13

From HSC to B-Lymphoid Cells in Normal and Malignant Hematopoiesis

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1. Introduction

Development of B-lymphoid cells is a highly ordered multi-step process that, in adult mammals, starts in bone marrow in a pool of self-renewing multipotential hematopoietic stem cells, which gradually commit to the lymphoid lineage and advance through high regulated differentiation pathways until formation of mature functional cells. Over the last few years, exceptional advances have been recorded in identifying primitive progenitors that lay the foundations of the lymphoid program while losing myeloid potential, along with patterns of transcriptional activity controlling lineage fate decisions and environmental cues that influence the differentiation pathway during normal hematopoiesis. Multicolor flow cytometry, controlled cell cultures, genetic marking systems, microarray technologies and xenotransplantation approaches are being extensively used to address fundamental questions on this regard. Of special interest is the stem cell research with relevance to hierarchy and early events in malignant lymphopoiesis, and to new insights into perspectives that may allow progress in means to protect and sustain the immune system during chemotherapy, inflammation, infection, and following hematopoietic transplantation. In this book chapter, we focus on the hierarchical structure of the early lymphoid system, the current knowledge about intrinsic and microenvironmental factors regulating the differentiation of lymphoid progenitors, and the emerging research to understand malignant lymphoid development.

2. The early steps in the lymphoid development

Mature blood cells are constantly replaced from a unique cell population of hematopoietic stem cells (HSC) residing in specialized niches within the bone marrow (BM), where the hematopoietic system is organized as a hierarchy of cell types that gradually lose multiple alternate potentials while commit to lineage fates and gain specialized functions (Baba et

al., 2004; Seita & Weissman, 2010). HSC possess two major characteristics: they are capable of maintaining their constant number by self-renewal and they are in charge of producing all mature blood cells through differentiation processes (Figure 1). Furthermore, HSC are mitotically inactive (quiescent) and divide very slow and intermittently under normal conditions, but are capable of proliferation and differentiation during recovery from chemotherapy or stress circumstances (Takizawa et al, 2011; Mayani, 2010; Passegue et al., 2005; Pelayo et al., 2006b). Movement into and out of a resting state might be crucial for ensuring that the correct number of new hematopoietic cells is produced.

The lymphoid pathway proceeds through critical stages of differentiation of HSC to multipotential early progenitors (MPP), which upon progressive loss of self-renewal capacity, give rise to oligopotent progenitors. Downstream, the production of lineage-committed precursors is crucial for cell maturation. Current knowledge about development of the lymphoid system is based, in great part, on the work done in animal models, demonstrating that lymphoid specification begins in the fraction of lymphoid-primed multipotent progenitors (LMPP). A series of studies using the transgenic RAG-GFP mouse (Igarashi et al., 2002) permitted us to determine that RAG^+ early lymphoid progenitors (ELP) are capable of differentiating into T, B, NK and conventional dendritic cells (cDC) (Pelayo et al., 2005a; Pelayo et al., 2006a; Welner et al., 2008a). Studies using defined co-cultures and short-term reconstitution assays have shown that ELP are also good producers of plasmacytoid dendritic cells (pDC) and of interferon-producing killer dendritic cells (IKDC), both being key components of the innate immune response to infections (Pelayo et al., 2005b; Welner et al., 2007). At the same time, ELP give rise to committed oligopotent common lymphoid progenitors (CLP), which are responsible for B- and NK- precursor cells production. CLP and lineage precursors have substantially lost the possibility of differentiating into the rest of the lineages.

Due to ethical reasons and technical limitations, human hematopoietic stem cell research has been slower than it has been in mouse models. In humans, the early hematopoietic progenitors are confined in bone marrow to a cellular compartment that expresses CD34 (Blom & Spits, 2006). The fraction of multipotent stem cells is characterized by the phenotype $Lin^-CD34^+CD38^{-/lo}CD10^-CD45RA^-$, whereas that of probably the earliest lymphoid progenitors is $Lin^-CD34^+CD38^{-/lo}CD45RA^+CD10^+$ and has been recently designated as multi-lymphoid progenitor (MLP) (Doulatov et al., 2010). According to Doulatov's studies, MLP may be directly derived from HSC. However, a precise precursor-product relationship needs to be determined (Figure 1). A description that fully matches the definition of mouse ELP is still missing, but cells with $Lin^-CD34^+CD38^+CD45RA^+CD7^+CD10^+$ phenotype seem to represent good candidates (Blom & Spits, 2006; and our unpublished observations). $Lin^-CD34^+CD38^+CD45RA^+CD10^+$ B/NK cells, which differentiate principally into B & NK cells, are considered the counterparts of CLP in mice (Figures 1 & 2) (Doulatov et al., 2010). Of special importance is the fact that increasing levels of CD10 correspond to B-lineage specification (Ichii et al., 2010). Downstream, the differentiation of fully committed precursors gives rise to B cells that eventually are exported to peripheral lymphoid tissues (see B cell development sections below).

