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Characteristics of Haemopoietic and Leukaemic stem cells and their regulatory pathways

This review aims to focus on the phenotypic markers of both haemopoietic stem cells (HSC) and leukaemic stem cells (LSC) in acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), and acute lymphoblastic leukaemia (ALL). The review also focuses on important pathways that regulate the proliferation of these stem cells, such as wingless type (Wnt), Notch, Hedgehog, janus kinase-signal transducer and activator of transcription (JAK-STAT), nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB), transforming growth factor beta (TGFβ), phosphatase and tensin homolog (PTEN), homeobox (HOX), polycomb ring finger oncogene (Bmi-1). While, the deregulated signals have been demonstrated in many types of leukaemias and cancers, however, these discoveries allow developing novel therapeutics targets, which can be targeted in combination with standard chemotherapy drugs to eradicate malignant cells.

INTRODUCTION

The haematopoietic system comprises of numerically expanding hierarchy of cells that are restricted in their proliferation and differentiation abilities. The mature functional cells of the haematopoietic and immune systems originate from a very small number of undifferentiated cells called haematopoietic stem cells (HSCs). HSCs are defined on the basis of three basic characteristics: their ability to undergo self-renewal, the ability of extensive proliferation, and the ability to differentiate into multiple distinct cell types. These dynamic features provide sufficient number of primitive cells to maintain a state of equilibrium and sustainability in haematopoiesis {1}. Till and McCulloch {2} were the first to identify the HSCs self-renewal in murine bone marrow cells. The asymmetric versus symmetric division of HSCs can be determined randomly through external signals such as cytokines. The asymmetric cell division is unpredictable; sometimes it results in the formation of both an identical stem cell and a more mature cell {3}. Bonnet at el., {4} and Hope et al., {5} reported the same hierarchical organization for the leukaemia stem cells (LSC). In other words, one can assume that LSCs exhibit similar characteristics to those of normal HSCs. However, the origin of LSCs is from normal stem cells through the accumulation of oncogenic insults, and differentiated progenitor cells that have reacquired the capacity for self-renewal {6, 7, 8}.

A. *Hematopoietic stem cells*

Stem cells differentiate into different multiple cell types {9}. They are capable to renew themselves through mitotic cell division and can be differentiated into a diverse range of specialized cell types (e.g. myeloid, erythroid, and lymphoid) through a range of lineage-committed progenitor cells. Generally, in mammals there are two types of stem cells; pluripotent stem cells and multipotent stem cells {10}. Pluripotent stem cells can be differentiated into endoderm, mesoderm, and ectoderm, e.g., embryonic stem cells. Whereas multipotent stem cells are lineage specific and include HSCs. HSCs are relatively rare cells in the bone marrow, and they are estimated to be between 3 x 10^5 and 4 x 10^6 in the humans {11}. It is documented that each HSC divides approximately 70 times during its lifetime {12}. Osawa et al., {13} and Kent et al.,{14} stated that a single HSC could generate the entire blood system for a lifetime. This is possible only if HSC at the time of cell division renews itself to produce at least one daughter cell with the undifferentiated characteristics of the parental stem cells {15}. Only a minority of the HSCs reconstitute the haematopoietic system and majority exist in an inactive state, which allows the resting HSCs to repair any DNA damage and maintain their integrity {16}. Mice models with immune deficiencies e.g., having severe combined immunodeficiency (SCID) (Lack B and T cells) and non-obese diabetic severe combined immunodeficient (NOD-SCID) (Lack B, T, and NK cells and have other immune deficiencies) were used to study normal HSC and LSC, which have long term repopulating potential and the ability to propagate and maintain the AML phenotype {17}. Leukaemia stem cells have different types and failure to eradicate these primitive cancer cells is a major reason of relapse of leukaemia {18}.

The transplantation of HSC into SCID or NOD-SCID murine models and reconstitution of haematopoiesis are used to define HSCs as SCID-repopulating cells (SRC), and SCID leukaemia-initiating cells (SL-IC), which are human leukaemia progenitor cells with the ability to reconstitute leukaemia in these murine xenotransplantation assays.

