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Pathways involved in the evolution of leukemic stem cells

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Abstract. – Understanding the evolution of the cancer cell from a normal cell holds the key to developing novel, potent therapies against cancer. Two hypotheses describing the origins of cancer cells have been developed: one stating that any normal cell can acquire the ability to replicate indefinitely and evade natural cell death signals by accumulating multiple mutations over time, and a second suggesting that adult stem cells, by virtue of their pre-existing capacity for differentiation, asymmetric division and self-renewal, are the more likely targets of carcinogenic mutation. The leukemic stem cell (LSC) was the first cancer stem cell described. Evolving from the aberrant regulation and mutation of hematopoietic stem cells (HSCs), LSCs are suggested to encompass the subset of tumor cells sufficient for continued tumorigenesis. LSCs were also found to differentiate into a variety of cancer cell progenitors in a manner reminiscent of HSC differentiation, also explaining the observed heterogeneity of leukemic cells. How these cells form from HSCs remains to be fully comprehended. However, over recent years, marked progress has been made in contributing to our knowledge of cancer stem cells and what signaling cascades are involved in their development. Therapeutics targeting the pathways allowing for LSCs to sustain proliferation and self-renewal may prove to be more effective treatments for lymphoblastic leukemia.

Key Words:
Leukemic stem cells, Hematopoietic stem cells, Lymphoblastic leukemia, cancer, Differentiation, Wnt-beta-catenin pathway, Notch signaling, BCR-ABL, PTEN, HOX family transcription factors.

Abbreviations

ALL = acute lymphoblastic leukemia; BM = bone marrow; CML = chronic myeloid leukemia; CSC = cancer stem cells; HSC = hematopoietic stem cells; LSC = leukemic stem cells; TCF/LEF = T-cell factor/lymphoid enhancing factor.

Introduction

The prevailing model of cancer cell development identifies key traits that allow a cell to evade built-in mechanisms that regulate the cell cycle. Carcinogenesis results from the accumulation of repeated genetic insults to a cell over a period of time that confers the ability to escape apoptosis and proliferate uncontrollably[1]. It has been hypothesized that cancer cells may arise from stem cells, which are characterized by their ability to regenerate and to differentiate into various cell types of their tissue of origin. The "cancer stem cell hypothesis" suggests that the cancer stem cell (CSC), while retaining many of the tissue-specific gene expression patterns and phenotype of the origin tissue, will mimic stem cells properties by multiplying indeterminately and differentiating into a variety of cell types within the tumor. However, unlike stem cells, CSCs no longer possess the checkpoints that limit the number of replications a cell can undergo[2,3]. Based on the observations made over a century ago that some tumors share close histological resemblances to fetal tissues[4,5], this perspective has had a significant impact on how we approach treatments for cancer. If the stem-cell-like properties of CSCs were indeed what sustain the indeterminate growth potential of tumors, then selectively eradicating the CSC population would represent an ideal target for the development of new cancer therapies. The existence of CSCs was first confirmed in the hematopoietic system, and in recent years, several studies have shown that such an approach may prove highly effective in the treatment of lymphoblastic leukemia[6]. Originating from hematopoietic stem cells (HSCs), leukemic stem cells (LSCs) were identified as the only sub-population of leukemic cells capable of unconstrained proliferation *in vitro*[7-9]. Understanding how LSCs evolve from HSCs,

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and how they differ from their normal counterparts, could provide invaluable insight into the treatment of lymphoblastic leukemia.

Identification of LSCs

More than fifty years ago, experiments using lethal irradiation in mice led to the discovery of a population of cells capable of replenishing depleted bone marrow[10], now known as HSCs. HSCs are critical for the continued replenishment of blood cells, hematopoiesis, sustained throughout the lifespan of an organism. HSCs divide into two daughter cells that can either become two HSCs, or progenitor cells that committed to differentiation into a specific cell type with limited replicative potential. The identity of the daughter cells is determined by regulatory signals that ensure the needed cells are produced (Figure 1). However, HSCs are also capable of asymmetric division where one daughter cell is a stem cell, while the other is a progenitor[11]. LSCs could utilize this capacity to persistently maintain proliferation via new stem cells, also synthesizing a variety of progenitors impervious to normal homeostatic signals[12,13] (Figure 1).

