Malaysian J Pathol 2012; 34(2) : 77 – 88

REVIEW

Leukaemic stem cells: Drug resistance, metastasis and therapeutic implications

Rebecca SY WONG and Soon-Keng CHEONG*

*Faculty of Medicine, SEGi University, Selangor, Malaysia and *Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, Bandar Sungai Long, Selangor, Malaysia*

Abstract

Although there have been many new developments in the treatment of leukaemia with the use of new anti-leukaemic agents and stem cell transplantation, drug resistance and treatment failure remain a great challenge for the attending physician. Several studies have suggested that leukaemic stem cells (LSCs) play a pivotal role in chemoresistance and metastasis and the mechanisms by which these cells do so have also been elucidated. There is increasing evidence to show that there exists a large pool of therapeutic targets in LSCs and that the eradication of these cells is feasible with some promising results. This article gives an overview of different types of cancer stem cells (CSCs) derived from various types of leukaemia, the mechanisms by which LSCs contribute to drug resistance and metastasis and some recent advances in targeted therapy against LSCs.

Keywords: Leukaemic stem cells, drug resistance, metastasis, therapeutic targets

INTRODUCTION

The leukaemias can be defined as malignant neoplasms involving cells that are originally derived from haematopoietic precursor cells. The bone marrow is diffusely replaced by abnormally proliferating neoplastic cells. The neoplastic cells may in turn escape into the blood where they may be present in large numbers, resulting in the clinical presentations of the disease. Generally, the leukaemias can be divided into four main groups, namely acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL) and chronic myeloid leukaemia (CML).[1] Although recent advances in stem cell transplantation have brought new hope to sufferers of the disease, the treatment of leukaemia still largely revolves around chemotherapy. However, researchers have noted considerable resistance even with the use of newly approved drugs such as the tyrosine kinase inhibitor, imatinib. Such resistance has been tied to the existence of a subpopulation of cells which are distinct from the less stubborn leukaemic cells and are called the leukaemic stem cells (LSCs).[2] The concept of cancer stem cells (CSCs) was proposed long before imatinib revolutionised CML treatment.[3] Although it remains debatable that CSCs exist and are important players of carcinogenesis, metastasis and drug resistance, literature on the existence of CSCs, particularly in leukaemia is abundant and ever-increasing. This review therefore gives an overview of LSCs from several types of leukaemia with an emphasis on their role in drug resistance, metastasis and their therapeutic implications

1. CANCER STEM CELLS AND LEUKAEMIC CANCER STEM CELLS

The concept of CSCs has been a topic of debate among researchers. While some believe that CSCs are responsible for carcinogenesis, metastasis and even drug resistance in certain cancers, others do not believe in the existence of CSCs to begin with. Although the theory of CSCs was first proposed more than 40 years ago,[3] the first conclusive evidence of CSCs was published by Bonnet and Dick in 1997. The authors reported the isolation of a subpopulation of leukaemic cells which lacked CD38 marker but expressed a specific marker, CD34. When injected into NOD/SCID mice, this CD34+/CD38- subpopulation of cells were able to cause tumour which was histologically similar

Address for correspondence and reprint requests: Rebecca SY Wong, Faculty of Medicine, SEGi University, No. 9, Jalan Tecknologi, Taman Sains Selangor, Kota Damansara, PJU 5, 47810 Petaling Jaya, Selangor, Malaysia Phone: +603- 6145 2777 (ext 3064). Fax: +603-6145 1666. Email: rebecca@segi.edu.my; rebeccawongsy@gmail.com

to that of the donor.[4] The discovery of CSCs in haematological malignancies had then led to exciting encounters of CSCs in solid tumours such as breast and brain CSCs.[5,6]

Currently, LSCs are believed to arise from three sources: (1) normal haematopoietic stem cells, (2) more differentiated progenitor cells that have become malignant and (3) de-differentiation of progenitor cells which have re-acquired stem cell-like properties.[7,8] Whatever the origin of LSCs, these cells retain some of the key features of a stem cell, i.e., they are capable of self-renewal and proliferation. However, they lack the ability to properly differentiate. Hence LSCs may play an important role in the pathogenesis of leukaemia. These unique properties of LCSs result in the production of abnormal leukaemic cells with uncontrolled proliferation. Increasing new knowledge of LSCs has at least two important implications: (1) it allows researchers to look into CSCs of other cancers, using LSCs as a template or foundation and (2) it gives new insights into the development of targeted therapy against LSCs and CSCs.

2. LEUKAEMIC STEM CELLS FROM VARIOUS TYPES OF LEUKAEMIA

The haematopoietic system is probably one of the best-studied systems as far as stem cell biology is concerned. The past few decades marked many important milestones in the development of haematopoietic stem cell research, and the resulting findings are useful and applicable in other areas of stem cell research. CSCs have been increasingly identified in various types of leukaemia especially in AML, CML and to a lesser extent, ALL.

2.1 Acute myeloid leukaemia stem cells

Studies have shown that when primary AML cells were transplanted into SCID or NOD/SCID mice, only very few cells were capable of initiating and sustaining the leukaemic clone *in vivo*.[4,9] These rare cells were termed SCID leukaemia-initiating cells (SL-ICs). Other than their ability to differentiate and proliferate, SL-ICs have also been reported to possess high self-renewal capacity. Therefore, they can be considered as AML stem cells. More importantly, these cells can be isolated and purified as CD34+/CD38- cells from AML patients and are the only cells capable of re-growing in recipient mice.[4,9] Although LSCs from AML share some similarities with normal haematopoietic stem cells (CD34+, CD38-, CD71-, HLA-DR-), at least three markers were reported to be unique to AML CSCs, i.e. CD90- , CD117- and CD123+.[10] Also, LCSs from AML demonstrated several molecular differences when compared to normal haematopoietic stem cells. For instance, in one study two tumour suppressor genes, interferon-regulatory factor-1 (irf-1) and death-associated protein kinase-1 (dapk-1), were consistently demonstrated in purified CD34+/CD38- from seven AML patients.[11] In another study, it was reported that the active form of NK-κB was expressed in LSCs from AML but absent in normal primitive haematopoietic cells.[12] However, the role of these molecular differences in AML awaits further exploration and evaluation.

