ABSTRACT

It has been shown throughout the years in numerous studies in cell biology that cancer stem cells have therapeutic implications that can be potentially used to revolutionize treatment of cancer. Using the information available to us from cancer stem cell research, a number of new therapies can be developed to provide a longer and better quality of life for patients suffering from cancer. This review provides an introduction to the biology of stem cells in general, explains the cancer stem cell concept and how stem cells behave in tumors, how this contributes to the thriving of tumor cells, and their heterogeneity. The review also provides examples of the mechanisms of resistance to conventional therapies widely used today, which are present in cells of various cancers such as breast cancer. It also goes through how some tumors acquire different subtypes that enable them to become resistant to therapy and how this contributes to tumor relapse. The various breakthroughs in potential targeted therapies are also explained, such as signaling pathways and tumor markers. Here we address the current issues involving selectivity of therapy and lack of availability of universal tumor markers, based on the pre-established understanding of cancer cell behavior when targeted for therapy.

KEYWORDS: cancer, cancer stem cells, therapeutic implication of cancer stem cells

INTRODUCTION

Stem cells are undifferentiated cells seen in the human body, which can potentially be stimulated to differentiate into more specific cells, with specific functions[1].

Primarily, there are two types of stem cells that reside naturally in the human body, pluripotent and multi-potent stem cells. In 2006 researchers have identified conditions that would allow some specialized adult cells to be genetically manipulated to act like stem cells. These types of stem cells are known as induced pluripotent stem cells[2].

Pluripotent or embryonic stem cells can produce, more or less, all human body cells, like those present in tissues such as the brain, bone, heart, and skin. Multi-potent stem cells reside in adult tissue, as well as fetal umbilical cord. Limitations to capacity of differentiation is explained by the fact that they are specific to the tissue of their origin[3]. For example, it is generally accepted that a hematopoietic stem cell (HSC) found in the bone marrow cannot give rise to cells of different tissue origin such as the brain.

Regardless of their primary source, stem cells share three unique characteristics. First, stem cells are capable of self-renewal for prolonged periods of time. Second, stem cells remain undifferentiated, and the last, their unique ability to generate diverse number of specialized cells.

Tumorogenesis and the cancer stem cell concept

The new approach to the explanation of tumorogenesis differs from the conventional model. The conventional model of tumorogenesis stems from the idea that tumor generation and growth are maintained by the growing number of dividing cells that make up tumors compared to normal tissues. The accumulation of mutations in the cells causes over expression of oncogenes and inhibition of tumor suppressor genes, and their ability to evade the natural process of apoptosis[3]. Bearing in mind that the traditional model of tumorogenesis has been for a very long time, the most widely accepted explanation for how tumors proliferate and thrive in different tissues, it fails to demonstrate how treatments designed to eliminate dividing cells, often fail to cure patients suffering from cancer. Therefore, a newer theory had to be proposed to cover the still unexplained features of tumorogenesis. The “cancer
stem cell (CSC) concept, the most recent theory for tumorigenesis, suggests that a small population of malignant cells is responsible for maintaining tumor growth. These cells exist as part of a heterogeneous tumor and are known as cancer stem cells (CSCs). The CSC concept proposes that the tumor population is hierarchically arranged with CSCs lying at the apex of the hierarchy. CSCs have been discovered in many hematopoietic malignancies as well as solid tumors, which reside within genetically heterogeneous tumors, along with distinct tumor cells known as sub-clones. Both cell types mentioned compete with the tumor mass. This means that on top of each sub clone resides a genetically distinct population of CSCs, which act as the building blocks of many complex tumors. The CSC concept is currently used to provide a model for the complex process of tumorigenesis and relapse of tumors, by demonstrating the universal traits of tumor cells. The universal traits of CSCs are: arrangement of tumor cells in a hierarchy, potential for unlimited renewal of cells, quiescent or slow-cycling states, and increased resistance to conventional antitumor therapies (Fig. 1).

There are a number of CSCs from common tumors such as breast carcinoma that have been reported to show mechanisms of resistance against conventional antitumor therapies. For example, CSCs from breast cancer in locally advanced disease were found after elimination of most of the tumor using cytotoxic agents. Another example is the BCR-ABL-driven leukemic stem cells (LSCs) that are resistant to tyrosine kinase inhibitors. CSCs sustain their resistance to a number of cytotoxic chemotherapies through diverse mechanisms, some of which have been demonstrated in different tumors. An example of one of these mechanisms is increased exportation of the drug from the cells, which is made possible by multidrug resistance (MDR) transporters.

The origin of cancer stem cells

When embryonic stem cells were transplanted into recipient mice, they were able to form teratomas (Sell et al., 1994). This suggests that CSCs share some of the properties seen in normal cells and might indeed originate from normal stem cells. It is also possible that CSCs originate from a more differentiated cell progeny, which have accumulated enough genetic abnormalities to allow them to develop stem cell like properties. This implies that each cell, of which the mutation originates, is stem cell in itself. However, different CSCs, which are responsible for the tumorigenic properties seen in the clone, is a more differentiated version. This is the case in acute myeloid leukemia (AML), as it has been demonstrated that the AML-ETO fusion protein was located in normal HSCs.

When the functional LSCs of AML were studied using an in vitro colony-forming assay, they were discovered to be in a progenitor cell state with the expression of the Thy1 surface protein, suggesting that the disease originated from a normal HSC. However, a contrast to the above case was found when studying CSCs in promyelocytic leukemia (APL), because the MLL-AF9 fusion protein responsible for the disease was not detected in the HSCs. This fusion protein was able to induce leukemia when it was introduced into mice. The occurrence of leukemia in those mice has demonstrated that the cell with the original mutation and the LSCs were more likely to be present in progenitor cells than HSCs.

Homing and T-Chimeric antigen receptors

Homing is the transfer of CSCs into new healthy tissue with implantation and possibility of growing new malignant foci. Homing is dependent on the ability of the CSC to implant and burrow in the new tissue as well as the natural features of the grounding tissue such as vascularization and chemotaxis. A modality of potential treatment of malignancies is targeting of stem cell homing with the use of T-cell Chimeric Antigen Receptor (CAR). CARs are synthetic, engineered receptors which target surface molecules in their natural conformation. CARs were most heavily studied in hematological malignancies, due to the T-cell innate homing ability with corresponding tissues and ease of sampling of tumor. Research into solid tumors has started and has good reported response in preliminary phases. Since the discovery of CARs, there have been three generations of CARs. First-generation CARs comprise of an extracellular portion that is a single-chain F-variable antibody fragment with tailored specificity to targeted population of cells as well as a transmembrane portion. The tests resulted in poor proliferation post-binding to the receptor. Second and third generation CARs were constructed to overcome this obstacle, by
adding intracellular portions of CD3z chain linked to one or two co-stimulatory receptors, for second and third generation CAR respectively[16,17].

The efficacy of CARs lies in its capacity to employ molecular structures independent of antigen processing by the target cell and of MHC. Despite the highly promising results, no comparative studies were conducted to compare different CARs and treatment is still only in phase 1 of testing. There are many reported side effects to the T-cell CARs infusion, such as cytokine release syndrome, tumor lysis syndrome and anaphylaxis. Autologous T-cells can be used to decrease rejection by recipient. Years of research are needed to start considering CARs as first line anticancer therapy[14,16,18].

