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Hematopoietic stem cells: cancer involvement and myeloid leukemia

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Abstract. – Hematopoietic stem cells (HSCs) are rare multipotent cells that possess ability to self-renew and differentiate to progenitor cells, which give rise to all blood cell lineages. The process involves specific regulation of gene transcription and its deregulation resulting in imbalance between self-renew and differentiation, can lead to cellular transformation and cancers. Substantial evidence indicates that accumulated mutations in HSCs contribute to the initiation and pathogenesis of at least some hematopoietic cancers. In particular, myeloid leukemias have been extensively characterized with regard to HSC and progenitor involvement. Thus, as a focal point for scientific and therapeutic endeavours, formation of cancer cells from HSCs represents a critical area of investigation. Consequently, understanding how HSCs function and how they undergo to transformation, is of fundamental importance to get insight in their contribution to the hematopoietic cancer development.

Key Words:
Hematopoietic stem cells, Progenitor cells, Leukemia, Myeloid, Mutation, Self-renewal, Differentiation.

Introduction

Hematopoietic stem cells (HSCs) are multipotent cells that generate progenitor cells, which differentiate to blood cell lineages[1]. They are capable of undergoing several cell fate decisions, including survival, death, self-renewal, differentiation and migration, which have critical role in the regulation of their number and life span. To varying degrees, these cell fates extent to progenitors at different levels of the hematopoietic differentiation hierarchy, and are regulated by transcription factors[2]. The key property that distinguishes HSCs from the downstream progenitors is their ability to self-renew. Thus, the strict regulation of correct balance between self-renewal and differentiation is essential for maintenance of the HSC pool and the normal hematopoietic development. Transforming events occurring in cells and leading to loss of control over cell fate decisions, may lead to cancer. For most cancers, the target cell of the transforming events is unknown, but evidence indicate that certain type of leukemias arise from mutations that accumulate in HSCs[3]. Alternatively, progenitors may also become transformed and cause leukemia. This review discusses the notion of HSCs as the first normal cell that becomes subverted in leukemia transformation, with emphasis on myeloid leukemia, where tumor stem and progenitor cells have been studied in detail. The review will begin with an overview on the functional proprieties of HSCs, and will, finally, describe the molecular mechanisms of HSCs transformation that may contribute to the leukemia development.

Functional Properties of HSCs

Since the discovery of HSCs in 1961 by Till and McCulloch[4] in bone marrow, considerable progress has been made toward the characterization of their functional properties and mechanisms controlling their fates. They can choose to self-renew, commit to differentiation, die by apoptosis, stay in the bone marrow or migrate to the periphery. These fate decision processes must be finely tuned to maintain a steady state level of functional HSCs in the bone marrow and to constantly provide progenitors for the different hematopoietic lineages.

Self-renewal: HSCs have the ability to generate daughter cells with the same properties as the parental stem cell. This process can be symmetrical, generating two daughter HSCs, or asymmetrical, resulting in one HSC and another downstream progenitor that possesses a reduced capacity for self-renewal, thereby, establishing a hierarchy to maintain blood production over time without depletion[5].

Mechanisms regulating HSC self-renewal activity are still poorly understood. However, evidence indicates that Notch, Sonic hedgehog (Shh) and

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Wnt signaling pathways[6-9] as well as Hox genes[10-12], are implicated in these mechanisms. Indeed, overexpression of certain Hox genes, such as HoxB4 and HoxA9 causes the selective expansion of the HSC population as well *in vitro* as *in vivo*. Activation of Shh pathway in HSC also results in increased self-renewal capacity, while activation of Notch in HSCs causes increased level of primitive progenitor. Similarly, activation of Wnt signaling by retroviral transduction of constitutively activated β-catenin into HSCs leads to HSC expansion, while retroviral transduction of the Wnt pathway inhibitor axin causes inhibition of their proliferation.

Differentiation: HSCs generates long-tern hematopoietic stem cells (LT-HSCs) capable of indefinite self-renewal, and a short-term subset (ST-HSCs) that self-renews for a defined interval. ST-HSCs differentiate into non-self-renewing multipotent progenitors (MPPs) from which are derived the common lymphocyte progenitors (CLPs) and the common myeloid progenitors (CMPs); two more restricted oligopotent progenitors that ultimately give rise to differential progeny through functional irreversible maturation steps. The CLPs are restricted to give rise to T lymphocyte, B lymphocytes, and natural killer (NK) cells, while the CMPs give rise to granulocyte/macrphage progenitor (GMPs), which generate monocytes, macrophages and granulocytes, and to megakaryotic/erythroid progenitors (MEPs), which produce magakaryocytes, platelets and erythrocytes. The surface markers that distinguish the different progenitor types and the transcription factors that are essential for each differentiation step are indicated in Figure 1.