2.2 Chronic myeloid leukaemia stem cells

As early as the 1970s, CML had been reported to have a stem cell origin.[13] However, with the use of long-term culture-initiating cell (LTC-IC) assays, it was further demonstrated that multipotent stem cells of malignant origins were present in patients with CML.[14] CML stem cells have also been demonstrated to engraft in SCID and NOD/SCID mice and they have similar phenotypic markers when compared to their counterparts.[15,16] Although the use of imatinib in the treatment of CML has resulted in an improved survival and an induction of complete cytogenetic responses (CCRs) in 80% of newly diagnosed chronic phase patients,[17] a vast majority of patients who have achieved CCRs have been found with RT-PCR detectable BCR-ABL transcripts.[18] Even in those who are negative for BCR-ABL by RT-PCR eventually experience a relapse upon discontinuation of imatinib.[19] The resistance to imatinib has largely been linked to the persistence of CML stem cells which renders the disease difficult to cure. The existence of CML stem cells has been further evidenced by the identification of BCR-ABL positive cells amongst CD34+ progenitor cells from CCR patients and the identification of LSCs in those who have achieved CCR.[2] In addition, CML stem cells have also been reported to be resistant to apoptosis which involved aberrant expression of apoptosis-regulatory proteins belonging to the Bcl-2 family such as Bcl-2 and Mcl-1 (anti-apoptotic) and Bad and Bim (pro-apoptotic).[20] As CML stem cells have been associated with disease progression, blast crisis and imatinib-resistance, further exploration of the

molecular aspects of CML stem cells may give new insights into targeted therapy of CML.

2.3 Acute lymphoblastic leukaemia stem cells

Although LSCs have been largely studied in myeloid leukaemias, these cells are not exclusive to myeloid leukaemias as they have also been identified in ALL. In one study, LSCs have been detected in Philadelphia-positive ALL.[21] The Philadelphia abnormality can be detected in 5% to 25% of ALL with the majority occurring in the B-cell lineage. Cobaleda *et al* demonstrated the presence SL-ICs in Ph+ ALL as evidenced by their ability to initiate Ph+ ALL in NOD/SCID mice, as well as the ability to differentiate, proliferate and self-renew. These cells were also found to be exclusively CD34+/CD38- and were phenotypically similar to their normal counterparts. The study therefore concluded that it was the normal primitive cells that were responsible for leukaemic transformation in Ph+ ALL instead of the committed progenitor cells.[21] In another study, George *et al* detected leukaemic cells in the CD34+/CD38- bone marrow progenitor population in children with ALL, which suggested that ALL may arise from a population of stem cells and these stem cells may in turn be the culprit of drug resistance.[22]

3. ROLE OF LEUKAEMIC STEM CELLS IN DRUG RESISTANCE AND METASTASIS

Drug resistance and metastasis are two important contributors of treatment failure in leukaemia whereas it is generally accepted that LSCs are one of the factors that maintain leukaemia.[23] Most of the conventional anticancer drugs have been designed to kill leukaemic cells based on the general biological properties of malignant blast cells. However, conventional anticancer drugs may not work on LCSs for the following reasons: (1) they are relatively quiescent,[23] (2) they exhibit an array of self-protecting mechanisms[24] and (3) they reside in a highly protective microenvironment.[25] In addition, LSCs may play a role in metastasis. This Section focuses on the role of LSCs in drug resistance and metastasis.

3.1 Leukaemic stem cells and drug resistance

3.1.1 Involvement of cell surface proteins

Several studies have been carried out to investigate the role of LCSs in drug resistance and the mechanisms by which they do so have also been elucidated. Figure 1 gives a summary of these mechanisms. In one study which aimed to investigate the relationship between multi-drug resistance and proportion of LCSs in drug-resistant K562/ADM cells, Yi *et al* demonstrated that the amount of CD34+, CD123+ and CD34+ CD38- cells in K562/ADM cells was 4.12-fold higher than that of drug-sensitive K562 cells.[26] In addition, the expression of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) was 11.25-fold higher in drug-resistant K562/ADM cells when compared to that of drug-sensitive cells. There was also a higher proportion of CD34+ CD38- CD123+ BCRP+ and CD34+ CD38- P-gp+ BCRP+ cells in K562/ADM cells (3.66- and 11.37-fold) when compared to that of drug-sensitive cells. The drug-resistant cells further demonstrated a 4.17-time greater colony-forming ability over the drug-sensitive cells. The study concluded that the ATP-binding cassette (ABC) transporters-overexpressing LSC population exists in drug-resistant K562/ADM leukaemic cells may contribute to chemoresistance in leukaemia.[26]

Jeong *et al* further confirmed the role of ABC transporters in LSC chemoresistance. The study demonstrated that SALL4, a stem cell factor which plays a critical role in embryonic and LSCs, was highly expressed in side populations of CSCs by 2-4 folds in various malignant haematopoietic cells lines. Its expression was higher in primary AML patients who experienced drug resistance than those who were drug responsive. Knock down of SALL4 showed a reduction in side population cells, suggesting that self-renewal was dependent on SALL4. The effects of SALL4 were mediated by regulation of the ABC drug transporter genes such as ABCG2 and ABCA3. The study concluded that SALL4 plays a role in drug resistance through the maintenance of side population CSCs.[27]