**Methods of evaluation of treatment efficacy**

Clinical trials involving antitumor therapies have typically relied on gross tumor regression as an evaluation of efficacy of antitumor therapy[19]. However, it is now accepted that the use of specific CSC tumor markers that can be reliably assigned to each tumor type, and the quantification of remnant CSCs within minimal residual disease, are more reliable in the evaluation of antitumor therapy[19]. The identification of CSC biomarkers has played an important role in showing that a significant inter-patient heterogeneity exists in tumor samples. Various studies involving the profiling of gene expressions along with the use of biomarkers and clinical approaches have deduced that solid tumors can be classified into subtypes of different molecular makeup[20]. This suggests that different CSC phenotypes produced from molecularly diverse tumor types, such as those seen in breast carcinoma, might be linked to different cancer subtypes of the same tumor. With regards to breast cancer a few hundred CSCs from mammary and pleural effusion samples, in the ESA+/CD44+/CD24 population were able to produce the original tumor in non-obese diabetic / severe combined immunodeficient mice, whereas cells from different phenotypes could not[21].

After identifying the CSC biomarkers, specific therapies can be used to target specific surface proteins on CSCs, and restrict tumor proliferation. The problem with this approach of targeted therapy is the severe side effects produced due to the expression of the same surface proteins seen on some normal tissue cells. One such surface protein is CD123, which is produced on LSCs in AML and also on normal HSCs[22].

**Molecular pathways and therapy resistance in CSCs**

CSC molecular pathways, such as the Wnt signaling pathway, function at different levels in a hierarchy to allow for the differentiation of specific tissues. This implies that it is possible that the self-renewal process in cells controlled by the Wnt signaling pathway in stem and progenitor cells is impaired in cancer cells, enabling malignant growth of cells[23]. This is why the pharmaceutical industry has put the impaired signaling pathways, in tumor cells, as their next target for the development of new treatments. A different pathway, known as the NFkB pathway, has been successfully targeted with a direct IkB kinase inhibitor (parthenolide), and as a result it can be used to selectively target AML and CML LSCs in vitro treated leukemic cells[24].

CSC resistance is a major factor in the initiation of several studies that have aimed to make CSCs more sensitive to chemotherapy. Recent reports have provided a useful strategy for targeting dormant CSCs, by demonstrating that quiescent as well as normal stems cells that are resistant to chemotherapy can be induced to become sensitive to such treatments[25]. Colon cancers CSCs are an example of CSCs that were successfully primed and sensitized to chemotherapy, by inhibition of the actions of IL-4[26].

**OBSERVED CANCER STEM CELL ACTIVITY IN SOME COMMONLY ENCOUNTERED TUMORS**

**Cancer stem cells: brain tumors**

Gliomas are aggressive incurable carcinomas of glial cells in the spinal cord, or more commonly the brain. They represent around 80% of the total incidence of primary tumors in the central nervous system[27]. In 2007, the World Health Organization (WHO) classified gliomas according to their histological similarities to normal glial cells such as astrocytes, oligodendrocytes, and oligoastrocytomas[28]. Glioblastoma multiforme (GBM, grade III or IV astrocytoma) represent more than half of the overall incidence of gliomas. GBM is a very aggressive cancer, and is known to have an estimated 5% five-year survival rate with multiple therapies of radio, chemo and adjuvant chemotherapy.

**Glioma core signaling pathways and glioma stem cells mechanism of resistance**

Three main pathways control the oncogenesis process in gliomas. They are receptor tyrosine kinase (RTK), p53, and retinoblastoma protein (Rb) pathways[29]. Through genetic alteration in the pathways mentioned above, cell proliferation and cell survival enhancement allow the GBM to escape checkpoints, senescence, and apoptosis. RTK pathway activation results in both EGFR and PDGFRA receptor dimerization and cross-phosphorylation, which activates PI3K and Ras, which in turn act on AKT, which controls proliferation, migration and survival. Rb binds transcription factors and seizes the trans-activation of primary genes in cell cycle, thus preventing proliferation[30]. The last major pathway is p53. It suppresses the propagation of the cell cycle by
slowing the G1 phase, or the initiation of the apoptosis process\textsuperscript{[33]}. These signaling pathways act on major parts not only seen in the natural evolution of the central nervous system, but also in glioma genesis, making them potential targets for therapy.

GSCs are one of the first kinds of CSCs to be isolated from solid tumors using the tumor marker CD133. When implanting 100 GSCs in immunodeficient mice, the full original tumor can be reproduced, while implantation of 1,000,000 non-GSCs could not achieve the same\textsuperscript{[32]}. This observation could explain the recurrence or relapses of treated tumors. In addition, it was found that GSCs with CD133 positive marker can activate DNA damage checkpoints and enhance the repair pathway through CHK1 / CHK2, therefore, making them more resistant to radiation compared to the CD133 negative cells. In fact, the fraction of CD133 positive cells was increased after radiation. It is suggested that targeting DNA damage checkpoints may be the therapy that overcomes the radio resistance of CD133 positive cells\textsuperscript{[33]}. Moreover, GSCs expressed chemoresistance by exporting chemotherapy agents from the cell such as temozolomide, by overexpression of ATP-binding cassette transporters (ABCG2) through the PTEN / PI3K/AKT pathway\textsuperscript{[34]}. GSCs can be targeted by compounds that affect differentiation promoters such as bone morphogenetic proteins, specifically (BMP4), which expresses the strongest effect on GSCs by inducing astrocytic differentiation of specific cell markers (CD133)\textsuperscript{[35]}. It was pointed out by multiple studies that the essential pathways of GSC maintenance are the Sonic hedgehog, Notch, and Wnt pathways\textsuperscript{[36-38]}. Furthermore, the key characteristic feature of stem cells in GSCs, the stemness, is controlled by the following factors: Olig2, Bmi1 and Nanog\textsuperscript{[39-41]}. When examining 305 GBM samples, the expression of BMI1 was detected in 99% of cases\textsuperscript{[42]}. BMI1 provides GSCs with the features of survival and self-renewal through transcript repression of p21\textsuperscript{Cip1}, p19\textsuperscript{Arf} and p16\textsuperscript{INK4a}. A new target for therapy is the JNK pathway, which acts on the processes of self-renewal, stem-like features and GSC differentiation. JNK-1 and JNK-2 were targeted with micro-molecules and JNK inhibitors (SP600125), which depleted the self-renewal feature and inhibited tumor formation, therefore possibly resulting in better prognosis and survival. Experiments involving different doses of SP600125 (such as 40 mg/kg/day) were conducted on nude mice affected with the tumor compared to the use of temozolomide at maximal dose, which displayed no inhibitory effect\textsuperscript{[43]}