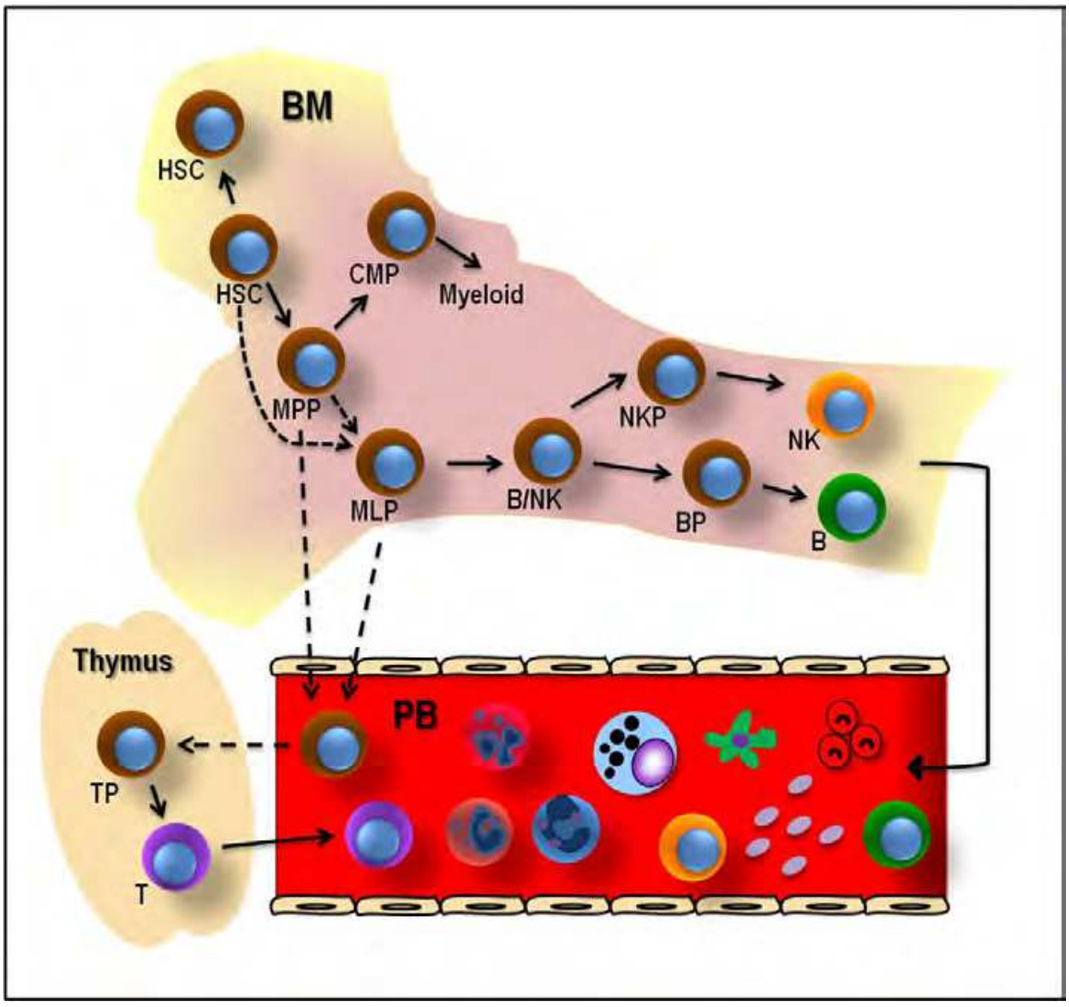

Fig. 1. Early lymphoid development in humans. Within bone marrow (BM), self-renewing hematopoietic stem cells (HSC) give rise to multipotent progenitors (MPP), which have the ability to differentiate into common myeloid progenitors (CMP) and into multi-lymphoid progenitors (MLP). MLP might alternately derive from HSC. NK and B-lymphoid cells are produced from B/NK-derived lineage committed precursors. Mature hematopoietic cells are exported to peripheral blood (PB). Early progenitor cells may colonize the thymus via circulation, and initiate the T-lymphoid development pathway. NKP, natural killer cell precursor; BP, B cell precursor; TP, T cell precursor.

The rigorous purification of human HSC and progenitor cell populations based on their surface phenotype has promoted the study of their biology in adult bone marrow, cord blood and G-CSF-mobilized peripheral blood (Figure 2). Importantly, some of their properties, including cell frequencies, developmental capacities, cell cycle status, transcription factors networks and growth factors production, show substantial differences between newborns and adults (Mayani, 2010). According to literature, we have found that most hematopoietic progenitors are more abundant in cord blood than in the adult tissues bone marrow and mobilized peripheral blood (Mayani, 2010). The implications of these discrepancies during haematological neoplastic diseases are not as yet clear.

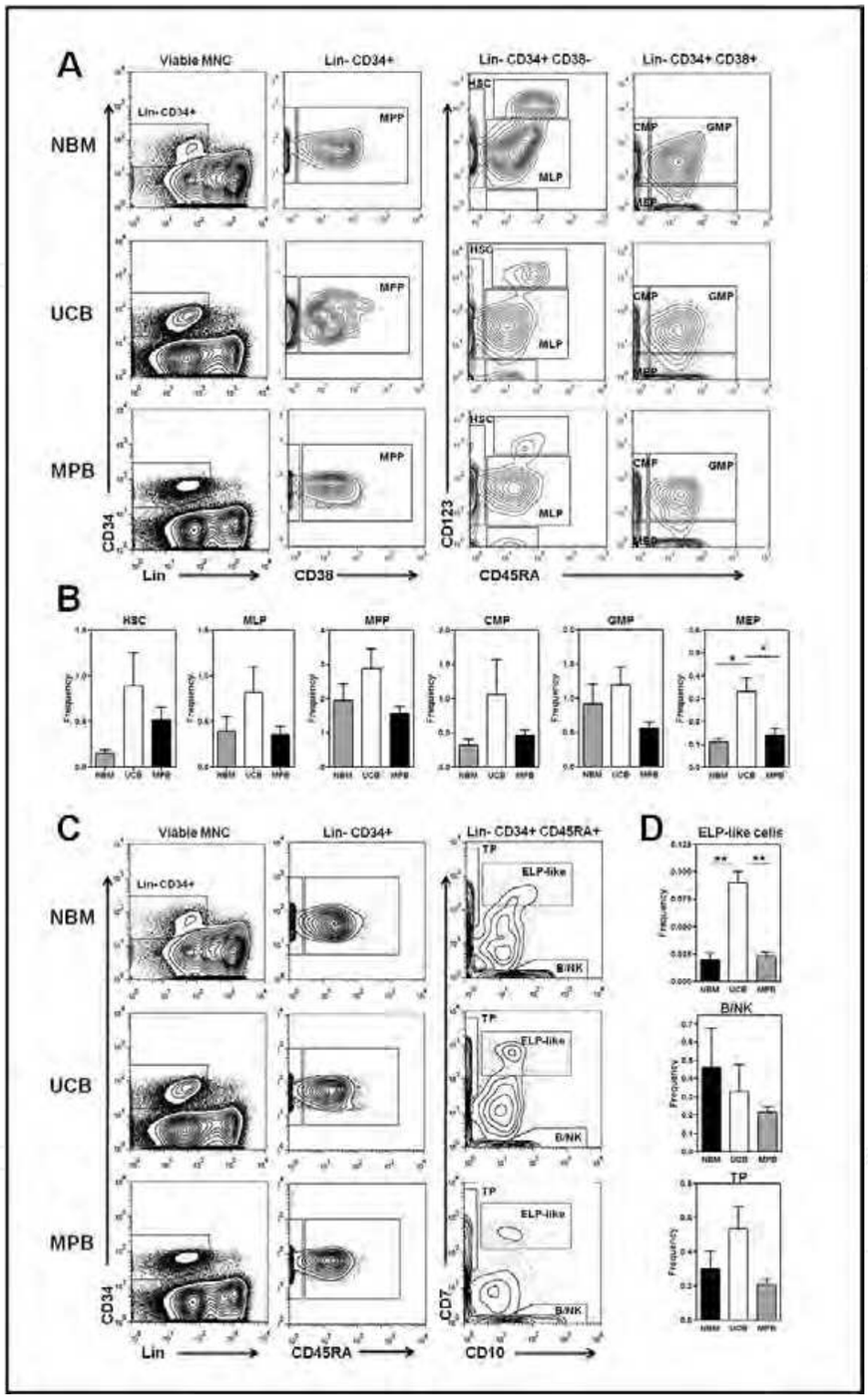

Fig. 2. Prospective identification of human myeloid and lymphoid progenitor cells by flow cytometry. HSC and early progenitor cells reside in the Lin$^-$ CD34$^+$ fraction of adult normal bone marrow (NBM), as well as in umbilical cord blood (UCB) and mobilized peripheral blood (MPB). Based on the surface expression of CD38, CD123 and CD45RA, multilymphoid progenitor cells (MLP) and most of the myeloid progenitors can be recognized (A). Further fractionation of Lin$^-$CD34$^+$CD45RA$^+$ cells into CD7 & CD10-expressing cells allows the

identification of T-cell progenitors (TP), B/NK progenitors and ELP-like cells (C). Cell frequencies for each population from the different sources are shown (B and D panels). CMP, common myeloid progenitor; GMP, granulocyte & monocyte progenitor; MEP, megakaryocyte & erythrocyte progenitor. The identity and functions of Lin$^-$CD34$^+$CD38$^-$ CD45RA$^+$CD123^{hi} cells still need more investigation.