3.1.2 Involvement of signaling pathways

β-catenin is a subunit of a cadherin protein complex and has also been shown to play a role in the Wnt signaling pathway,[28] which involved a large number of proteins responsible for cell differentiation and cell polarity generation.[29] In mixed lineage leukaemia (MLL), it has been demonstrated that β-catenin was activated during the development of MLL LSCs. However, MLL LSCs could be reversed to a pre-LSC-like stage with the suppression of β-catenin and such suppression reduced the growth of MLL

leukaemic cells. Suppression of β-catenin also re-sensitised MLL LSCs which were resistant against GSK inhibitors whereas deletion of β-catenin eliminated the oncogenic potential of MLL-transformed cells.[30] In addition, CD27, a member of the TNF receptor family, has also been found to be involved in the Wnt signaling pathway in chronic myelogenous LSCs by activating Wnt target genes and promote disease progression.[31]

Besides the Wnt signaling pathway, several other pathways have also been implicated in LSC chemoresistance. Two examples of such pathways are the Phosphoinositide 3-kinase(PI3K)/akt and the Hedgehog (HH) pathways. The PI3K/akt pathway is an intracellular pathway that plays a critical role in apoptosis and cancer and its components are often altered in cancer, leading to dysregulated apoptosis and chemoresistance.[32] Mammalian target of rapamycin (mTOR), a substrate of PI3K has been reported to regulate the survival of AML stem cells after etoposide treatment. Xu *et al* showed that inhibition of mTOR with rapamycin significantly decreased AML cell survival and the effects of etoposide in these cells were enhanced by rapamycin.[33]

The Forkhead Box O class (FOXO) proteins are transcription factors belonging to the Fox superfamily.[34] They are involved in many signaling pathways and biological activities, including cellular differentiation, tumour suppression, metabolism and cell death.[35, 36] In CML, it is believed that BCR-ABL activates Akt signaling that suppresses FOXO, which supports the proliferation and inhibits apoptosis in CML cells. Naka *et al* observed enrichment of cells with nuclear localisation of FOXO3a and decreased Akt phosphorylation in leukaemic-initiating cell (LIC) population. In the absence of FOXO3a, there was a reduced ability of LIC to cause disease. In addition, TGF-β was found to regulate Akt signalling and control FOXO3a localisation and that imatinib could efficiently deplete CML cells with TGF-β inhibition and FOXO3a deficiency, suggesting that TGF-beta-FOXO signaling maintains LICs in CML and may play a role in drug resistance.[37]

Hedgehog molecules were first discovered in Drosophila. Currently there are three types of HH molecules, namely Desert Hedgehog (Dhh), Indian Hedgehog (Ihh), and Sonic Hedgehog (Shh). Several cell proliferation processes like pattern formation, stem cell maintenance and tumorigenesis are regulated by the HH pathway.[38] Kobune *et al* reported that there was a high level of HH signaling in several AML cells, especially the CD34+ cells, as evidenced by the expression of the downstream effectors glioma-associated oncogene homolog (GLI)1 or GLI2.[39] As the HH pathway controls the cell cycle fate during cell proliferation, its activation in LSCs may promote tumour repopulation post chemotherapy, contributing to chemoresistance.[40]

The role of the expression of various microRNAs (miRNA) which are important in LSC differentiation and drug resistance has also been investigated. In one study, miRNA microarray analysis was carried out in childhood ALL cells by Han *et al*.[41] Three miRNAs, miR-708, miR-223 and miR-27a were found to be associated with the risk of relapse in childhood ALL and miR-708 was further found to be associated with glucocorticoid therapy response. These miRNAs are known to be associated with oncogenesis, classical multidrug resistance pathways and LSC self-renewal and differentiation pathways.[41] For example, the study identified BMI1 as a bona fide target of miR-27a. This coincides with findings of an earlier study, as BMI1 was demonstrated to play a critical role in the regulation of self-renewal in LSCs and that the deletion of BMI1 could prevent such self-renewal as well as recurrence of leukaemia.[42] Other targets of the miRNAs validated by Han *et al* include FOXO3 and E2F1.[41]

3.1.3 Involvement of the leukaemia stem cell niche

The LSC niche has been an area of intensive research. It has been previously reported that chemotherapy-resistant human AML stem cells home to and engraft within the bone marrow endosteal region.[43] Adhesion molecules, such as CD44, which is a key regulator of AML stem cells, has been reported to play a part in LSC niche and that when CD44 was targeted, there was a reduction in leukaemic population.[44] Using the cobblestone area -forming culture system, in which LSCs adhere to bone marrow-derived stem cells, Funayama *et al* demonstrated adhesion-induced drug resistance in LSCs via various mechanisms. The adhering leukaemic cells were less sensitive to drugs such as cytarabine, etoposide and daunorunicin. Other associated changes observed include an increased proportion of the G0/G1 phase, increased upregulation of cyclin-dependent kinase inhibitors, and increased levels of Bcl-2. However, the expression of BAX or drug transporters like ABCG2 and MDR1 remained unchanged.[45]

3.1.4 BCR-ABL independent mechanisms

The BCR-ABL gene is a fusion gene found almost in all CML patients and it gives rise to constitutive tyrosine kinase activity of ABL and results in dysregulation of multiple signaling pathways involved in cell proliferation and apoptosis.[46] The use of the BCR-ABL selective kinase inhibitor, imatinib, in chronic-phase CML has revolutionised CML treatment and has greatly improved the median survival.[47] However, it has been demonstrated that CML stem cells are resistant to imatinib therapy. Corbin *et al* reported that human CML stem cells do not depend on BCR-ABL activity for survival and are therefore, not eliminated by imatinib therapy. Despite inhibition of BCR-ABL activity, the growth and survival of CML stem cells were supported by cytokines.[48] Therefore, targeting BCR-ABL activity alone may not be efficient in eliminating CML stem cells.