Controversies about glioma stem cells

Although many studies identified and isolated GSCs using the transmembrane glycoprotein CD133, the use of CD133 as a universal marker is still controversial. In two different studies the value of CD133 as a marker for GSCs was doubted. Moreover, more than 40% of freshly isolated cells from tumors did not contain CD133 markers; this suggested that CD133 could not be the enhancement marker for GSCs. It was suggested by one study that CD133 positive cell in isolation is insignificant for the progression of tumors. It is the general condition surrounding these cell that are the important determining factors of the tumorigenesis process. It was found that CD133-negative glioma cells subcultured under similar GSCs conditions could also have the characteristics of self-renewal, differentiation and formation of tumors if implanted in a xenograft. All these are features of CSCs in non-CD133 cells\textsuperscript{[44]}. Another observation was the formation of floating spheroids in cultures positive for CD133, whereas negative cultures grew as adhesive spheres with some even giving rise to CD133-positive cells\textsuperscript{[45]}. Other stem cell markers were suggested but not validated such as CD44, CD15 and integrin α\textsuperscript{6}\textsuperscript{[46-48]}. The niche of GSC consists of endothelial and ependymal cells located in subventricular zone and subgranular layer\textsuperscript{[49]}. The proliferation of endothelial cells increases in cultures with CD133 positive cells in relation to the angiogenesis process and it is slowed when GSC are eradicated\textsuperscript{[50]}. However, GSC themselves can express angiogenic effects by producing SDF-1 and VEGFA factors. This leads to the controversy of what the cause is, and what the effects of it might be. In relation to new research into GBM, CARs are being explored as therapeutic options. This however, is proving difficult as the tumor in its natural habitat is immune-privileged and xenografted human brain tumors are not as invasive and usually would grow in a well-circumscribed manner. In an article published in 2013, two cell lines were yielded and implanted in mice with monitoring of tumor growth. Mice were given T-Cell CARs targeting the specific EGFRVIII\textsuperscript{+}. The results were supportive that there might be a role of adoptive immunotherapy in the future in the case of GBM\textsuperscript{[14]}

Cancer stem cells: breast tumors

Transplant experiments involving CSCs in the 1950s led to the development of two theoretical models that may explain which group of cells within a certain tumor is responsible for tumorigenesis. The hierarchy model predicts that there is a unique group of cells within a tumor that are capable of regenerating the tumor. On the other hand, the stochastic model proposed that every cell within a certain tumor has the potential to initiate and sustain a tumor; however, this property is regulated by a number of variables or “stochastic events” that make the cell enter the cell-cycle\textsuperscript{[51,52]}. Research has supported the hierarchy theory which entails that a tumor is composed of heterogeneous groups of cells and only a limited number of cells have the tumor-initiating property.
Pioneering work in the area of CSCs involved studies of hematopoietic malignancies and was later extended to solid tumors such as brain and breast cancer. The study that was carried out in 2003 by Al-Hajj et al represents an important landmark in breast CSC research[21]. A summary of the study is mentioned below.

Al-Hajj et al used a model of immune-deficient mice called “non-obese diabetic / severe combined immunodeficient (NOD / SCID) mice. Special xenograft assays were prepared in such a way that enabled the implantation of single cell suspensions of human breast cancer tissue into the mammary fat pads of these mice. Samples from both primary and metastatic breast cancers (from pleural effusions) were efficiently implanted and grown in the mammary fat pads of the NOD / SCID mouse model[21].

Breast cancer cells express a variety of cell surface markers. Among these markers are two adhesion molecules called CD24 and CD44. Flow cytometry was used to separate cells that were positive or negative for these markers. It was found that injections of cells which carried the phenotypes CD44+ or CD44−low into the breasts of the NOD / SCID mice led to the formation of visible tumors within two weeks of injection; whereas none of the CD44− cell injections gave rise to tumors and only two out of 12 mice that were injected with CD24+ developed palpable growths at injection sites (the authors have provided explanations for the latter)[21].

The human cancer specimens that were used invariably contained some normal cell types like leukocytes and fibroblasts. Several antigens called “lineage markers” were found to be associated with those normal cells, like CD2, CD3, CD18 and others. Cancer cells did not express these antigens. Therefore, one of the main final results of the study by Al-Hajj et al was the identification and isolation of the tumorigenic cells which carry the phenotype CD44+ CD24−low lineage. This was found in eight out of nine patients. Another important observation was that a small number of cells with this phenotype, not even exceeding a hundred cells, were able to initiate and sustain tumors, and the formed tumors were phenotypically diverse and complex. On the other hand, thousands of cells which carried different phenotypes were not capable of forming neoplasms[21]. Pece et al suggested that the heterogeneity of breast cancer might be attributed to CSCs. Furthermore, their studies showed that poorly differentiated breast cancers (grade 3) are enriched with CSCs compared to more differentiated tumors[53].

Tumorigenic breast cancer cells referred to as “breast cancer stem cells” (Br CSCs) overexpress a detoxifying enzyme called aldehyde dehydrogenase (ALDH 1). This enzyme is found in both normal stem / progenitor cells of normal human breast as well as the breast CSCs. The function of this enzyme is to regulate the oxidation of intracellular aldehydes and it may be involved in the early differentiation of stem cells[54,58].

Epithelial mesenchymal transition (EMT) is an essential part of normal development through which epithelial cells transform into cells that possess properties that resemble mesenchymal cells[54,56]. Recent studies show that EMT plays a significant role in the pathogenesis of some cancers, including breast cancer, and contributes to the acquisition of malignant and stem cell properties. The acquired mesenchymal features include motility, invasiveness and increased resistance to apoptosis. These features are consistent with high grade malignancy. EMT may also contribute to metastatic dissemination[57].

The “Claudin-low” is one of the molecular subtypes of breast cancer that have been identified using gene expression analyses. Most of the Claudin-low tumors are triple negative breast cancers (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative and human epidermal growth factor receptor 2(HER2)-negative) and most of them have poor prognosis. A study by Prat et al has shown that the Claudin-low breast cancer subtype has characteristic stem cell-like features. These include the CD 44+ / CD24- low phenotype which has been found to be enriched in breast CSCs[21]. Also, ALDH-1 enzyme has been noted to be highly expressed in these Claudin-low tumors. This enzyme, as mentioned previously, is found in both normal cells and CSCs. In addition, it was discovered that the Claudin-low subtype was enriched with epithelial-mesenchymal transition features[58].

Studies by Korkaya et al suggested that the HER2 pathway plays a role in carcinogenesis and tumor growth through its effect on mammary stem / progenitor cells. Their studies showed that HER2 receptor overexpression may contribute to the amplification of the mammary stem cell population. Furthermore, their studies provided evidence that the HER2 pathway may be involved in increasing the population of ALDH1-expressing CSCs. These ALDH1-expressing cells demonstrated increased invasion in vitro as well as increased tumorigenesis in immunocompromised mice. In addition, the studies by Korkaya et al showed that the effect of trastuzumab (Herceptin), the humanized anti-HER2 antibody, might be mediated through its ability to reduce the number of CSCs in HER2-amplified tumors[59].

The CSC model has important therapeutic implications. Designing therapies that specifically target tumorigenic cells may lead to better treatment results and prevent relapse. Conventional cancer therapies merely lead to tumor regression. According to the stem cell model, these therapies mainly target non-tumorigenic cells which represent the bulk of any tumor, and fail to affect CSCs. This results in tumor...
regression; however, in this case there is a strong chance for tumor recurrence due to the ability of the surviving CSCs to create new tumors. Furthermore, recent preclinical studies showed that current breast cancer treatment modalities may actually lead to CSC enrichment and contribute to chemotherapeutic and radiotherapy resistance\cite{21,54}.