Proliferation: HSCs divide infrequently before differentiation. But when they mature from LT-HSC into MPP, they progressively lose their self-renewal ability and become more mitotical-

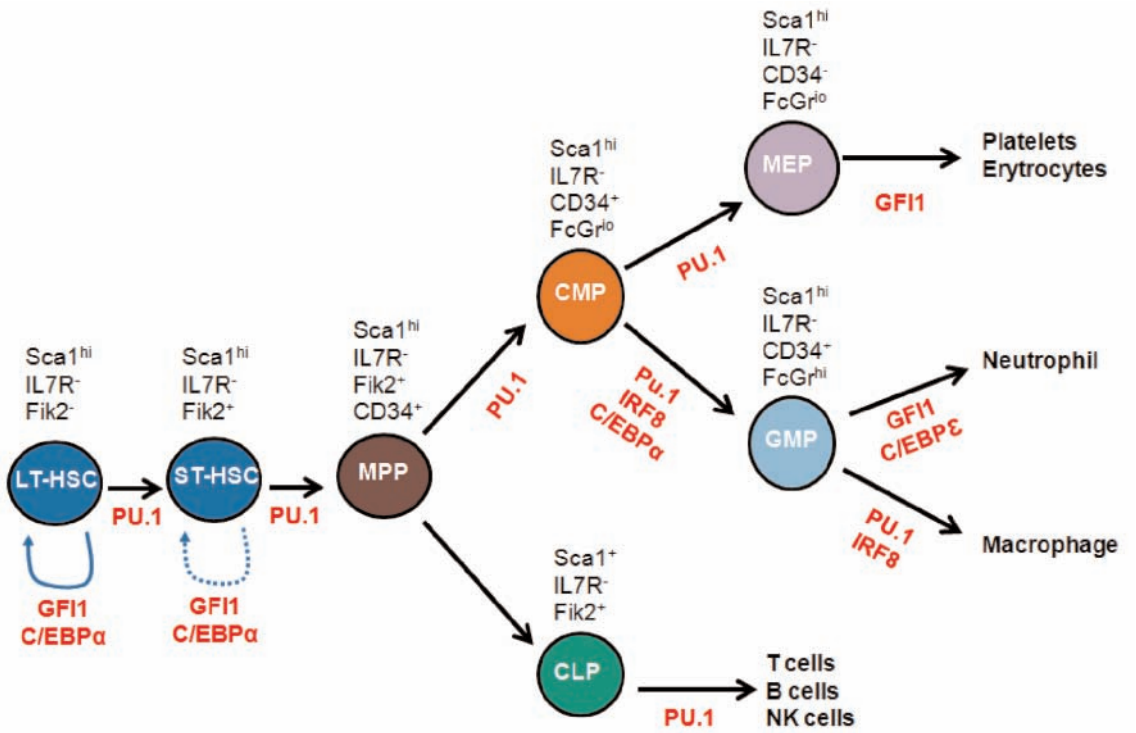

Figure 1. Formation of hematopoietic and progenitor cell lineages and requirements for transcription factors. The differentiation of stem cells into hematopoietic cells through functionally irreversible maturation steps is orchestrated by a hierarchical network of transcription factors. Individual transcription factors are essential for specific step. Growth-factor independent 1 (GFI1) and CCAAT/enhancer binding protein-α (C/EBPα) are required for the self-renewal of HSCs. But C/EBP has another indispensable role in conferring the transition of common myeloid progenitors (CMPs) into granulocyte/monocyte progenitors (GMPs), whereas GFI1, and similarly C/EBPε, are crucial for late-stage neutrophil production. Macrophage production depends on PU.1 and interferon-regulatory factor 8 (IRF8). In this process, PU.1 seems to be essential for all intermediate steps starting from HSCs, whereas IRF8 seems to fulfil a role in later progenitor myeloid cell lineages. The surface marker phenotypes that distinguish the different progenitor types are indicated in the figure.

ly active. Usually, HSCs enter the cell cycle one a month and about 8% of LT-HSC population has been estimated to randomly enter asymmetrical division per day. Their ability to become quiescent protects them from uncontrolled proliferation and warrants their genomic integrity, as frequent chromosomal replications may induce oncogenic DNA mutations. In addition, HSCs express the enzyme telomerase, which maintains the ends of chromosomes during successive rounds of DNA[13]. Telomerase activity serves to enhance life span by protecting chromosomal integrity at the telomeres during exhaustive replication. HSCs possess a non-exhaustive replication and proliferation activity that can be initiated by cytokine produced under stress conditions. However, these cells express receptors for cytokines and chemokines, which allow them to respond to signal from immune cells to adapt their cycling[14].