Lentivector mediated clonal tracking displayed the existence of short and long term repopulating capacity (ST-SRCs and LT-SRCs) {19}. The SRC xenotransplantation assay helped researchers to identify many cell surface markers that

define the developmental hierarchy of human haematopoiesis. Passegue et al., {20} have used different surface markers to identify human HSC. Human HSCs that have been identified are Lin^-, $Thy1^+$, $CD34^+$, and $CD38^{Neg/Low}$. Mazurier et al., {21} have also demonstrated heterogeneous repopulation potential of different HSC subsets by $Lin^-CD34^+CD38^-$ (SRC), $Lin^-CD34^+CD38^{Low}$ (ST-SRC), and $Lin^-CD34^+CD38^{Hi}$ (Progenitors).

B. *The cell of origin in AML*

AML is a heterogeneous disease and it is important to explain from which leukaemia cells are originated {22}. AML blasts are heterogeneous in their differentiation stage and vary in characteristics from patient to patient, whilst the mechanism is not fully understood. There are two hypotheses that may explain this mechanism. The first hypothesis emphasizes on the difference in cell types within the stem/progenitor system that are more prone to transformation {23}. The leukemogenic events alter the normal differentiation process to promote the production of abnormal cells by blocking a particular stage of differentiation {24}. The second hypothesis focuses on mutations responsible for transformation and progression occurring in primitive cells only {25}. The difference in characteristics results from the ability of these primitive LSCs to differentiate or acquire lineage markers depending on the influence of progression related genes {23}. Transplantation experiments in SCID and NOD-SCID have given the idea to develop methods of choice for assessing the engraftment potential and self-renewal capacity of HSC and LSC {17, 18}. Leukaemic cells from patients with different FAB subtypes transfected into SCID and NOD-SCID resulting in successful engraftments representing the original patient's disease having identical morphology with the exception of M3 type {4, 26}. Turhan et al., {27} have explained the failure to engraftment M3 cell using FISH method, and shown that the characteristic PML-RARα translocation in M3 subtype were present only at $CD34^+CD38^+$ fraction and were undetectable in the more primitive $CD34^+CD38^-$ population. Although $CD34^+CD38^-$ cells represent a small proportion of the AML cells (0.2–1%), they are the only cells capable of transferring human AML to NOD/SCID mice. Bonnet and Dick {4} demonstrated that the cells capable of initiating human AML into NOD/SCID mice are known as SL-IC. Regardless of the heterogeneous characteristics of the leukaemic blasts, SL-ICs ($CD34^+CD38^-$) were exclusively similar to the normal SRC phenotype. However, it was surprising to see that SL-ICs were able to differentiate *in vivo* into leukaemic blasts, indicating that the leukaemic clone has organized hierarchy. Bonnet and Dick {4} also showed that LSC expands at a greater extent than normal HSCs, probably because of increased symmetric self-renewal cell divisions.

C. *The cell of origin in CML*

CML is a clonal myeloproliferative disorder; CML stem cells are present in $CD34^+$ $CD38^-$ cells and contain the Philadelphia chromosome {28}. The Philadelphia chromosome is the result of reciprocal translocation between BCR gene on chromosome 22 and ABL gene on chromosome 9, and is specifically designated as t(9;22)(q34;q11) {29}. The protein products of the BCR-ABL chromosomal translocation are $p210^{BCR\text{-}ABL}$ and $p190^{BCR\text{-}ABL}$. LSCs in CML appear to be very similar in characteristics to the normal HSCs. BCR-ABL transcripts in CML bone marrow and HSC in normal marrow both are detected in $CD34^+CD38^-CD90^+$ cells {6, 30}. Studies using SCID and NOD/SCID mouse transplant models have been used to determine the engraftment potential of CML stem cells, which share phenotypic markers with their normal counterparts {31}. A series of studies have defined the CML stem cell immunophenotype as $CD34^+$ and $CD38^-$ {28}. However, higher levels of $CD34^+Lin^-$ cells were found in bone marrow from patients with CML in the accelerated or blast phase than in normal bone marrow {6}. Moreover, some of CML LSCs culture with reduced levels of growth factors display elevated BCR-ABL expression and changes in the expressions of the cytokines interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF), drug transporters ATP-binding cassette-1 (ABC1)/multi-drug resistance-1 (MDR1), ABCG2 and the transcription factor Oct-1 {32}.