During biological contingencies -chemotherapy, infections and transplantation procedures-, the replenishment of the innate immune system from hematopoietic stem/progenitor cells appears to be critical. Interestingly, these seminal cells can proliferate in response to stress conditions and systemic infection by using mechanisms that apparently involve interferons and tumor necrosis factors, among others (Baldridge et al., 2011). Moreover, they are capable of self/non-self discrimination through Toll-like receptors (TLR), which recognize microbial components. Mouse stem cells and early B-cell progenitors express and use TLR, a mechanism that facilitates their differentiation to the innate immune system (Nagai et al, 2006; Welner et al., 2008b; Welner et al., 2009). Recent work suggests that, as in mice, human primitive cells, including MLP, also express functional TLR (Kim et al., 2005; Sioud & Fløisand, 2007; De Luca et al, 2009; Doulatov et al, 2010). In shape with those findings, we have found that BM lymphoid progenitor-enriched fractions display TLR9 (Figure 3) and their differentiation potentials bias toward NK and DC production upon TLR9 ligation (RP & EV, unpublished observations). Thus, plasticity in primitive cells is vulnerable to extrinsic agents that can modify early cell fate decisions during infections or stress, suggesting that the stages of lineage restrictions are less abrupt than previously assumed (Welner et al., 2008a).

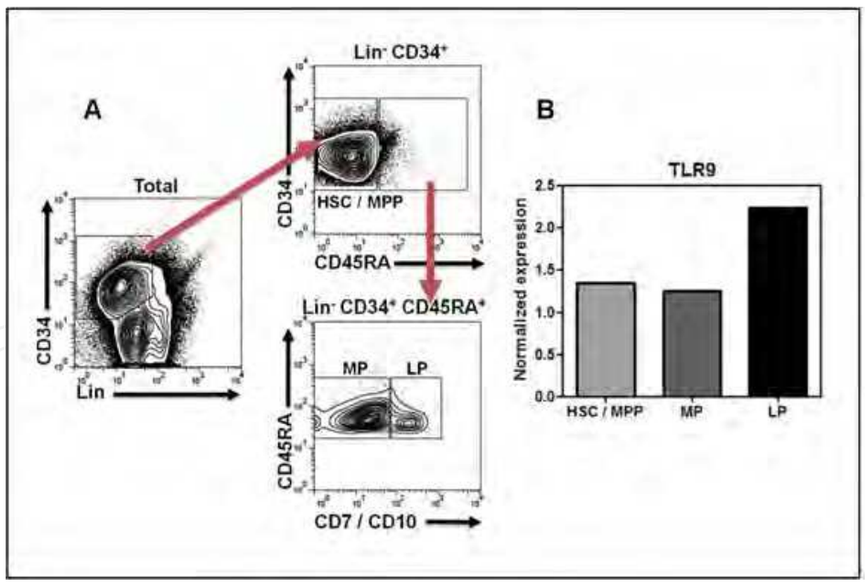

Fig. 3. Lymphoid progenitors from human bone marrow express TLR9. Adult bone marrow is fractionated according to cell surface expression of lineage markers, CD34, CD45RA and CD7/CD10 (A). Lin$^-$CD34$^+$CD45RA$^-$ HSC/MPP, Lin$^-$CD34$^+$CD45RA$^+$CD7/CD10$^-$ myeloid progenitors (MP) and Lin$^-$CD34$^+$CD45RA$^+$CD7/CD10$^+$ lymphoid progenitors (LP) were tested for their intracellular expression of TLR9 by flow cytometry using a specific anti-TLR9 antibody (B).

3. The B cell antigen receptor (BCR) and bone marrow B cell development

The main function of mature immunocompetent B cells is to make antibodies upon recognition of particular new or recurrent antigens by the B cell receptor (BCR). The BCR is a membrane-bound complex of proteins, consisting of a heterodimer of identical pairs of immunoglobulin (Ig) heavy and light chains, which are responsible for the clonal diversity of the B cell repertoire and the antigen identification, but are unable to generate signals and trigger biological responses after antigen binding. This function is mediated by the disulfide-coupled heterodimer of Igα (CD79a) and Igβ (CD79b), which is non-covalently associated with the Ig antigen recognition unit (Figure 4A). Igα/Igβ signaling is dependent on distinct tyrosine-based activation motifs localized in the cytoplasmic tails of these proteins. It is the sequential expression and assembly of the BCR components that defines each developmental stage of the B cell pathway, and, therefore, each stage is characterized by a particular form of BCR, reflecting the progression of receptor assembly (Fuentes-Pananá et al., 2004a).

To achieve BCR clonal diversity, the Ig heavy and light chain genes are composed of constant and variable regions. The variable region is formed by a series of segments V (variable), D (diversity) and J (joining) (Figure 4B), which are brought together by a highly ordered process of VDJ recombination accomplished by the products of the recombinase-associated genes 1 and 2 (RAG1 and RAG2) occurring first in the heavy and then in the light chain loci (Thomas et al., 2009). ProB and PreB stages are characterized by rearrangements of the Ig heavy and light chains, respectively (Figure 5) (Fuentes-Pananá et al., 2004b), and further divided according to the status of the recombination. In mice, ProB-A is the sub-stage during which the heavy chain is in germ line state, whereas during ProB-B the heavy chain D and J fragments are recombined, and in ProB-C, V-DJ is recombined. In large PreB cells, the preBCR is already expressed in surface and the light chain V and J fragments are in germ line state, while in small PreB cells light chain V-J is recombined (Hardy et al., 1991). These stages are better known in humans as Early ProB or Pre-proB (A), ProB (B), PreB I (C), large and small PreB II (Figure 5). In the ProB stage Igα and Igβ are expressed at cell surface in association with chaperon proteins such as calnexin (the proBCR). As soon as the heavy chain is successfully recombined, it is assembled with Igα and Igβ and the surrogate light chains λ5 and VpreB to form the preBCR. Surface expression of this receptor marks the transition to the preB stage (Figure 5) (Fuentes-Pananá et al., 2004a; 2004b).