Indeed, cancer cells isolated from patients with acute or chronic lymphoblastic leukemia were discovered to be a very heterogeneous population[14-16], exhibiting clonal diversity and a varied expression of cell surface antigens. Isolating the LSCs amongst these cells was achieved due to advances in antibody technology – elegant ex-

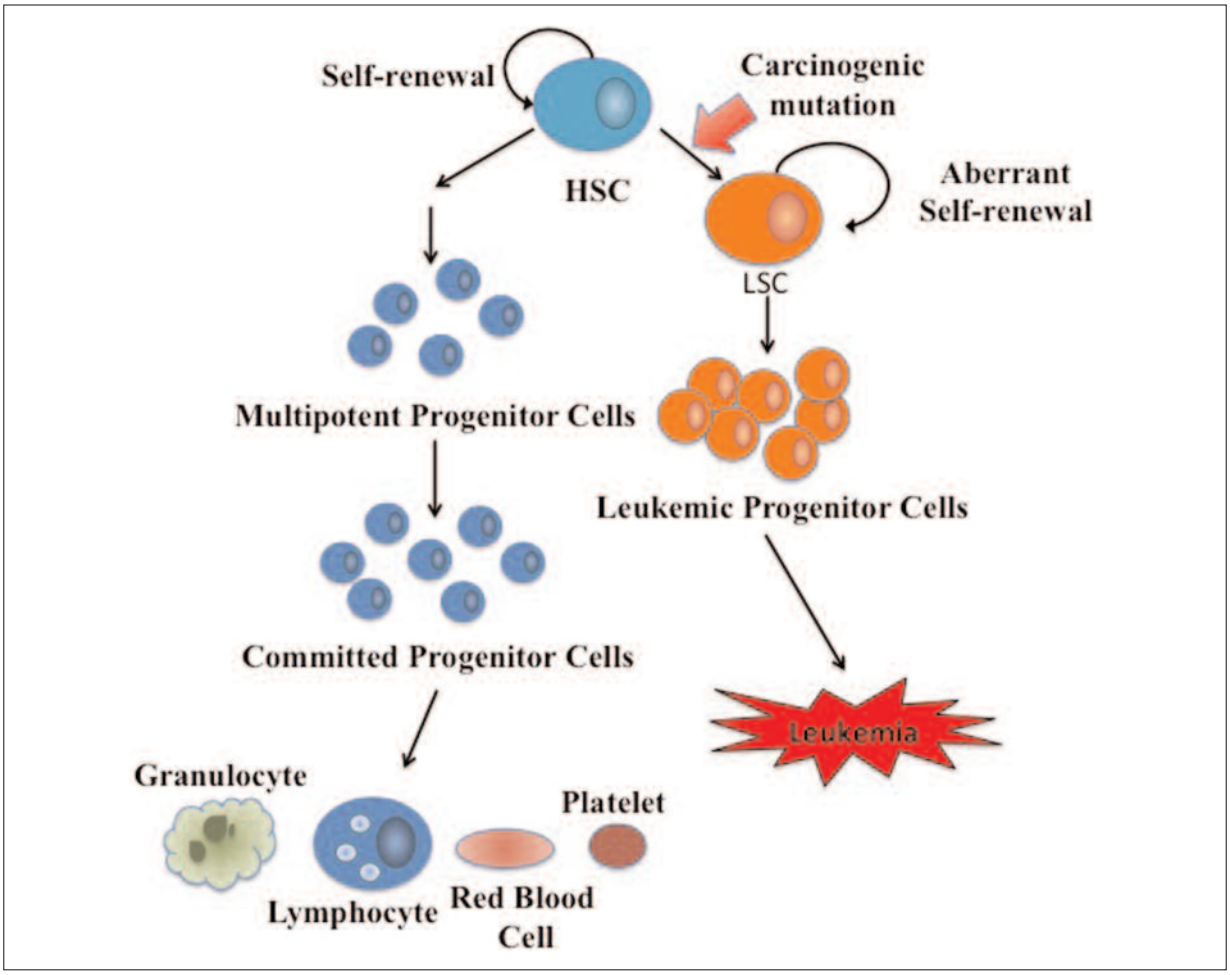

Figure 1. LSC formation from HSCs. LSCs are phenotypically similar in many ways to their normal counterparts, the HSCs. Mutations that allow an HSC to escape the normal regulatory mechanisms used to maintain quiescence and limit the number of replicative cycles will lead to formation of LSCs. They also self-renew, and form progenitors sharing many of the cell surface markers seen in normal progenitor cells. While HSCs ultimately replenish certain cell types of the hematopoietic system, LSCs will multiply and partially differentiate, forming heterogeneous leukemia tumors. Adapted from Reference 4.

periments fractionating leukemic cells from patients into marker-specific groups, and grafting these cells into sublethally irradiated mice, found that it was only the $CD34^+CD38^-$ cell immunophenotype, similar to that of normal HSCs, that was capable of proliferating and causing human leukemia in mice. In patients with acute lymphoblastic leukemia (ALL), it was observed that leukemic cells capable of recapitulating human cancer in mice also expressed the marker pattern of $CD34^+CD10^-CD19^-$ – the absence of both CD10 and CD19 suggests that the transformation events leading to cell cycle dysregulation occurred in a target cell that was less mature than a B-lymphoid progenitor cell.