D. *The cell of origin in ALL*

ALL is characterized by the malignant expansion of immature cells from lymphoid lineages. Approximately 85% of diagnosed ALL cases result in response to the expansion of B-cell precursors, and 15% of them correspond to T-cell precursor aberrancies {33}. Just like CML, ALL can also result from the rearrangement of BCR and ABL genes in Ph^+ cells. NOD/SCID xenotransplantation studies were used to assess the functional characteristics of $CD34^+CD38^-$ cells in ALL patients carrying the BCR/ABL translocation. Cobaleda et al., {34} showed that only $CD34^+CD38^-$ subfraction from Ph^+ ALL cases could engraft NOD/SCID mice. While another study on leukaemia initiating cells proposed a $CD19^{Neg}$ phenotype in ALL ($CD34^+CD19^-$ and $CD34^+CD10^-$) {35}, Hong et al., {36} demonstrated that leukaemia cells with a committed progenitor phenotype $CD34^+CD38^{Low}CD19^+$ have SL-IC potential. Later on, Kong et al., {37} reported serial transplants of three different primary ALL patient samples $CD34^+CD38^+CD19^+$, $CD34^+CD38^-CD19^+$, and $CD34^+CD38^-CD19^-$ into NOD/SCID IL2rγnull mice. Kong et al., {37} found that $CD34^+CD38^+CD19^+$ and $CD34^+CD38^-CD19^+$ initiate B-ALL in primary recipients.

E. *Leukaemia Stem Cell Properties*

1- *Phenotype.*

It is important to differentiate between HSC and LSC phenotype markers to guarantee therapeutics strategies targeted against the LSC. LSC and HSC share many of the cell surface markers such as CD34, CD38, HLA-DR, and CD71. Blair et al., {38} demonstrated that the majority of AML blasts lack the expression of Thy1 (CD90), which differentiate primitive AML progenitor cells from normal haematopoietic progenitor cells. Similarly, LSC lacks the c-kit (CD117) expression that is not shared by normal HSC.

Jordan et al., {39} showed that the interleukin-3 receptor α chain (IL-3α) (CD123) acts as a unique marker for human AML stem cells. Majority (98%) of the $CD34^{+}CD38^{-}$ AML cells express this marker. Hosen et al., {40} identified CD96 as a surface marker on the majority of $CD34^{+}CD38^{-}$ AML cells with minimal expression on normal $CD34^{+}CD38^{-}$cells. Previous studies suggested that LSCs from most AMLs express early myeloid antigen CD33 {41} and the novel antigen C-type lectin- like molecule- 1 (CLL-1) {42, 43}. However, CD33 might also be expressed by normal HSCs {41}.

2- *Cell cycle*

Cell cycle status is one of the essential parameters that can be used as a marker to differentiate among LSC and other AML blast, as most of the AML-colony forming units (AML-CFU) are active cells and LSCs are quiescent cells. Guan et al., {44} showed that AML stem cells reside mostly in a quiescent cell cycle state, which are analogous to the normal haematopoietic stem cells. This is one of the most important observations to the therapeutic approaches to leukaemia and is directed towards actively cycling populations. The quiescent nature of LSC indicates that standard chemotherapy drugs will not generally be effective against AML stem cells. Furthermore, the relative quiescence of LSCs may be a major factor contributing to relapse {45}. **Guzman et al., {46}** isolated $CD34^{+}CD38^{-}CD123^{+}$ cells to analyze nuclear factor-B (NF-κB) in primitive AML cells; these cells were not stimulated to reflect the status of primitive AML cells. Analysis with flow cytometry revealed that the average proportion of cells in G_0 correspond to 96% ± 2.3%. Terpstra et al., {47} reported evidence for quiescent LSCs, and found that the treatment with the cycle active drug 5-fluorouracil was not effective in ablating AML cells in SCID mice transplanted with primary leukaemic specimens. Moreover, the treatment of CML patients with imatinib mesylate leave behind only the non proliferating quiescent fractions, which are the main cause of the CML {48, 49}.