In addition to their VDJ recombination status and pattern of surface marker expression, ProB and PreB stages can be recognized by their proliferative state (Hardy et al., 1991). RAG-1 and RAG-2 enzymes are tightly regulated during the cell cycle, being highly active in G_0 and degraded before the cell enters S phase (Li et al., 1996). By assuring that proliferation and recombination are mutually exclusive mechanisms, the developing B cell guarantees that no events of non-homologous recombination will occur during DNA replication, thus avoiding an increase in the mutation rate.

3.1 Self-recognition and peripheral B cell development

Once the mature BCR is present in the surface of immature B cells, it is finally able to interact with conventional polymorphic ligands, and selection at this stage is designed to test the receptor-ligand interaction. Intimate contact between the immature B cell and the

stromal cells of the bone marrow allows those receptors capable of recognizing self-antigens to be identified and eliminated through a variety of mechanisms collectively termed "tolerance". Non-self-reactive B cells exit to the periphery and reach the spleen where they are again tested for reactivity against self-antigens before they transition to the mature stage (Figure 5) (von Boehmer & Melchers, 2010). Three main mechanisms of B-cell tolerance are known: receptor editing, deletion of auto-reactive clones (negative selection) and anergy. Only those B cells that carry receptors without self-specificity are allowed to exit the bone marrow and become mature B cells in peripheral lymphoid organs.

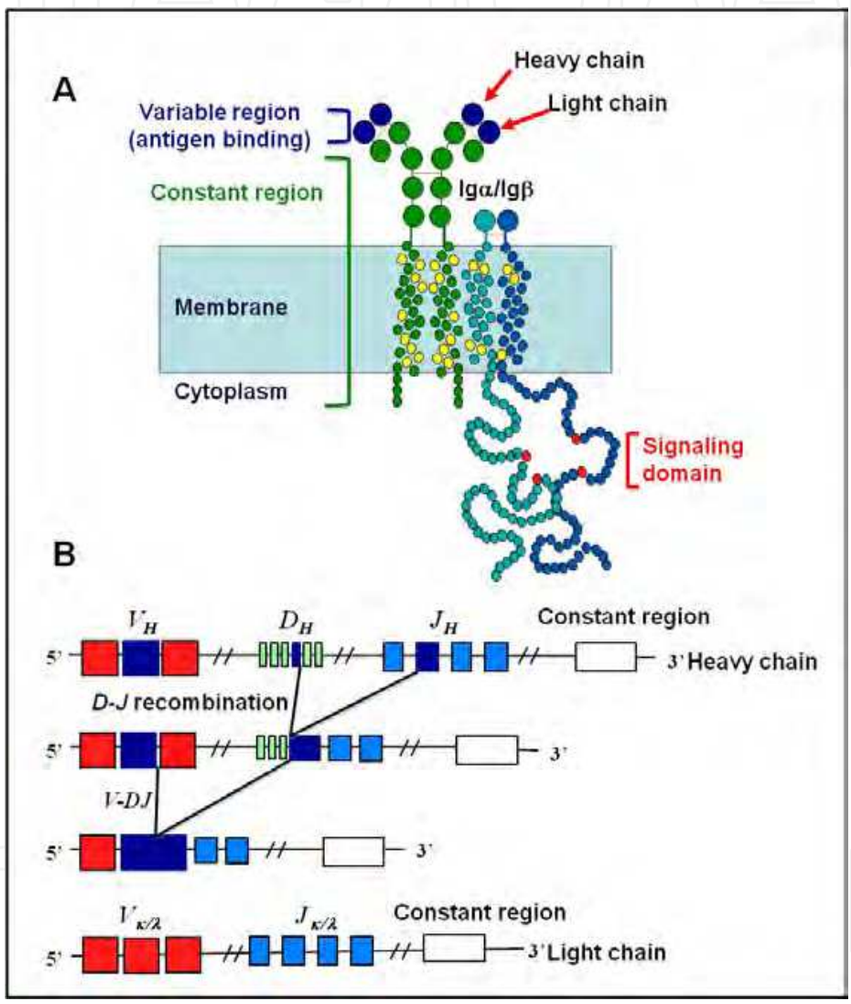

Fig. 4. The B cell antigen receptor (BCR). A) Heavy and light chains are comprised of variable regions where VDJ recombination occurs (shown in dark blue) and constant regions (green). The signaling domains are present in the cytoplasmic leaflet of Igα and Igβ. B) Variable regions are formed by a number of segments termed V (variable), D (diversity) and J (joining) within the heavy chain, and by segments V and J within the light chain, which are brought together by a VDJ recombination process. Randomly, D and J segments recombine at first, followed by V segments joining the DJ fragment (shown in dark blue squares is an example of segment choice). This mechanism is responsible for the extensive repertoire of BCR specificities.

