kDa protein, consisting of a N-terminal PH-domain, a proline-rich Tec homology domain, a SH3 domain, a SH2 domain and a kinase domain (Figure 2)[23]. Btk is expressed at all stages of B cell differentiation, is selectively down-regulated in plasma cells [24-26], and is a crucial mediator BCR signaling [27-29]. The mechanism of Btk activation will be discussed later.

Mutations in Btk cause the inherited X chromosome-linked humoral immunodeficiency disease X-linked agammaglobulinaemia (XLA) [30], due to an almost complete arrest in B cell development at the pre-B cell stage. This results in a severe reduction of mature B cell numbers and decreased immunoglobulin serum levels of all isotypes, and as a consequence an increased susceptibility to infections caused by extracellular bacteria. XLA was first described by Bruton in 1952, has an incidence of 1 in 200,000 births, and can be recognized by markedly decreased or absent tonsils and cervical lymph nodes [31,32]. Between 1950 and 1980 most affected patients died before 20 years of age. Currently, the majority of XLA patients are diagnosed to have immunodeficiency during or shortly after their first hospitalization for infection, and are treated with use of antibiotics and monthly infusions of intravenous gammaglobulin [32,33], allowing them to be moderately healthy and leading productive lives [33].
In mice, a missense mutation of a conserved arginine residue within the PH domain of Btk results in the X-linked immunodeficiency (Xid) phenotype [34,35]. Xid mice have a ~50% reduction in B cell number and low levels of serum IgM and IgG3, but normal levels of other isotypes. Those B cells that are formed in Xid mice have an immature IgM\textsuperscript{high}/IgD\textsuperscript{low} phenotype and are functionally impaired, since they hardly respond to BCR cross linking, IL5, IL10, CD38 or LPS. Although both in XLA patients as well as Xid mice the T cell independent antibody responses are lacking, the T cell dependent antibody responses are only affected in XLA patients. Targeted deletion of the Btk gene in mice causes a similar phenotype as that of Xid mice [36,37]. The less severe defect in B cell development observed in Xid mice, compared to that found in human XLA, is apparently due to that in mice B cells Tec may partly compensate for the Btk defect. Tec is also expressed in B cells, activated upon BCR triggering, and mice deficient for both Tec and Btk exhibited a block at the pro-B cell stage and displayed a severe reduction of peripheral B cell numbers [38,39].

### 2.2. Ras superfamily of small GTPases

Proteins belonging to the Ras superfamily of small GTPases are small (20-30 kDa) signaling molecules involved in regulating numerous cellular responses, which all share some sequence homology to the Ras proto-oncogene. The Ras superfamily consists of several subfamilies, including the Ras family, which has been typically implicated in controlling proliferation and differentiation, and the Rho family, which is typically involved in modulating the actin cytoskeleton. All small GTPases are considered to be molecular switches: they cycle between an inactive GDP-bound conformation and an active GTP-bound conformation (Figure 3). Activation of the GTPases is catalyzed by guanine nucleotide exchange factors (GEFs), which release GDP from the GTPases, so they can bind the more abundantly present GTP. Upon GTP binding the conformation of the effector domain, consisting of the so-called switch-1 and -2 regions, is altered, which allows binding of effector proteins thereby modulating their biological activity. GTPases can be inactivated by the action of GTPase activating proteins (GAPs), which catalyze the intrinsic GTP-hydrolyzing activity of the GTPases.
Figure 3. Regulation of the GTPase cycle.
Guanine nucleotide exchange factors (GEFs) release GDP from the GTPase so it can bind the more abundantly present GTP and becomes activated. In its GTP-bound active conformation, GTPases are able to bind downstream effectors. GTPases can be inactivated again by the actions of GTPase activating proteins (GAPs), which catalyze the intrinsic GTPase activity of the GTPases.

Most members of the Ras superfamily are anchored to the membrane via a lipid-modification at their C- or N-terminus. Ras GTPases are farnesylated, whereas Ral, Rap and Rho are geranylgeranylated at their C-terminus. The difference in modification causes these proteins to localize to different membrane regions of the cell.

**Ras GTPases**
Ras GTPases become activated by most, if not all, receptor tyrosine kinases (RTKs) and GPCRs, and exert their wide variety of biological effects by controlling the activity multiple effectors, including Raf [40], phosphoinositide 3-kinase [41] and guanine nucleotide exchange factors for the small GTPase Ral [42,43]. In mammalian cells 3 ras genes are expressed: H-ras, K-ras and N-ras. Although *in vitro* all three isoforms can bind the same effector proteins, *in vivo* there are marked quantitative differences in the ability of K- and H-Ras to activate Raf-1 and PI3K [44]. The different biological function of the different Ras proteins is also reflected by the fact that whereas mice deficient for H-Ras and/or N-Ras are viable [45,46], targeted deletion of K-ras results in embryonic lethality [47,48]. The major differences between the Ras proteins are located in the C-terminal 25 amino acids comprising the hypervariable region, in which sequence homology is less than 15% between any two isoforms. The hypervariable region contains the protein sequences required for Ras to associate with the inner plasma membrane [49]. The differences in the hypervariable region cause localization of the Ras proteins to spatially distinct microdomains of the plasma
membrane, which can probably explain the functional differences between the Ras proteins [49].