F. *Mechanism of self-renewal in LSC: Therapeutics targets*

1- *Wnt pathway*

Wnt ligands are glycoproteins that are critical for normal development {50}. β-catenin is a key downstream mediator of the canonical Wnt signaling pathway. Retention of β-catenin at the stem cell membrane may prevent precocious activation of the Wnt signaling pathway which further hinder the ability of the cells to self renew{51}. Wnt stimulation results in accumulation of β-catenin and of which translocation to the nucleus of cell interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF1) to regulate genes that are important in embryonic development and cell proliferation. Initial experiments that studied the role of Wnt signaling in haematopoiesis showed haematopoietic progenitors from mouse fetal liver. It has been noticed that *in vitro* Wnt signaling results in an increase of haematopoiesis by three to four folds when cultured with conditioned media containing Wnt5a {52}. Reya et al., {51} isolated Lin^{-}, c-kit^{Hi}, Sca-1^{Hi} and Thy1.1^{Low} (KTSL) cells from mice over-expressing the anti-apoptotic *BCL-2* gene and transducing them with a constitutively active form of β-catenin. This resulted in 20 to 48 folds *in vitro* expansion of KTSL cells for up to 2 months compared to control KTSL cells that did not survive past 48 hours. Reya et al., {51} also showed that over-expression of activated β -catenin expanded the pool of HSC in long-term culture. Activation of Wnt signaling increases the expression of other transcription factors like HOXB4 and Notch1. These two factors are important for cell cycle regulators in HSC renewal. Moreover, Wnt pathway not only promotes the normal stem cell renewal but also results in the enhancement of LSC proliferation {53}. Many researchers have proved the important role of Wnt pathway signaling in leukaemia and LSC biology {6, 51, 54}. In AML, studies have shown that there is constitutive activation of the Wnt pathway and translocation products, e.g., AML1-ETO, PML-RARa, and PLZF- RARa activate the Wnt signaling pathway in haematopoietic cells {55, 56}. Abrahamsson et al., {57} have shown that progression of blast crisis CML is associated with missplicing of GSK-3β in granulocyte-macrophage progenitors that allows unphosphorylated β-catenin to contribute to self-renewal. In another study, Majeti et al., {58} compared the expression profile of HSC and LSC from patients with AML and identified 3,005 differentially expressed genes. Several pathways were aberrantly regulated in LSCs including Wnt pathway, suggesting possible contribution to pathogenesis. Hu et al., {59} showed, suggesting the role of β -catenin in regulating survival of LSCs, that expression of the β-catenin gene was 2.5 fold up-regulated in LSCs in CML mice. The Wnt pathway can be regulated by different inhibitory factors that play important roles in oncogenesis. The Wnt inhibitory factors-1 (Wif-1) and Cerberus both bind to Wnts and change their ability to bind to the Wnt receptor complex, resulting in inhibition of the Wnt pathway {60}. Aberrant methylation of Wnt antagonists was detected in four AML cell lines and AML marrow samples. Treatment of the cell lines with 5-aza-2′-deoxycytidine induced re-expression of methylated Wnt antagonists by down-regulation of Wnt pathway genes cyclin D1, TCF1 and LEF1 and reducing nuclear localization of β-catenin {61}.

2- *Notch*

Notch signaling pathway has been demonstrated to play crucial regulatory role for self-renewal of HSCs {62}. Studies *in vitro* and *in vivo* have shown that activation of Notch signaling results in increased numbers of HSCs and haematopoietic progenitors. Activation of Notch through ligand binding results in proteolytic cleavage of the intracellular domain of Notch, which subsequently transfers to the nucleus where it acts as a transcriptional regulator. It has been shown that Notch signaling is mediated by both Delta and Jagged ligands through proteolytic cleavage involving α-secretase and γ-secretase. This mediation in Notch signaling expands the HSC compartment by blocking or delaying terminal myeloid differentiation with a decreased interval in the G1 phase of the cell cycle {63}.

Retrovirus mediated expression of activated Notch1 enhances HSC self-renewal and a similar effect of differentiation inhibition and progenitor HSC expansion was reported with activated Notch4 {64}. Varnum et al., {65} and Delaney et al., {66} demonstrated that the incubation of murine BM precursors with the Notch ligand Delta1 extracellular domain fuse to the Fc portion of human IgG1 and promote an increase in precursors capable of short term lymphoid and myeloid repopulation. Duncan et al., {67} showed that the inhibition of Notch signaling leads to accelerated differentiation of HSC *in vitro* and depletion of HSC *in vivo.* Whilst Notch signaling is active in HSCs *in vivo* and down-regulates as HSCs differentiate. It is found that Notch pathway promotes normal stem cell self-renewal and LSC proliferation {53}. Notch1 is found to be constitutively activated in patients with the t (7:9) chromosomal translocation. This involves high expression of a constitutively activated form of Notch1 by the promoter and enhancer elements regulating the β-chain of the T-cell receptor. This distinctive chromosomal translocation is found in <1% of T-cell acute lymphoblastic leukaemia cases and >50% of T-cell acute lymphoblastic leukaemia patients carry somatic activating point mutations of Notch1 {68, 69}. Although Notch has been implicated in many lymphoid leukaemias, no evidence explains the Notch mutations in myelogenous leukaemias {70}. It has been proved previously that γ-secretase is necessary for Jagged and Notch signaling {71}. Inhibition of γ-secretase induced growth arrest and apoptosis of T-ALL cells by two inhibitors: presenilin and mastermind-like-1 (MAML1) through blocking ligand induced Notch proteolysis and signaling {72}. It is proved γ-secretase inhibitor (GSI) MK-0752 inhibits Notch1 pathway, however patients have shown significant gastrointestinal toxicity {73}.

