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Cancer stem cells and resistance to chemo and radio therapy

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1. ABSTRACT

Cancer stem cells (CSCs, or tumor initiating cells) are responsible for tumor initiation. If cancer treatment kills most of cancer cells in the stage of transit amplifying and differentiation without killing the stem cells, the surviving CSCs will eventually lead to recurrence of tumors. Studies have suggested that CSCs may be the primary mediators of resistance to chemo- and radio-therapy, leading to failure in cancer therapy. Numerous targets are being investigated for their potential involvement in the self-renewal and chemo- and radio-resistance of cancer cells. However, despite the intensive efforts invested into characterizing the role of cancer stem cells, there is a sense of uncertainty regarding the identity and number of these cells as well as the implications in cancer treatment. In this review, we will discuss the identification of CSCs by cell surface markers, the biology of CSCs, and the role of CSCs in resistance to radio- and chemo-therapy. This review will discuss the advances in targeting CSCs to improve the efficacy of chemo- and radio-therapy.

2. INTRODUCTION

According to statistics published by the National Cancer Institute, the Center for Disease Control and Prevention, and the North American Association of Central Cancer Registries in 2010, a total of 1,529,560 new cancer cases and 569,490 deaths from cancer are projected to occur in United States of America. Cancer is a disease where a cell or a group of cells display unregulated growth, leading to expansion beyond normal limits which, sometimes, manifest in the form of invasion and metastasis (1). Even though chemo- and radio-therapies have been devised and improved, cancer related deaths have not declined, since malignant tumors display resistance to chemo- and radio-therapy. Recent studies suggest that cancer stem cells (CSCs) may be the primary mediators of resistance to radio- and chemo-therapy, responsible for tumor recurrences after treatment. In this review, we discuss the implications of cancer stem cells in cancer treatment.

3. HISTORY OF CANCER STEM CELLS

3.1. Cancer stem cell hypothesis

There is a general belief that a tumor is composed of distinct types of cancer cells which interact with multiple surrounding stromal cells such as myofibroblasts, fibroblast, endothelial cells and inflammatory cells. However, the heterogeneity in tumor tissues is not only limited among cancer cells and stroma, but also extends to intra-tumor population (2).Whereas the contrary stochastic model proposes that all cancer cells within the same tumor are presumed to take part in the generation of new tumors; tumors that are grown by a small subpopulation of stem cell-like cancer cells, have been postulated to explain the intra-tumor heterogeneity. A cancer stem cell (CSC) has been defined as a small subpopulation of cells within a tumor that possesses the capability to renew itself and give rise to heterogeneous cell lineages of cancer cells that, in turn, comprise the tumor (1). CSCs can give rise to the different cells found within a tumor tissue, and when compared to normal stem cells, CSCs often have an aberrant mitosis due to mutations in genes controlling DNA repair, cell division and signal transduction. (3)

3.2. Cancer stem cells role in carcinogenesis

The role of stem cells in cancer pathogenesis and their potential involvement in the resistance to chemo- and radio-therapy has been under investigation since last 20 years. Scientists have been able to define them as highly plastic cells capable of adapting to their environment, they are responsible for giving rise to similar progeny as well as diverse heterogeneous progeny, together, forming a tumor mass (4). It has been observed that the CSCs share the same activation of signaling pathways that regulate self renewal and stem cell properties, including the Wnt, Notch, and Hedgehog Signaling pathways (5). The scientific interest in CSCs has been arising from the current reports that scientists are able to isolate CSCs using specific cell surface markers (6). The growing evidence has suggested that the cancer stem cell concept gives new explanation of the origins of cancer as well as the therapeutic challenges encountered so far in the field of oncology.

