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A BRIEF REVIEW ON THE THERAPEUTIC RESISTANCE AGAINST CANCER BY CANCER STEM CELL (CSC)

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Review Article

ABSTRACT

Cancer stem cells (CSCs) are small subpopulation of cells within tumours which possess characteristics associated with normal stem cell such as self-renewal and differentiation. CSCs play crucial role in cancer development by regulating the cancer cell survival, metastatic potential, disease relapse and poor prognosis. There are many cell markers like CD44, CD24, EpCAM, CD133, CXCR4, cMet, ALDH1 which can identify the CSCs.These cells are poorly regulated through cell cycle and also have metastatic ability as well as long life span. Chemotherapy is a part of successful cancer treatment. But the CSC's multidrug resistance mechanism (MDR) like high expression of ABC transporter, histone lysine demethylase, suppression of apoptosis, progesterone receptor membrane component-1(PGRMC1) and increased expression of aldehydrogenase-1 are responsible to resist chemotherapy. Combined therapies targeting CSCs and their progenies may represent the most promising approach for the future treatment of cancer patients. This review summarizes the characterisation and identification of CSCs, different multidrug resistance mechanism of CSCs and the advanced types of treatment mechanism.

Keywords: Cancer stem cell (CSC); CSC markers; disease relapse; metastasis; multidrug resistance; selective therapy; tumours.

1. INTRODUCTION

Cancer stem cell is a general term referring to the cancer cells capable of differentiation and self-renewal which is the role of CSCs chemotherapy resistance. The definition of CSCs does not determine their origin and the term "Cancer Stem Cell" does not mean that cancer begins from stem cell. CSCs are more differentiated than stem cells including a more limited spectrum of the cells existing in a tissue [1,2]. Some cells in a tumour may undergo some sort of genetic or epigenetic changes in the signalling pathway which results in phenotype similar to that of

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stem cells [3,4]. These changes may happen in different types of cells such as stem cells, progenitors, and differentiated cells [5,6]. In 1994, CSCs were isolated for the first time. In 1855, German pathologist stated that cancers arise from the activation of dormant embryonic-like cells present in mature tissue and argued that cancer does not simply appear spontaneously [3,7]. One and half century later, Lapidot and colleagues came up with the CSC hypothesis [4]. The initial cell that develops cancer is not necessarily a cancer stem cell, though cancer-initiating cell and cancer stem cell are sometimes used interchangeably. The existence of CSCs was first proposed 40 years ago, though analysis of its details remains a mystery until the evolution of advanced research tools [2,8]. The best evidence to support the existence of CSCs came from the study of haematological malignancies [6]. Considering the role of embryonic stem cells and self-renewal in mature cells like blood cells, the definition of CSCs was revealed [7,9]. TPC (Tumour Propagating Cell) is the other term which has been used very often for cancer stem cells [8].

Tumour stem cell research is progressing rapidly in various fronts including studies of their origin, organization and characterization [2,10]. Cellular mechanism of their propagation, communication and signalling as well as immunological aspects are also discussed in detail after thorough studies [1,11-13]. The origin of human acute myeloid leukaemia is engraved in primitive hematopoietic cell [8,4]. The evolution of cancer stem cell has been described with detailed investigation in leukaemic cancer cells [7]. On the other hand, the nature and composition of tumour stem cells microenvironment is enlightened by Resetova et al. [14]. Cancer cells also exhibit cellular resistance against therapeutic avenues achieved through chemical and physical therapeutic modalities. In this connection, drug resistance by tumour stem cells in the form of chemoresistance is achieved by cancer stem cells [15,16]. Modulation of cell death pathways in such stem cells is also studied [17]. Thyroid cancer resistance to hemotherapeutic drugs via anticrime production of interleukin-4 and interleukin-10 is discussed by Stassi et al. [18]. Despite chemo resistance by cancer stem cells, various modalities have been under development to tame the uncontrolled propagation of stem cell populations hiding inside a cancerous tissue [19,20,21,22]. Ubiquitination is one of such pathways which is essential in targeted cancer therapy of cancer stem cell [3]. Targeting apoptosis pathways in cancer stem cells is another possibility [18]. So, cellular chemotherapy against cancer stem cellsis the need of the hour [20]. Detailed understanding and working on cancer stem cells is required to uproot the origin of the cancer cell population and thus also helpful for their management in cancer therapy [23].