3. *Hedgehog*

Hedgehog (Hh) family of proteins comprises three proteins: Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) and two primary receptors Smoothened (Smo) and Patched (Ptc). Hedgehog signaling plays essential role in HSC regulation, while previous studies reveal that Hedgehog is a cell cycle regulator in HSCs that control haematopoietic regeneration {74}. SHH treatment in CD34 cultures induced expansion of human HSC. Noggin, a specific inhibitor of bone morphogenic protein-4 (BMP-4) inhibits SHH induced proliferation via HSC regulation mechanisms that are dependent on downstream BMP signals {75}. Microarray studies by Graham et al., {76} demonstrated that SHH pathway is active in CML stem cells and becomes more active in progressive phases. Similarly Radich et al., {77} also showed that the activation of SHH pathway correlated with CD34 expression, and suggested up-regulation within CML stem and primitive progenitor cells. Abnormal Hedgehog signaling may also be a feature of AML and MDS. Dierks et al., {78} showed that Hedgehog signaling is activated in Bcr-Abl positive leukaemic stem cells and differentiated haematopoietic cells via up-regulation of Smo. Many natural and synthetic inhibitors have been used to target the Hedgehog pathway such as Cyclopamine, CUR61414, and GDC-0449. Cyclopamine is a plant-derived steroidal alkaloid that binds directly to the trans-membrane helices of Smo and inhibits Hedgehog signaling {79}. Kobune et al., {80} tested Cyclopamine against various leukaemic cell lines including Kasumi-1, Kasumi-3, U937 and HL-60, and found that Cyclopamine caused apoptosis after 48 hrs in Kasumi-1, Kasumi-3 where Hedgehog signaling were active and the downstream effectors glioma associated oncogene homolog GLI1 or GLI2 were expressed. However, cyclopamine failed to affect growth or survival in U937 and HL-60 cell lines that lack expression of Hedgehog receptor components. These findings confirm that the effect of Hedgehog inhibition is specific. Other inhibitors like CUR61414 and GDC-0449 are still in clinical trials.

4. *JAK-STAT*

The JAK-STAT pathway is a common downstream pathway from cytokine receptors and plays important roles in transmitting a variety of biological functions by activating transcription of various target genes. STAT proteins form homodimers or heterodimers upon tyrosine phosphorylation, which are usually mediated by JAKs. Dimerized STAT proteins immediately enter the nucleus and bind to specific DNA sequences in promoter regions of various genes, resulting in gene activation or repression {81}. The STAT5 pathway is activated strongly following ligand binding to the erythropoietin and IL-3 receptors. However, constitutively activating mutants of Flt3 in human AML are associated with activation of approximately 30% of STAT5 {82}. Constitutively activated double mutants of STAT5a transduced into CD34 cells result in enhanced HSC self-renewal and increase erythroid differentiation relative to myeloid {83}. To activate STAT signaling selectively in HSCs and to evaluate the role in normal and leukaemic stem cells, Kato et al., {84} transfected constitutively active STAT5 mutants. They also found that activation of STAT5 in HSCs led to a dramatic expansion of multipotential progenitors and promoted HSC self-renewal *ex-vivo*. In an animal model of myeloproliferative disease (MPD) in mouse revealed that sustained STAT5 activation in HSCs induces fatal MPD, indicating that the capacity of STAT5 to promote self-renewal of HSCs is crucial to MPD development. These findings indicate a specific role of STAT5 in self-renewal of normal as well as LSC. More tractable stem cell systems such as mouse (ES) cells and *Drosophila* germ line stem cells identified a role for STAT transcription factors in promoting self-renewal {85, 86}. STAT transcription factors are the effectors of the JAK-STAT signaling pathway, suggesting that an instructive mechanism can be important for the regulation of self-renewal. Several effective inhibitors have been proposed to target JAK-STAT pathway such as Licochalcone A and Homoharringtonine. Licochalcone A is a flavonoid isolated from the root of Glycyrrhiza induced apoptosis of TEL-Jak2-transformed cells and inhibited the phosphorylation and nuclear localization of STAT3, which is essential for TEL-Jak2 induced cell transformation {87}. Homoharringtonine (HHT) inhibited primary AML cells and AML cell lines induced apoptosis, and the expressions of p-JAK2, p-STAT5, and p-AKT were down-regulated while the total JAK2, STAT5 and AKT protein levels were stable {88}.