3.2 Role of leukaemic stem cells in motility and metastasis

Many studies have investigated the role of CSCs in metastasis. It has been suggested that CSCs are not just responsible for tumourigenesis, there are subpopulations of cells which express C-X-C chemokine receptor type 4 (CXCR4), which are capable of disseminating.[49] The specific ligand of the CXCR4 chemokine receptor is known as stromal-derived-factor-1 (SDF-1, also called CXCL12). Both CXCR4 and SDF-1 are expressed in various tissues and cell types which regulate cell mobilisation, migration, proliferation and survival.[50] The SDF-1/CXCR4 axis has also been implicated in the migration of stem cells, whether they are normal stem cells or cancer stem cells, as it is believed that the trafficking of normal and cancer stem cells share many similar mechanisms.[51] Hence, it

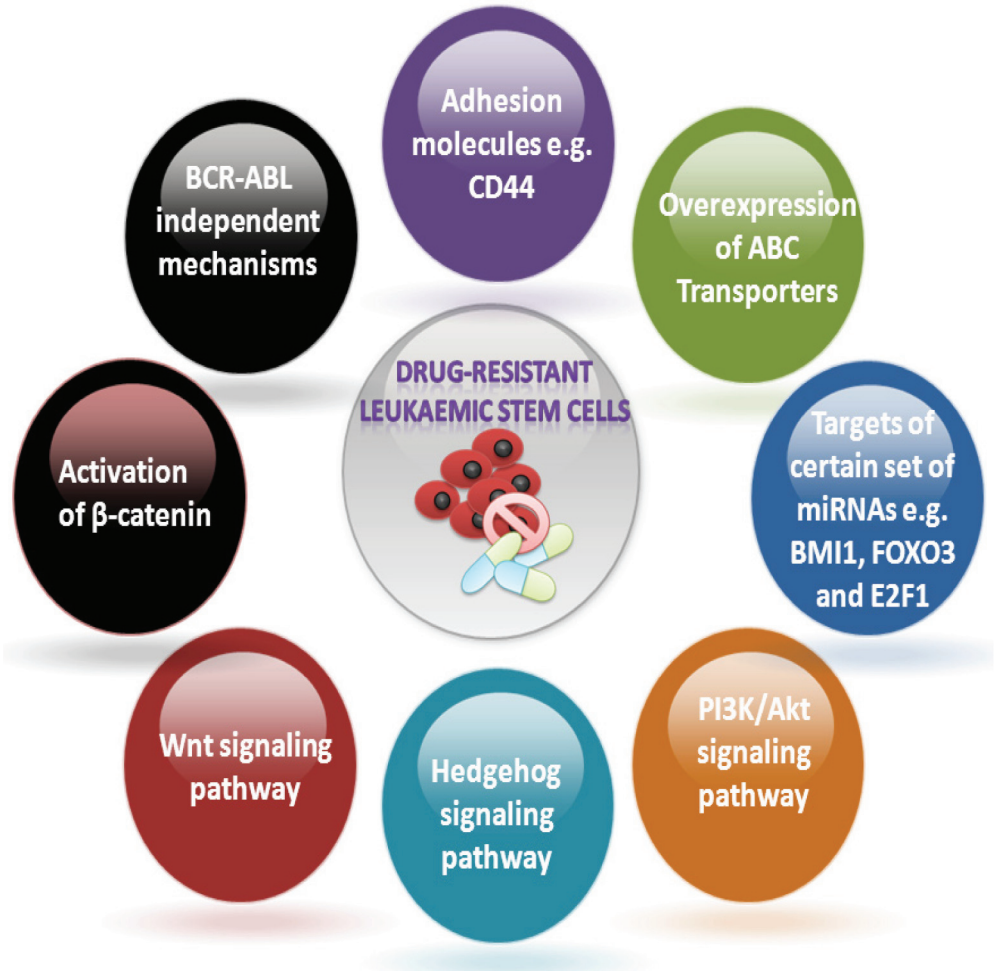

FIG. 1: Mechanisms involved in drug resistance induced by leukaemic stem cells.

can be said that the expression of CXCR4 leads to CSCs being retained in SDF-1-rich tissues, enhancing growth and metastasis.

The exact mechanism by which LCSs contribute to metastasis is largely unknown. Currently, direct evidence linking LSCs to metastasis is lacking. Despite the scarcity of literature on the role of LSCs in metastasis, there are a few sporadic reports that suggest that LSCs may play a role in metastasis. In AML patients, it has been demonstrated that a low level of CXCR4 expression was correlated with a better prognosis, a longer relapsed-free period and overall survival. It was also suggested that CXCR4 is an independent prognostic predictor for disease relapse and survival.[52] Tavor *et al* demonstrated that the SDF-1/CXCR4 axis was important in the regulation of *in vivo* motility and development of human AML stem cells. When cultured with CXCR4 antibodies, SDF-1 antibodies or AMD3100, a significant decrease in AML cell survival was observed. In addition, homing into the bone marrow and spleen of transplanted NOD/SCID/B2m^{null} mice was blocked. There was also a dramatic decrease in human AML cells in the bone marrow, blood and spleen with weekly administrations of antihuman CXCR4 to mice engrafted with primary AML cells.[53]

Several studies have suggested that the stem cell niche may be involved in metastasis. Deng and Zhang hypothesized that there exists a "metastatic niche" which facilitate the survival, proliferation and metastasis of LSCs[54] whereas Kaplan *et al* demonstrated that vascular endothelial growth factor receptor 1 (VEGFR1) was involved in the initiation of a pre-metastatic niche and that cells expressing VEGFR1 home to tumour-specific pre-metastatic sites and form cellular clusters before the arrival of tumour cells. On the other hand, antibodies targeting VEGFR1 could prevent the formation of pre-metastatic clusters and tumour metastasis, suggesting that a permissive niche facilitates metastasis.[55] The influence of the LSC niche on leukaemogenesis has been reviewed by Lane *et al.*[25]

4. THERAPEUTIC IMPLICATIONS OF LEUKAEMIC STEM CELLS

There is ample evidence to show that LSCs indeed play a role in drug resistance, metastasis, relapse and treatment failure in leukaemia. This means that killing the leukaemic cells by conventional anticancer agents alone may not be sufficient to treat leukaemia. Instead, killing the differentiated cancer cells often enrich the survival of the LSCs and preferentially preserve these cancer stem cells. In one study, it has been shown that the application of differentiation therapy for the treatment of acute promyelocytic leukaemia showed reduced self-renewal capacity and improved patient survival.[56] Each of the contributing factors of LSC drug resistance and metastasis mentioned in Section 3 can be viewed as a double-edged sword. On one hand, they are the obstacles in leukaemia treatment; on the other hand, they give rise to a large pool of therapeutic targets which may be specifically aimed at while designing treatment for leukaemia. Hence attention should be turned to the stubborn LSCs which are the culprit of the problem. Table 1 gives a summary of the therapeutic strategies targeting LSCs.