A study by Li et al used suspension cultures combined with chemotherapeutic agents (paclitaxel and epirubicin) in order to isolate and identify breast CSCs. Their findings showed that after one week of administering this chemotherapeutic combination to a group of breast cancer cells, the majority of the remaining cells which survived had the phenotype CD44+ CD24+, which has been repeatedly shown to be associated with CSCs as mentioned previously. This finding supports the view that current chemotherapeutic agents merely target the rapidly dividing “differentiated” cells and miss the relatively dormant stem cells, therefore contributing to future relapse of cancer due to the ability of CSCs to generate new tumors\cite{60}.

Studies carried out by Cicalese et al provided evidence that the tumor suppressor factor P53 plays an essential role in regulating self-renewal divisions in mammary stem cells. Their results demonstrated that P53-null SCs (with targeted mutation in P53) are almost immortal in culture, and undergo an unlimited number of symmetric self-renewing divisions thus expanding the pool of stem cells. The role of P53 mutation / loss in different types of cancer has been suggested by many studies over the past decades, and it has been found that a significant proportion of breast cancers involve mutations in P53. Theoretically, restoration of the tumor suppressor P53 function in CSCs is an appealing approach in cancer therapy; however, research is still lacking in this area\cite{61}.

As noted previously, CSCs may play a role in cancer relapse after treatment. There is emerging evidence that CSCs are involved in chemoresistance as well as resistance to radiotherapy. Furthermore, they may contribute to resistance against endocrine therapy.

RESISTANCE TO CHEMOTHERAPY
CSCs are not actively dividing cells; this contributes to their resistance against chemotherapeutic agents which mainly target rapidly proliferating cells. Another factor which might explain CSCs chemoresistance is that they are rich in anti-apoptotic proteins which resist cell apoptosis\cite{52}. Recent data suggest that primary breast cancers that contain a higher proportion of Br CSCs are associated with poor clinical response to neo-adjuvant chemotherapy. Furthermore, a high expression of ALDH-1 may be associated with chemoresistance and a poor clinical outcome\cite{62}.

Experiments by Shafee et al which were focused on Brca-1/P53-mutated mouse mammary tumors showed that CSCs contribute to resistance against platinum compounds, namely Cisplatin. Their findings showed that cells carrying phenotypes, supposedly expressed by mammary CSCs were enriched in BRCA-1-positive tumors that were refractory to platinum treatment\cite{63}.

RESISTANCE TO RADIOTHERAPY
Breast CSCs that express CD44+ and CD24low were discovered to be resistant to radiotherapy and it has been proposed that this may be due to enhanced activation of the ataxia-telangiectasia mutated kinase (ATMK) signalling pathway which is involved in DNA damage repair. A recent study demonstrated that targeting the ATM kinase with a specific inhibitor called KU55933 has led to increased sensitivity to radiotherapy and decreased survival of CD44+ CD24low cells after being exposed to radiation\cite{64}.

RESISTANCE TO ENDOCRINE THERAPY
A group of steroid receptor-negative cells (namely ER- PR- CK5+) that carry the phenotype CD44+ which have the characteristics of CSCs have been identified in ER+ / PR+ breast cancer xenografts. Endocrine therapy in breast cancer targets ER+ / PR+ cells; therefore, this rare population of steroid receptor-negative cells would survive endocrine therapies and lead to future relapse due to their CSC-like property\cite{65}.

NOTCH-1 and NOTCH-4 are breast oncogenes which are involved in stem cell self-renewal; hence they play an important role in breast cancer tumorigenesis. NOTCH-1 and NOTCH-4 were found to be over-expressed in triple-negative breast cancers which are considered to have the worst prognosis among the breast cancer subtypes. It has been shown that NOTCH-4 is an essential factor that is involved in breast CSCs survival\cite{66,67}.

A recent study demonstrated that PEA3 (polyomavirus enhancer activator 3), an ETS (E-Twenty Six) transcription factor, plays an important role in the transcription and regulation of NOTCH-1 and NOTCH-4 in certain subtypes of breast cancer; therefore, PEA3 represents an important target for future breast cancer therapeutics\cite{66}.

NOTCH-1 and NOTCH-4 are activated through several ligand-binding steps which involve enzymatic cleavage. Gamma-secretase is considered to be an essential enzyme that is involved in the activation of NOTCH signalling. The inhibition of gamma-secretase is an appealing approach for targeted cancer therapy. Gamma-secretase inhibitors (GSIs) are currently being investigated in several trials as a novel therapy for certain breast cancer subtypes\cite{66}. A study by Grudzen et al supported this novel approach by showing that MRK003, a GSI induced apoptosis is effective in Br CSCs\cite{67}.
Interesting recent data provided evidence that metformin, the first line drug used for type 2 diabetes mellitus, may have a role in breast cancer therapy. Studies by Iliopoulos et al showed that metformin selectively targets and kills Br CSCs in mammary xenografts. Furthermore, combination therapies which include metformin with different chemotherapeutic agents like doxorubicin, paclitaxel or carboplatin is more effective in suppressing tumor growth and preventing relapse than each of these drugs when used as monotherapy. Another interesting observation is that metformin reduced the dose of the chemotherapeutic agent doxorubicin that is required to delay cancer relapse and prolong survival.

Cancer stem cells: Haematological malignancies

CSCs resemble normal HSCs in their cell-surface markers, multipotency of progenitor cells and hierarchical self-renewal properties. This led researchers to hypothesize that leukemic stem cells are derived from normal HSCs, or from more differentiated cells that acquired the normal HSC properties. The point of mutation that confer carcinogenic properties to stem cells, is believed to be a slight alteration in normal signalling pathways acting on specific stem cell niche, resulting in cancerous stem cell phenotype. Understanding the basis of the stem cell carcinogenesis renewal provided two targets for therapeutic agents. The first is the identification of specific leukemic cell surface markers, and the use of anti-monoclonal antibodies acting on surface antigens such as Anti-CD44 and TIM-3, as seen in the treatment for AML.[69] The second is blockade and manipulation of signalling pathways identified in CSC renewal including Bmi-1, Wnt, Notch and Hedgehog pathways.[70]

Retrospective analysis of collective data, concerning surface markers and pathways of stem cells, specifically in myeloid malignancies reveal an organizational hierarchy similar to that of normal myelopoietic cells in terms of progenitor cell formation, self-renewal and maturation. This is mainly due to the shared expression of CD34 and CD45 and the lack of CD38 surface markers, in both normal and malignant myeloid cell phenotypes alike. The discriminative distinction between normal and neoplastic myeloid stem cell lines was made by the discovery of additional surface markers, i.e., CD123 (alpha chain of IL-3 receptor). The year 2006 marked the emergence of many clinical articles confirming that neoplastic stem cells of the CD34+ / CD38- progeny found in AML and MDS patients all express CD123. Furthermore, chronic myeloid leukemia (CML) patients and systemic myelocytosis (SM) patients co-expressed CD123 markers on multi-colour flow cytometry of bone marrow cells, making CD123 a reliable marker for neoplastic progenitor stem cells.[71] Other targeted antigens discovered in the same paper include CD13, CD33 and CD44.