Apoptosis: Preventing apoptosis by transgenic expression of the anti-apoptotic protein Bcl-2 in LT-HSCs has been show to cause a gradual increase in LT-HSC frequency, which takes place without malignancy manifestation despite decreased LT-HSC entry into the cell cycle[15]. Different mouse strains also show differences in the number and cell cycle status of HSCs, likely indicating differences in susceptibility to apoptosis.

Transforming Mutations Leading to Myeloid Leukemia

Although target cell of transforming mutations is still unknown for most leukemia, considerable evidence indicates that certain subtypes of myeloid leukemia arise from accumulation of mutations in HSCs. These cells possess both self-renewal propriety and extensive proliferative capacity that would be advantageous for malignant growth. Also, HSCs persist throughout life and, therefore, have much greater opportunities to acquire and accumulate the requisite mutations than more mature cells, which only persist for a short period. However, only HSCs with $CD34^+CD38^-$ phenotype are capable of initiating acute myeloid leukemia (AML) in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, whereas the $CD34^+CD38^+$ leukemic blast cells cannot transfer the disease to mice, although displaying a leukemic blast phenotype[16]. This suggests that normal HSCs rather than progenitors are the target for transforming mutations leading to AML.

The most frequent chromosomal abnormalities associated with this form of myeloid leukemia is the t (8,21) translocation, which generates AML1-ETO transcripts[17]. These transcripts were detected in leukemic blast cells and in normal HSCs in bone marrow from AML patients[18]. However, the prospectively isolated HSCs and their progeny were not leukemic, and could differentiate into normal myeloerythroid cells *in vitro*. This indicates that the translocation occurred originally in HSCs and suggests that additional mutations in a subset of these HSCs or their progeny subsequently led to leukemia[18].

In chronic phase of disease (CML), approximately 95% of patients possess the Philadelphia (Ph) chromosome, a shortened chromosome 22 arising from the t(9q34;22q11) reciprocal translocation containing the BCR-ABL fusion gene that produces the BCR-ABL chimeric protein[19]. The presence of this protein in the majority of CML patient suggests that the original translocation takes place in HSCs.

Collectively these observations support the idea that HSCs are a common target of transforming mutations. However, there is evidence that progenitors may also become transformed. In patients with the M3 subtype of AML (APML), it has been shown that the APML-associated fusion gene PML-RARα, was present in $CD34^-CD38^+$HSC-enriched cell population but not in $CD34^+CD38^-$ HSC-enriched cell population[20]. This suggests that in this AMPL subtype, the transformation process may involve a more differentiated cell than HSCs and pluripotent progenitors.

Further evidence indicating that progenitors can also be the target cells for transforming mutations are from analysis of leukemia-associated genes in the mouse. Indeed, several AML mouse models have been generated with the promoter of the human MRP8 (hMRP8) gene, which is only expressed in neutrophil, monocyte, and their immediate progenitors CMP and GMP, but not in HSCs[21]. In addition, purified populations of CMP or GMP transduced with a retroviral vector encoding the MLL-ENL gene could generate AML *in vivo*[22].

Overall, these observations indicate that, in the case of spontaneously occurring human leukemia, it is possible that mutations occur in HSCs but these mutations change properties of downstream progenitors, which in turn generate leukemic cells. It is also possible that transforming mutations take place in pluripotent progeni-