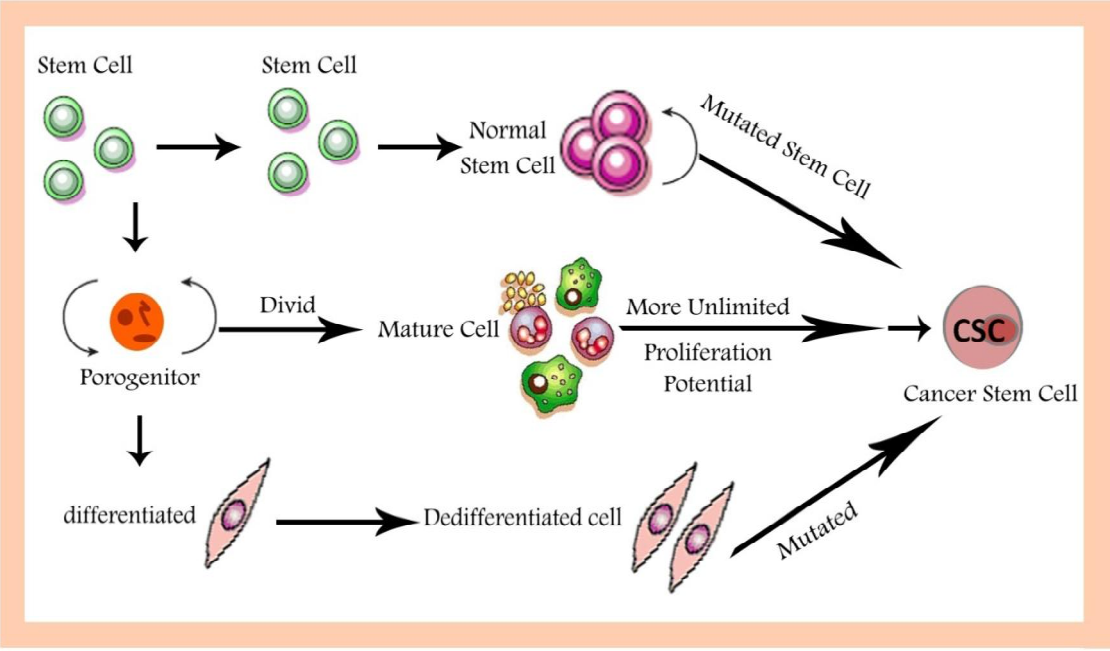

Fig. 1. Proposed model for the formation of cancer stem cells shows the origin of CSCs in different tissues [2]

2. CANCER STEM CELLS

Cells with stem-cell qualities have been identified in malignancies of haematopoietic origin and in some solid tumours. The existence of such a population would imply that the stem cell represents the cell of origin for the tumour, as illustrated in Figs. 1 and 2. One can predict that such cancer stem cells represent only a small fraction of a tumour, as they possess the capability to regenerate a tumour, and most cancer cells lack this regenerative capability. For example, when plated in soft agar or injected into mice, most tumour cells do not give rise to colonies. Similarly, in experiments performed in humans in the 1950s, unthinkable by today's ethical standards, 35 patients had an estimated one billion of their own tumour cells injected into their thigh or forearm. Only seven of these autotransplants resulted in tumour growth at the injection site. Furthermore, studies of acute myelogenous leukaemia have shown that only 0.1–1% of all cells have leukaemia-initiating activity. These leukaemia-initiating cells have many markers and properties of normal haematopoietic stem cells. So it is believed that leukaemia arises from a stem cellthat becomes transformed and gives rise to a large population of clones that proliferate but cannot self-renew or fully differentiate [12]. Similar populations of self renewing cells, such as those that carry the chromosomal translocation t(9; 22)(q34; q11), which forms the *bcr–abl*fusion gene, have also been identified in patients with chronic lymphocytic leukaemia and chronic myelogenous leukaemia (CML).

3. ISOLATION OF CSCS BY VARIOUS MARKERS

Long term cell culture, FACS (Fluorescence-activated cell sorting), and MACS (magnetic cell sorting) are the main techniques used to isolate CSCs. CSCs enrichment can be done using the FACS technique. We can also isolate cells based on the expression of special proteins of cellular-level, cell culture, epigenetic changes and expression pattern of such cellular-level markers as CD 24, CD133, ALDH1 and CD44. CSC characteristics can be determined through mRNA and miRNA expression analysis, copy number variation, etc. Then phenotypic and genotypic characteristics can be associated with *in-vitro* and *in-vivo* clinical data. Magnetic Cell Sorting (MACS) technology isolates cells with a high quality and is regarded as a standard method for cell isolation. This technique can isolate cells based on expression of special stem cell markers like CD133. Before isolation, cell markers are labelled using special monoclonal antibody or magnetic micro bead like anti

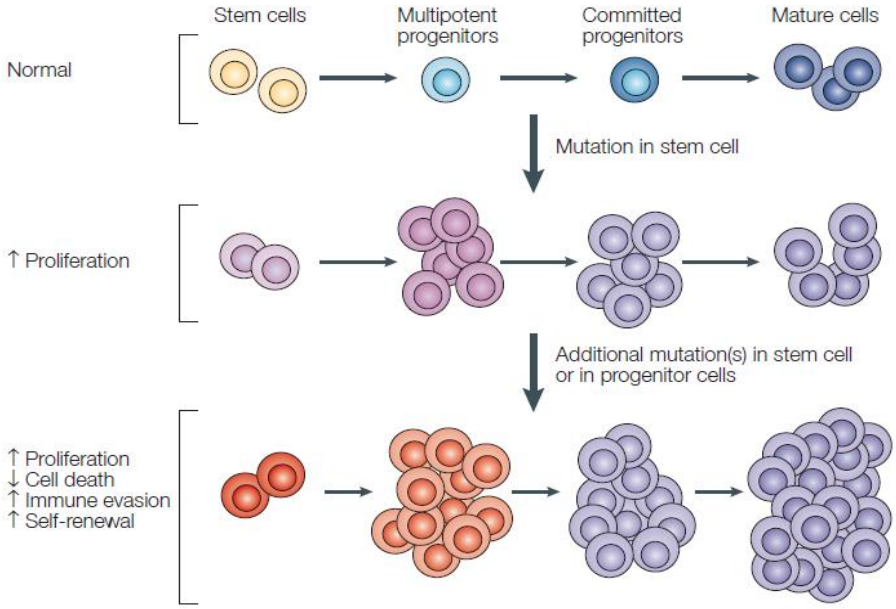

Fig. 2. Cancer stem cells and tumour progression, Normal stem cells give rise to multipotent progenitor cells, committed progenitors and mature, differentiated cells. Mutations in a stem cell give rise to a stem cell with aberrant proliferation and result in a pre-malignant lesion. Additional mutations lead to the acquisition of further increased proliferation, decreased apoptosis, evasion of the immune system, and further expansion of the stem-cell compartment that is typical of malignant tumours [7]