This literature review will examine the molecular targets (CD44, CD33, and CD123 “alpha chain of IL-3 receptor”) expressed as surface antigens of CD34+ / CD38-stem cells. The findings of this literature review were refined to highlight the application of these surface markers as targeted pathway therapies for hematological malignancies in general and myeloid neoplasms in particular. Quoted clinical trials are limited to AML patients. Meta analysis of laboratory based articles was restricted to HSCs of the CD34+ / CD38- progeny.

Molecular targets on CD34+/CD38- stem cell surface markers

Minimal residual disease (MRD) cells reside in the bone marrow, and are believed to be responsible for the recurrence of AML relapses after chemotherapy. In fact, MRD parameters are proven to be highly reliable in predicting survival rates in AML. Immunophenotypic detection of MRD frequencies through flow cytometry, helped tailor chemotherapeutic requirements of AML patients, as well as predict morbidity and mortality.[72] According to MRD parameters only 30 - 40% of patients with AML survive despite high dose chemotherapy. Besides, the prognostic value of MRD cells, detection of relapse and survival prediction is also useful in the identification of stem cell-targeted therapy. Some of the stem cell therapeutic agents identified so far include Anti-CD123, Anti-CD44, and Anti-CD33.

CD44+ Leukemic stem cells in AML patients

Studies in 2004 explored the effects of Anti-CD44 monoclonal antibodies (mAbs) on normal myelopoiesis by and large and defective myelopoiesis seen in AML blasts.[73] They illustrated the hierarchical organization of the leukemic clones, through antigenic and cytological features defining the well known AML subtypes (AML 1/2, AML3, AML4, AML5, AML6, and AML7). Like normal HSCs, AML stem cells of all subtypes are found in the primitive CD34+ / CD38-fractions. Thus, they allow Anti-CD44 mAbs to induce terminal differentiation of primary blasts, especially in AML1 to AML5 subtypes. Another mechanisms of action employed by Anti-CD44 mAbs includes inhibition of AML cell proliferation by stabilizing p27kip-1 (a specific cyclin dependent kinase inhibitors, CKI), and finally inducing apoptosis in NB4 cell line and AML3 subtype. Anti-CD44 mAbs have successfully utilized the molecular basis of dysregulated self-renewal features of AML stem cells as a therapeutic target.

Further studies in 2006 questioned the niche dependency aspect of leukemic stem cells (LSC), and utilized the unique requirements of AML LSCs for
interaction with a niche. This resulted in the successful eradication of AML LSCs by solely targeting CD44 receptors\[74\]. Here, the activation of CD44 by H90 treatment resulted in effective block of the homing of leukemic cells, including primitive CD34+ / CD38-SL-ICs in both bone marrow and spleen. H90 treatment was found to work in two different modes of action; first is alteration of AML LSCs through the manipulation of CD44 function, as the main regulator of leukemic cell proliferation and second, is the abrogation of AML LSCs homing, which resulted in collective eradication.

Receptor CD33 (Siglec-3) expressed in AML LSC

The functional criterion of AML LSCs is their ability to repopulate NOD / SCID irradiated mice with leukemic cells. European Journal of Clinical Investigation experimented with the capabilities of CD33+ progenitor cells to repopulate NOD / SCID mice with leukemic stem cells, and was also successful in presenting evidence demonstrating the co-expression of CD33 cell surface markers in CD34+ / CD38-LSCs in AML patients\[75\]. However, the study failed to specify the AML-repopulating abilities of all CD34+ / CD38- LSCs that expressed CD33. The importance of this discovery stems from targeting CD33 cell surface receptors for treatment of AML.

Since then, collective meta-analysis of cell surface markers in AML patients confirmed that the expression of CD33 as a myeloid marker is apparent in 75% of AML patients. Thus, making CD33 antibodies a frequently used agent in bone marrow (BM) purging strategies and in Ab-targeted therapies. Reports also depict a discrepancy in AML patients response to CD33 Abs based treatments, reflected in variable rates of relapse, and modifiable rates of AML morbidities and mortalities. This was later explained by the CD33+ under expression in CD34- AML primitive leukemic progenitors, when compared to most CD34+ leukemic progenitors\[76\].

CD123-targeting mAb

The discovery of CD123 cell surface markers explained the extensive rate of proliferation of leukemic progenitors, when compared to normal hematopoietic cells. The significance of CD123 expression comes from its correlation with IL-3 stimulated and spontaneous signal transducer and activator of transcription 5 (STAT5) levels, cycling cell proportions, primitive cell-surface phenotypes and resistance to apoptosis. The over-expression of CD123 in AML LSCs advocates for the efficacy of CD123-targeted treatments in the form of specific monoclonal antibody (7G3)\[77\]. An extensive body of evidence supports the clinical potential for in vivo 7G3 treatment for CD34+ / CD38- leukemic cells. The therapeutic benefit of 7G3 treatments is substantiated by three main lines of evidence. The first, 7G3 specifically targets IL-3 receptor (consisting of CD123- CD131) cell surface markers of LSCs seen in AML. The second line of evidence examines levels of toxicity of the chimeric IgG1 variant of 7G3, and proceeds to emphasize the lack of interference with normal hematopoiesis (confirmed by measured parameters over 70 days). The third and last line of evidence is the application of 7G3 application in phase 1 clinical trials\[78\], where incidence of adverse events did not increase with escalating doses and reports of adverse events did not correlate with complications and risks of biochemical profile of the infused material\[79\].

T-Cell Immunoglobulin mucin-3 (TIM-3): Novel therapeutic target for AML

TIM-3 as a cell surface marker uniquely expressed in AML LSCs, is reported to be absent in normal bone marrow HSC. TIM-3 is a negative regulator Th1-T-cell immunity that was recently discovered to synergize with Toll-like receptor signalling via cross linking with Galectin-9, when expressed on innate immune cells. It has also been reported to mediate phagocytosis of apoptotic cells by binding phosphatidylserine\[80\].

The successful reconstitution of Anti-TIM-3 monoclonal antibody, as the established clone (ATIK2a) was effective in eliminating TIM-3 expressing cell lines via both antibody dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The therapeutic effect of ATIK2a on normal and leukemic AML hematopoiesis was found to be substantial in leukemic development in NOD / SCID mice, with near complete sparing of normal hematopoietic monocytes. Collective data indicates the combination of novel Anti-TIM-3 and known Anti-CD44 or Anti-CD33 as a successful practical approach to eradicate AML LSCs, with minimal incidence of relapse. This is currently an active area for clinical trial design, and patient recruiting is currently ongoing\[81\].

Manipulation of signalling molecules in AML pathways

Another strategy to induce longer remissions, in AML patients with higher rates of relapses, is leukemic hematopoiesis pathway blockage and manipulation. A prominent example of application in this field is Notch pathway manipulation, a pathway strictly involved in CSC renewal. Recent interest in Hes1 induction as a critical stem / progenitor cell signalling molecule potentiated by hypoxia inducible factor (Hif1-alpha), lead to the discovery of Hif1-alpha as a therapeutic target in hematological malignancies\[82\]. This is achieved by the Hif1-alpha dose dependent autoregulation of Hes1 via blockade of its expression by blocking a negative-feedback autoregulation loop. Researchers continued identifying pharmacological
agents for Hif1-alpha knockdown such as echinomycin. However, the limitations of these studies were delineated by utilizing an exogenic model of human AML and from the short-term nature of echinomycin therapeutic effects reported.