4.1 Targeting signaling pathways

Various signaling pathways have been targeted in the eradication of LSCs and have been previously reviewed.[57,58] For example, hedgehog inhibitors have been found to dramatically reverse drug resistance in CD34+ leukaemic cells. Using naturally-derived Smoothened antagonist cyclopamine, hedgehog-interacting protein or anti-hedgehog neutralising antibody, induction of apoptosis in CD34+ leukaemic cells was observed with cyclopamine, showing a marked reduction in drug resistance to cytarabine (Ara-C).[39]

Another pathway which has been targeted is the PI3K/Akt/mTOR pathway. A dual PI3K/mTOR inhibitor, PI-103, has been reported to target CD34+/CD38-/CD123+ AML cells, a subpopulation that is enriched in LSCs.[59] It has also been indicated that blocking CD27, which is involved in Wnt signaling in CML stem cells, could delay disease progression and prolong patient survival[31] whereas the targeting of Rac signaling pathway by pharmacologic or genetic methods led to rapid and specific apoptosis of MLL-AF9 expressing CD34+ cells.[60]

4.2 Targeting cell surface proteins

Several proteins such as IL-1 receptor accessory protein (IL1RAP) have been targeted in published literature. Järås *et al* reported that using an anti-IL1RAP antibody, it is possible to target CML CD34+ CD38- cells and induce cell-mediated cytotoxicity against these cells.[61] Siglect-3 (CD33), a cell surface antigen, is another popular therapeutic target. Thus far, CD33 has

been found in AML stem cells,[62] and CML stem cells.[63] Several attempts to eradicate CD33 expressing LSCs have been made and the results are encouraging. For example, AML patients who derived most long-term benefits from the immunoconjugate gemtuzumab ozogamicin (Mylotarg) were found to have a better response to conventional anti-leukaemic treatment.[64] Such CD33-targeted immunotherapy has also been applied to CML stem cells. Herrmann *et al* reported that CD34+/CD38- cells from CML patients abundantly expressed CD33 and that gemtuzumab ozogamicin was capable of inhibiting growth in leukaemic progenitor cells obtained from these patients. *In vitro* studies revealed dose-dependent apoptosis induction and growth inhibition with the use of low concentrations of gemtuzumab ozogamicin. Synergistic effects with nilotinib and bosutinib were also observed.[63]

However, not all attempts to eradicate LSCs were successful. Although several studies have demonstrated the pivotal role of the ABC transporters in multidrug resistance in LSCs, targeting these transporters is challenging. Several studies have explored the feasibility of the use of ABC transporters inhibitors such as cyclosporine and zosuquidar. However, their clinical efficacy was found to be limited, largely because the normal haemotopoietic stem cells also highly express these transporters, making them equally susceptible to the ABC transporter inhibitors.[57]

4.3 Targeting the leukaemia microenvironment

Another potential battlefield for anti-leukaemia treatment is the leukaemia microenvironment. Evidence has shown that cross talks between LSCs and the bone marrow microenvironment exist and that targeting the latter may be a feasible approach in leukaemia treatment. For example, studies have shown that the expression of CXCR4 leads to CSCs being retained in SDF-1-rich tissues, enhancing growth and metastasis. Using the CXCR4 inhibitor AMD3465, Zeng *et al* demonstrated that kinase resistance could be overcome in AML patients. In animal studies, AMD3465 induced mobilisation of AML cells and progenitor cells into circulation where the anti-leukaemic effects of chemotherapy were enhanced, reducing leukaemia burden and prolonging survival of the animals. Hence disruption of the CXCR4 interactions with the microenvironment may be strategic in reversal of chemoresistance in leukaemia.[65]

In addition, adhesion molecules like CD44 are also therapeutic targets in the eradication of LSCs. Jin *et al* reported that an activating monoclonal antibody directed to CD44 in mice with human AML decreased leukaemic repopulation. It was evidenced that the AML LSCs were directly targeted as there was absence of leukaemia in serially transplanted mice. CD44 was identified as a key regulator and it was postulated that targeting CD44 resulted in interference with transport to stem cell-supportive microenvironmental niches, indicating the maintenance of LSCs requires an interaction with these niches.[44]

4.4 Targeting leukaemic stem cells using immunotherapy

More recently, researchers have explored the feasibility of treating leukaemia using cancer vaccines. As LSCs are difficult to be eliminated with conventional chemotherapy due to drug resistance, the use of immunotherapy may be a potential therapeutic option as its antileukaemic mechanism is different from that of conventional antileukaemic drugs (reviewed by Kadowaki and Kitawaki, 2011).[66] It has been shown that T lymphocytes play a critical role in the rejection of leukaemic cells via the graft-versus-leukaemia (GVL) effect in allogeneic stem cell transplantation and donor lymphocyte infusions (reviewed by Grenier *et al*., 2006).[67] Hence, elimination of minimal residual disease after chemotherapy or enhancement of the GVL effect in stem cell transplantation may be achieved by triggering specific immune response against leukaemic cells and LSCs.

One of the many ways LSCs may be suppressed is by inducing differentiation, which bypasses genetic abnormalities that give rise to malignancy. [68] Differentiation therapy for the treatment of acute promyelocytic leukaemia has been shown to reduce self-renewal capacity and improved patient survival.[56] In addition, differentiation of CML blasts into dendritic cells has been associated with the downregulation of BCR-ABL.[69] Such differentiation of leukaemic blasts into dendritic cells not only reduces malignancy in CML by bypassing the BCR-ABL abnormality, more importantly, it increases immunogenicity and may also open doors for anti-CML vaccines. Hence, targeting LSCs by differentiating them into dendritic cells may be a worth-while exploration.