CD133 which is 106 times smaller than the cell's size. After labelling, magnetic isolation is carried out. Washing cells is the third step and after positive selection, marked cells are separated from unmarked ones. Positive selection is one of the best and most direct ways to isolate target cells from cell suspension. CSCs have a set of markers for detection and determination. For instance, CD133 known as Prominin 1 or AC133 is an intermembrane protein and a special surface antigen in blood stem cells and a marker for Murin neureopithelial. Although the function of CD133 is yet to be discovered, it is known as a marker for cancer tissues and isused individually or combined with other markers to isolate stem cells from many tumours like brain, prostate, colorectal, etc. In pancreatic cancer, surface markers such as ESA, CD24+, CD44+, etc. have been detected. The only selected marker identified for T ALL (T-acute Lymphoblastic Leukaemia) was CD34+ and further studies on T ALL cell lines have led to the detection of other markers like CD110 (C-MP1), CD90 (ty-1), CD44+, CD49+, CD133+ and the ALDH enzyme in colorectal cancer. ALDH1 is introduced as a stem cell marker (Table 1). Expression of ALDH1 may be associated with clinic pathologic feature in Esophageal squamous cel carcinoma patients. In the following table, some of the known markers are indicated [8,4].

4. CANCER STEM CELL RESISTANCE AGAINST DRUG AND CHEMO-THERAPY

The therapeutic strategies against cancer are facing too many problems. One of them is drug resistance. Recent researches have revealed that CSCs, especially pancreatic CSCs, play crucial roles in drug resistance which often impairs the successful use of chemotherapies. In pancreatic cancer stem-like cells were found to be more resistant to gemcitabine, commonly used against pancreatic carcinoma, and those cells were more invasive. Pancreatic CSCs were proved to contribute to drug resistance of gemcitabine as well. Another problem is that most therapies for pancreatic cancer do not affect pancreatic CSCs, which canthen re-establish tumours after treatment. The cancer stem cell developed many mechanisms to resist the drug and chemotherapy.

5. DRUG TRANSPORTERS IN STEM CELLS

Stem cells have many properties that separate them from mature, differentiated cells. In addition to their ability to self-renew and differentiate, they are quiescent, dividing infrequently. They also require specific environments comprising other cells, stroma and growth factors for their survival. One particularly intriguing property of stem cells is that they express high levels of specific ABC drug transporters. For example, haematopoietic stem cells express high levels of ABCG2, but the gene is turned off in most committed progenitor and mature blood cells [5,9]. The two ABC transporter-encoding genes that have been studied most extensively in stem cells are ABCB1, which encodes P-glycoprotein30, and ABCG2. Along with ABCC1, they represent the three principal multi drug-resistance genes that have been identified in tumour cells. These genes, members of the ABC-transporter super family, are promiscuous transporters of both hydrophobic and hydrophilic compounds (Table 2). These transporters also have important roles in normal physiology in the transport of drugs across the placenta and the intestine (more accurately, there tention of drugs in the intestinal lumen), and are important components of the blood–brain and blood–testis barriers. By using the energy of ATP hydrolysis, these transporters actively efflux drugs from cells, serving to protect them from cytotoxic agents. Mice deficient in either Abcg2, Abcb1 or Abcc1 are viable, fertile and have normal stem-cell compartments. This indicates that none of these genes are required for stem-cell growth or maintenance. However, these knockout mice are more sensitive to the effects of drugs such as vinblastine, ivermectin, topotecan and mitoxantrone, consistent with a role for these ABC transporters in protecting cells from toxins [9,24]. The drug-transporting property of stem cells conferred by these ABC transporters is an important marker in the isolation and analysis of haematopoietic stem cells. Most cells accumulate the fluorescent dyes Hoechst 33342 and rhodamine 123, but stem cells do not, as these compounds are effluxed by ABCG2 and ABCB1, respectively. Because they don't accumulate these fluorescent dyes, stem cells can be sorted by collecting cells that contain only a low level of Hoechst 33342 fluorescence. These cells are referred to as 'dull cells' or 'side population' (SP) cells. The term side population was coined because during flow-cytometry analysis, SP cells are visualized as a negatively stained 'side population' to one side of the majority of cells on a density dot plot. A large fraction of haematopoietic stem cells is found in the SP fraction40 and when isolated from mice and transplanted into irradiated mice, small numbers of these SP cells can reconstitute the bone marrow, demonstrating that these cells are pluripotent. SP cells can be isolated from many tissues including the brain, breast, lung, heart, pancreas, testes, skin and liver, and these cells might represent lineage- specific stem cells. Hoechst-33342 staining of bone marrow from ABCG2-null mice fails to detect SP cells. However, the lack of staining for SP cells occurs not because

Table 1. List of cell surface markers found on various tumour cells [4]

Tumour type	Cell surface marker references
Acute myeloid leukaemia (AML)	CD34+, CD38−
Breast cancer	EPCAM (ESA)+, CD44+, CD24−, ALDH, CD29, CD133
Ovarian cancer	CD133+, CD44+, CD117+, CD24+
Glioblastoma	CD133+, CD15+
Medulloblastoma	CD133+, CD15+
Small cell and non-small cell lung cancer	CD133+
Hepatocellular carcinoma	CD45−, CD90+
Colon cancer	CD133+, CD44+, CD26+, ALDH
Prostate cancer	CD44+, CD133+, CD49
Melonoma	CD20+, CD271+
Pancreas adenocarcinoma	CD44+, CD24+,
Renal carcinoma	CD133+
Head and neck squamous cell carcinoma (HNSCC)	CD44+, ALDH1
Lung cancer	CD133+, CD90, CD117, ALDH1