The ras genes were first recognized as human oncogenes when DNA derived from human carcinoma cell lines able to transform murine fibroblasts appeared to be homologous to the transforming genes of Harvey (H-ras) and Kirsten (K-ras) sarcoma viruses [50]. Ras oncogenes are found to be mutated in about 20% of human tumors [51]. These point mutations in codon 12, 13 or 61 of one of the three ras genes results in expression of Ras mutants which have lost their ability to hydrolyze GTP to GDP, and thus renders the proteins constitutively active, leading to autonomous growth stimulation [51,52]. The incidence of Ras mutations is found in a variety of tumor types, including pancreas, colon and lung carcinomas, thyroid tumors and myeloid leukemia [52]. Remarkably, no oncogenic Ras mutations have been found in lymphomas, except for MM. Although rare in MGUS, activating mutations in N-Ras and K-Ras have been reported to occur in 40-50% of patients with MM at diagnosis [53-55]. Mutated Ras-containing MM cell lines demonstrated constitutive activation of the Raf-MEK-Erk, PI3K-PKB and nuclear factor-κB (NF-κB) pathways, which are required for proliferation and survival of MM cells [56]. In addition to the occurrence of oncogenic Ras mutations, Ras is found to be activated in MM cells by numerous signals provided by the BM microenvironment [57-59].

**Ral GTPases**

The Ral GTPases belong to the Ras branch of small GTPases and were identified as proteins being highly homologous to Ras GTPases [60], with which they share 58% identity. Several Ral-specific GEFs have been identified, of which several bind GTP-bound Ras, resulting in membrane localization and their activation [42,43]. Ral proteins have been implicated in controlling a multitude of cellular processes, including proliferation, survival, endo- and exocytosis, cytoskeletal rearrangements, migration, gene transcription and transformation [61]. The two highly similar Ral GTPases, RalA and RalB, show 85% identity. The main differences between RalA and RalB is found in their C-terminal variable regions, causing different subcellular localization of the two isoforms, which causes that RalA and RalB differ in function [62,63]. In addition, despite the high degree of sequence homology, the affinity for effectors varies between the two Ral isoforms [62].

Ral binds and regulates the activation of the Cdc42/Rac GAP Ral binding protein-1 (RalBP1) [64,65], the actin binding protein filamin [66], the Sec5 and Exo84 subunits of the exocyst complex [67,68], and phospholipase D1 [69]. The first identified Ral-effector was RalBP1 [64], which by binding Epsin and Eps15 mediates Ral-dependent endocytosis of the receptors for EGF and insulin [70]. However, as mentioned, RalBP1 was originally identified as a GAP for CDC42 and Rac [64,65], and as such is potentially involved in cytoskeletal reorganization. Furthermore, by recruitment of filamin, activated Ral induces filopodia formation [66], which are slender cytoplasmic projections, extending from the leading edge of migrating cells. Indeed, Ral is involved in EGF-induced motility of human bladder cancer cells [71], and mediates migration of skeletal myoblasts [72,73] and of border cells during *Drosophila* oogenesis [74]. Moreover, Ral was also found to bind Sec5 and Exo84 subunits of the exocyst complex [67,68]. The exocyst is an evolutionary conserved multi-subunit complex involved in the docking of exocytic vesicles [75]. By binding the exocyst Ral regulates vesicle trafficking, and thereby is implicated in cell polarity, neurite differentiation and regulated exocytosis [67,68,76]. Interestingly, filopodia formation and motility was shown to depend on Ral-mediated recruitment of the exocyst-subunit Sec5 [77,78]. Since the Exocyst controls transport of secretory vesicles, Ral-mediated migration could involve coordinated delivery of secretory vesicles to the sites of dynamic plasma membrane
expansion that specify directional movement. In addition, the RalGEF-Ral pathway regulates gene transcription by modulating the activity of various transcription factors. For example, Ral is involved in the transcriptional activation of the c-fos promoter [79-81], and mediates Ras-controlled phosphorylation of JUN [82] and ATF2 [83]. Furthermore, Ral signaling modulates the transcriptional activity of the FOXO family member FOXO4 [84-86], mediates EGF-induced Stat3 activation via Src [87], and regulates the relief of transcriptional repression by ZO-1-associated nucleic acid-binding protein (ZONAB) [88]. Moreover, Ral is able to activate NF-kB, resulting in an increase of cyclin D1 expression in fibroblasts [89].