**Other hematological malignancies and possible therapeutic pathways**

CARs have been greatly explored in the region of hematological malignancies. However, study designs in all available data are variable in many aspects including CAR design, in vivo preparation and pre-administration of lymph depletion mechanisms used[83].

Both chronic and acute lymphocytic leukemias (CLL and ALL) have been looked at as models to use T-cell CARs. The most used is anti-CD19 to bind receptor CD19 which is expressed on almost all B-cells, whether they were normal or B cells ALL[83-85].

With all data available, we conclude that the higher the CAR activity preinfusion and the longer its residence in vivo, the more effective is the treatment. However, with the higher titers in the body, there is a greater chance of exhibiting toxicity. Results were also more promising in ALL than in CLL[83-85].

A small study has shown a good response in 30 patients with different types of B-cell malignancies who received second generation CARs anti-CD19 with two subjects achieving complete remission[105].

A case report published in NEJM, reported a patient with refractory CLL who exhibited positive results with low dose T-cell with anti-CD19 combined with CD137 antigen infusion[83].

A brief report was published by Grupp and colleagues in 2013, in which they studied CD19 in a patient population of B-cell malignancies reaped good results in CLL with no need of concomitant chemotherapy to achieve remission. A subsection of relapsed ALL patients developed CD19 blast cells and speculations were that this is specific to a subset of ALL patients[84].

However, due to the nature of CD19 antigen being present on all B cells, B cell deficiency is expected. It cannot be speculated, if normalization of cell population would occur or remain deficient. Further studies are required to assess both therapeutic and complication profile of CARs in hematological malignancies[14,83,84].

**Cancer stem cells: Colorectal tumors**

Colorectal cancer (CRC) is one of the most well understood and researched cancers. Yet, it still remains as one of the leading causes of deaths worldwide. It was the third highest estimated incidence of cancer in the United States in 2012. Over 50,000 estimated deaths makes colorectal cancer, the 2nd highest of cancer related deaths[86]. Methods of treatment and therapy such as chemotherapy, radiotherapy and surgery do not cover the metastatic nature of the tumor in the later stages of cancer growth.

The mechanism of tumor development still remains one of the most elusive. With better understanding, we may one day be able to create a targeting therapy to disrupt cancer growth and metastasis. The CSC theory provides a new base of research in understanding colorectal cancer mutagenesis. The CSC theory attempts to open new insight regarding mutagenesis, discovery and the treatment of colon cancer. This review will show the developments and setbacks of recent research in colorectal CSC targeting.

The CSC model states that malignancies are derived from cancer cells with stem cell characteristics[83]. This model puts into perspective that existing stem cells within the human body are mutated and in those stem cells, there is a mechanism in which cancer cells may proliferate. Colonic stem cells are located at the bottom of the colon crypts. These cells normally replicate and produce progenitor cells for goblet cell, enteroocyte, and endocrine cell formation. There is also a belief that these cells exist around the base sparsely placed throughout paneth cells, possibly implicating the surrounding tissues.

Stem cells have the ability to regenerate themselves as well as the ability to form differentiated specialized cells. This pluripotency may enable colorectal stem cells to form the malignant nature of tumor development. These mutated stem cells would then be capable of mass proliferation and creation of other progenitor cells. The colorectal stem cells are normally capable of asymmetric proliferation. This asymmetric division allows for a division in which a stem cell regenerates as a new cell, while the other cell develops as a progenitor cell. Due to this, the stem cell is more constant through the cell cycle. This provides a location in which targeted therapy may benefit as now it is understood that CSCs are implicated in the development, metastasis and resistance of cancer[87]. It was previously thought that six mutations were needed to cause a cell to become cancerous. Now it might possible that only one mutation is needed[88].

Due to the long lasting and proliferative nature of the stem cells, a mutation in those cells provides a strong foothold for the tumor to develop.

Chemoradiotherapy (CRT) tends to target rapidly growing mutated cells. It may be plausible that the therapies don’t target the CSC. This could be the reason behind recurrence of tumor growth or resistance to treatment post CRT[89]. Many studies have investigated different ways to target tumorigenesis. One focus of recent research has been usage of markers. Markers are molecules on the cell surfaces of these mutated cells. These markers are used in attempts to identify CRC and potentially target therapy.
Implications of tumor markers in colorectal tumors

CD133, one of the more commonly known markers has been used to note multiple different types of cancer growths. It is a 5- transaminase glycoprotein found on cell surface on many cells, but more prominently and in greater numbers on CSCs. The function of CD133 is still unknown. It came with promise for radio or cytotoxic targeting. However, targeting cells with CRT using CD133+ would not be applicable as the CD133 expression is found within normal tissue as well[90]. It has also been shown to exist in normally functioning cells such as acinar and islet cells[91]. Although it has been shown not to be a sensitive marker for targeting therapy, CD133+ cells have been able to produce and maintain tumor growth on xenografts, while CD133- were not capable of initiating tumor growth[92]. CD133 serves as a prognostic factor for colorectal cancers as it becomes over expressed in metastasis. Although the majority is present in mature cancer cells, identifying CD133+ in patients with colorectal cancer served to kick-start the CSC theory[5]. It has also been shown to have a role in chemotherapy resistance[93]. In a study by Kunming Wen, two chemo-resistant cell lines were found. Both of these lines had an over expression of OCT4. These lines were characteristically similar to CSCs. The study also showed that when the OCT4 gene was not expressed, there was an increase of apoptotic rate within cells. It was also discovered that higher levels of STAT3 and surviving proteins were also found in these cell lines[100]. This provides new evidence to prove that OCT4 has a hand in anti-apoptosis in CSCs.

Targeting colorectal cancer stem cells signaling pathways

Another form of targeting cancer growth is via pathways. The regulation of the stem cells survival is through the Wnt pathway. Markers for the mentioned pathways may enable us to locate stem cells and monitor progression. To understand tumor development we must understand the signaling pathway. Disturbing this pathway has shown to affect the stem cells’ ability to replicate[101]. The Wnt pathway is the pathway in which stem cells are capable of regenerating, or differentiating. Destabilizing this pathway, along with TGF pathway is what is believed to be the CSC’s ability to proliferate and cause tumor growth[102]. ALDH is seen to be more specific towards CSCs than CD44 and CD133 towards cancers, although all are raised in malignant cells.

Some are even thought to be used in combination to increase monitoring ability and to determine more specific targeting. These have only offered a minute effect in understanding tumor initiation[96]. ALDH is proving to be beneficial for marking metastasis progression[96]. Another marker with potential promise is BCL-2. BCL-2 acts as an anti-apoptotic gene. When tested on mice lacking the gene, rates of apoptosis were significantly higher. This gives rise to the idea that the gene is resistant to apoptosis[103,104].