Another way LSCs may be targeted is by active specific immunisation. Many studies and clinical trials have been carried out on the use of antigen-specific immunotherapy in the treatment of leukaemia and have been reviewed by Kadowaki and Kidowaki (2011),[66] Grenier *et al*. (2006),[67] Anguille *et al* (2012).[70] For example, Van Tandeloo *et al* demonstrated that the use Wilm's tumour 1 antigen-targeted dendritic cell vaccination induced complete and molecular remissions in AML and was found to increase WT1- specific IFN-γ–producing CD8+ T cell levels and features of general immune activation,[71] suggesting that the use of dendritic vaccination may be a feasible approach to prevent relapse in AML patients and may be useful in targeting the stubborn LSCs, which frequently contribute to drug resistance and relapse.

CONCLUSION

There is a growing body of research that supports the role of LSCs in drug resistance and metastasis via various mechanisms. Many studies have also been conducted to eradicate LSCs using different therapeutic targets, some of which are still in the *in vitro* and *in vivo* stage while others have moved into clinical trials. The abundance of literature suggests that targeting LSCs is feasible and the results are very encouraging. However, finding a therapeutic target that is specific to LSCs and finding antigens that are expressed abundantly and specifically in LSCs for immunotherapy remains a big challenge in this field of research. Therefore, further exploration and more clinical trials are needed before treatment strategies that target LSCs can be used safely and widely in the treatment of leukaemia.

TABLE 1: Therapeutic targets in leukaemic stem cells

Therapeutic target	Remarks	Author/ Reference
Targeting signaling pathways		
Hedgehog signaling pathway	Hedgehog inhibitors such as naturally-derived Smoothened antagonist cyclopamine, hedgehog-interacting protein or anti-hedgehog neutralizing antibody led to induction of apoptosis in CD34+ leukaemic cells with cyclopamine showing a marked reduction in drug resistance to cytarabine (Ara-C).	Kobune et al., 2009[39]
PI3K/Akt/mTOR pathway	A dual PI3K/mTOR inhibitor, PI-103, has been reported to target CD34+/CD38-/CD123+ AML cells, a subpopulation that is enriched in LSCs	Martelli et al., 2010[59]
Wnt signaling pathway	Blocking CD27, which is involved in Wnt signaling in CML stem cells, delayed disease progression and prolonged patient survival.	Schürch et al., 2012[31]
Rac signaling pathways	Targeting of Rac signaling pathway by pharmacologic or genetic methods led to rapid and specific apoptosis of MLL-AF9 expressing CD34+ cells.	Wei et al., 2008[60]
Targeting cell surface proteins		
CD33	AML patients treated with the immunoconjugate gemtuzumab ozogamicin targeting CD33 had a better response to conventional chemotherapy	Walter et al., 2012[64]

CD33	Gemtuzumab ozogamicin was capable of inhibiting growth in CD34+/CD38- cells obtained from CML patients. *In vitro* studies revealed dose-dependent apoptotic induction and growth inhibition. Synergistic effects with nilotinib and bosutinib were also observed.	Hermann et al., 2012[63]
IL-1 receptor accessory protein (IL1RAP)	Using an anti-IL1RAP antibody, it was possible to target CML CD34+ CD38- cells and induce cell mediated cytotoxicity against the IL1RAP of these cells.	Järås et al., 2010[61]
ABC transporters	Several studies have explored the feasibility of the use of ABC transporters inhibitor such as cyclosporine and zosuquidar. However, their clinical efficacy was found to be limited, largely because the normal haemotopoietic stem cells also highly express these transporters, making them equally susceptible to the ABC transporter inhibitors	Styczynski and Drewa, 2007[57]
Targeting the leukaemia microenvironment		
CXCR4	In animal studies, the CXCR4 inhibitor AMD3465 induced mobilisation of AML cells and progenitor cells into circulation where the anti-leukaemic effects of chemotherapy were enhanced, reducing leukaemia burden and prolonging survival of the animals.	Zeng et al 2009[65]
CD44	An activating monoclonal antibody directed to CD44 in mice with human AML decreased leukaemic repopulation. AML LSCs were directly targeted as evidenced by the absence of leukaemia in serially transplanted mice. Such targeting resulted in interference with transport to stem cell-supportive microenvironmental niches, indicating the maintenance of LSCs requires an interaction with these niches.	Jin et al., 2006[44]
Targeting leukaemic stem cells using immunotherapy		
Differentiation therapy	Differentiation of CML blasts into dendritic cells has been associated with the downregulation of BCR-ABL Such differentiation reduces malignancy and increases immunogenicity and may open doors for anti-CML vaccines	Lindler et al., 2003[69]
Antigen-specific immunotherapy	The use Wilm's tumour 1 antigen-targeted dendritic cell vaccination induced complete and molecular remissions in AML and was found to increase WT1- specific IFN-γ–producing CD8+ T cell levels and features of general immune activation, suggesting that it may be a feasible approach to prevent relapse in AML patients and may be useful in targeting the stubborn LSCs, which frequently contribute to drug resistance and relapse.	Van Tandeloo et al, 2010[71]