The hypothesis that the genetic mutations conferring survival advantages and ultimately leading to cancer occur in HSCs differs from the model that tumors form from differentiated somatic cells that acquire several mutations giving them stem-cell-like properties. For a cell to undergo such a transformation, it must be long-lived so that it has enough time to be exposed to repeated genetic insult. As HSCs are maintained throughout adulthood and undergo a higher number of divisions, the likelihood that an HSC could accumulate the necessary mutations to become malignant is greater than for differentiated cells[17].

The Molecular Mechanisms Promoting LSC Survival

Though our understanding of normal HSC regulation and LSC formation has progressed considerably, what pathways are responsible for inducing HSC to LSC transformation, and how LSCs maintain their survival, are questions that require further elucidation. Crucial evidence that suggests that HSCs are indeed the source of LSC formation includes the finding that both cell types express comparable amounts of telomerase. Telomerase activity is needed to preserve the length of telomeres, maintaining the integrity of chromosomes through each division and allowing a cell to evade senescence. Though absent in normal cells, telomerase activity has been shown in various tumor types and may be important for maintaining high rates of proliferation in the advanced stages of lymphoblastic leukemia[18,19]. LSCs have also been shown to harbor the BCR-ABL fusion gene associated with both chronic myeloid leukemia (CML) and ALL[20]. The fusion of BCR with ABL stimulates its tyrosine kinase activity and releases it from negative regulation[21,22]. Increased oncogenic BCR-ABL activity can inhibit apoptosis by down-regulating tumor suppressors such as PTEN[23]. PTEN activity is decreased in LSCs and PTEN loss promotes LSC formation[24], while activation of the PTEN pathway has been demonstrated to hamper ALL development[25]. Though LSCs are resistant to most drugs targeting BCR-ABL[26,27], therapeutics designed to bolster the PTEN pathway may prove to be effective in eliminating the LSC population and curbing tumorigenesis.