Importantly, Ral was found to be a critical mediator of Ras-induced tumorigenesis [90]. Recently, it was discovered that the mechanism of Ras transformation may differ between mice and humans. Whereas Raf is generally the most potent transforming Ras-effector in murine cells, Ral appears to have this role in human cells [91,92]. Knockdown of RalA, but not of RalB expression, impaired the ability of human cancer cells to form tumors in immunocompromised mice, indicating that activation of RalA is a crucial step in Ras-induced tumorigenesis of human cells [63]. The subcellular localization of either Ral isoform appears to be of great importance for their specific function. For example, by swapping the C-terminal variable regions which control localization of these GTPases, RalB gains and RalA loses transforming potential [63]. Notably, RalA was frequently activated in a panel of pancreatic cancer cell lines, most of which known to have an activating mutation in the K-ras gene [63]. In addition, the RalGEF RalGDS was shown to be required for tumor formation in a mouse model of skin carcinogenesis [93].

Interestingly, RalGDS was also found to bind GTP-bound TC21, a highly oncogenic member of the Ras superfamily, and expression of constitutively active TC21 leads to the activation of RalA [94,95]. Activation of the Ral pathway by TC21 is required for TC21-stimulated DNA synthesis [95], suggesting that the RalGEF-Ral pathway might, besides being involved in Ras-induced tumorigenesis, function in TC21-controlled transformation as well.

Rap1 GTPases
There are two Rap1 isoforms, Rap1A and Rap1B, which are different in only a few amino acids, and will be referred to collectively as Rap1. Rap1 is one of the closest relatives of Ras, sharing ~50% amino acid identities with Ras GTPases. Rap1 was originally identified as a protein, designated Krev-1, able to revert the phenotype of K-Ras transformed fibroblasts to a flat morphology [96]. Since the effector-binding domain of Rap1 is identical to that of Ras, it was believed that Rap1 acted as a Ras-antagonist by binding Ras effector molecules, thereby trapping them in an inactive complex [97]. However, Zwartkruis et al. have shown that activation of endogenous Rap1 does not influence Ras-mediated activation of Raf or Erk [98]. Moreover, in transgenic mice expressing endogenous levels of constitutively active Rap1 in thymocytes, Ras-mediated Erk activation was not affected [99]. At present, it is well established that Rap1 is a very potent activator of β1 and β2 integrins in pro-B-cells and T cells, and required for integrin-mediated adhesion [100,101]. By regulating the adherence of integrins to their ligands, Rap1 modulates T-cell responses and plays a decisive role in the interaction of T-cells with antigen-presenting cells critical for T-cell activation [99,102]. Although Rap1A-deficient T and B cells show impaired integrin-mediated adhesion, Rap1A deficiency does not result in defective immune functions, most likely due to redundancy with Rap1B [103].

Cytokine-induced receptor activation leads to either membrane recruitment and phosphorylation of the RapGEF C3G, which then is activated [104,105], or of activation of
GENERAL INTRODUCTION

certain RapGEFs via common second messengers such as cyclic AMP (cAMP), Ca\(^{2+}\) and diacylglycerol (DAG) [106-108], which subsequently activate Rap1. As a consequence Rap1 can be activated by various cytokines [98] and upon activation of both the B cell and the T cell antigen receptors (TCRs) [109,110]. Interestingly, stress induced by turbulence was found to be sufficient for Rap1 activation in megakaryocytes, suggesting that Rap1 activity is also influenced by mechanical and shear stress [111]. Once activated, Rap1 binds its effector RAPL (regulator of adhesion and cell polarization enriched in lymphoid tissues) that mediates Rap1 signaling to integrins [112].

2.3. Transcription factors

The regulation of gene transcription underlies many biological responses. Extracellular stimuli simultaneously modulate the action of an extensive repertoire of transcriptional activators and repressors. Ultimately, the combined action of all the transcription factors binding promoter regions and enhancers of genes controls their transcription. Here, some families of transcription factors involved in B cell development and function are described.

**NF-κB transcription factors**

In the absence of stimuli, the NF-κB complex resides in the cytoplasm due to binding of IκB (inhibitor of NF-κB), which masks nuclear-localization sequences (NLSs) on NF-κB subunits and mediates nuclear export via its nuclear-export signal (NES) [113]. Upon stimulation, IκB proteins are phosphorylated on conserved serine residues by IκB kinase (IKK), leading to the subsequent polyubiquitylation and proteasomal degradation of IκB. No longer bound by IκB, NF-κB translocates into the nucleus and binds NF-κB responsive elements of promoters of target genes and controls their expression.

Besides controlling differentiation, survival and proliferation of several cell types, NF-κB is an important mediator of tumorigenesis. Apart from being implicated in progression of several solid tumors [114], NF-κB was found to play a crucial role in lymphomagenesis [4]. Furthermore, NF-κB was described to be constitutively activated in several MM cell lines and primary MM cells [115]. Inhibition of NF-κB signaling in MM cells, by means of the proteasome inhibitor PS-341 or the specific NF-κB-inhibitor SN50, results in down-regulation of anti-apoptotic proteins, activation of caspases and induction of apoptosis [116,117]. Furthermore, growth of oncogenic Ras-expressing MM cell lines was inhibited by viral transduction of the IκB superrepressor, a non-degradable IκB mutant which prevents NF-κB activation [56].