REFERENCES

1. Porth CM. Disorders of white blood cells and lymphoid tissues. In: Porth CM, Matfin G, editors. Pathophysiology: Concepts of altered health states. 8th ed. Philadelphia: Wolters Kluwer Health; 2009.p. 313-18.
2. Bhatia R, Holtz M, Niu N, *et al*. Persistence of malignant haematopoietic progenitors in chronic myelogenous leukaemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003; 101(12):4701–7.
3. Bruce WR, Van Der Gaag H. A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. Nature. 1963; 199: 79-80.
4. Bonnet D, Dick JE. Human acute myeloid leukaemia is organized as a hierarchy that originates from a primitive haematopoietic cell. Nat Med. 1997; 3 (7): 730–7.
5. Al-Hajj M, Clarke MF. Self-renewal and solid tumuor stem cells. Oncogene. 2004; 23: 7274-82
6. Singh SK, Clarke ID, Terasaki M, *et al*. Identification of a cancer stem cell in human brain tumours Cancer Res. 2003; 63: 5821-8.
7. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med.2006; 355: 1253-61.
8. Krause DS, Van Etten RA. Right on target: eradicating leukaemic stem cells. Trends Mol Med. 2007; 12:470-81.
9. Lapidot T, Sirard C, Vormoor J, *et al*. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994; 367: 645-8.
10. Guzman Ml, Jordan CT. Considerations for targeting malignant stem cells in leukaemia. Cancer Control. 2004; 11 (2): 97-104.
11. Guzman ML, Upchurch D, Grimes B, *et al*. Expression of tumour-suppressor genes interferon regulatory factor 1 and death-associated protein kinase in primitive acute myelogenous leukaemia cells. Blood. 2001; 97: 2177-9.
12. Guzman ML, Neering SJ, Upchurch D, *et al*. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukaemia cells. Blood. 2001; 98: 2301-7.
13. Fialkow PJ, Jcobson RJ, Papayannopoulou T. Chronic myelocytic leukaemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med. 1977; 63:125-30.
14. Udosakdi C, Eaves CJ, Swolin B, *et al*. Rapid decline of chronic myeloid leukaemic cells in long-term culture due to a defect at the leukaemic stem cell level. Proc Natl Acad Sci USA. 1992; 89:6192-6.
15. Sirad C, Lapidot T, Vormoor J, *et al*. Normal and leukaemic SCID repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukaemic SRC are detected in blast crisis. Blood. 1996; 87: 1539-48.
16. Wang JC, Lapidot T, Cashman JD, *et al*. High level engraftment of NOC/SCID mice by primitive normal and leukaemic haematopoietic cells from patients with chronic myeloid leukaemia in chronic phase. Blood. 1998; 91: 2406-14.
17. Druker BJ, Guilhot F, O'Brien SG, *et al*. Five-year follow-up of patients receiving imatinib for chronic myeloid leukaemia. N Engl J Med. 2006; 355(23):2408–17.
18. Hughes TP, Kaeda J, Branford S, *et al*. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukaemia. N Engl J Med. 2003; 349(15):1423–32.
19. Merante S, Orlandi E, Bernasconi P, Calatroni S, Boni M, Lazzarino M. Outcome of four patients with chronic myeloid leukaemia after imatinib mesylate discontinuation. Haematologica. 2005; 90(7):979–81.
20. Jamieson CH. Chronic myeloid leukaemia stem cells. Haematology Am Soc Haematol Educ Program. 2008; 436-42.
21. Cobaleda C. Gutie´rrez-Cianca N, Pe´ rez-Losada J, *et al*. A primitive haematopoietic cell is the target for the leukaemic transformation in human Philadelphia-positive acute lymphoblastic leukaemia. Blood. 2000; 95:1007-13.
22. George AA, Franklin J, Kerkof K, *et al*. Detection of leukaemic cells in the CD34(+)CD38(-) bone marrow progenitor population in children with acute lymphoblastic leukaemia. Blood. 2001;97(12):3925-30.
23. Hope KJ, Jin L, Dick JE. Acute myeloid leukaemia originates from a hierarchy of leukaemic stem cell classes that differ in self-renewal capacity. Nat Immunol. 2004; 5: 738-43.
24. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005; 5: 275-84.
25. Lane WS, Scadden DT, Gilland DG. The leukaemic stem cell niche-current concepts and therapeutic opportunities. Blood. 2009; 114(6): 1150-7.
26. Yi J, Chen J, Sun J, Wei HL. The relationship between multi-drug resistance and proportion of leukaemia stem cells and expression of drug transporters in drug-resistant leukaemia K562/ ADM cells. Zhonghua Yi Xue Za Zhi. 2009; 89(25): 1741-4.
27. Jeong HW, Cui W, Yang Y, *et al*. SALL4, a stem cell factor, affects the side population by regulation of the ATP-binding cassette drug transport genes. PLoS ONE. 2011; 6(4): e18372.
28. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009; 17 (1): 9-26.
29. Logan C.W, Nusse R. The Wnt signaling pathway in development and disease. Cell Dev Bio. 2004; 20: 781–810.
30. Yeung J, Esposito MT, Gandillet A, *et al*. -Catenin mediates the establishment and drug resistance of MLL leukaemic stem cells. Cancer Cell. 2010; 18(6):606-18.
31. Schürch C, Riether C, Matter MS, Tzankov A, Ochsenbein AF. CD27 signaling on chronic myelogenous leukaemia stem cells activates Wnt target genes and promotes disease progression. J Clin Invest. 2012; 122(2): 624–38.
32. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt