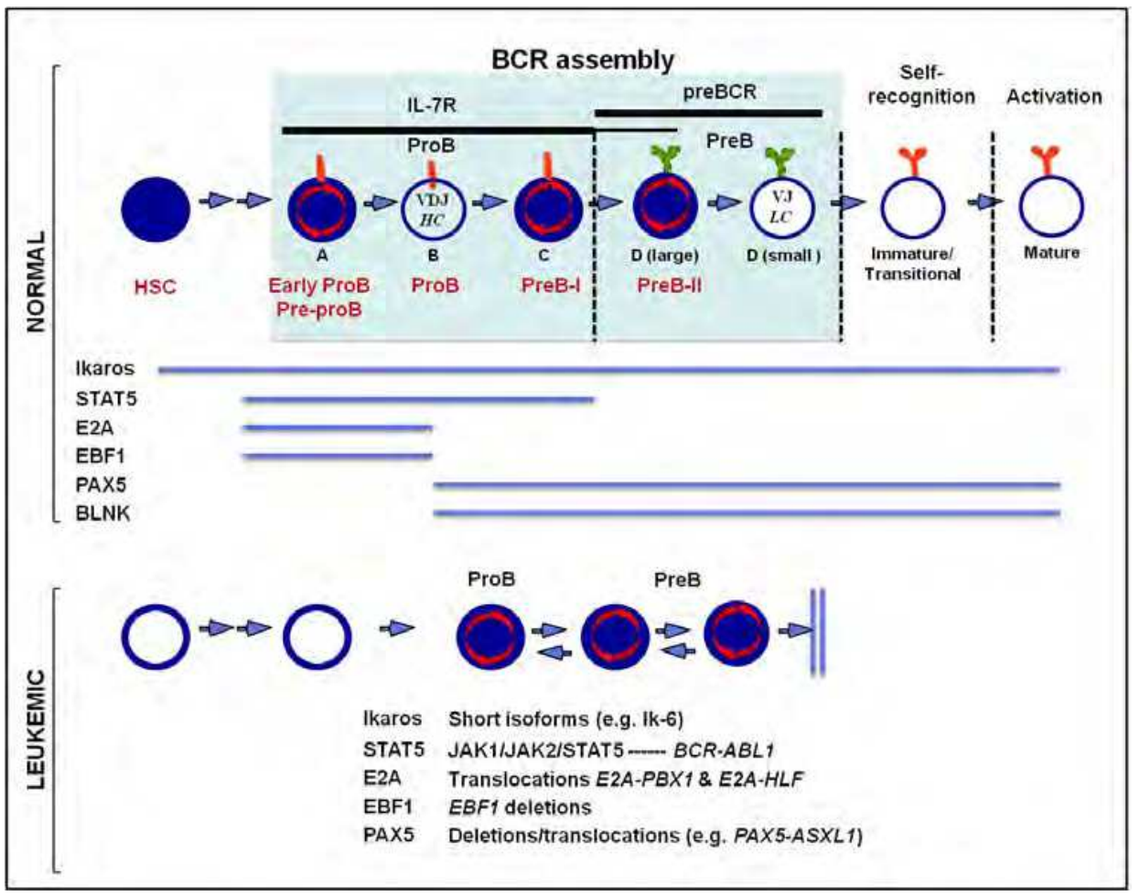

Fig. 5. Normal and leukemic B cell development. B cell stages can be divided according to the main processes guiding development: receptor assembly, self-recognition and activation (top panel). Receptor assembly occurs in bone marrow (light blue box) by VDJ recombination in the Pro-B and Pre-B stages, whereas self-recognition starts in bone marrow and ends in periphery, and activation takes place at peripheral level. Nomenclature for each sub-stage in mice is shown in black letters while the most common nomenclature for their counterparts in humans is shown in red letters. The dashed lines separating all stages indicate checkpoints at which signaling from the preBCR and BCR is required for positive selection and progression along the B-cell maturation pathway. The proBCR, preBCR, and mature receptor are also illustrated in their respective stages. Replication and recombination processes are mutually exclusive as denoted by the circular arrows and VDJ signs inside the cell. The replication stages are also frequently compromised in pediatric B cell acute leukemia. Black lines under IL-7R and preBCR indicate the stages where these receptors are most required. The differential thickness in the IL-7R line shows the sub-stages where a higher (nanograms) or lower (picograms) concentration of the IL-7 is required. Homeostatic and leukemic expression of transcription factors along the B cell pathway are shown in the middle and bottom panels. Blue bars mark normal gene expression, and the most common modified forms of the transcription factors associated with B cell acute lymphoblastic leukemia are revealed. HSC, hematopoietic stem cell.

On the basis of their cell-surface phenotype, peripheral immature B cells are further divided into transitional 1 (T1, $AA4^{+}IgM^{high}CD23^{-}$) and transitional 2 (T2, $AA4^{+}IgM^{high}CD23^{+}$). T1 cells inhabit the spleen's red pulp and give rise to T2 cells (Allman et al., 2001). There is an additional population designated T3, but it is controversial whether this is a population in line in the progression to the mature stage or whether it represents a population of anergic cells (Merrell et al., 2006).

3.2 Innate and adaptive mature B cell populations

Following antigen binding, mature B cells activate pathways that lead to proliferation and further differentiation into antibody-producing B cells (plasma cells) or memory B cells. In the spleen, mature B cells are sub-divided into follicular (FO, $AA4.1^{-}CD21^{int}CD23^{high}$) and marginal zone (MZ, $AA4.1^{-}CD21^{high}CD23^{-}$) B cells according to both their location and their cell-surface phenotype. A distinct subset of mature B cells is preferentially present in the peritoneal cavity; these are known as B1 cells [$B220^{+}CD11b^{+}CD5^{+}$ (B1a) or $CD5^{-}$ (B1b)]. Among them, FO B cells are responsible for adaptive antibody responses, whereas MZ and B1 mature populations respond rapidly to antigenic stimulus but do not go through germinal-center reactions and thus their response can be independent of T cell help (Martin et al., 2001). Therefore, MZ and B1 B cells are thought to be part of an innate–like response. The origin of both of these populations is not well understood. While MZ B cells share part of FO pathway, the fetal liver was thought to originate a large fraction of the adult B1 B cells (Tung et al., 2006). Recently, a novel developmental model suggests that some B1 cell progenitors can be produced in bone marrow (Esplin et al., 2009).

3.3 Regulation of B lineage commitment: The critical role of preBCR tonic signaling, IL-7R and transcription factors in context

Limitation of lineage choice during development is regulated by a combination of signaling pathways and transcription factors (TF). In mice, the main receptor controlling the ProB stage is the IL-7R, which is composed of a α chain (IL-7Rα) and the common cytokine receptor γ chain (γc). Deletion of IL-7Rα or γc leads to developmental arrest at the early ProB stage (von Freeden-Jeffrey et al., 1995; Cao et al., 1995).

IL-7 activates the major signaling pathway JAK–STAT, with STAT5 being the essential mediator of IL-7 signals in early B cell development (Yao et al., 2006).

By the other hand, an important characteristic of the developmental process that distinguishes B and T lymphocytes from other cell lineages is the continuous selection of these lymphoid cells for their ability to express a competent, non-self receptor. B cells that fail to express a receptor are eliminated. Thus, BCR and BCR-like receptors must generate active permissive signals that allow differentiation through the different developmental stages. Because the preBCR lacks of the light chain and therefore of the capacity to bind polymorphic ligands, it has been proposed that this receptor is able to signal constitutively and independently on ligand, an activity also known as tonic signaling. Although there is little understanding of how tonic signals are generated, the view is supported by receptor-less B cells able to differentiate into mature B cells by expression of a chimeric construct of Igα and Igβ positioned in the cell surface membrane (Bannish et al., 2001).

Once the preBCR is expressed at the end of the proB stage, it can take over many of the functions performed by the IL-7 receptor signaling. Both receptors act individually and together to allow B cell development (Figure 5). Like IL-7R, the preBCR promotes mechanisms of positive selection, survival and proliferation (Ramadani et al., 2010; Yasuda et al., 2008). The CCND3 gene, which encodes for cyclin D3, is essential for PreB cell expansion and integrates IL-7R and preBCR signals (Cooper et al., 2006).