Cancer stem cells: Lung tumors

Lung cancer is the second most commonly encountered cancer in the United States as well as the major cause of cancer-related deaths. Lung cancer makes up the world’s highest incidence of malignant tumors, and has become a burden because of the increase in mortality rate[105]. In men, the age-adjusted incidence of lung cancer is 84.9 in every 100,000 men. Regarding cancer deaths, the age-adjusted incidence is 68.8 in every 100,000 men. In women, the age-adjusted incidence is 55.6 in every 100,000 women, while the age-adjusted lung cancer deaths incidence is 40.6 in every 100,000 women[106]. About 95% of all lung cancer cases have been reported in men that are above 40 years old, especially those who are between the ages 55 and 65 years. In non-smokers, the male and female incidence
is similar\textsuperscript{107}. Treatment failures occur whenever the cancerous cells in the affected tissues develop resistance to chemotherapy. Ideally, the function of the chemotherapy interventions is to induce apoptosis in the affected area. Whenever this is unachievable at clinically relevant drug concentrations, resistance can be said to have set in\textsuperscript{108}. Apoptosis fails to happen when there is increased DNA repair in resistant cells, increased cytoplasmic detoxification, and reduced cellular drug accumulation\textsuperscript{108}. Specific cancer cell populations that are resistant to chemotherapy are able to produce progenitor cells, metastasize and repopulate. These cells are known as CSCs. In recent years, when the concept of stem cells was introduced in oncology research, in addition to a variety of tumor tissues, and cancer cell lines, tumor stem cells have been successfully isolated and CSCs identified\textsuperscript{109}.

CSCs are characterized by self-renewal and proliferation similar to normal somatic stem cells, which makes them resistant to chemotherapy and radiotherapy. Therefore, they prevent chemo treatment from inducing apoptosis. This leads to the hypothesis that chemotherapy is not as effective in killing CSCs as compared to a treatment that specifically targets CSCs. Therefore more studies are needed to find out the origin of lung CSCs as well as new markers, to lead us to a new target therapy.

The lung CSC research breakthrough occurred in 2007. Ho et al\textsuperscript{110}, found for the first time that Hoechst dye efflux method from a variety of human lung cancer cell lines and human lung cancer clinical samples in the separated side populations cells showed high tumorigenicity \textit{in vitro}, fine ABC transporter cell surface protein expression, human telomere terminal transferase, and enzyme expression increased similar to the rate of stem cells. This indicated that this part of the side population had cells with CSC characteristics. The signalling pathways of Hedgehog (Hh), Notch, and Wnt / \(\beta\)-catenin signalling pathways all play an important role in tumorigensis by acquisition of unregulated self-renewal\textsuperscript{111}. These unregulated pathways are involved in inappropriate proliferation, tumor occurrence, invasion, and the metastasis process. They can potentially account as targets for lung cancer treatment.

The Hh / Patched pathway is taking part in embryonic development and cell fate determination. Throughout lung growth, Hh signalling is a major factor in lung bud branching morphogenesis. Hh signalling has been identified in regulating self-renewal of cancers cells in a number of solid tumors, like hematopoietic stem cells\textsuperscript{112} and myeloid leukemia cells\textsuperscript{113}. Hh overexpression may result in out of control proliferation of tissue stem cells, producing a sufficient number of target cells for further oncogenic occurrences, resulting in acquisition of CSCs. Modifications in the Hh pathway have been documented in a number of cancers such as glioma, stomach, colon, breast, prostate, and lung cancer\textsuperscript{114}.

The Notch family of transmembrane signalling proteins (Notch-1 , -2 , -3 , and-4 ) determine cell fate and are identified in stem cells\textsuperscript{115}. Notch signalling stimulation is involved in the up keep of self-renewal and plasticity \textit{via} Jagged-1, for hematopoietic stem cells\textsuperscript{116}. Inappropriate stimulation of Notch signalling induces proliferation, limits differentiation and inhibits apoptosis in cancer cells, and is associated with a variety of human cancers including lung cancer\textsuperscript{117}. Furthermore, Notch signalling has been proven to maintain lung somatic stem cells in an undifferentiated form, blocking terminal epithelial differentiation.

The Wnt pathway is responsible for cell fate determination in a number of organs for the period of embryonic growth. The Wnt pathway consists of an enormous range of proteins in a stream that eventually results in regulating the quantity of \(\beta\)-catenin that reaches the nucleus to stimulate gene expression. Wnt signalling has been demonstrated to control self-renewal in stem cells\textsuperscript{118}. Data from transgenic mouse models showed Wnt signalling pathway stimulation in stem cells resulting in epithelial cancers\textsuperscript{119}. This indicates the contribution of Wnt signalling pathway members in the deregulation of stem cells into CSCs. Wnt signalling might have a part in lung tumorgenesis\textsuperscript{120}.

### Lung cancer stem cell surface markers: CD133 and CD44

CD133 is a five-transmembrane glycoprotein also known as prominin-1. The function of CD133 has not been clearly explained and its ligand is unidentified at this point. At first it was described as a marker for isolating CD34+ human hematopoietic progenitor cells\textsuperscript{121}. It has been documented that CD133 might contribute to cell cycle regulation and proliferation of cells, but not always tumor initiation\textsuperscript{122}. CD133 has been used as a marker to analyze lung CSCs.

Chen et al showed larger expression of Oct-4 in CD133+ cells in comparison to CD133- cells in a research involving the examination of CD133 in lung cancer cells from patients and cell lines\textsuperscript{123}.

In that research, CD133+ cells were also reported to have enhanced resistance to conventional treatments and enhanced \textit{in vivo} tumor-restoration capability and proliferation compared to CD133- cells. Also Oct-4 expression was shown to be important for keeping stem cell-like characteristics, for instance self-renewal capacity and invasiveness. Eramo et al showed that the expression of CD133 ranged between 0.32% and 22% of tumor cells. In that research, the CD133+ cells isolated...
from lung cancer patient samples can mature and have a tumorigenic capability that was not present in CD133− cells\cite{124}. CD133+ cells have been documented to have a high rate in most lung cancers, with a relatively less rate in normal lung cells (< 1%) samples. Cells expressing CD133 have been identified as having a self-renewal capacity, chemotheraphy resistance in vitro and in vivo, and greater tumorigenicity\cite{125}. In spite of this, CD133 is probably not a widely used marker for all lung CSCs. In one of the studies, CD133 was not identified in most of the non-small cell lung cancer cell lines\cite{126}. Likewise, another study showed that both CD133+ and CD133− sub-populations of two human lung cancer cell lines have similar CSCs features\cite{127}. The unique reliance of this marker needs to be further evaluated.

CD44 is a cell-surface glycoprotein receptor that is involved in cell-cell interaction, cell adhesion, migration, and is associated with multi-drug resistance\cite{128}. Cancer associated factors such as chemokines, cell adhesion molecules, genes associated with Wnt signalling and TGF-β have been found to be up-regulated in cells expressing CD44\cite{129,130}. CD44, by means of its actions as a co-receptor with EGFR and ErbB family receptor tyrosine kinases, can influence cell proliferation\cite{131}. Furthermore, via the PI3K / AKT cascade CD44 has been associated with increasing anti-apoptosis\cite{132}. Leung et al showed that CD44 expressing sub-populations of some lung cancer cell lines have stem cell like properties. More studies are needed to justify the function of CD44 in tumor cell renewal and proliferation in the in vivo\cite{126}.