**AP-1 transcription factors**

Activator protein-1 (AP-1) transcription factors are homo- or heterodimeric complexes of FOS, JUN, or ATF family proteins, that stimulate transcription of genes containing AP-1 regulatory elements, controlling proliferation, apoptosis, transformation, differentiation and development [118-120]. The FOS and JUN proteins are encoded by the proto-oncogenes \(c-fos\) and \(c-jun\), respectively, and control transcription of genes containing AP-1 sites or TPA-responsive elements (TREs). ATF2 binds to cAMP response elements (CREs), forms a homodimer or heterodimer with JUN, and stimulates CRE-dependent transcription. The different AP-1 proteins are differentially expressed and regulated, resulting in that every cell type has a complex mixture of AP-1 dimers with different functions [120]. In addition, the
functional consequence of AP-1 activity is often determined by the interaction with other transcription factors.

**NFAT transcription factors**
In resting cells, NFAT (Nuclear Factor of Activated T cells) transcription factors are cytoplasmic and highly phosphorylated on conserved serine residues in their regulatory domain. An increase of intracellular Ca\(^{2+}\) results in calmodulin-dependent activation of the phosphatase calcineurin, which dephosphorylates NFAT proteins [121]. Dephosphorylation of NFAT is required to mask a NES, cause full exposure of a NLS, resulting in translocation from the cytoplasm into the nucleus and expression of target genes [122,123]. NFAT binds the DNA in complex with other transcription factors on so-called composite binding sites present in the regulatory regions of NFAT target genes [124,125]. Especially FOS and JUN heterodimers have been found to cooperate with NFAT in controlling expression [126]. Several kinases, including GSK3, casein kinase 1, p38 and JNK, have been described to phosphorylate NFAT proteins and thereby control their nuclear exclusion [123,124].

**FOXO transcription factors**
FOX transcription factors of the O class (FOXOs) regulate transcription of genes involved in cell cycle arrest, protection against oxidative stress and apoptosis [127]. Upon growth factor stimulation, FOXOs are phosphorylated by the PI3K-regulated kinases PKB and serum- and glucocorticoid-induced kinase (SGK), which results in nuclear exclusion of the transcription factors, and thus inhibits their activity [20,128]. Overexpression of FOXO1 (FKHR), FOXO3A (FKHRL1), or FOXO4 (AFX) was found to cause growth suppression in a variety of cell lines, including a Ras-transformed cell line and a cell line lacking the tumor suppressor PTEN [129]. FOXOs control transcription of p27KIP1 [129,130] and cause downregulation of Cyclin D1 and D2 [131], resulting in cell cycle arrest. In addition, FOXOs regulate expression of the Bcl-2 interacting mediator of cell death (Bim) [132], Fas ligand [20], tumor necrosis factor-related apoptosis inducing ligand (TRAIL) [133] and promote downregulation of FLICE-inhibitory protein (FLIP) [134], and as a consequence activate the apoptotic pathway. Thus, FOXOs are involved in cell cycle regulation and survival and inactivation of these proteins may be an important step in oncogenic transformation. However, on the other hand, FOXOs mediate protection from reactive oxygen species-induced apoptosis caused by glucose deprivation, by transcriptional activation of manganese superoxide dismutase (MnSOD) [135].

**2.4. B cell antigen receptor signaling**
The BCR consists of the clonally restricted antigen-binding membrane immunoglobulin, which is a tetrameric complex of Ig heavy (H) and light (L) chains, and the signal transduction component consisting of a disulfide-bonded heterodimer of the Igα (CD79α) and Igβ (CD79β) molecules. The BCR plays an essential role in determining the fate of B cells by regulating proliferation, survival and differentiation. As a consequence of encountering antigen recognized by the BCR, various signaling molecules are activated which transduce the signal leading to the several biological responses, required to differentiate in either memory B cells or plasma cells to mediate clearance of the antigen. Upon antigen binding, activation of both the Src-family protein tyrosine kinase Lyn, and of the protein tyrosine kinase Syk occurs. These kinases subsequently mediate activation of PI3K, Btk, PLCγ2 and
members of the Ras superfamily of small GTPases (Figure 4). These signaling molecules have an important function in BCR signaling, as will be discussed below.

PI3K is activated within seconds of BCR triggering and has been proven to play a prominent role in BCR signaling. Its importance is reflected by the defects in B cell development and activation observed in the various PI3K-gene-deficient mice, and the essential function fulfilled by its effectors PKB and Btk in mediating BCR-controlled responses [13]. Given that PKB inhibits several pro-apoptotic pathways and activates the pro-survival NF-κB pathway, it is not surprising that PKB is activated by several signals which induce survival of B cells, including signaling by the BCR (Figure 4).