Downstream the IL-7 and preBCR receptors, a handful of transcription factors (TF) are critical for commitment to the B cell lineage and early development; these include E2A/TCF3 (immunoglobulin enhancer binding factors E12/E47/transcription factor 3), EBF1 (Early B cell Factor 1) and PAX5 (Paired box 5) (Figure 5). Loss of E2A and EBF1 blocks entry into the B cell lineage, while loss of PAX5 redirects B cells into other lineages (Nutt et al., 1999; O'Riordan & Grosschedl, 1999). Acting together with E2A, EBF1 and STAT5, one of the main molecular functions of PAX5 is to allow VDJ recombination (Hsu et al., 2004). Also, E2A, PAX5, IKZF1 and RUNX1, among other TF, are responsible for RAG expression (Kuo & Schlissel, 2009). Moreover, IL-7R signaling fulfills an essential role in early B cell development, with STAT5 participating in the activation of the B cell regulatory genes E2A, EBF1 and PAX5. E2A encodes two TF via alternative splicing, E12 and E47. In mice lacking the E2A gene, the B cell lineage is lost, there is no heavy chain recombination, and the expression of the B cell-restricted genes EBF1, PAX5, CD79A/B and VPREB1 (CD179A) is also affected.

Enforced expression of EBF1 and PAX5 is sufficient to overcome the developmental block in mice deficient in E2A, IL-7 or IL-7Rα, further illustrating the transcriptional hierarchy of the B cell-specific program triggered by IL-7 receptor signaling (Nutt & Kee, 2007). EBF1 acting together with PAX5 drives the expression of many genes critical for early B cell development and B cell function, including FOXO1, MYCN, LEF1, BLNK, CD79A (MB-1), RAG2, CD19 and CR2 (CD21) (Nutt & Kee, 2007; Smith & Sigvardsson, 2004).

Although PAX5 is a positive regulator of B-cell specific genes, also functions as a repressor of non B-lineage genes such as M-CSFR, NOTCH1 and FLT3 (Cobaleda et al., 2007) so B cell development is unidirectional and mostly irreversible in homeostatic conditions.

Also important for lymphoid development are members of the Ikaros family of TFs, mainly IKZF1 (which encodes Ikaros) and IKZF3 (which encodes Aiolos). Ikaros activates B cell genes and represses genes that are unrelated to the B lineage. Expression of IKZF1 and IKZF3 is regulated by alternative splicing, which produces long isoforms (Ik-1, Ik-2, Ik-3, Aio-1, Aio-3, Aio-4 and Aio-6) that efficiently bind to DNA, and short isoforms (Ik-4, Ik-5/7, Ik-6, Ik8, Aio-2, Aio-5) that are unable to bind DNA with high affinity and do not activate transcription (Liippo et al., 2001). Ikaros is activated in early stages of lymphopoiesis and is required for both early and late events in lymphocyte differentiation. Aiolos is not required during the early specification of the B and T lineages but is essential during further B cell maturation. They also act in concert to promote preB cell cycle exit and transition to small PreB stage (Ma et al., 2010).

3.4 Human B cell development

Selection processes operating on developing B cells are similar in all mammals. Thus, early B cell development in humans is also mainly guided by VDJ recombination and by the

proliferative expansion of clones that have successfully completed the rearrangement of their receptors, whereas late development is led by mechanisms of tolerance to self-antigens. All these processes in humans are less well understood than are their counterparts in mice. Importantly, human B cells can still be generated in severe combined immunodeficiency (SCID) patients with mutations in the IL-7R gene, suggesting that IL-7 signaling is not essential for human B cell development (Puel et al., 1998) although a recent study has demonstrated that in vitro human B cell production is dependent on IL-7 (Parrish et al., 2009). The fine regulatory mechanism separating proliferation and differentiation might explain why the proliferating ProB and PreB sub-stages are the ones generally found to be compromised in human pediatric B-cell acute lymphoblastic leukemia (B-cell ALL) and why this disease is characterized by leukemic blast cells that are often unable to progress through the differentiation pathway. This tendency to be arrested in proliferative states might result in an increased rate of mutations, leading to formation of neoplastic cells. Supporting the later, mice expressing B cell mutants in the adaptor protein BLNK are arrested in the large PreB stage and often develop B cell malignancies (Flemming et al., 2003). Proliferative stages occur in the early ProB, PreB-I and large PreB-II fractions (Figure 5).

4. Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is a disorder characterized by the monoclonal and/or oligoclonal proliferation of hematopoietic precursor cells of the lymphoid series within the bone marrow. At present, ALL is the most frequent malignancy in children worldwide and a serious problem of public health, constituting 25% of all childhood cancers and 75%-85% of the cases of childhood leukemias (Perez-Saldivar et al., 2011). Near to 80% of ALL cases have precursor B-cell immunophenotype, while approximately 15% show T-cell immunophenotype. Even when a relatively high efficiency of therapeutic agents has been demonstrated (Pieters & Carroll, 2010), there has been a slight but gradual increase in the incidence of ALL in the past 25 years, and appears to be highest in Hispanic population, which also show superior rates of high risk patients (Fajardo-Gutiérrez et al., 2007; Abdullaev et al., 2000; Perez-Saldivar et al., 2011; Mejía-Aranguré et al., 2011). Factors such as drug resistance, minimal residual disease, cell lineage switch, and the rise of mixed lineages often put the success of treatment at risk and change the prognosis of the illness. The molecular mechanism involved in these phenomena and the identities of the target hematopoietic populations have not been completely defined, due in part, to the fact that neither the precise origin of the disease, nor the susceptibility of primitive leukemic cells to extrinsic factors, is known.

4.1 The origin of ALL

Over the last two decades, cancer stem cells (CSC) have been defined as cells within a tumor that possess the capacity to self-renew and to cause heterogeneous lineages of cancer cells that comprise the tumor (Clarke et al., 2006). According to MF Greaves, who proposed the original hypothesis for leukemogenesis, multiple consecutive carcinogenic hits in hematopoietic cells may drive the malignant transformation (Greaves, 1993; Greaves & Wiernels, 2003), where the second oncogenic event on pre-leukemic clones could be indirectly promoted by delayed infections (Greaves, 2006; Mejía-Aranguré et al., 2011). Our general current view suggests the occurring of oncogenic lesions in early development or in

a primitive cell that result in the abnormal differentiation of leukemic stem cells. Among the various factors that hit the HSC fraction, anomalous microenvironmental cues may contribute to trigger and support the leukemic behaviour of precursor cells (Figure 6).

Although CSC in myeloid leukemias have been strictly depicted as the responsible cells for tumour maintenance, which clearly keep the biological hierarchy within the hematopoietic structure (Dick, 2008), identification of a rare primitive and malignant cell with intrinsic stem cell properties and the ability to recapitulate the acute lymphoblastic leukemia has been more complicated (Bomken et al.,2010), particularly due to the genetic diversity of the disease and the lack of appropriate *in vitro* and *in vivo* models.