5. *NF-κB*

As it is shown that LSC and normal HSC are capable to self-renew, thus to understand the relapse of AML cases, it is essential to explore how HSC and LSC maintain normal haematopoiesis and leukemogenecity, respectively**.** **Guzman** et al., {89} showed that primitive AML cells unusually express the tumour suppressor genes interferon regulatory factor 1 (*IRF1*) and death associated protein kinase (*DAPK*) with an increase in expression of transcription factor NF-κB. This behaviour is not obvious in $CD34^{+}CD38^{-}$ cells from normal specimens. The activation of NF-κB is the phenomenon seen obviously by the leukaemia cells. Guzman reasoned that transcriptional activators NF-κB might lie upstream of IRF-1. Furthermore, it is also seen that NF-κB is activated in the majority of primary AML specimens and LSC quiescent cells. Normal cells do not show this phenomenon, suggesting this transcription factor as a key survival factor and a leukaemia specific phenomenon for the LSC {90}. Several drugs have been investigated to target NF-κB such as proteosome inhibitor (salinosporamide A and bortezomib) and parthenolide. **Guzman** et al., {91, 92} treated AML and CML stem cells with parthenolide and found them to be more specific to leukaemia cells than the standard chemotherapy drug cytosine arabinoside. The molecular mechanism of parthenolide mediated apoptosis was associated with great inhibition of NF-κB through inhibition of IκB degradation. Salinosporamide (NPI-0052) was used by Miller et al., {93} and showed the inhibition of proteolytic activities in proteosome with an increase in apoptosis in Ph+ ALL leukaemia.

6. *TGF-β*

The TGF-β superfamily of growth factors regulates a wide variety of biological functions such as proliferation, differentiation, migration, and apoptosis. TGF-β is the key member of the TGF-β superfamily and exists in mammals in three isoforms: TGF-β1, TGF-β2, and TGF-β3. Factors such as TGF-β1 and macrophage inhibitory protein-1α (MIP-1α) are found to play a vital role in dampening of haematopoietic cell growth kinetics {94, 95}. Particularly, TGF-β1 has been found to selectively inhibit the growth of HSCs and progenitor cells {96, 97, 98, 99}. TGF-β2 has demonstrated to function as a positive regulator of HSCs, while TGF-β3 only functions as an inhibitor on primitive haematopoietic cells {100, 101}. Inhibition of the cell cycle by TGF-β is thought to be mediated in part by down-regulation of proliferative proteins, such as c-*myc*, coupled with up-regulation of cell cycle inhibitory proteins, such as p15, p21, or p27 {102}. Studies have shown that p27 is essential for TGF-β mediated proliferation inhibition of primitive haematopoietic cells {103}. However, using a siRNA it was shown that p57 is crucial for TGF-β mediated cell cycle arrest in haematopoietic cells {104}. Up-regulation of TGF-β/Smad pathway has been shown in many types of leukaemia and cancers. Bcr-Abl expression increases TGF-β/ Smad transcription activity in Cosl cells, and this may be due to enhancement of Smad promoter activity that results in leukaemic cells escaping the TGF-β mediated inhibition of cell proliferation {105}.

Several products have been used to target TGF-β1 such as Geldanamycin and SM16. Geldanamycin (GA) inhibitor has been shown to suppress TGF-β signalling, and degrade TGFβ type I and type II receptors through a proteasome dependent pathway {106}. SM16 small molecule ALK5 kinase inhibitor has been also tested against TGF-β in murine mammary carcinoma cell line (4T1) and mice model. SM16 was able to blockade TGF-β signal transduction, prevented TGF-β induced morphological changes and inhibited TGF-β induced invasion {107}.