While BCR-ABL confers a survival advantage, this mutation alone does not induce malignant transformation. An additional mutation leading to activation of the Wnt-β-catenin pathway (Figure 2) is necessary for carcinogenic transformation[28]. Mutations allowing both escape from apoptotic signals as well as enhanced self-renewal collectively foster the transition from HSC to LSC. First discovered as a proto-oncogene, Wnt is a regulatory factor that functions in either a paracrine or autocrine manner. Wnt binding to its receptor Frizzled leads to recruitment of Dishevelled, sequestering the APC-Axin-GSK3 complex and releasing β-catenin. In the absence of the Wnt signal, β-catenin is bound to the APC-Axin-GSK3 complex and targeted for proteasomal degradation. β-catenin release will allow it to translocate to the nucleus where it induces the expression of several genes involved in growth and proliferation, such as TCF/LEF (T-cell factor/lymphoid enhancing factor)[29,30]. The Wnt-β-catenin pathway is required for differentiation and regeneration of normal HSCs; however, increased cytoplasmic and nuclear β-catenin levels found in LSCs promote their survival, and drugs inhibiting β-catenin may sensitize LSCs to treatment with other inhibitors[31-33] (Figure 2).

Up-regulation of the Wnt-β-catenin pathway has also been found to occur concomitantly with increased HOX gene expression. Dysregulation of the HOX family of transcription factors has also been demonstrated to play a role in the development and progression of ALL. These transcription factors are known to be significant regulators of HSC self-renewal – their expression is increased in HSCs that divide to produce new HSCs as opposed to progenitors, and expression of HOX family proteins is decreased upon differentiation[34]. T-cells from ALL patients were found to overexpress various HOX family proteins such as HOX11L2, and their elevated levels are associated with poor prognosis[35]. Furthermore, HOXB4 overexpression was found to ex-