3.3. Evidence in favor of cancer stem cell hypothesis

Understanding the patho-physiology behind cancerous cells has generated diverse views. Various hypotheses have been proposed in order to ascertain the origin of the CSC. According to one of these hypotheses which reiterates that CSC maintains some of the biological properties of normal stem cells such as indefinite self replication, resistance to toxic agents due to elevated expression of drug resistance transporters and asynchronous cell division so the normal stem cells, which are essential for most tissue survival in living beings are believed to transform into CSCs during aberrant physiological mechanisms. All mechanisms are physiologically regulated, but rare evasions of regulation steps can lead to the appearance of the CSCs (7). Later, this hypothesis led to oncogenic theories which hypothesize that aberrant signaling in tumor suppressor genes are responsible for oncogenesis (8). Now there is general belief that defilement of cell-to-cell communication is a potential cause for cancer (9).Recently environmental factors such as chemical carcinogens or life style factors (alcohol, tobacco consumption or drug abuse) have been known to play a pertinent role in producing mutations and other genetic abnormalities observed in cancer cells (10). Indeed, if normal stem cells could endure the mutations observed in tumor cells, this would ultimately compromise the genetic stability of the organism .It is generally believed that tumor formation and progression favors the concept of a tiny population of pluripotent cells that are capable of initiating and proliferating cancerous growth. Acute myelogeneous leukemia provided first experimental evidence on the existence of cancer stem cells (6). A subpopulation of leukemic cells, which express the CD34 surface maker but lack the CD38 marker, was able to recapitulate leukemia in NOD/SCID mice. The cells exhibited a cell surface phenotype similar to normal hematopoietic stem cells. Although the presence of CSC in cancer cells has been a subject of debate, CSCs have been isolated from different types of cancers, with different but sometimes overlapping profiles of cell surface markers (Table 1). For example, in breast cancer, as few as 200 CD44 positive/CD24 negative/low cells were able to form tumors in immunodeficient mice, whereas injections of 20,000 cells from the remaining population failed to form tumors. The tumorigenic population gave rise to additional CD44 positive/CD24 negative/low epithelial tumors, these cells were able to form tumors in other immune deficient mice when they were serially transplanted, which is a hall mark for CSC.

4. ROLE OF CANCER STEM CELLS IN RADIO-RESISTANCE

4.1. Mechanism of radio-resistance in cancer stem cell

Radiotherapy utilizes ionizing radiation to control and cease malignant cell proliferation for curative, therapeutic, adjuvant and palliative treatment. It works by damaging the DNA of the cells using ionizing radiation. Since only healthy cells can lead to a certain degree of repair and rebuild damaged nuclear material, cancerous cells remain damaged and slowed. Since malignant cells consist of a number of rapidly dividing cancerous cells, radiotherapy is effective in controlling the rapid growth of tumors. (11)

Radiotherapy has been in use for more than 100 years, since it has helped to improve the survival of patients with malignant cancers (59). Some cancers like leukemia, lymphoma and germ cell tumors are highly sensitive to radiotherapy as compared to melanoma and renal cell carcinoma that are resistant to radiotherapy. As mentioned above, DNA is prone to damage by radiation beams. However, different doses of radiation therapy are required to slow down the progression of cancers depending on the source of cell origin. Malignant cells lack the near-perfect DNA damage repair mechanism, and hence, are more likely to scathe from radiotherapy than normal cells (12). This DNA damage in a group of cells within a tumor is responsible for slowed tumor growth. Fractionating the total dose helps to prevent damage to normal cells and the effectiveness in specifically targeting malignant cells is increased multifold (12).

Table 1. Various CSCs isolated on the basis of cell surface markers by cell sorting techniques

Cancer	CSC phenotype	Reference
AML	$CD34^{positive}CD38^{negative}CD90^{negative}$	(51)
Breast cancer	$ESA^{positive}CD44^{positive}CD24^{negative/low}Lin^{negative}$, ALDH1	(52, 53)
Brain cancer	$CD133^{positive}$	(54) (10) (11) (12) (13) (14)
Colon cancer	$CD133^{positive}$, $CD44^{positive}EpCam^{positive}CD166^{positive}$	(55, 56)
Osteosarcoma	$CD133^{positive}$	(57)
Pancreatic cancer	$CD44^{positive}$ $CD24^{positive}ESA^{positive}$	(58)
Prostate cancer	$CD44^{positive}\alpha2\beta1^{positive}CD133^{positive}$	(59)
CNS	$CD133^{positive}$	(60)
Head and Neck	$CD44^{positive}$	(61)
Melanoma	$ABCB5^{positive}$	(62)