signalling pathway and cancer. Cancer Treat Rev. 2004; 30(2):193-204.
33. Xu Q, Thompson JE, Carroll M. mTOR regulates cell survival after etoposide treatment in primary AML cells. Blood. 2005;106(13):4261-8.
34. Kaestner KH, Knochel W, Martinez DE. Unified nomenclature for the winged helix/ forkhead transcription factors. Genes Dev. 2000; 14(2):142-6.
35. Accili D, Arden KC. FOXOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell. 2004; 117(4):421-6.
36. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene. 2005; 24:7410-25.
37. Naka K, Hoshii T, Muraguchi T, *et al*. TGF-beta-FOXO signaling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature. 2010; 463(7281):676-80.
38. Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. Genes Dev. 2008; 22:2454-72.
39. Kobune M, Takimoto R, Murase K, *et al*. Drug resistance is dramatically restored by hedgehog inhibitors in CD34+ leukaemic cells. Cancer Sci. 2009;100(5):948-55.
40. She MR, ChenXL. Targeting signal pathways active in leukemic stem cells to overcome drug resistance, In: Shostak S, editor. Cancer stem cells theories and practice. Croatia; InTech; 2011. p. 404. [cited 2012 July 6] Available from: http://www.intechopen.com/books/cancer-stem-cells-theories-and-practice
41. Han BW, Feng DD, Li ZG, *et al*. A set of miRNAs that involve in the pathways of drug resistance and leukemic stem-cell differentiation is associated with the risk of relapse and glucocorticoid response in childhood ALL. Hum Mol Genet. 2011; 20(24):4903-15.
42. Lessard, J, Sauvageau, G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature.2003; 423, 255–60.
43. Ishikawa F, Yoshida S, Saito Y, *et al*. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007; 25: 1315–21.
44. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE.Targeting of CD44 eradicates human acute myeloid leukaemic stem cells. Nat Med. 2006;12(10):1167-74.
45. Funayama K, Murai F, Shimane M, Nomura H, Asano S. Adhesion-induced drug resistance in leukaemia stem cells. Pharmacology. 2010;86(2):79-84.
46. Welch JS, Martin MG. Leukaemias. In: Cashen AF, Wildes TM, Henderson KE, De Fer TM, editors. The Washington manual subspecialty consult series: Haematology and oncology subspecialty consult. Philadelphia; Lippincott Williams & Wilkins; 2008. p. 308-22.
47. Shah NP. Medical treatment of chronic myeloid leukaemia. Haematology Am Soc Haematol Educ Program. 2007; 371-5.
48. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukaemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest. 2011; 121(1):396-409.
49. Herman PC, Huber SL, Heeschen C. Metastatic cancer stem cells: A new target for anti-cancer therapy? Cell Cycle. 2008; 7(2): 188-193.
50. Kucia M, Jankowski K, Reca R, *et al*. CXCR4-SDF-1 signaling, locomotion, chemotaxis and adhesion. J MolHistol. 2004; 35: 233-45.
51. Kucia M, Reca R, Miekus K, *et al*. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells. 2005; 23: 879-94.
52. Spoo AC, Lübbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in acute myelogenous leukaemia. Blood. 2007; 109(2): 786-91.
53. Tavor S, Petit I, Porozov S, *et al*. CXCR4 regulates migration and development of human acute myelogenous leukaemia stem cells in transplanted NOD/SCID mice. Cancer Res. 2004;64(8):2817-24.
54. Deng CH, Zhang QP. Leukaemia stem cells in drug resistance and metastasis. Chin Med J. 2010; 123(7):954-60.
55. Kaplan RN, Riba RD, Zacharoulis S, *et al*. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438(7069):820-7.
56. Zhou BG, Zhang J, Wang ZY, Chen SJ, Chen Z. Treatment of acute promyelocytic leukaemia with all-trans retinoic acid and arsenic trioxide: a paradigm of synergistic molecular targeting therapy. Philos Trans R Soc London. 2007; 362 (1482):959–71.
57. Styczynski J, Drewa T. Leukaemic stem cells: from metabolic pathways and signaling to a new concept of drug resistance targeting. Acta Biochimica Polonica. 2007; 54(4): 717-26.
58. Chumsri S, Matsui W, Burger AM. Therapeutic implications of leukaemic stem cell pathways. Clin Cancer Res. 2007;13(22 Pt 1):6549-54.
59. Martelli AM, Evangelisti C, Chiarini F, Grimaldi C, McCubrey JA. The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in cancer stem cell biology. Cancers. 2010; 2: 1576-96.
60. Wei J, Wunderlich M, Fox C, *et al*. Microenvironment determines lineage fate in a human model of MLL-AF9 leukaemia. Cancer Cell. 2008;13(6):483-95.
61. Järås M, Johnels P, Hansen N, *et al*. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. Proc Natl Acad Sci U S A. 2010; 107(37):16280-5.
62. Hauswirth AW, Florian S, Printz D, *et al*. Expression of the target receptor CD33 in CD34+/CD38-/ CD123+ AML stem cells. Eur J Clin Invest. 2007;37(1):73-82.
63. Herrmann H, Cerny-Reiterer S, Gleixner KV, *et al*. CD34(+)/CD38(-) stem cells in chronic myeloid leukaemia express Siglec-3 (CD33) and are responsive to the CD33-targeting drug gemtuzumab/ ozogamicin. Haematologica. 2012;97(2):219-26.
64. Walter RB, Appelbaum FR, Estey EH, Bernstein

ID. Acute myeloid leukaemia stem cells and CD33-targeted immunotherapy. Blood. 2012;119(26):6198-208.
65. Zeng Z, Shi YX, Samudio IJ, *et al*. Targeting the leukaemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood. 2009;113(24):6215-24.
66. Kadowaki N, Kitawaki T. Recent advances in antigen-specific immunotherapy for acute myeloid leukaemia. Clin Dev Immunol. 2011; 2011: Article ID 104926, 7 pages.
67. Greiner J, Döhner H, Schmitt M. Cancer vaccines for patients with acute myeloid leukemia--definition of leukaemia-associated antigens and current clinical protocols targeting these antigens. Haematologica. 2006; 91(12):1653-61.
68. Thomas X. Targeting leukaemia stem cells: The new goal of therapy in adult acute myeloid leukemia. World J Stem Cells. 2009; 1(1): 49-54.
69. Lindner I, Kharfan-Dabaja MA, Ayala E, *et al*. Induced dendritic cell differentiation of chronic myeloid leukaemia blasts is associated with down-regulation of BCR-ABL.J Immunol. 2003; 171(4):1780-91.
70. Anguille S, Willemen Y, Lion E, Smits EL, Berneman ZN. Dendritic cell vaccination in acute myeloid leukaemia. Cytotherapy. 2012;14(6):647-56.
71. Van Tendeloo VF, Van de Velde A, Van Driessche A, *et al*. Induction of complete and molecular remissions in acute myeloid leukaemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci U S A. 2010; 107(31):13824-9.