Table 2. List of ABC transporter proteins responsible for drug resistance [10]

ABC transporters involved in drug resistance			
Gene	**Protein/ alias**	**Chemotherapeutic drugs effluxed by transporter**	**Other drugs and substrates**
ABCA2	ABCA2	Estramustine	-
ABCB1	PGP/ MDR1	Colchicine, doxorubicin, etoposide, vinblastine, pacitaxel	Digoxin, saquinivir
ABCC1	MRP1	Doxorubicin, daunorubicin, vincristine, etoposide, colchicine, camptrothecins, methotrexate	Rhodamine
ABCC2	MRP2	Vinblastine, cisplatin, doxorubicin, methotrexate	Sulfinpyrazone
ABCC3	MRP3	Methotrexate, etoposide	-
ABCC4	MRP4	6-mercaptopurine, 6-thioguanine and metabolites; methotrexate	PMEA, Camp, cGMP
ABCC5	MRP5	6-mercaptopurine, 6-thioguanine and metabolites	PMEA, Camp, cGMP
ABCC6	MRP6	Etoposide	-
ABCC11	MRP8	5-fluorouracil	PMEA, Camp, cGMP
ABCG2	MXT/ BCRP	Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, imatinib, methotrexate	Phenoforbide A, Hoechst 33342, rhodamine

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; MDR, multidrug-resistance-associated protein; MXR, mitoxantrone resistance protein; PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine

these cells are absent, but because the lack of ABCG2 expression allows these cells to accumulate Hoechst dye and become fluorescence [24,10].

6. MULTIDRUG RESISTANCE MECHANISMS

6.1 Histone Lysine Demethylase and Cell Death Pathways in Cancer Stem Cells

His tone lysine demethylases promote tumourigenicity, they modulates cell death pathways in two possible ways-1) Epigenetic regulation by H3K4, H3K36 demethylation, thus repressing the transcription of pro-apoptotic or anti-proliferation related genes and H3K9, H3K27 demethylation, thereby activating anti-apoptotic or proliferation related genes; 2) Modulation of cell signalling pathways by direct lysine methylation mediated activation and inactivation of targeted proteins. His tone demethylases are known to repress mRNA expression of Bcl2, p21, ERBB2, CCNA2, BRCA1, miRlet-7e and regulate p53 functions [17].

6.2 Epigenetic Regulation of Cell Death and Proliferation

Epigenetic regulation of cell death and proliferation by histone lysine demethylases is mediated mainly

through repression of p21 by LSD1 and KDM5b. In MLL- AF9 leukaemia stem cells LSD1and p21 are essential for maintaining the properties of oncogenic potential and self-renewal. p21 is a cyclin-dependent kinase (cdk) inhibitor and is a key mediator of DNA damage induced p53-dependent cell cycle arrest and apoptosis. In leukaemic cells, p21 is necessary for self-renewal of leukaemia stem cells. LSD1 and KDM5b regulate mRNA expression of anti-apoptotic gene CDKN1 (p21). LSD1 also regulates expression of cellular proliferation genes CCNA2 and ERBB2 by binding directly to the promoters of these genes. KDM5b interacts with TFAP2C and Myc to form a complex leading to transcriptional repression of p21. As LSD1 represses the expression of p21, knockdown of LSD1 in MDA-MB 231 cell model decrease the occupancy of LSD1 on the p21 promoter and significantly increase in the repressive mark of methylated H3K9 on CCNA2 and ERBB2 promoter regions. CCNA2 encodes Cyclin A2 that functions as CDK2 kinase activator and promotes progression of cell through G1/S and G2/M phases of cell cycle. ERBB2 (HER2) is a member of epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. It forms heterodimer with other members of EGF receptor family, stabilizing ligand binding and enhances downstream mitogen-activated protein kinase and phosphatidylinositol-3 kinase mediated downstream signalling pathways. Over-expression of cyclin A2 and ERBB2 corresponds to a drug resistant or aggressive phenotype of tumour cells. LSD1 can also be linked to the aberrant regulation of Wnt signalling pathways in cancer cells. Wnt signalling is important to maintain cancer stem cell state in various cancers. Treatment of colon cancer cells with LSD1 oligoamine inhibitor SL111144 led to increases in H3K4Me3, restoring expression of secreted frizzled-related proteins 2 (SFRP2). SFRP2 is a Wnt signalling pathway antagonist and it enhances the expression of the epithelial marker E-cadherine, through inhibition of the expression of SLUG, TWIST and SNAIL. SNAIL, SLUG and Twist are transcription factors involved in the epithelial mesenchymal transition (EMT) program. KDM6b act on H3K27 and is responsible for activation of anti-apoptotic gene Bcl2 transcription in hormone dependent breast cancers. Apart from normal antiapoptotic functions Bcl2 is thought to be involved in resistance to conventional cancer therapies, suggesting role of decreased apoptosis may play a role in the development of cancer. KDM5A-mediated H3K4 demethylase activity plays an important role in maintaining the proliferative capacity of breast cancer cells through repression of tumour suppressor genes, including BRCA1. Another histone lysine demethylase JARID1B leads to repression of let-7e which then increases expression of cyclin D1 [17,25]. Cycline D1 is a target gene of mir let-7e mediated gene regulation. JARID1B demethylase contributes to tumour cell proliferation through the epigenetic repression of a tumour suppressor miR let-7 that has been reported to be a direct regulator of RAS expression in human cells. In lung cancer patient samples, expression of RAS and let-7 showed reciprocal pattern, which has low let-7 and high RAS in cancerous cells, and high let-7 and low RAS in normal cells. Other targets of let-7 are some oncogenes like high mobility group A2 (HMGA2) and MYC. Histone lysine demethylase mediated epigenetic gene regulation thus can drive tumourigenesis in cancers and inhibit programmed cell death to support cancer stem cells state [26].