The current advances in radiological techniques have brought down cancer death but the resistance to radiotherapy presented by some cancer cells remains to be a challenge to the therapeutic efficacy. Recent researches suggest that CSCs are resistant to radiotherapy. CD133 positive CSCs have been found to have better and more efficient DNA repair mechanism (13). Moreover, increased expression of VEGF has been observed in tumors associated with CSCs which correlates to increased angiogenesis. Signaling pathways such as Wnt, Notch, Hedgehog, which play important roles in downstream signaling of the CSCs, may contribute to the radio resistance capacity of tumor initiating cells (14).

Wnt-β-catenin over-expression in CSCs has been associated with better repair of DNA after radiotherapy. Constitutively active form of Wnt/β catenin leads to formation of multiple mammary carcinoma in mouse (15).It has been observed that mutated form of β catenin leads to its stabilization by activating cyclin D transcription which results in proliferation of colorectal cancers (16, 17). Wnt 2 is upregulated in gastrointestinal cancer and can be used as biological marker (18).In general increased expression of Wnt/B catenin pathway has been associated with cancers such as breast, melanoma, sarcoma, myeloid leukemia, multiple myeloma and brain tumors (19).

A notch protein on activation causes release of intracellular components by proteolysis and causes binding with the HLH transcription factor. Notch pathway has been integrated with the regulation of neuronal stem cell renewal and differentiation (20).Activation of Notch signaling in primary human mammary epithelial cells causes attenuation of Wnt induced oncogenic alteration (21).Inhibition of Notch signaling pathway by γ secretase inhibitor (a multi-subunit protein complex that cleaves transmembrane protein at residues within the transmembrane domain) decreases *in vivo* tumor growth by annihilating proliferation and inducing apoptosis (22).

Hedgehog signaling cascade modulates various developmental process such as procreation, differentiation and morphogenesis (23).Granule cell precursors present in cerebellum crave for hedgehog downstream signaling cascade for proliferation during the early embryonic period (24).Association of hedgehog signaling and cancer was elaborated with the studies done in Gorlin's syndrome (autosomal dominant disorder associated with malignancies of skin and cerebellum).Individuals suffering from this disease has presence of mutation in PTCH which is a member of hedgehog pathway hence it results in formation of medulloblastoma and sporadic basal cell carcinoma (25, 26).It has been observed that ligand associated hedgehog signaling cascade causes formation of various cancers such as small cell lung cancer (SCLC), oesophagus, stomach, pancreas, liver, ovary, melanomas and intestinal stromal tumors (27, 28).Inhibition of the hedgehog signaling cascade either by using cyclopamine (inhibits the hedgehog signalinghttp://en.wikipedia.org/wiki/Hedgehog_signaling_ pathway by influencing the balance between the active and inactive forms of the smoothened protein) or GLI1 (These are effectors of hedgehog signaling) siRNA led to decreased cell procreation rate in ovarian carcinoma cells (29).

Radiotherapy effectiveness declines when cells are hypoxic because it enhances the self-renewal capacity of CD133-positive human glioma-derived cancer stem cells (CSCs) (30). It has been seen that CSCs proliferated at increased rate and maintained undifferentiated phenotype under hypoxic conditions whereas on the contrary CSCs cultured at normoxic conditions did not. Proliferation of the glioma-derived CSCs in a hypoxic environment also enhanced the expression of cell surface markers such as CXCR4 (CD184), CD44 (low) and A2B5 (57). The CD133-positive CSCs in hypoxia were accompanied with upregulation of HIF-1alpha. Knockdown of HIF-1alpha abolished the hypoxia-mediated CD133-positive CSC proliferation (57). These results suggest that under hypoxic conditions CSCs causes enhanced activity of HIF-1 alpha which leads to increased self renewal activity of CD133-positive cells and causes inhibition of CSC differentiation hence causes radio-resistance in CSCs.