Figure 4. B cell antigen receptor signaling.
Schematic representation of BCR-controlled signal transduction cascades. See text for more details.
In addition, PI3K, together with Lyn, facilitate Btk activation upon BCR stimulation [27-29]. The PI3K-product PIP3 has been implicated in membrane recruitment of Btk, enabling phosphorylation at tyrosine residue 551 in the catalytic domain of Btk by Lyn, resulting in Btk activation (Figure 4) [136-138]. Subsequently, Btk becomes autophosphorylated at tyrosine 223 [136,137], which is believed to disturb the intramolecular interaction between the proline-rich and SH3 domains [23], affecting Btk binding to the signaling molecules c-Cbl, WASP and Syk (Figure 2) [139]. PLC\(\gamma\)2 is a substrate for Btk that, via diacylglycerol (DAG) and inositol-1,4,5-trisphosphate(IP3)-mediated Ca\(^{2+}\) release, mediates various BCR-controlled, Btk-mediated cellular responses [140-142]. Syk has been implicated in phosphorylation of the adaptor BLNK (also known as SLP-65 or BASH), thereby creating docking sites for both Btk and PLC\(\gamma\)2, which facilitates phosphorylation and activation of PLC\(\gamma\)2 by Btk (Figure 4) [142]. PLC\(\gamma\)2-mediated Ca\(^{2+}\) release, among others, results in activation of PKC\(\beta\). PKC\(\beta\) phosphorylates Btk at serine 180 in the Tec homology domain, causing altered Btk membrane localization, thereby providing a negative feedback loop [143,144].

BCR-induced PLC\(\gamma\)2-mediated DAG production is involved in Ras activation, by inducing membrane recruitment and PKC-mediated activation of the DAG-dependent RasGEF RasGRP3, and to a lesser extent RasGRP1 (Figure 4) [145-147]. Furthermore, B cell proliferation induced by BCR ligation was found to depend on RasGRP1 and RasGRP3 [148]. Ras was found to be essential in B cell development and differentiation. For example, expression of a dominant-negative Ras mutant arrests B cell development at a very early stage, prior to formation of the pre-BCR [149]. Similarly, B cells deficient for RAG proteins are blocked in their development at the pro-B cell stage, due to their inability to express a functional pre-BCR. However, introduction of a constitutive active Ras mutant into RAG1-deficient cells induces differentiation of pro-B cells to pre-B cells and subsequent mature B cells [150,151]. Furthermore, inhibition of Ras impairs affinity maturation in memory B cells and prevents terminal differentiation of memory B cells in response to recall antigen [152]. RasGRP3\(^{-/-}\) mice show isotype-specific deficiencies in antibody induction in immunized young mice and exhibit hypogammaglobulinemia [148]. Besides Ras activation, BCR stimulation also results in DAG-dependent activation of Rap1 [109]. Blocking Rap1 activation by overexpression of RapGAPII inhibits BCR-controlled ICAM-1 and VCAM-1 binding, and B cell spreading [153].

**Transcriptional regulation by BCR signaling**

BCR signaling influences the transcriptional activity of several transcription factors, among which the NF-\(\kappa\)B, AP-1, NFAT and FOXO transcription factors. BCR-controlled activation of NF-\(\kappa\)B [154] is critically involved in controlling immune responses including B cell proliferation, activation, cytokine production and isotype switching [155]. The requirement for NF-\(\kappa\)B in B cell development is illustrated by the fact that mice deficient in several NF-\(\kappa\)B signaling components display lack of immunoglobulin class switching, lack of germinal centres or disruption of splenic microarchitecture leading to B cell abnormalities [155]. For example, mice deficient in IKK\(\alpha\) as well as NF-\(\kappa\)B1/NF-\(\kappa\)B2 double deficient mice have no mature B cells [156,157].

BCR activation results in expression of the c-fos proto-oncogene [158]. FOS regulates Blimp-1 expression and terminal differentiation of activated B cells [159], and plasma cells display up-regulation of c-fos and other AP-1 transcription factors [160]. Moreover, induction of germline epsilon transcripts, an essential step preceding Ig isotype switching to IgE, requires FOS and JUN activity [161]. On the other hand, forced overexpression of FOS in
mouse GC B cells inhibits cell cycle progression [162] and induces apoptosis [163], thus emphasizing the importance of tight regulation of FOS expression in B cells.

NFAT transcription factors play crucial roles in the development and function of the immune system by regulating proliferation, apoptosis and cytokine production [124]. Up to now, most studies addressed the function of NFAT in T cells. NFAT proteins were found to regulate T cell activation and are also involved in the control of thymocyte development, T-cell differentiation and self-tolerance [123]. However, NFAT was also found to be activated upon BCR activation (Figure 4) [164,165]. In B cells, NFAT regulates expression of the pro-inflammatory cytokine TNFα [166], Igκ [167] and CD5, a negative regulator of BCR signaling [168]. Furthermore, NFAT2 is required for development of B-1a cells [169], which plays an important role in T-cell-independent antigen responses. Moreover, mice repopulated with B cells deficient for both NFAT1 and NFAT2 showed increased levels of IgG1 and IgE and expanded populations of plasma cells [170], indicating NFAT proteins are involved in normal B cell homeostasis and differentiation.