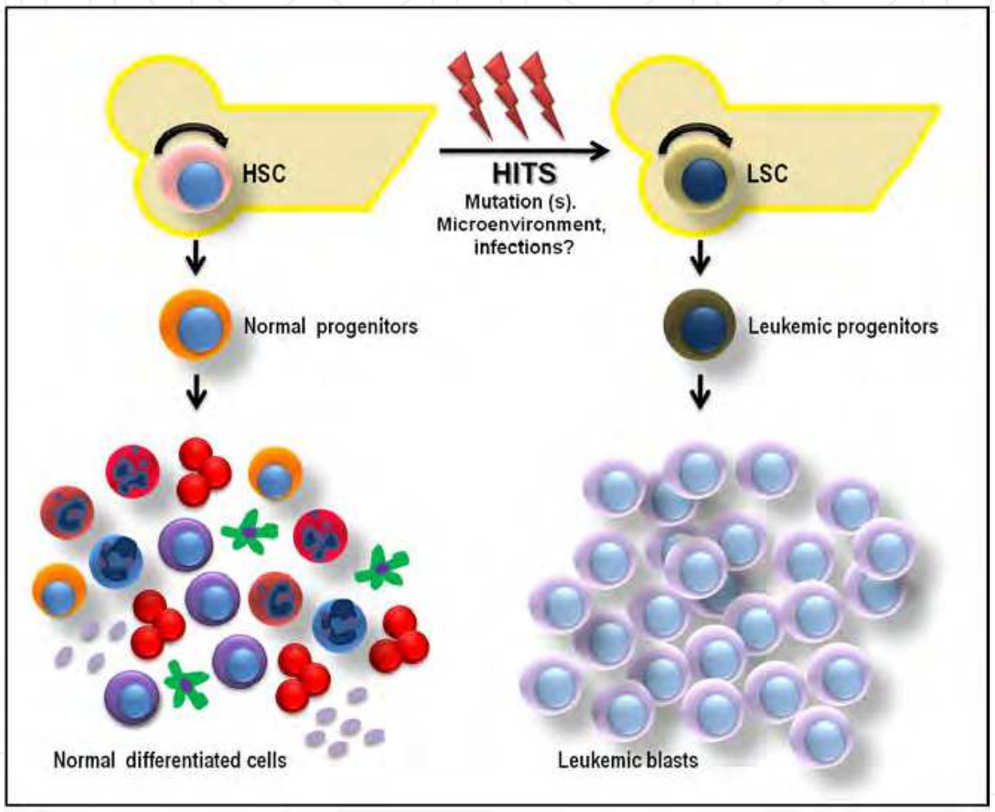

Fig. 6. Leukemic stem cell model. Normal hematopoietic stem cells (HSC) give rise to progenitors and mature blood cells within a hierarchical structure in the bone marrow. As a result of multiple and consecutive oncogenic hits on HSC including genetic and microenvironmental alterations, a malignant counterpart (the leukemic stem cell, LSC) emerge, which maintains some degree of developmental potential, generating the leukemic progenitor and blast cells.

Cell culture systems revealing alterations in early hematopoiesis, the existence of leukemic clones with unrelated DJ rearrangements and cytogenetic abnormalities on cells lacking lineage markers, have strongly suggested the participation of primitive cells in ALL. Moreover, data showing cells with immature phenotypes capable of engrafting and reconstituting leukemia in immunodeficient mice, lead to believe that, as in AML & CML, the hierarchy structure of the hematopoietic system is kept in ALL, and infant B cell-

leukemia initiating cells have undifferentiated characteristics (Espinoza-Hernandez et al., 2001; Cobaleda et al., 2000; Cox et al., 2004; Cox et al., 2009). To characterize ALL progenitor cells, Blair and colleagues have purified by flow cytometry a number of cell fractions based on the expression of CD34 and the B-lymphoid marker CD19. Regardless the risk stratum of the patient, $CD34^{+}CD19^{-}$ cells, but not committed B precursors, were able to reconstitute the disease in NOD/SCID models (Cox et al., 2004). Moreover, $CD133^{+}CD38^{-}CD19^{-}$ primitive cells residing in ALL BM are suggested to be the leukemia-initiating cells and responsible of drug-resistant residual disease (Cox et al., 2009). However, recent studies have remarkably shown that precursor blasts can also reestablish leukemic phenotypes *in vivo*, conferring them stem cell properties (Heidenreich & Vormoor, 2009; Bomken et al., 2010). Using novel intrafemoral xenotransplantation strategies, Vormoor's Lab has found that all differentiation stages of B precursor cells within $CD34^{+}CD19^{+}$ and $CD34^{-}CD19^{+}$ fractions are able to successfully engraft and recapitulate the original patient's disease in long-term systems, suggesting that committed cells in ALL do not lose the self-renewal stem cell property while they mature (le Viseur et al., 2008) (Figure 5), though their multi-lineage potential is uncertain.

These discordant results unveil that key questions regarding leukemic stem cells and the earliest steps of the lymphoid program in ALL still to be solved. Recently, the combination of clonal studies and alterations on genetic copies along with xenotransplant models, showed unsuspected genetic diversity, supporting multiclonal evolution of leukemogenesis rather than lineal succession (Dick, 2008). Thus, a less rigid structure of CSC models should further take account of functional plasticity and clonal evolution to understand CSC biology and to develop novel, stem/progenitor cell-directed therapies (Bomken et al., 2010).

4.2 Genes, cytogenetic alterations and transcription factors in B-cell leukemogenesis

The leukemogenic program is characterized by arrest of differentiation pathways, increased cell proliferation, enhanced self-renewal, decreased apoptosis rates and telomere maintenance. It is thought that together these alterations result in production of highly proliferative clones of immature leukemic blast cells with intrinsic survival advantage and limitless replicative potential (Warner et al., 2004).

Gain or loss of function of transcription factors such as E2A, EBF1, PAX5 and Ikaros affect homeostatic B cell lymphopoiesis in murine models, and are often associated with malignant transformation in humans, supporting conserved roles for these TFs and their activating signaling pathways (Figure 5) (Pérez-Vera et al., 2011).

A high frequency of ALL patients has genetic lesions -mostly chromosomal translocations- associated with leukemic cells. E2A is often translocated with several partners, including PBX1 [t(1;19)(q23;p13)] and HLF [t(17;19)(q22;p13)], which are detected in 5-6% and 1% of ALL children, respectively. E2A-PBX1 is a potent transcriptional activator of the WNT16 oncogene (McWhirter et al., 1999), while E2A-HLF functions as a survival factor of early B cells by activating expression of the anti-apoptotic genes SNAI2 (SLUG) and LMO2. Accordingly, gene silencing of LMO2 in an $E2A\text{-}HLF^{pos}$ cell line induced apoptotic cell death (Hirose et al., 2010). RUNX1 is also a frequent target for chromosomal rearrangements and mutations in ALL. 25% of children and 2% of adults of ALL patients carry the ETV6/RUNX1 fusion as a result of the translocation t(12;21)(p12;q21), which may play a role

regulating the B lineage-specific transcriptional program at an early stage (Durst & Hiebert, 2004). SNP array analysis of ETV6-RUNX1 samples has recently identified multiple additional genetic alterations, but the role of these lesions in leukemogenesis remains undetermined (van der Weyden et al., 2011).