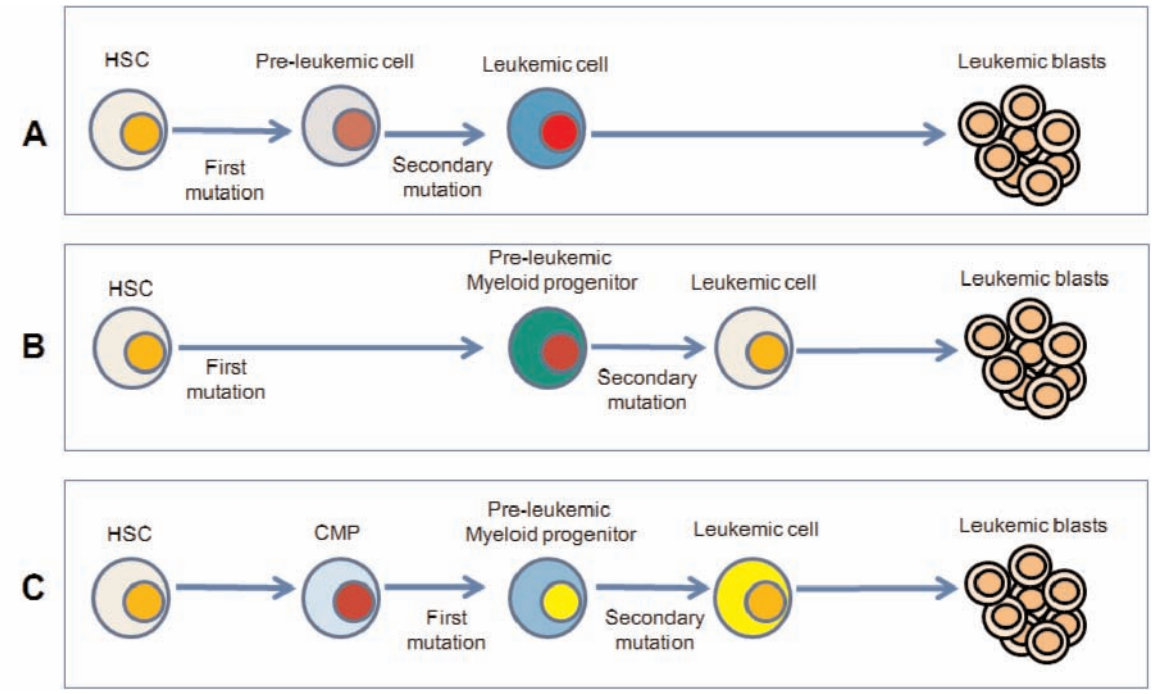

Figure 2. Scenarios of leukemic cell formation. **Panel A** depicts the first scenario in which leukemic cells arise directly from normal HSC. The initial mutation occurs in an HSC, leading to the formation of a pre-leukemic stem cell. Secondary mutation(s) in the pre-leukemic cells then gives rise to leukemic cells. Both the initial and secondary mutation(s) in this scenario are at the stem cell level. **Panel B** shows the second scenario in which an initial mutation occurs at the HSC level, followed by differentiation to a preleukemic MP stage and subsequent secondary mutation(s) leading to the leukemic cells. **Panel C** shows the third possible scenario, which suggests that HSCs first differentiate to normal MP, and then undergo primary and secondary mutations to ultimately generate leukemic cells. In all three scenarios, once leukemic cells are formed, subsequent differentiation generates the leukemia blast population.

tors and in more differentiated cells. Figure 2 depicts three possible scenarios by which transforming mutations can occur and lead to leukemia. It should be kept in mind that the bone marrow microenvironment also contains innate and adaptive immune cells, which paradoxically contribute to disease progression in leukemia[23]. Thus, cancer or the myeloid leukemia can occur not necessarily due to gene or other mutations.

Deregulated Processes Leading to Myeloid Leukemia

Transforming mutations can impact a wide range of cellular pathways and processes including self-renewal, differentiation, proliferation and apoptosis that may cooperate to induce myeloid leukemia (Figure 3).

Increased proliferation: Ras, NF-kB, PI3 kinase and two mitogenic growth factor receptor tyrosine kinases, Flt-3 and c-kit, are frequently mutated in AML[24]. Increased proliferation of AML progenitor cells but not leukemic HSCs has been associated with activating mutation of c-kit in these cells. In contrast, analysis of leukemic cells has revealed constitutive activation of NF-kB in HSCs but not in progenitor of a large percentage of specimens[25]. This transcription factor has been the focus of numerous studies in the cancer field[26]. In the vast majority of cases, activation of NF-kB was directly linked to increased growth and survival of tumor cells. Flt-3 and Ras also exhibited activating mutations in AML[27], and evidence indicate that Flt-3 can activate Ras[28], which in turn may stimulate NF-kB[29]. Similarly, constitutive PI3 kinase activity has been reported in HSCs for a large percentage of AML specimens[30].