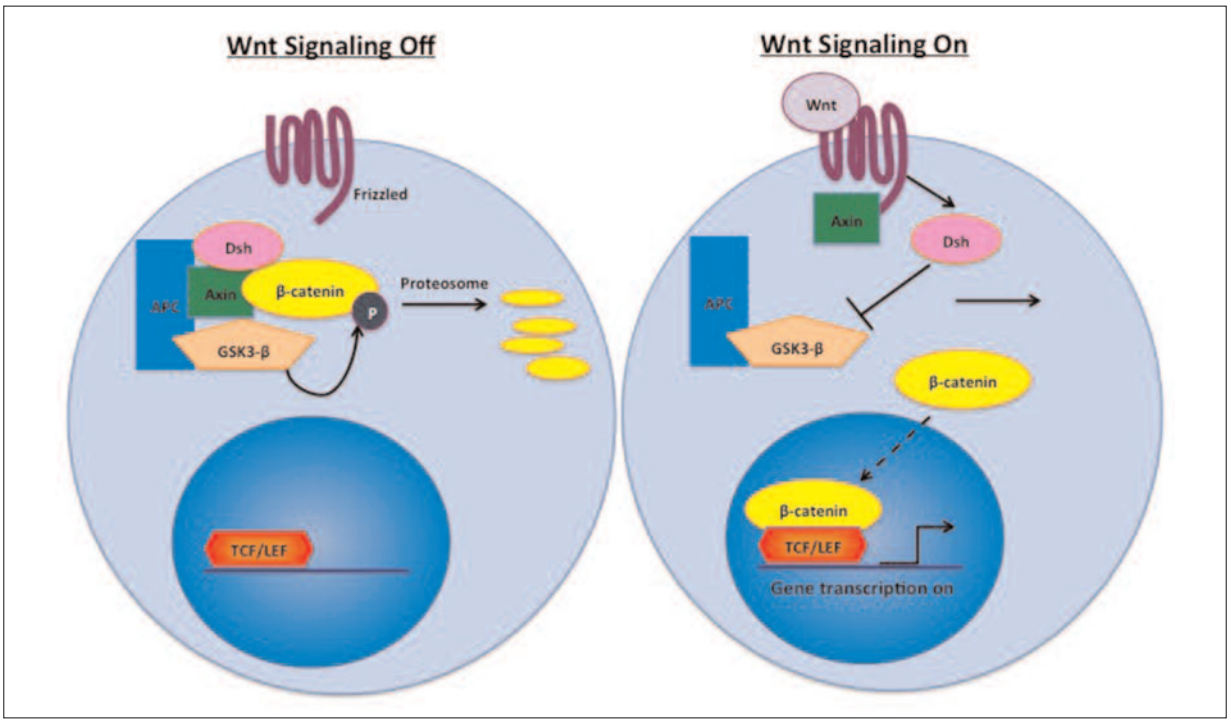

Figure 2. The Wnt-β-catenin Signaling Pathway: β-catenin is sequestered in a complex with APC, Axin and Dsh in the basal state. GSK3-β, also present in this complex, phosphorylates β-catenin and targets it for proteosomal degradation. However, upon binding of the Wnt ligand to the extracellular domain of the Frizzled G-protein coupled receptor, β-catenin is released from the complex and GSK3-β phosphorylation activity is inhibited by the now active Dsh. β-catenin can then translocate to the nucleus where is binds to TCF/LEF and turns transcription of target genes on. Adapted from Reference 32.

pand HSC numbers *ex vivo*[36,37]. Upstream regulators of the HOX family have also been reported to be involved in leukemic transformation. MLL is a histone methyltransferase required for regulation of the expression of HOX transcription factors[38]. Loss of MLL function was shown to result in increased HOX gene expression in leukemic cells, and hypomethylation of HOX gene promoters[39-41]. Interestingly, numerous MLL gene rearrangements and oncogenic fusions have been detected in patients with ALL[42,43]. Normally responsible for regulating self-renewal in HSCs, it is possible that mutation or aberrant regulation of HOX family transcription factors could sustain self-renewal in LSCs as well, and the presence of a large array of MLL and HOX gene rearrangements suggests that de-regulating this pathway is critical for LSC sustenance.

Another family of proteins implicated in promoting LSC self-renewal is the Notch family. Activation of Notch signaling (Figure 3) has been shown to enhance both HSC and LSC self-renewal, and is known to be necessary for progenitor differentiation into T-cells[44,45]. The four Notch genes code for single transmembrane receptors that are activated upon extracellular binding with a Delta/Jagged ligand. This will trigger cleavage of the extracellular domain of Notch by γ-secretase, releasing the intracellular domain (IC-Notch). IC-Notch will then translocate to the nucleus where it interacts with the transcription factor CSL, ultimately promoting expression of genes required for self-renewal as well as T-cell differentiation[46]. The tumorigenic potential of Notch has also been described, Notch1 mutations allowing increased cleavage and release of the IC-Notch or decreased targeting of IC-Notch to the proteasome, were found in patients with chronic lymphoblastic leukemia as well as T-cell ALL[47-49]. Down-regulating the Notch signaling pathway may, therefore, also prove to be an effective method to target LSCs in the treatment of leukemia (Figure 3).

Niche Environment Interactions Sustaining LSC Growth

While numerous intracellular pathways are important to HSC replication and self-renewal, and mutation of components in these pathways are sufficient for the formation of LSCs, the ex-