BCR engagement triggers PI3K-dependent phosphorylation and nuclear export of FOXO1 (Figure 4) [171]. Furthermore, expression of FOXO mutants which lack PKB phosphorylation sites in activated B cells induces partial arrest in G1 phase of the cell cycle and increases apoptosis [171]. In contrast, BCR-induced growth arrest and apoptosis of murine WEHI 231 immature B lymphoma cells is due to reduced PI3K/PKB signaling and an consequent increased nuclear accumulation of FOXO3a and p27KIP1 expression [172]. Furthermore, PI3K-dependent activation PKB suppressed isotype switching, in part, through the inactivation of the FOXO transcription factors [173]. Taken together, these findings suggest that FOXOs play an important role in BCR signaling and B cell development.

2.5. Chemokine signaling

Chemotaxis is the process of induced directional migration towards a gradient of chemotactic cytokines, or chemokines. Chemokine receptors belong to the large family of seven transmembrane (7-TM) GPCRs and are expressed by a wide variety of cell types. In particular the role of the chemokine stromal-derived factor-1 (SDF-1, also known as CXCL12) in lymphocyte trafficking has been well established. SDF-1 was originally identified as a growth-stimulatory factor for pre-B cells [174], and is a potent chemotactic factor for different cell types. Its cognate receptor, CXCR4, is broadly expressed in cells of the immune system, and was also shown to be an important co-receptor for the human immune-deficiency virus HIV-1. Mice deficient in either SDF-1 or CXCR4 die perinatally with cardiac, cerebellar, vascular and hematopoietic defects [175-177]. In these mice, B cell lymphopoiesis and myelopoiesis are reduced in fetal liver and virtually absent in the BM [175-177]. SDF-1 is constitutively expressed by bone marrow (BM) stromal cells [178-180], and retains multipotent hematopoietic progenitors and pre-pro-B cells in specific niches of the BM microenvironment [181]. Also GC B cells respond to SDF-1, and CXCR4 expression by B cells was found to regulate GC organization [182]. CXCR4, together with CCR7, is the major chemokine receptor involved in B cell entry into lymph nodes [183]. Finally, SDF-1 induces plasma cell migration, and CXCR4 is required for accumulation of long-lived plasma cells in the BM [184].

SDF-1/CXCR4-signaling has been implicated in hematological malignancies as well. SDF-1-induced homing of malignant cells to the BM, where they receive protective survival signals, is believed to be an important feature of B-cell chronic lymphocytic leukemia (B-CLL), precursor-B-cell acute lymphoblastic leukemia (ALL), acute myelogenous leukemia...
(AML) and MM [185]. Notably, CXCR4 is also expressed by a broad range of solid tumors. These include breast, small-cell lung, and colorectal cancer and melanomas, and CXCR4 expression in these tumors is frequently associated with relatively poor patient survival [185]. The distinct pattern of chemokine receptor expression by these tumor cells has a critical role in metastasis.

Several proteins involved in the signaling pathways underlying chemokine-induced lymphocyte adhesion and migration have been identified. These proteins mainly include kinases, adapter proteins and small GTPases. Of the small GTPases, especially members of the Rho family have drawn much attention due to their capacity to regulate cytoskeletal rearrangements [186,187], which is required for cell polarity and motility. Members of the Ras family, like the Ras and the Rap1 GTPases, have also been found to be involved in chemotaxis [188,189]. Since Rap1 activity is required for integrin function, it is not surprising this GTPase is involved in regulating migration. In Drosophila, lack of Rap1 function disrupts cell migration and causes abnormal cell shape, leading to severe defects in morphogenesis [190]. In addition, Rap1 was found to be involved in adhesion, spreading and cell migration of mouse embryonic fibroblasts, and to be required for early mouse embryogenesis [191]. In B cells, SDF-1-induced Rap1 activation mediates integrin activation and migration (Figure 5).
[153,189], and SLC- and SDF-1-induced Rap1 activation is required for under flow adhesion to endothelial cells and transendothelial migration of lymphocytes [192]. Furthermore, Dock2, a hematopoietic cell-specific CDM family protein that is indispensable for lymphocyte chemotaxis, mediates SDF-1-induced Rap1 activation, suggesting that defective migration of Dock2<sup>−/−</sup> B cells is, at least in part, due to impaired Rap1-mediated integrin activation [193]. The Rap1 effector RAPL induces distribution of LFA-1 to the leading edge associated with lymphocyte polarization, which is an essential event for LFA-1 activation [112]. Moreover, impaired B lymphocyte adhesion and migration underlies the defect in B cell homing to lymphoid tissues and decreased B cell maturation in RAPL-deficient mice [194], demonstrating the importance of the RAP1-RAPL signaling pathway in B cell differentiation and function. In addition to the Rap1 GTPases, Rap2 GTPases have also been suggested to function in B cell migration [189].