7. *PTEN*

PTEN (Phosphatase and tensin homologue) is a tumour suppressor gene that negatively regulates signaling through the phosphatidylinositol-3-OH kinase (PI-3K) pathway. Zhang et al., {108} showed that PTEN maintains the haematopoietic stem cells. It is also seen that PTEN deficient HSC successfully engrafted in recipient mice but they were unable to maintain the haematopoietic reconstitution. These findings reflect the dysregulation of their cell cycle and decreased retention in the bone marrow niche. To examine the role of PTEN in HSC, Yilmaz et al., {53} have deleted the PTEN tumour suppressor gene in HSC and showed that this deletion caused proliferation of HSC and led to myeloproliferative disease within days and ALL and AML leukaemias within weeks. It is likely that these effects were modulated by mammalian target of rapamycin (mTOR), which is a serine/threonine protein kinase that regulates cell growth, proliferation, motility, survival, protein synthesis, and transcription. Rapamycin not only depleted LSCs but also restored normal HSC function, providing a mechanism through which LSCs can be selectively targeted, while maintaining the function of normal HSCs {53}. The observation of eliminating LSC by rapamycin has no effect on HSC and this suggests that rapamycin and its analogues may be used to treat cancers that exhibit increased PI-3K pathway activation.

8. *HOX*

HOX genes (including A, B, C, D clusters) encode transcription factors that are important regulators of hematopoiesis. HOXB4 over-expression in particular is identified to drive high level *ex vivo* HSC expansion. It was seen that the number of HSC in mice transplanted with virally HOXB4 transduced HSC is significantly higher (14-fold on average) than in mice transplanted with normal untransduced mice {109}. Antonchuk et al., {110} demonstrated the potency of HOXB4 to enable high level *ex vivo* HSC expansion. Over-expression of HOXB4 can result in a rapid and extensive *in vitro* expansion of polyclonal HSCs that retain full lympho-myeloid repopulating potential and result in enhanced *in vivo* regenerative potential. It has been seen that co-cultures of human stem cells on stromal cells secreting HOXB4 underwent a 20-fold increase in long term initiating cells (LTC-IC) and a 2.5-fold increase in SRC {111}. Similar results were obtained when mouse BM cells were grown on stromal cells engineered to express HoxB4 preceded by the human immunodeficiency virus (HIV)-transactivating protein, TAT {112}.

Retroviral-mediated ectopic expression of HOXB4 resulted in a rapid increase in proliferation of murine HSC both *in vivo* (1000-fold increase in transduced HSC in a murine transplant model) and *in vitro* (40-fold expansion of murine HSC*)* with retention of lymphomyeloid repopulating potential and enhanced regenerative capability in mice {113}. However, high levels of HOXB4 expression in human umbilical cord blood (CB) CD34 cells have recently been reported to either increase proliferation of HSC or inhibit differentiation {114} in order to direct the cells toward a myeloid differentiation program rather than increasing proliferation {115}. These studies suggest that in human haematopoietic progenitors, HOXB4 affects cell fate decisions (self-renewal, differentiation, or differentiation block) in a concentration dependent manner. Many HOX genes are linked with the development of acute leukaemia, and chromosomal translocations between NUP98 and HOXA9 or HOXD13 are reported in AML {116, 117} with over-expression of HOXA9 carrying a particularly poor prognosis {118}. Over-expression of HOX11 has been reported in T-ALL {119}. It has been suggested that self-renewal in LSCs may be regulated by HOX dependent pathways {120}. A recent study by Andreeff et al., {121} has demonstrated that low levels of HOX expression are characteristic of favourable cytogenetic AML t(15;17) and t(8;21), while they tend to be higher in intermediate cytogenetic AMLs inv(16) or t(16;16) with both NPM and Flt3 mutations as compared to NPM mutation alone. Melanoma B16 cells, having over-expressed Hox genes, have been treated with HXR9 that antagonizes the interaction between HOX and a second transcription factor (PBX) that modifies HOX activities. This led to increase apoptosis and blocked the growth in B16, primary cells and mice model. Results also showed that HXR9 has no toxicity on mice and HSCs continued to grow and proliferate {122}.