The regeneration of tumors after initial effective treatment is considered as resistance to chemotherapy and radiotherapy. Resistance to conventional therapies of cancer seen in CSCs compared to non-stem cells is likely because like their slow division, they have decreased apoptosis rate and the enhanced capability for DNA repair\cite{7}. This resulted in the concept that treatment targeting differentiated cells, but missing CSCs, results in tumor relapse, while new therapies focusing on CSCs could leads to tumor elimination. Discovering new therapies is a dedicated concept. The lack of specific and universal markers for lung CSCs makes it difficult to tackle.

Targeting lung cancer stem cells self-renewal pathways

There is rising curiosity to study the self-renewal pathways used by lung CSCs as therapeutic targets. These pathways include Hh, Notch, and Wnt. There is also an increased interest in using a combination of targeted lung CSC therapies with traditional therapies.

Taipale et al showed that the use of cyclopamine, a plant veratum alkaloid, inhibits the stimulation of Hh abnormal cell growth and the response pathway in basal cell carcinoma, rhabdomyosarcoma, and medulloblastoma. Other tumors with abnormal Hh signalling pathways may benefit from the use of Cyclopamine\cite{133}. The primitive features of pulmonary neuroendocrine cells are found in small cell lung cancer (SCLC) and activation of Hh signalling pathway in SCLC stimulates tumor growth. In the growth of SCLC and in the normal differentiation of pulmonary neuroendocrine precursor cells, Hh signalling is a major factor. Cyclopamine has shown to inhibit Hb signalling pathway as result enhanced apoptosis for SCLC\cite{134}. A new novel semi-synthetic cyclopamine analogue, IPI-926, with enhanced pharmaceutical properties and potency has been identified. It is now being subjected to clinical trials for numerous tumors\cite{135}.

Inhibition of Notch signalling pathway in lung cancer has demonstrated confirmation of reducing tumor growth and enhancing apoptosis via inhibition of the self-renewal effectiveness of CSCs\cite{136,137}. Konishi et al showed that a gamma-secretase inhibitor, MRK-003, decreased growth and enhanced apoptosis of human lung cancer cell lines in vitro and in vivo, from utilizing xenograft models and inhibiting Notch-3 signalling\cite{138}. Haruki et al showed that a dominant-negative Notch-3 receptor was useful in decreasing soft agar growth of human lung cancer lines by inhibiting the Notch-3 pathway\cite{139}. Tumor inhibition and inhibition of self-renewal in progenitor cells / adult stem cells by antibodies directed towards Notch signalling pathway is an undergoing investigation\cite{140}.

A wide range of mechanisms can be used to inhibit Wnt pathway. Down-regulation of β-catenin signalling has been achieved by using retinoic acid (RA) and tyrosine kinase inhibitors\cite{139,140}. A good result has been achieved against an NSCLC cell line using monoclonal antibodies targeted towards Wnt-1 by preventing the Wnt / β-catenin signalling pathway as well as inducing apoptosis\cite{141}. The monoclonal antibody may have effects on normal cells. For this reason, the benefit has to be weighed against treatment efficacy with CSCs.

The use of monoclonal antibodies (mAbs) targeting CSCs is a fairly new method. Direct mAb therapy particularly aiming towards lung CSCs has not been clearly studied. In spite of this, two of the markers (CD133 and CD44) expressed by lung CSCs, do have the possibility of being treated by mAb therapy. AC133 is a monoclonal antibody that identifies CD133. Display of tumor growth inhibition in hepatocellular cancer cells in vitro and in vivo has been achieved by the use of AC133 conjugated to a cytotoxic drug in a pre-clinical study\cite{142}. Likewise, H90 is another mAb that identifies CD44. H90 has shown to be effective in an AML model by being particularly aimed towards
CSCs, resulting in inhibition of tumor proliferation and niche localization[24].

CONCLUSION

The identification of CSCs represents a turning point in cancer research. Breast CSCs have been studied widely and recent studies have been successful in isolating Br CSCs that bare distinctive phenotypes like CD44+ and CD24low. Breast CSCs-targeted therapy represents a new era in breast cancer therapeutics. These targeted therapies have potential advantages in preventing relapse and prolonging survival, compared to conventional therapies.

Our understanding of CSCs has completely revolutionized how new treatments aimed at curing cancer patients should be designed. As is seen with many conventional anti-tumor therapies and as mentioned before, it is a fact that they merely target the proliferating population of cells that make up the tumor. This results in a failure to cure many patients suffering from cancer, and necessitates the use of approaches that not only target the feature of proliferation, but also other unique characteristics seen in cancer cells like resistance and novel survival strategies.

Although research has uncovered multiple new vulnerable features in cancer cells which can be exploited for new treatments, there is still the issue that mainly involves the selective and direct targeting of cells. The CSC concept has even allowed us to understand and model resistance to conventional therapies, which is an important factor that influences treatment outcome. Using the information gathered from analysis of CSCs, the mechanisms of resistance to CRT and endocrine therapy are now better understood.

The mechanism of how cancer cells are generated as progeny from stem cells and the fact that they can be reproduced, when transplanted into other hosts, is further proof that stemness is a property exhibited by cancer cells, and plays a role in survival and tumor relapse. The uncovering of the stem cell signalling pathways is breaking new ground in the designing of anti-tumor therapies by providing us with a better understanding of how tumor cells thrive, and new targets to focus treatment on. By exploiting such signalling pathways, it is also possible to make cancer cells less resistant to conventional treatments, making it possible to combine newer emerging treatments with current cytotoxic therapies. Finally, the identification of tumor markers has provided us with other explanations as to how cancer cells confer resistance, because with such markers we now know that cancers such as breast carcinoma exhibit subtypes which are more resistant to treatments than other subtypes due to differences in their molecular make-up.

As promising as the these markers seem to be towards potential monitoring progression of tumors and targeting cancer cells, the outlook for many offers a grim reality. For example, CD133, although a marker of colonic tumor cells, has shown an increase post-CRT, not only in the crypts but throughout the intestine. This tends to show a dimmer outlook toward effective targeting. CD44, OCT4, ALDH, BCL2 and CD133 have all shown to have potential as CSC markers. They shed light into the novel world of colorectal CSCs.

Colorectal cancer can be treated in the early stages via surgery, however if not diagnosed and treated in time, the rate of survival diminishes rapidly. All colorectal CRT treatments target the proliferative nature of the cancer cells in an attempt to minimize the growth. It seems as if the stem cells are not being targeted or perhaps provide resistance to the therapies themselves. The CSC may be capable of regenerating cells resistant to the therapy. The future of treatment of colorectal cancers lies in the new and upcoming research of CSCs. The potential in CSC targeting via markers is vast in account of the benefits regarding treatment. If these CSCs can be found and pathways better understood, CRT resistance cancers can be targeted, offering a better prognosis than the current situation.

This review article is a retrospective review of medical theory and clinical practice style, utilizing all medical publications excluding historical novelty references. The following databases are quoted: BMJ publishing Group Ltd, Cochrane Library, New England Journal of Medicine (NEJM), Medline (EBSCO), and PubMed.

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