PI3K signaling was also found to be involved in chemotaxis (Figure 5). Treatment of T lymphocytes with the PI3K inhibitors wortmannin and LY294002 inhibits SDF-1-induced migration [195], and mouse neutrophils deficient in PI3K<sub>γ</sub> show impaired chemotaxis [196]. However, whereas PI3K<sub>γ</sub>-deficient T cells show reduced chemotactic responses towards CCL19, CCL21 and SDF-1, migration of B cells lacking expression of this PI3K isoform towards these chemokines was normal [197]. B cells deficient in PI3K<sub>δ</sub>, however, did show reduced CXCL13-induced migration, although also in these cells migration towards CCL19, CCL21 and SDF-1 was unaffected [197]. Thus, depending on the chemoattractant and the cell type, certain PI3K isoforms can operate as mediators of chemotaxis.

### 2.6. HGF/Met signaling

Hepatocyte Growth Factor (HGF, also known as Scatter Factor) is a pleiotropic cytokine, which is involved in proliferation, survival, motility and differentiation of target cells. It is secreted as an inactive single-chain precursor, and can be converted to a biologically active heterodimer by the serine protease HGF activator (HGFA) [198,199]. All known biological effects of HGF are transduced via the receptor tyrosine kinase Met, which is encoded by the <i>c-met</i> proto-oncogene [200]. Met is expressed on a wide variety of epithelial cells, whereas its ligand HGF is expressed by mesenchymal cells. HGF and Met play an important role in epithelial-mesenchymal interactions underlying differentiation of a wide variety of lumenerforming organs, such as lungs, kidney and mammary glands, leading to the formation of polarized epithelial cell layers and, depending on the type of tissue, tubulogenesis and branching morphogenesis [201,202]. Indeed, HGF and Met are indispensable for mammalian development, as inactivation of the <i>hgf</i> or <i>met</i> genes in the mouse causes embryonic lethality between E12.5 and E15.5 [203-205]. <i>hgf<sup>−/−</sup></i> and <i>met<sup>−/−</sup></i> mice have abnormal development of the liver and placenta, and show disrupted migration of myogenic precursors into the limb bud [203-205]. When epithelial cells are grown in a monolayer, HGF stimulation causes cytoskeletal reorganization and dissociation of cells, followed by active migration or scattering. Scattering reflects the first phase of epithelial morphogenesis through mesenchymal induction, underlying the complex, coordinated formation of branched organs.

The HGF/Met pathway plays an important role in tumor growth, invasion and metastasis [206]. In several tumors aberrant Met signaling occurs, which can be due to amplification or overexpression of Met, chromosomal translocation, or the existence of an autocrine loop. Overexpression of Met can also occur in absence of gene amplification. For example, in colorectal carcinoma (CRC), the Wnt signaling pathway, which plays an crucial role in CRC tumorigenesis, regulates expression of Met, which is suggested to be implicated
in the transformation process [207,208]. HGF was shown to induce invasion into collagen gels of several tumor cell lines, including mammary, colon and squamous cell carcinoma, and melanoma cell lines [209,210]. Studies using in vivo models confirm the involvement of HGF and Met in tumorigenesis. Autocrine stimulation of Met-transfected NIH3T3 cells with HGF, enhanced the tumorigenic and metastatic capacity of these cells in nude mice [211,212]. Moreover, MET signaling was found to increase tumor formation of colon epithelial cells in nude mice independently of aberrant Wnt signaling [208]. Furthermore, HGF transgenic mice were shown to develop a broad range of primary tumors and metastases of mesenchymal as well as epithelial origin, including malignant melanoma, fibrosarcoma, and mammary carcinoma [213,214]. In addition, in human hereditary papillary renal carcinomas (HPRCs) potentially activating Met mutations are found, which when expressed in NIH3T3 cells are transforming in vitro and tumorigenic in vivo [215,216].