6.3 Non-Epigenetic Regulation of Cell Death and Proliferation

Non-epigenetic regulation by lysine histone demethylases is mediated by their potential to demethylate various cellular proteins. E2F1-p53 axis is the major target of non-epigenetic regulation of cell death and proliferation. p53 transcriptional activity is necessary to inhibit cancer stem cells growth and proliferation. His tone lysine-specific demethylase LSD1 interacts with p53 to repress p53-mediated transcriptional activetion and to inhibit p53 mediated apoptosis. LSD1 removes both mono and di-methylation at K370 of p53. Mono-methylation K370me1 represses p53 func- tion and prevents interaction of p53 with TP53BP1 (p53- binding protein 1), thus represses p53-mediated transcriptional activation in p53 negative cells (p53-/-) LSD1 removes methylation mark from E2F1 at lysine- 185. Lysine-185 methylation leads to E2F1 accumulation during DNA damage and activation of its pro-apoptotic target genes p73 and Bim (Fig. 3). E2F1 promotes DNA damage-induced apoptosis in p53 dependent as well as p53 independent manner. LSD1 mediated demethylation leads to dysregulation of the E2F1 function and promotes survival in many cancer cells [15,17].

7. INCREASED EXPRESSION OF ALDEHYDE DEHYDROGENASE IN CSC

Stem cell-like populations in normal female breast tissue are characterized by the expression of aldehyde dehydrogenase 1 (ALDH1). ALDH1, an enzyme responsible for oxidation of retinol to retinoic acid, is important for normal development and homoeostasis in several organs and is crucial during embryogenesis. Breast cancer stem cells also express ALDH1 and breast CSCs have been isolated on the basis of increased ALDH1 [24]. Indeed, expression of ALDH1 by breast tissue is considered to be a marker, in breast,

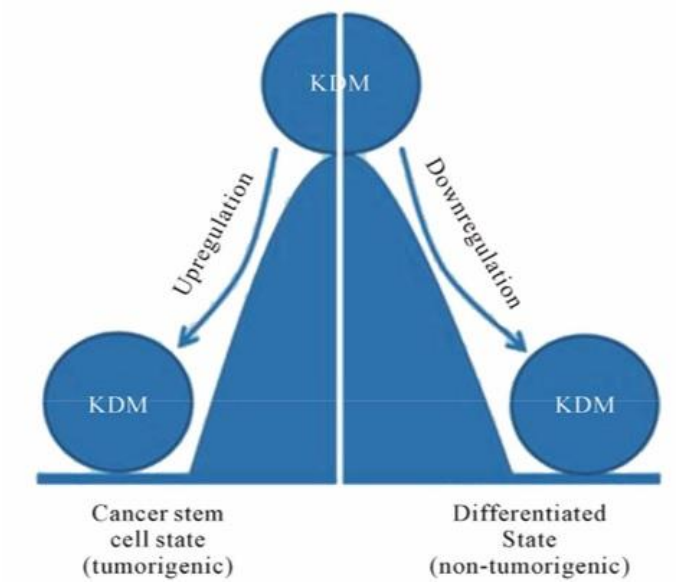

Fig. 3. General role of lysine histone demethylases in maintaining cancer stem cell state. Cancer stem cells over-express histone lysine demethylases and low expression is associated with differentiated state. KDM stands for lysine demethylase [17]

for both normal and malignant stem and progenitor cells. This finding appears to Breast cancer cells with a CD44+/CD24− phenotype have also been suggested to have tumour-initiating properties with stem cell-like features and have been shown to be associated with basal-like cancers and BRCA1 hereditary breast cancers in women [10]. The recent study showed increased invasion and tumourigenicity capacity of CD44+/CD24− breast cancer MCF7 cells *in vitro* and in nude mice [15]. A partial overlap between the CD44+/ CD24−/low population and the ALDH1+ population has been reported. The combined CD44+/ALDH1+ phenotype shows an especially high tumourigenic capacity, be- ing able to form tumours in nude mice from as few as 20 transplanted cells [10,15]. In this study, we are going to use CD44 and ALDH1 as cancer stem cell marker. The tumour microenvironment also affects cancer development and plays a significant role in prognosis. This appears to be true for the effect of stem/progenitor cells on the prognosis of human breast tumours. Stromal cells of breast tumours have been shown to be positive for ALDH1 [17]. Several studies have investigated the correlation between the expression of CSC markers and prognosis and drug resistance in female breast cancer. However, the expression of CSC markers in male breast cancer has not been well studied. Accordingly, in this study, the expression of two CSC markers—ALDH1 and CD44—in 19 male breast cancers, clinically important as ALDH1 expression has been associated with poor clinical outcome and resistance to chemotherapy in female [25].