4.2. Potential targets for improving radio-sensitivity

Theoretically, the best approach appears to be marking the CSC accurately in the malignant zones. The hypoxic zones need to be evaluated before finalizing the radiation doses. Unlike present techniques of radiation therapy where homogenous spatial radiations are applied, a higher dose of radiation should be given to the hypoxic and CSC rich areas so that resistance can be managed aggressively (1).

4.3. Role of environmental niche in radio-resistance

It has been proposed that transformation of tumor from benign to malignant cells is dependent on many factors. One such factor that regulates the growth of tumor cells is the microenvironment called "niche". This microenvironment is responsible for generating factors that

help in regulation of proliferation of stem cells, since it has been reported that hematopoietic stem cells (HSCs) are controlled by their osteoblastic niche, which provide the proliferation through the activation of signaling pathways (31, 32).However, the niche is responsible for the homeostatic balance between self-renewal and differentiation in normal stem cells since they are activated by the release of stimulating signals from the micro environment by a group of cells from niche. A delicate balance exists between proliferation and anti-proliferation signaling. Any genetic mutation that disturbs the balance between proliferation and anti-proliferation signaling causes the niches to be functionally deregulated and allow the escape of stem cells from the natural mechanism to excessive self-renewal. The normal cells embracing tumor cells affect tumor development via secreting molecule, such as cytokine and chemokine. A combination of both programs may be necessary in some cases. However, the stem cells with uncontrolled proliferation and improper differentiation results in the proliferation of tumor (33).

Recently it has been reported that massive cellular deregulations in tumor microenvironment results in the promotion of genetic and epigenetic alteration which in turn, results in tumor progression by generating tumor tissue containing aberrant stem cells (34). In myeloid tumors, Jun B (a well known tumor suppressor) deficiency results in the deregulation of a large pool of genes associated with cell matrix and cell-cell interactions. The JunB-deficient HSCs ends up losing self-renewal ability and show impaired migratory ability. On the contrary, the loss of JunB in HSCs is related to myelo proliferative disorders. These results indicate that micro environment regulations are imminent for the emergence of CSC activity during blood cancer initiation (2).In addition, the vascular niche is essential to provide a crucial bed for brain tumor stem cells (2, 35). Increased number of blood vessels in orthotopic brain tumor xenografts increased the portion of self-renewing cells and accelerated the formation and growth of tumors. On the contrary, the depletion of blood vessels by bevacizumab abolished self-renewing cells from the tumors and suppressed tumor growth (2).

Normal stem cells are usually under predominant inhibition of proliferation and differentiation from the routine niches; CSCs are more self-sufficient to undergo deregulated proliferation due to changed niches in cancerous conditions, which causes proliferation and growth of tumor stem cells. A combination of both programs may be necessary in some cases. However, the stem cells with uncontrolled proliferation and aberrant differentiation results in accumulated genetic mutations and tumor promotion.

5. ROLE OF CANCER STEM CELLS IN CHEMO-RESISTANCE

Mechanism of action of most chemotherapeutic drugs is the impairment of cell division or mitosis; hence they regulate the cell cycle proliferation by completely abolishing the rate of cell growth. Although the chemotherapeutic drugs kill most cells in a tumor, it is currently believed that CSCs may survive and maintain mechanisms to resist chemotherapies. Potentially there are 15 different types of drug efflux pumps however ABCB1, ABCG2 and MRP1 confer the most Multiple Drug Resistance (MDR). P-glycoprotein, MRP1, MRP2, and BCRP/ABCG2 are generally expressed in organs important for absorption, metabolism and excretion (36).When these genes are expressed in tumor cells, they allow cancers to resist the chemotherapies by increasing the efflux of drugs, deactivation of enzymes, decreasing permeability of drugs, altering the drug binding sites as well as changing metabolic pathways (37).