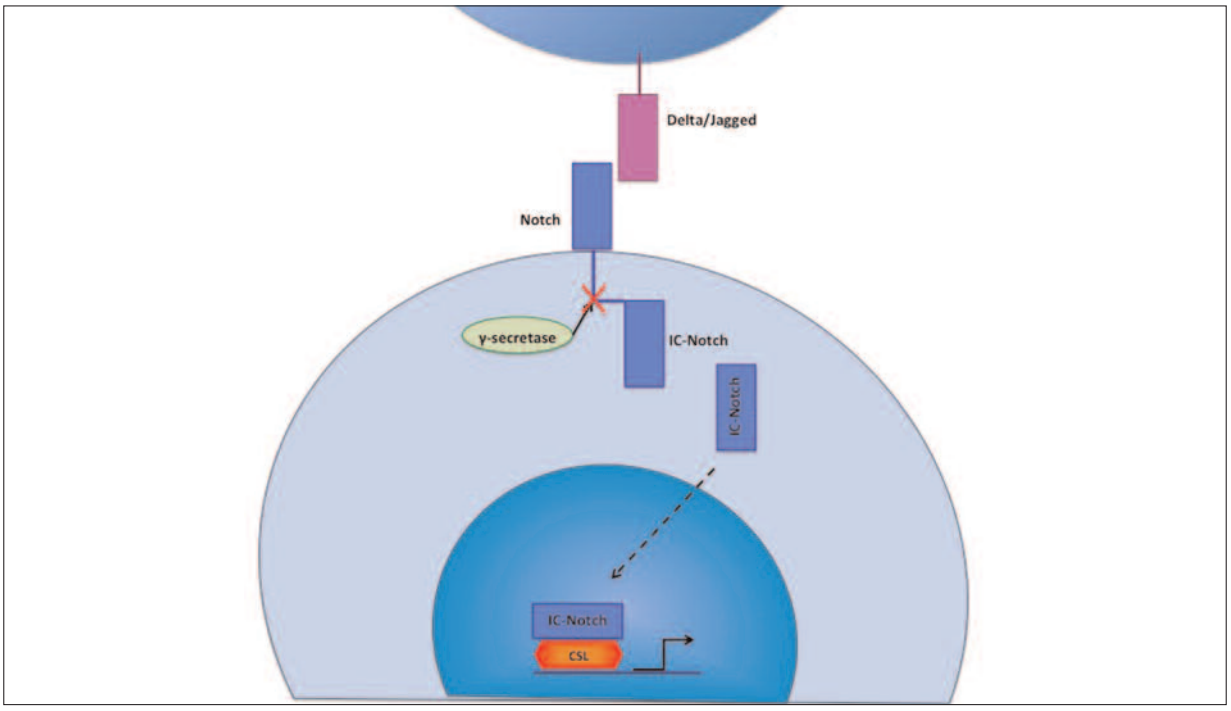

Figure 3. The Notch Signaling Pathway: Binding of Delta/Jagged expressed on a neighbouring cell surface to the Notch receptor leads to recruitment of γ-secretase, which will cleave the extracellular domain from the intracellular domain of Notch (IC-Notch). IC-Notch will then translocate to the nucleus where it interacts with CSL to induce transcription. Adapted from Reference 48.

tracellular environment also plays a significant role in the homeostatic control of HSC differentiation and population[50]. A large fraction of adult HSCs reside in the bone marrow (BM) where interactions with osteoblasts are critical for regulation. Osteoblasts expressing N-cadherin have been shown to affect the number of HSCs in the BM niche, an increase in their numbers associated with a greater number of HSCs[51,52]. The expression of cell-adhesion proteins, such as N-cadherin and β1-integrin, has been demonstrated to be important for proper retention of HSCs in the bone marrow. About 75% of HSCs in the BM are quiescent – maintenance of quiescence is achieved by expression of the Tie2 tyrosine receptor kinase in HSCs. This receptor binds strongly to the Ang1 ligand produced by osteoblasts, anchoring the HSCs within the BM. Ligand binding will also trigger Tie2 signaling which will promote N-cadherin expression downstream, which is also required for quiescence[53]. Importantly, it has been shown that c-Myc negatively regulates this pathway, decreasing N-cadherin expression and allowing HSCs to proliferate[54]. Many c-Myc mutations have been detected in LSCs from leukemia patients, and may be involved in transformation of HSCs to LSCs by hindering regulation by the BM niche environment[55,56].

Conclusions

Mounting evidence in support of the cancer stem cell hypothesis is leading to a better understanding of carcinogenesis. Many treatment strategies fail to eliminate the entire tumor cell population, often leading to resurgence of tumor growth, largely due to the continued survival of LSCs. The discovery of LSCs and the crucial pathways mutated in these cells allowing them to evade the many layers of control in place to regulate normal HSC function will lead to the development of more effective therapeutics to treat lymphoblastic leukemia.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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