The Met protein consist of an α- and a β-subunit which are linked by a disulfide bridge (Figure 6). The cytoplasmic tail of the β-subunit contains the tyrosine kinase domain and a docking site which can interact with several signaling molecules [217]. Upon binding of HGF to Met, Met is believed to dimerize, and becomes strongly autophosphorylated on cytoplasmic tyrosine residues. Phosphorylation of docking site tyrosine residues subsequently enables downstream effectors to bind the receptor and to become activated. For example, upon Met activation a complex consisting of Grb2 and the RasGEF SOS is recruited to the Met protein, resulting in activation of Ras (Figure 6). By activating the Raf-MEK-ERK
pathway, Ras mediates a variety of responses elicited by HGF/Met, including mitogenesis, motogenesis and morphogenesis, as well as Met-induced transformation, invasion, metastasis and tumorigenicity [218-221]. Another important substrate for Met is the docking protein Gab1, which has the ability to directly bind with several signaling molecules such as Grb2, PI3K, PLCγ and SHP2, and mediates most of the complex cellular responses following Met activation [206,222]. Besides that PI3K can associate with Gab1 and activated Ras, PI3K was also found to directly interact with Met. PI3K by activating PKB mediates HGF-induced survival. In addition, PI3K has a prominent regulatory function in Met-induced mitogenesis, motility and morphogenesis, as well as invasion and metastasis [221]. Moreover, the ability of HGF/Met signaling to regulate these complex processes by activating a wide range of signaling molecules that modulate the cytoskeleton and the activity of integrins underlies HGF/Met-induced scattering, branching morphogenesis, motility and invasion. For example, by activating the small GTPases Rho, Rac and Cdc42, which are involved in modulating the actin cytoskeleton, HGF induces scattering of MDCK cells [221]. Besides mediating HGF-induced survival, PI3K has been found to regulate HGF-induced cytoskeletal rearrangements and scattering, possibly by activating Rac GTPases. Furthermore, the HGF/Met pathway activates the small GTPase Rap1 [223], which has been shown to control integrin-mediated adhesion [224], and HGF-induced activation of Ras and Ral are involved in migration of skeletal myoblasts [72]. Thus, by regulating the activation of various signaling molecules mediating affinity and avidity of integrins, and cytoskeletal rearrangements, the HGF/Met pathway controls motility, migration and invasion of target cells.

Studies from our laboratory have provided evidence for a role of the HGF/Met pathway during antigenic-specific B cell differentiation. Met is expressed by centroblasts and plasma cells, and HGF is produced by FDCs and tonsillar stromal cells [225,226]. Here, HGF/Met signaling regulates integrin-mediated adhesion of B cells to FDCs by activating the integrin α4β1, which binds VCAM-1 and fibronectin [225]. Additionally, HGF and Met function in the progression of a subset of B cell neoplasia. In MM cell lines and patient material expression of both HGF and Met was found, suggesting the existence of an autocrine loop [227,228]. Syndecan-1 expressed on MM cells binds via its heparan sulfate proteoglycan-side chains HGF, and is by increasing the effective HGF concentration promoting Met signaling [229]. Since MM cells are also able to secrete HGFA, which catalyzes proteolytic conversion of HGF into its active form, MM cells express all components of the HGF/Met signaling cascade required for autocrine signaling [230]. Furthermore, HGF induces PI3K-dependent α4β1 integrin-mediated DLBCL adhesion to VCAM-1 and fibronectin, which may very well contribute to lymphomagenesis in DLBCL [231].

3. Aim and outline of this thesis

The aim of the studies described in this thesis was to identify and molecularly dissect signaling pathways that are in control of survival, proliferation, adhesion and migration of normal and malignant B cells. This will provide a more detailed insight into the molecular aspects of B cell development, differentiation and function, as well as the pathogenesis of B cell malignancies and immunological disorders. We anticipate that this will ultimately lead to improved diagnosis and/or treatment of cancer and immunological disorders.
Coordinated lymphocyte trafficking is essential for the control and integration of systemic immune responses. In chapter 2, it is discussed that the adhesion molecules and chemokine receptors regulating the trafficking of normal lymphocytes are also expressed and functionally active in their malignant counterparts. By enhancing lymphoma dissemination and transducing signals into the cell, adhesion molecules and chemokine receptors promote cell growth and survival, which might contribute to lymphoma aggressiveness.

In chapter 3, we identify the small GTPase Ral as a mediator of BCR-signaling involved in controlling AP-1- and NFAT-mediated gene transcription. BCR-controlled activation of Ral was found to be mediated by Lyn and Syk, Btk, PLCγ2, Ras and IP3-receptor-mediated Ca2+ release. Next, we demonstrate that several of these mediators of BCR signaling are also involved in chemokine-induced adhesion and migration. In chapter 4, we show that SDF-1 and CXCL13 induce activation of Btk and PLC-γ2, and that these signaling molecules control chemokine-induced adhesion, migration and homing of B cells. In chapter 5 we demonstrate that Ral is also activated in response to SDF stimulation. This activation is not mediated by Lyn and Syk, Btk, PLC-γ2 or Ras. SDF-1-induced Ral activation is required for migration of both B cells and MM cells, suggesting that Ral is involved in regulating B cell homeostasis, trafficking, and function, as well as homing of MM cells to the BM microenvironment. In chapter 6, we show that HGF, a growth factor secreted by BM stromal cells, controls growth and survival of MM cells. Finally, Chapter 7 summarizes and discusses the results presented in this thesis, and provides suggestions for further studies and potential therapeutic applications of these findings for the treatment of lymphoma and MM patients.
References


GENERAL INTRODUCTION


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