Blocked differentiation: A common feature to all AML cases is arrested differentiation leading to accumulation of blast cells in the bone marrow [31]. Many studies of human AML suggest that cancer-associated differentiation is caused by mutated or deregulated transcription factors. Various fusion proteins, including RUNX1-ETO, CBFβ-MYH11, AML1-ETO, PML-RARα, MLL-ENL and BCR-ABL have been

observed in AML[32]. The 210-kDa BCR-ABL fusion protein differs from the normal 145-kDa c-ABL in its preferential location in the cytoplasm and its constitutive elevated tyrosine kinase activity. Structural analysis has revealed that both of these properties are critical for the transforming activity of BCR-ABL[19]. Regarding other AML-associated fusion proteins, one of the two components of each fusion protein is generally a transcription factor (AML1, CBFβ, or RARα) whereas the other partner is more variable in function, but is often involved in the control of cell survival and apoptosis such as the nuclear structure PML[33]. Moreover, AML-associated fusion proteins have been shown to affect hematopoietic differentiation in a variety of experimental models, and the specific stage of myeloid maturation arrest appears to direct dependent on the nature of the fusion protein expressed. The abnormal network of transcriptional regulation induced by these leukemia-associated fusion genes seems to occur through common mechanisms, including recruitment of aberrant co-repressor complexes, alteration in chromatin remodelling, and disruption of specific sub-nuclear compartments[33,34].

Acquired self-renewal: Some genes that mediate increased self-renewal appear to be benign with regard to subsequent mutational events. As an example, constitutive expression of the AML1-ETO translocation product has been shown to increase the self-renewal frequency of stem cells, but results in no apparent pathogenic consequences[35,36]. Presumably, only subsequent random mutations provide a molecular context in which a leukemic role for AML1-ETO becomes apparent. In contrast, other genes with known self-renewal potential, such as HoxA9, have been demonstrated to induce cytogenetic aberrations when expressed in an unregulated fashion. While not being sufficient to generate overt disease, it is possible that activation of HoxA9 predisposes stem cells to subsequent oncogenic events by virtue of decreasing genomic stability. Interestingly, HoxA9 up regulation is commonly observed in AML[37,38]. It is also possible that the progression of the Fas$^{lpr/lpr}$hMRP8^{Bcl-2} mice to AML is

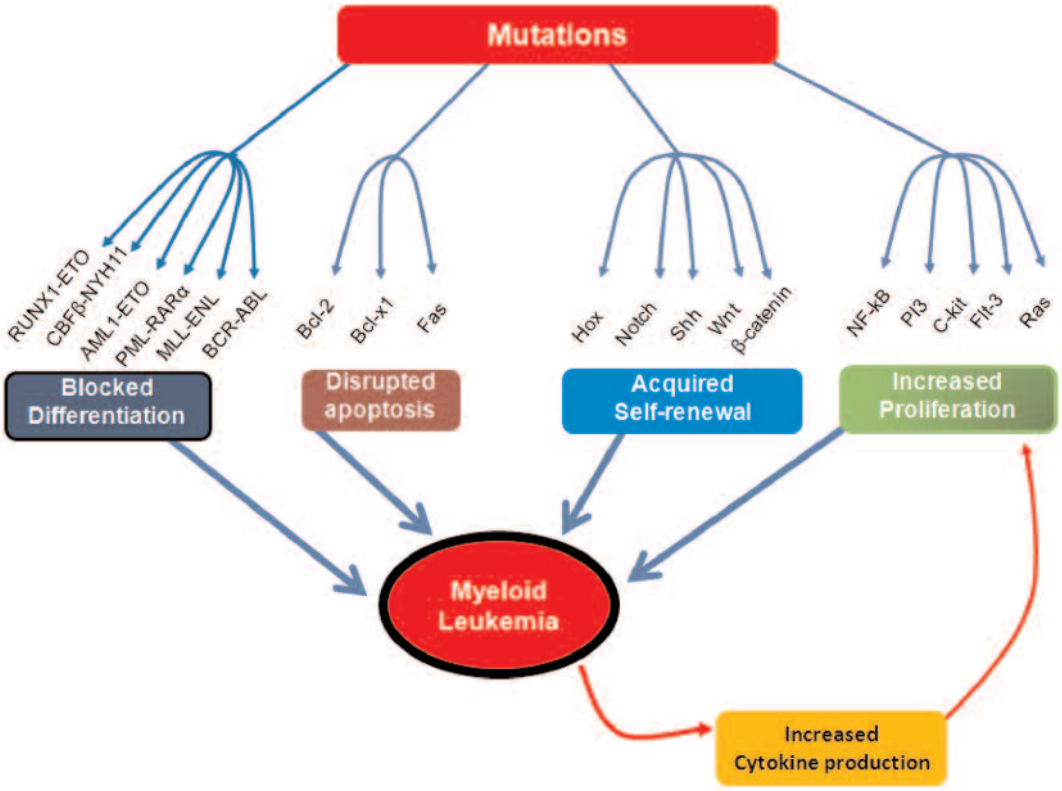

Figure 3. Cooperation between different mutations leading to causative processes of myeloid leukemia. Please refer to the text for details.