8. ROLE OF PROGESTERONE RECEPTOR MEMBRANE COMPONENT (PGRMC1)

PGRMC1 is induced in a number of cancer types, including breast, ovarian and lung cancers, and a small study indicated that PGRMC1 is associated with poor survival in lung adenocarcinoma. PGRMC1 is also expressed in sebaceous carcinomas. PGRMC1 plays a causative role in cancer progression, because *in vitro*, PGRMC1 increases tumour cell proliferation, chemotherapy resistance and invasion, and *in vivo*, PGRMC1 increases tumour growth, angiogenesis and metastasis. There are a number of potential mechanisms through which PGRMC1 might promote tumour growth. PGRMC1 associates with the epidermal growth factor receptor (EGFR) and regulates susceptibility to the EGFR inhibitor erlotinib by increasing plasma membrane pools of EGFR. PGRMC1 also increases EGFR levels in Zebrafish. In lung cancer cells, the EGFR-PGRMC1 complex drives invasion, at least in part, by activating matrix metalloproteinases [19]. PGRMC1 is also detected in the nucleus in some cell types, where it regulates transcription and in the centromeric region of chromosomes during oocyte meiosis. PGRMC1 also localizes to the actin cytoskeleton and binds actin. In lung cancer cells, the prominent localization for PGRMC1 is cytoplasmic puncta, including early endoscopes, and numerous groups have reported similar findings in other cell types. Finally, PGRMC1 is secreted by lung cancer cells, where it has a pro-

proliferative function, and is detected in the plasma of lung cancer patients. There is a growing consensus that PGRMC1 is critical for the transport of specific receptors to the plasma membrane. The receptors include EGFR, GLP1R, glucagon-like peptide 1 receptor, and mPR1α, membrane progesterone receptor α. PGRMC1 binds to mPR1α and transports it to the plasma membrane. Indeed, PGRMC1 was originally identified as a putative hormone receptor or "receptor membrane component". Partially purified PGRMC1 binds to progesterone, and recently, progesterone binding by recombinant PGRMC1 was reported, suggesting a direct role for PGRMC1 in progesterone function. PGRMC1 has anestablished role in progesterone signalling, and in some diseases, such as breast cancer, this contributes to hormonal growth and anti-apoptotic signalling. However, PGRMC1 shares no homology with hormone receptors but has motifs that are structurally related to cytochrome *b5*, and PGRMC1 binds heme, an evolutionarily conserved function that is distinct from progesterone binding. According to the cancer stem cell theory, tumours contain a sub-population of cells with extended replicative potential that contribute to drug resistance. Cancer stem cells are thought to arise from mutations to either normal stem cells or transit amplifying cells, with key signalling contributions from the tumour microenvironment.PGRMC1 is detectable in amniotic-derived mesenchymal cells and has been identified as an important hormonal signalling intermediate in neuronal stem cells, but its expression and function in cancer-derived stem cells have not been determined. Is has been demonstrated that PGRMC1 is elevated in multiple tumour types, including head and neck cancer and in oral cancer. Using immunohistochemistry of paraffin-embedded tissue, we also confirm previous findings from western blots of frozen tissue that PGRMC1 staining correlated with survival in lung cancer patients. According to the stem cell theory, cancer stem cells are critical for the long term survival of a tumour population and its therapeutic resistance. We report here that PGRMC1 is abundant in lung cancer-derived stem cells from patients, and PGRMC1 inhibition triggered cell death in lung cancer stem cells where other therapeutic classes failed [25].

9. SUPPRESSION OF APOPTOSIS IN CSC

There are several methods of cancer treatment under discussion such as inhibiting kinases using small molecules, monoclonal antibodies, and other new treatment methods. These factors are designed individually or combined with chemotherapy to prevent the dysregulated signalling pathways which cause disorder through blocking the tumour growth or sensitizing cancer cells to death. One of the factors that let cancer cells overcome stress is their ability to avoid apoptosis. CSCs share some of their features with stem cells including dormancy, activation of DNA-repair machinery, expression of drug transporter (ABC), and natural resistance to apoptosis [19]. In thyroid cancer, therapy resistance leads to the induction of cell death, as a result of which the expression of anti-apoptotic proteins is increased along with the production of autocrine products such as (IL4) [27,14]. It has been proved that IL4 causes resistance to apoptosis in chronic lymphocytic Leukaemia and increases the anti-apoptotic proteins' expression in normal cells [18,28]. In pancreatic cancer, IL4 boosts the growth and its blockage has inhibitive effects [20]. The efficacy of chemotherapy may be enhanced when combined with anti-IL4 adjuvant treatment [29]. Several different molecular changes can regulate apoptosis among which are the activation of anti-apoptotic factors (Bcl-2, Bcl-xl, Bfl1/A1), inactivation of factors driving apoptosis such as p53. Most effective therapeutic strategies are based on special molecular biomarkers which respond to treatment in a group of patients. Apoptotic signal is a background that discovered in tumour biology and efforts have been made to activate organized death in CSCs [18,16]. According to the CSC hypothesis, manipulation of apoptotic machinery in order to eradicate tumour-initiating cells requires a huge therapeutic potential [28].

10. HEDGEHOG SIGNALLING AND CANCER

The Hedgehog molecules (SHH, IHH and DHH) are important signalling proteins in the development of embryonic stem cells and in the differentiation of many tissues. Hedgehog (HH) binds to the cell-surface receptor Patched (PTCH) and signals through the Smoothened (SMO) and GLI proteins. This pathway has a clear role in tumour formation in patients with nevoid basal-cell carcinoma syndrome, in which *PTCH* mutations have been described. Additional members of the HH pathway have also been found to be tumour suppressors or oncogene. Recently, components of the HH–PTCH pathway have been shown to be disrupted or over expressed in a large number of tumours, including sporadic medulloblastomas, breast, prostate, stomach, colon and pancreatic cancers. Most sporadic medulloblastomas have either germline *PTCH* mutations or *PTCH* silencing through methylation. Treatment of medulloblastomas with the SMO inhibitory compound cyclopamine resulted in reduced proliferation and changes in gene expression consistent with differentiation. Small-cell lung tumour cell lines show high expression of *SHH*, and their growth can be inhibited by SHH antibodies

orcyclopamine. Similarly high levels of HH expression and HH–PTCH pathway activation have been found in oesophageal, stomach, pancreatic, prostate and biliary tumours and in cell lines. Treatment with cyclopamine led to regression of pancreatic and prostatic tumours in mice, providing a model system for therapeutic development. HH over expression could lead to the unregulated growth of tissue stem cells (Fig. 4). This would result in a pre-malignant lesion in which abnormal stem-cell growth drives hyper proliferation. These unregulated stem cells would be the target for genetic events that drive the stem cells into the formation of tumour stem cells. Continued evolution of the tumour stem cells could occur to give rise to metastatic cells or further drug resistance [4,10].