9. *Bmi-1*

The Polycomb-group transcriptional repressor gene Bmi-1 was implicated in HSC maintenance, and loss of function showed profound defects in HSC. Park et al., {123} showed that Bmi-1 is highly expressed in purified HSC and its expression declines with differentiation. Bmi-1 represents one of the best-characterized Polycomb family members with respect to function within multiple stem cell types. Bmi-1 deficient mice exhibited a reduction in phenotypically defined HSCs. These cells engraft poorly and exhaust prematurely in serial transplantation experiments. It has been seen that both gain and loss of function experiments have demonstrated that Bmi-1 plays a vital role in HSC self-renewal, with an initial focus on the role of Bmi-1 as a negative regulator of the CDK inhibitor, $p16^{INK4\alpha}$ and/or $p19^{ARF}$ {123}. Bmi-1 has an essential role in regulating the proliferative potential of LSC. Previous experiments with Bmi-$1^{-/-}$ mice showed that leukaemic (AML) stem/progenitor cells lacking Bmi-1 were unable to engraft and proliferate and displayed signs of differentiation and apoptosis. Conversely, the reconstitution of the Bmi-1 gene was found to completely abrogate these proliferative defects {124}.

Bhattacharyya et al., {125} investigated the expression of Bmi-1 in CML $CD34^{+}$ cells at each of the chronic phase (CP), the accelerated phase (AP), and blastic crisis (BC) by flow cytometry, and found that the level of Bmi-1 expression was significantly higher in CP than in controls and was further increased during the course of the disease progression.

Functionally, Bmi-1 forms a heterodimeric complex with another PcG protein. PcG complexes bind to chromatin and initiate/maintain gene repression that is thought to be mediated by methylation, deacetylation, and ubiquitination of core histones. Bmi-1 and Ring1b reconstitute an ubiquitin E3 ligase activity with histone H2A as their ubiquitination substrate {126}. Thus, inhibition of methylation, histone deacetylase inhibitors, or inhibitors of the ubiquitin proteasome system could be exploited as anti–Bmi-1 strategies in LSCs.

10. *Telomere*

Telomeres are regions of double stranded DNA, consisting of repetitive T2AG3 sequences. They are found at chromosome ends, and were first described in 1930s as essential components that stabilize chromosomal ends {127}. Three critical observations have been noted; first, telomere length decreases with every cell division, second, telomeres from older tissues are shorter than younger tissues, and third, telomere length is reduced in tumours as compared to adjacent normal tissue {128}. Telomere length is maintained by a balance of processes that lead to shortening or lengthening of the telomere sequence. The main factor, which elongates telomeres, is the enzyme telomerase. Low levels of telomerase expression are seen in primitive HSC and in lymphocytes {129}. However, in tissue culture, when human cell lines are immortalized, telomerase expression is greatly upregulated and high levels of telomerase are seen in 90 percent of human cancers {130}. Studies have shown that telomeres are shorter and telomerase activity higher in CML LSCs as compared to normal HSCs {131}. This raises the possibility of exploiting differences in telomerase activity to target LSCs. Drugs regulating telomerase activities in clinical trials include arsenic trioxide, GRN163L, and hTERT vaccines. In one study, GRN163L was used to inhibit lung cancer. Using A549-luciferase′ GRN163L effectively inhibited telomerase activity, resulting in progressive telomere shortening {132}.

CONCLUSION

The SRC, SCID and NOD/SCID xenotransplantation assays have helped researchers to identify many cell surface markers to understand the biology of human haematopoiesis and leukomogenesis. Intensive studies with microarray experiments have demonstrated that the deregulated pathways such as Wnt, STAT, and NF-κB play critical roles in sustaining leukomogenesis and self-renewal activity. Chemotherapy drugs such as cytarabine and daunorubicin, and other kinase inhibitors such as imatinib have been approved for patients with AML or CML. Unfortunately, many of these patients tend to have a relapse since standard chemotherapy are not effective

against LSCs because majority of LSCs exist in a quiescent state, which may be a major factor contributing to relapse.

The era of targeted therapy has almost arrived to eradicate leukaemic and other cancer stem cells. Several targeted therapies have been proposed to target the deregulated targets. Some of them have been approved by US Food and Drug Administration (FDA) such as proteasome inhibitor bortezomib, while others are still in clinical trials such as parthenolide, and GRN163L. The combination of standard chemotherapy and targeted therapy may be a good strategy to eradicate leukaemia stem cells.

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