due to the fact that the leukemic cells acquire an additional mutation that causes deregulated self-renewal. Research focusing on gain-of-function mutations that promote constitutive self-renewal, such as stabilization of β-catenin, has revealed that stabilized β-catenin promotes the self-renewal of stem cells and other types of progenitor cells[8,39], and that the activation of β-catenin and deregulation of the Wnt signaling pathway is a common phenomenon in cancer. Mutations in other signaling pathways that promote progenitor self-renewal, such as Notch and Shh, are also likely to contribute to unregulated self-renewal of leukemic cells.

Disrupted apoptosis: The Bcl-2 family members, such as Bcl-2, Bcl-xl, Mcl-1, and A1, function as cell death antagonists against a wide array of apoptotic stimuli, whereas their binding partners, like Bax, Bad, and Bak, promote apoptosis [40]. Thus, gain-of-function mutations in Bcl-2 family members or loss-of-function mutations in Bax family members would be expected to predispose toward cancer. The fusion protein AML1-ETO was shown to directly up regulate expression of Bcl-2 by binding its promoter elements (91). This protein is present in leukemic blast cells from most AML patients[18], and these cells have been shown to express Bcl-2 at much higher levels than their normal counterparts[41]. In addition, activating mutations by retroviral integration in the murine Bcl-xl gene have been reported for myeloid leukemia[42]. Collectively, these data support the idea that gain-of-function mutations in Bcl-2 gene family members may be important in the multistep process leading to myeloid leukemia transformation. Hence, the increased survival provided by enforced Bcl-2 family member expression may allow sufficient time for the acquisition of additional oncogenic mutations, a mechanism thought to underlie the transition from chronic to acute leukemia[43]. Accordingly, transgenic mice constitutively expressing Bcl-2 in myeloid cells by the hMRP8 promoter develop a disease that is similar to human chronic myelomonocytic leukemia as the mice age[44].

However, despite decreased survival compared with littermates, these mice rarely progress to acute leukemia and they require additional mutations to promote AML, as has been shown for lymphoid leukemia[45].

It is known that granulocytes and their myeloblastic progenitors express high levels of Fas[46], and several patients with granulocytic leukemias have been shown to have blasts with functional deficiencies in the Fas signaling pathway[47]. Interestingly, intercrosses between $hMRP8^{Bcl\text{-}2}$ and Fas-deficient $Fas^{lpr/lpr}$ mice lead to the development of AML in 15% of the $Fas^{lpr/lpr}hMRP8^{Bcl\text{-}2}$ mice, with an expansion of myeloblasts and substantially lower numbers of granulocytes in the bone marrow and blood[44]. This indicates that Bcl-2 and the Fas receptor regulate two distinct apoptosis pathways in the myeloid lineage and that alteration of these two pathways may be required for transformation of myeloblasts in both mouse and human. Moreover, increased survival induced by enforced Bcl-2 expression greatly increases the incidence of CML-like disease in $hMRP8^{BCR\text{-}ABL}hMRP8^{Bcl\text{-}2}$ double transgenic mice[48], as well as the incidence of APML development in $hMRP8^{PML/RAR\alpha}$ $hMRP8^{Bcl\text{-}2}$ double transgenic mice[49].

Overall, these observations demonstrate that prevention of programmed cell death is one of the crucial events in the development myeloid leukemia and may even be the first step that sets the stage for additional mutations.

Conclusions

Myeloid leukemia is typically a disease of stem or progenitor cell origin. It can be initiated either by HSCs that have become leukemic as the result of accumulated mutations or by more restricted progenitors that have reacquired the stem cell capacity for self-renewal. Identifying the leukemic cell for each given leukemia, is therefore needed to fully understand their specific biology and get further insight into the key elements of the leukemogenic process, many of which might be relevant to other human cancers.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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