ABCB1 and ABCG2 transporters have the capability to cause efflux of unmodified drugs and drugs conjugates whereas MRP1 causes efflux of conjugates having glutathione and un- conjugated free glutathione drugs (38). ABCB1 is an ATP -dependent efflux pump which is expressed primarily in certain cell types such as liver, pancreas, kidney, colon, and jejunum (39). ABCB4 gene has 78% sequence homology with ABCB1 gene which functions to cause the efflux of phosphatidylcholine from the liver canalicular cells into the bile acting as a lipid flippase (play an essential role in this transport process) (40).MRP1 tend cause the efflux of metal oxyanions such as arsenite (41).ABCG2 transporters are present at the apical surface of liver and body and enhances excretion of xenobiotics (42).

CSCs are said to be involved in chemotherapy resistance. As the CSCs form more differentiated progeny, MDR decreases. So, the presence of CSCs in the tumor zone is proportional to the chemo resistance of the tumor. ATP binding cassette (ABC) transporters are energy dependent transporters that have been found to play pivotal role in cytotoxic drug resistance (43). These transporters translocate solutes across membranes, and their role in drug resistance is of extreme interest. For example ABCB5, a super-family of ABC, has a significant role in chemo resistance in human melanoma. When ABCB5 mRNA levels are intentionally reduced using siRNAs, sensitivity of melanoma tumor cells to various drugs, including 5-Flourouracil, camptothecin, and mitoxantrone, improves considerably. (44)

(Figure 1) defines the ways by which cancer cells can develop drug resistance (43) (a) Cisplatin (water soluble compound) shows reduced accumulation due to cross resistance to methotrexate and nucleoside analogs. (b) Cyclophosphamide are administered as prodrugs, which must be converted to their cytotoxic forms by the targeted tumor or by other tissues, decreased conversion of these analogs to their cytotoxic nucleoside results in resistance. (c). ABCB1 and MRP1 belong to the ATP - binding cassette (ABC) super family of transport proteins.

It has been experimentally shown that ABCG2 and other MDR transporters are responsible for the transporting fluorescent dye out of normal murine and human cancer cells (20). It has been observed that cancerous cell lines such as glioma, breast cancer and retinoblastoma, show stem-cell like characteristics as well

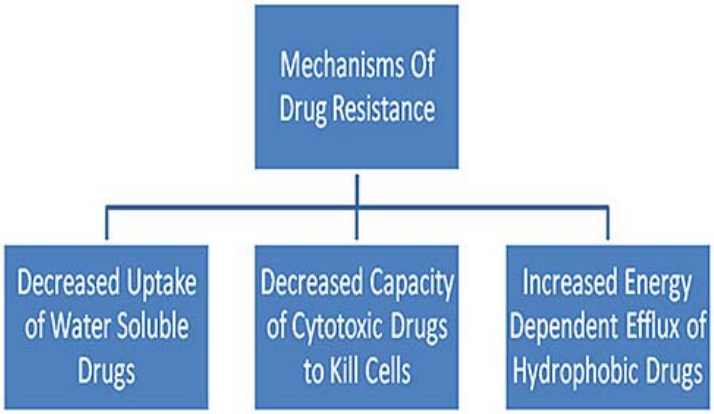

Figure 1. Mechanism of chemoresistance. Tumor cells can decrease the uptake of drugs and/or increase efflux of drugs in their resistance to chemotherapy.

as inherent drug resistance. (26) Moreover, the CD133-positive cells isolated from the glioblastoma patient sample has exhibited the significant resistance to the chemotherapeutic agents including temozolomide, carboplatin, paclitaxel (Taxol), and VP16 (45).