11. RECENT APPROACHES FOR TARGETING PANCREATIC CSCs

Minnelide, a water-soluble prod rug of triptolide, is currently under phase I clinical trial. By decreasing CD133+tumour-initiate cells (TICs or CSCs) as well as non-TIC population, Minnelide could reduce tumour burden, which might point out a potential and effective therapy against PC [20]. Sulforaphane could inhibit the growth of pancreatic CSCs orthotopically implanted in NOD/SCID mice by inhibiting SHH pathway and also inhibits the marker of EMT in human pancreatic CSCs [16]. The dual endothelin1/VEGF signal peptide receptor, DEspR, is detected in micro vessels and tumour cells in PDAC. It can be found in CSCs isolated from PDAC-Panc1 cells as well. Researches demonstrated that DEspR inhibition could decrease Panc1-CSC xenograft tumour growth in nude rats by impacting CD133+ CSCs, suggesting that DEspR-inhibition defines a novel targeting therapy for pancreatic cancer [30]. Disulfiram, an irreversible inhibitor of ALDH, was found to play a key role in resistance to anticancer therapies for PDAC. Kim et al found human PDAC-derived cells, expressing high levels of ALDH, could show CSC features. Disulfiram is sensitive to this gemcitabine-resistant subpopulation and removes ALDH-high cancer cells and inhibits tumour growth [31]. CSCs are enriched in the side proportion (SP) cells, which over expresss stem cell markers as well as pluripotency maintaining factors, such as Nanog, Sox2, Oct4, c-Myc, signalling molecule Notch1, and drug resistant gene ABCG2. Some scientists established a combination of Sox2/Oct4/c-*myc* targeting agent, which could suppress all CSC properties and phenotypes, and reduce the tumourigenic capability of the SP cells and the resistance to conventional chemotherapy [32]. Inhibiting c-Met with XL184 or Alk-4/7 with SB431542 [33] reduces the number of CSCs in tumours and has synergistic effects with gemcitabine. While Gemcitabine treatment results in an increase of the c-Me-thigh CD44+ population, c-Met inhibition with XL184 leads to a decrease in c-Met high CD44+ cells. Combination treatment prevents the increase in the CSC population observed with Gemcitabine alone and also contributes toa decrease in c-Met high CD44+ population, suggesting that XL184 targets the CSC population specifically.

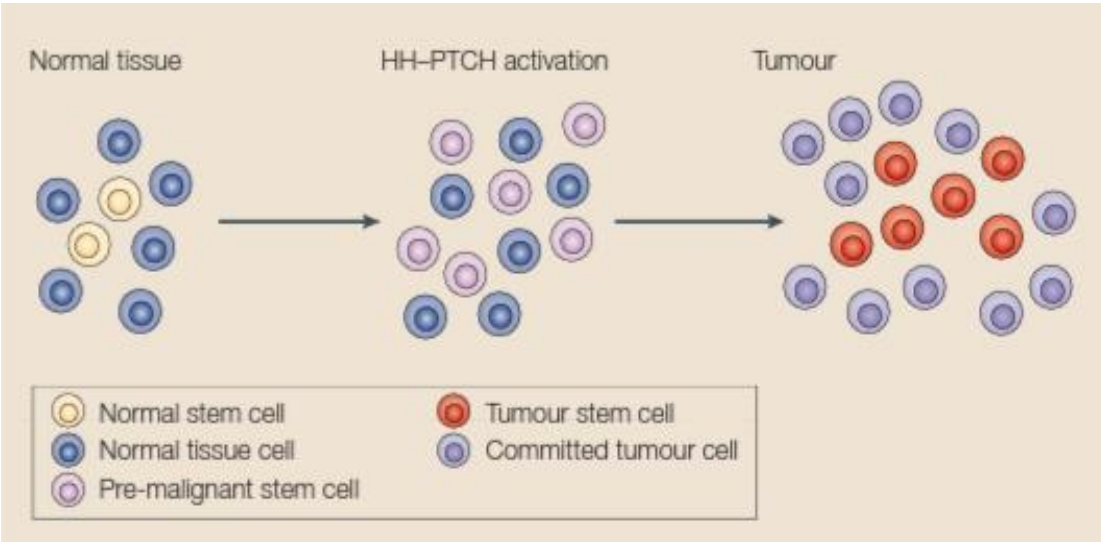

Fig. 4. In the above figure, normal stem cells (blue) undergo transformation to a stem cell with abnormal malignant. Subsequent genetic events give rise to a tumour stem cell (red) that can generate additional stem cells with abnormal signalling [10]