Genome-wide analysis has recorded abnormalities in PAX5 and EBF1 in up to 32% of children and 30% of adults with B ALL, and in 35% of relapsed cases (Mullighan et al., 2007). Currently, five PAX5 fusions have been identified with the gene partners LOC392027 (7p12.1), SLCO1B3 (12p12), ASXL1 (20q11.1), KIF3B (20q11.21) and C20orf112 (20q11.1), with the resulting chimeric proteins expressing lower levels of PAX5 and its target genes (An et al., 2008). EBF1 alterations are common in patients with poor outcomes and are particularly frequent (25%) in relapsed children (Harvey et al., 2010).

The MLL (mixed lineage leukemia) gene is often rearranged in leukemias with myeloid and lymphoid phenotype, probably indicating a very early multipotent progenitor origin. More than 50 fusions involving MLL have been documented. Among them, the MLL-AF4 [t(4;11)(q21;q23)] translocation is present in 80% of infant, 2% of children, and 5-10% of adult ALL (McCarthy, 2010).

The BCR-ABL1 translocation [t(9;22)(q34;q11), also known as Philadelphia chromosome] is found in 5% of pediatric and 25% of adult B cell ALL. An important consequence for this translocation is the over-expression of STAT5. STAT5 inactivation results in cell cycle arrest and apoptosis of BCR-ABL^{pos} malignant B cells and BCR–ABL1^{pos} STAT5 knockout mice do not develop leukemia (Malin et al., 2010). Interestingly, genome-wide analysis of B cell ALL has identified mutations in the STAT5 upstream regulators JAK1 and JAK2 in up to 10% of patients, and patients BCR-ABL^{pos} or with JAK1&2 mutations have a similar gene expression profile and prognosis (Malin et al., 2010). JAK2 mutations lead over-expression of CRLF2 (also known as thymic stromal lymphopoietin receptor) which forms a heterodimeric complex with the IL-7R (Harvey et al., 2010). In a subset of cases, CRLF2 promotes constitutive dimerization and cytokine-independent proliferation. Finally, high expression levels of the short Ikaros isoforms, particularly the dominant negative Ik-6, are also associated with high risk leukemia (Sun et al., 1999). Most of the BCR-ABL^{pos} B ALL patients have deletions in IKZF1 and increased levels of the short isoforms; however, Ik-6 has also been found to be elevated in BCR-ABL^{neg} patients (Mullighan et al., 2008). It has been proposed that the high level of Ikaros short isoform expression is due to genetic lesions. Supporting this idea, IKZF1 somatic deletions have been found in a number of recurrences and are strongly associated with minimal residual disease (Mullighan et al., 2009). A summary of homeostatic and leukemic expression of transcription factors along the B cell pathway is shown in Figure 5.

Despite these important advances in the definition of genetic abnormalities that are prevalent in ALL, the disease is heterogeneous at the molecular level, and possibly it is the result of combination of genetic and epigenetic alterations. Furthermore, high frequencies of ALL cases seem not to be associated to intrinsic genetic abnormalities, opening the possibility of microenvironmental cues leading to disease.

4.3 Leukemic microenvironmental cues?

The complexity of leukemogenesis increases when we consider the indubitable influence of the bone marrow microenvironment in the hematopoietic development, which is a network of

cells (mesenchymal cells, osteoblasts, fibroblasts, adipocytes, endothelial cells, etc) and their products (extracellular matrix molecules, cytokines and chemokines) that support hematopoiesis. Under physiological conditions, the appropriate production of mature blood cells throughout life is sustained by special niches that provide stem and progenitor cells with regulatory signals essential for their maintenance, proliferation and differentiation (Nagasawa et al., 2011). Among secreted factors, CXCL12, FLT3-L, interleukin 7 and stem cell factor are critical for commitment to the lymphoid program and normal B cell development is supported by two stage specific cellular niches within central bone marrow: a CXCL12/SDF1 expressing niche, and a IL-7 expressing niche. B cell precursors are thought to move from one to another as differentiation progresses (Tokoyoda et al., 2004; Nagasawa, 2006). The role of the bone marrow microenvironment in carcinogenesis has been conceived through three possible mechanisms: competition of tumor cells for normal HSC niches, which may allow their maintenance and survival; manipulation of the environment to promote tumor progression and disruption of hematopoietic-niche communication that drives oncogenesis (Raaijmakers, 2011). Although these potential mechanisms are tempting, their contribution to ALL remains formally unexplored. It has been proposed by Sipkins and colleagues that the leukemic cells derived-tumor microenvironment impairs the behavior of normal hematopoietic cells (Colmone et al., 2008). Furthermore, a number of alterations have been recorded in the marrow microenvironment of ALL, including chromosomal aberrations in mesenchymal stem cells, anomalous expression of adhesion molecules, abnormal levels of CXCR4 and growth factors, as well as prevalence of pro-inflammatory cytokines (Menendez et al., 2009; Geijtenbeek et al., 1999; Juarez et al., 2009; and our unpublished results). Whether an abnormal microenvironment anticipates the leukemic stage or is a consequent fact, is still an open issue.

5. Conclusion

Much has been learned about identity, function and intercommunication of seminal cells within the hematopoietic system from animal models. However, our understanding of the hierarchy and regulation of human stem/progenitor cells is still incomplete and the hematopoietic charts have been in constant re-construction over the last few years. Furthermore, while it has long been recognized that intrinsic abnormalities in primitive hematopoietic cells may cause hematological disorders, it has also become clear that changes in both cell composition and function of the bone marrow microenvironment might govern stem cell activity and lead to disease. Future progress in these areas will be decisive to suggest novel classification, prognosis and treatment venues.

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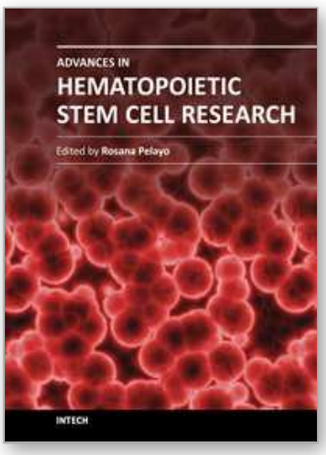

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This book provides a comprehensive overview in our understanding of the biology and therapeutic potential of hematopoietic stem cells, and is aimed at those engaged in stem cell research: undergraduate and postgraduate science students, investigators and clinicians. Starting from fundamental principles in hematopoiesis, Advances in Hematopoietic Stem Cell Research assemble a wealth of information relevant to central mechanisms that may regulate differentiation, and expansion of hematopoietic stem cells in normal conditions and during disease.

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