Serious malignant potential of tumors depends on the presence of CSCs within the tumor, proven by the fact that even a small number of CSCs transplanted into an animal host can generate a full grown tumor. Moreover, this newly regenerated cancer, after implanting from a primary source is highly resistant to therapy. (46, 47).Recent studies have identified hyaluronan receptor CD44 on leukemia and other CSC cells. In myeloma, another hyaluronan protein, Rhamm, has been implicated. Another protein that closely related in these structures is Emmprin, a surface glycoprotein that promotes hyaluronan production. This receptor interacts with hyaluronan and has an unconfirmed role in malignant activity of tumor cells. Moreover, the presence of CD44 receptors is closely associated with CSCs, tumor spread and chemo resistance. The drugs have shown better results when hyaluronidase treatment is given, implicating CD44 receptor-hyaluronan role in drug resistance in tumors (27,28).The proteins and genes identified to have a role in resistance are mentioned in the (Table 2) (48).

Stem cell marker CD133 has been mentioned, underlining the fact that CD133 is considered the strongest marker of stem cell presence within the glioblastoma (brain cancer stem cells) (29) though CD133 expression is not only restricted to stem cells. It has been shown that both CD133 positive and CD133 negative in colon cancer cells augments tumor growth (49). In neural cells, NG2 (cell surface neuron–glial 2) is considered a progenitor neural cell marker. NG2 positive cells have been found to have all the features of stem cells, as they differentiate into oligodendrocytes, astrocytes, and other sub population. NG2 positive cells having the capacity of neural progenitor cells are found throughout life in the human brain. It is believed that it is these neural stem cells that have the capability to form the tumor initiating stem cells in patients with brain tumors (4).

Like all stem cells, CSCs have trans-differentiation capacity, i.e. they can modulate according to the environmental requirements and differentiate into vascular, lymphatic, angiogenic and neurogenic derived cells. This provides the tumor with ample opportunities to blossom in its physical environment. In cases of melanoma, markers for vascular, angiogenic, neurogenic and lymphatic cells have been isolated from the tumor zones. This trans-differentiation potential of CSCs within the tumor, gives rise to an adamant tumor, as the progenitors within the tumor bulk keep modifying the ever forming, new cells according to their environment, in our case, a chemotherapeutic regime (50).

6. CONCLUSIONS AND FUTURE DIRECTIONS

The presence of quiescent stem cells in tumor bulk has been proposed to be associated with multi-drug resistance and radioresistance. In cases of melanoma, the cancer progenitor cells display a series of cascade reactions at molecular level, including some increased DNA repair mechanisms, which prevent effective drug response in patient with malignant melanoma. Studying hyaluronan and its CD44 receptors closely would help understand their role in CSCs malignant proliferation and development of hyaluronan-CD44 antagonists may help prevent tumor recurrence and decrease tumors with chemo resistance potential (46, 47). The role of specific antibodies and tyrosine kinase inhibitors has become more significant, as they can inhibit and slow down these signaling cascades predominantly taking place in the CSC of the tumor bulk (50). Since MDR associated genes/proteins are responsible for CSC's capacity to resist chemotherapy, inhibitors of P-gp, Bcl-2 and multi drug resistant proteins (MRP) probably would result in better drug response in cancer patients in the coming years (48).

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Table 2. Expression of molecules involved in chemoresistance in CSCs

Genes	Proteins	Normal tissue expression	Expression in tumors
mdr1	P-GP	Brain, Kidney, Liver, Adrenal Gland, Stem Cells	Glioma, Leukemia, Breast, Lung, Prostate
mrp1	MRP1	Normal tissues except liver	Gliomas, Neuroblastoma, Breast Cancer
abcg2	BCRP/MXR1	Brain, Placenta ,Liver, Ovary, Testis, Stem Cells	Gliomas, Breast, Colon, Lung, Ovarian, Fibrosarcoma
lrp/mvp	LRP/MVP	Brain, Epithelium	Gliomas, Acute Myeloid Leukemia, Lung Cancer
bcl-2	Bcl-2	Brain, Ovary, Breast, Skin	Gliomas, Leukemia, Melanoma, Breast, Lung
cd133	CD133	Stem cells	Glioma, Leukemia, prostate cancer, colon cancer

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