12. IMMUNOTHERAPY AGAINST CSCs

Nowadays, a series of immunotherapies are induced and directly targeting towards specific antigens expressed by tumour cells including CSCs. A recent study by Huang and colleges shows an anti-CD3/anti-CD133 bispecific antibody (BsAb) bounding with cytokine-induced killer (CIK) cells could target and kill CD133 high CSCs. The killing of CD133 high pancreatic (SW1990) by the effect cells (BsAb-CIK cells) was significantly ($p < 0.05$) higher than the killing by the parental CIK or by CIK cells bound only with anti-CD3 (CD3-CIK) and inhibited CD133 high tumour growth significantly. The findings introduce a novel immunotherapy for patients with cancer containing CD133 high CSCs by selectively targeting this population [23,34]. Immunotherapy with unconventional T cells such as $\gamma\delta$T cells is based on their potent HLA-nonrestricted cytotoxicity against different tumour entities and their additional capacity to recognize and present antigens to αβT cells [21,22]. Oberg demonstrated how bispecific antibodies that selectively recruit $\gamma\delta$T cells to tumour antigens expressed by cancer cells illustrate the tractable use of endogenous $\gamma\delta$T cells for immunotherapy [35]. They isolated $\gamma\delta$T cells from patients with PDAC tumour infiltrates lyse pancreatic tumour cells after selective stimulation with phosphorylated antigens and designed bispecific antibodies that bind CD3 or Vγ9 on $\gamma\delta$T cells andHer2/neu (ERBB2) expressed by pancreatic tumour cells [36,11]. Both antibodies enhanced $\gamma\delta$T-cell cytotoxicity with the Her2/Vg9 antibody also selectively enhancing release of granzyme B and perforin and reduced growth of pancreatic tumours grafted into SCID-Beige immunocompromised mice. As mentioned above, high level of ALDH was related with pancreatic CSCs. Visus et al. used ALDH asa marker for identifying and selectively targeting pancreatic CSCs as well [12,35,37]. They generated ALDH1A1-specificCD8+ T cells in order to eliminate ALDH+ CSCs, which induced growth inhibition of CSCs and reduction of metastasis. However, ALDH1A1-specific CD8+ T cells are not CSCs-specific. They could target normal ALDH+ stem cell as well [13].

13. DISCUSSION

Acquiring genetic change, clonal evolution, and the tumour microenvironment promote progression of cancer, metastasis and therapeutic resistance. These events correspond to the establishment of the great phenotypic heterogeneity and plasticity of cancer cells that contribute to tumour progression and resistance [23]. Cancer stem cells are likely to share many of the properties of normal stem cells that provide for a long lifespan, including relative quiescence, resistance to drugs and toxins through the expression of several ABC transporters, active DNA-repair capacity and resistance to apoptosis [38]. Therefore, tumours may have a built-in population of drug-resistant pluripotent cells that can survive chemotherapy [20] and repopulate the tumour. Cancer stem cells can acquire resistance to chemotherapy by a range of mechanisms, including the mutation or over expression of the drug target [31], inactivation of the drug, or elimination of the drug from the cell [15]. Typically, tumours that recur after an initial response to chemotherapy are resistant to multiple drugs (HDR) [6,15]. In the usual view of drug resistance, one or several cells in the tumour population obtain genetic alterations that confer drug resistance. These cells have a selective advantage that allows them to overtake the population of tumour cells following cancer chemotherapy [39]. Current therapies target proliferating cells and quickly achieve tumour mass reduction, but leave the CSCs unaffected and these, over time, originate tumour recurrence [23]. But, CSC-targeted therapies attack the root of the tumour by killing the CSCs. Since the rest of the tumour population, although highly proliferative, is short-lived and lacks self-replicative capability, eventually the tumour dries out and is cured [39]. Amalgamation of both kinds of approaches should be feasible to achieve quick reduction that would be also definitive. Cancer stem cells (CSC) are associated with the mechanisms of chemo resistance to different cytotoxic drugs or radiotherapy, as well as with tumour relapse and a poor prognosis [26]. Despite managing chemo resistance of cancer stem cells, various other modalities have also been under development to deal with the uncontrolled propagation of CSC populations hiding inside a cancerous tissue [19,20,21,22]. Ubiquitination is one of such pathways which is essential in targeted cancer therapy of cancer stem cell [3]. Targeting apoptosis pathways in cancer stem cells is another possibility [18]. So, cellular chemotherapy against cancer stem cells is the need of the hour [20]. Detailed understanding and working on cancer stem cells is required to uproot the origin of the cancer cell population and thus also helpful for their management in cancer therapy [23]. Some studies have shown that mitochondria sometimes play a central role in the propagation and maintenance of CSCs because of the ability of this organelle to modify cell metabolism, allowing survival and avoiding apoptosis clearance of cancer cells [40]. Thus, the whole mitochondrial cycle, from its biogenesis to its death, can be targeted by different drugs to reduce mitochondrial fitness, allowing for a restored or increased sensitivity to chemotherapeutic drugs. Once mitochondrial misbalance is induced by a specific drug in any of the processes of mitochondrial metabolism, an augmentation in reactive nitrogen/oxygen species is

achieved and, subsequently, activation of the intrinsic apoptotic pathway in such cells is accomplished [40].

14. CONCLUSION

Finally, it is to be expected that CSCs from different cancer types share many similarities in their basic biology, implying that similar therapeutic approaches could be used in many different cancers. The challenge is now to find a way to specifically target CSC without causing toxicity to normal cells. Cancer cells are themselves unique in their properties which confer them immortality and spreading ability throughout the body. Nonetheless the CSCs hiding inside cancer tissue microenvironment is much more lethal in such activities which ultimately make the tumours more destructive. Prevailing therapeutic procedures target only proliferating cell mass, thus quickly reducing the tumour, but leaving the CSCs untouched causing tumour recurrence over time. The repeated exposure of such chemotherapies may develop resistance in CSCs against an array of chemicals. Wining over or bypassing chemo resistance is one of the possible pathways to combat CSCs.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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