Review Article

Cancer stem cells: Recent advances, signaling and molecular markers

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ABSTRACT

Cancer stem cells (CSCs) are tumor cells with stem-like features that possess the ability to self-renew, but can also give rise to more differentiated progeny. CSC can be identified based on increased *in vitro* spheroid- or colony formation, enhanced *in vivo* tumor initiating potential, or expression of cell surface markers. Since CSCs are thought to be required for the maintenance of a tumor cell population, these cells could possibly serve as a therapeutic target. Therapeutics based on CSC markers, epithelial mesenchymal transition, developmental pathways, or tumor micro-environment could potentially be used to target CSC which may lead to a reduction of tumor growth, metastatic events and chemoresistance in various cancers.

Key words: Cancer stem cells, epithelial mesenchyme transition, self-renew, stem cell

INTRODUCTION

Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Stem cell biology has provided a platform to address many questions in developmental biology of inflammation and cancer. Carcinomas, which represent the most prevalent malignancies in humans, arise from normal epithelial tissues in a multistep progression from benign precursor lesions. Metastasis, the final step in malignancy, is the main cause of death for cancer patients.[1,2]

Stem cells are divided into three main categories: (1) Embryonic stem cells (ESCs) (2) germinal stem cells (GSCs) and (3) somatic/adult stem cells (ASCs). ESCs originate from the inner cell mass of blastocyst. They are omnipotent and have indefinite replicative life span. GSCs are derived from primary germinal layers of embryo. They differentiate into progenitor cells to produce specific organ cells. Somatic/ASCs are progenitor cells having less replicative activity then ESCs. They exist in mature tissues such as hematopoietic, neural, gasterointestinal and mesenchymal tissues. The most commonly used ASCs derived from bone marrow are hematopoietic stem cells (HSCs) and other primitive progenitor cells including mesenchymal stem cells and multipotent adult progenitor cells.[3]

ESCs were the source of stem cells for therapeutic purposes due to higher totipotency and indefinite life span compared to ASCs with lower totipotency and restricted life span. However, use of ESCs has ethical constraints (Department of Health, UK, National Institutes of Health and International Society for Stem Cell Research) and their use for research and therapeutic purposes are restricted[4,5] and prohibited in many countries throughout the world. Thus, for ease of availability and lesser constrained on ethical issue, ASCs are the stem cells most commonly used for research and therapeutic purposes. The other reason for the use of ASCs is their easy accessibility compared with ESCs.[5] ASCs can be obtained from many tissues including bone, synovium, deciduous teeth, adipose tissue, brain, blood vessels, blood and umbilical cord blood.[6-9]

Advances in stem cell biology over the last two decades have provided new insights into cancer biology.[10-12] Tumors show marked heterogeneity in morphology, proliferation

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rates, genetic lesions and therapeutic response. Thus, not all tumor cells are equal. It is now believed that most tumors contain a small subpopulation of cells with stem cell properties, namely cells with the ability to perpetuate through self-renewal and the ability to generate diverse mature cell types by differentiation.[13] These cells, termed cancer stem cells (CSC), have the ability to produce the entire distinct cell types found in their original tumor.

CSCs, have been identified in many types of cancer, including colorectal, breast, ovarian, pancreatic, prostate, head and neck, melanoma, hematopoietic, brain tumors and also proved to be responsible for minimal residual disease, recurrence of tumors and tumor resistance to chemotherapy.[14-16] They have also been identified in. The CSC concept postulates that, similar to the growth of normal proliferative tissues such as bone marrow, skin or intestinal epithelium, the growth of tumors is fueled by limited numbers of dedicated stem cells that are capable of self-renewal. The bulk of a tumor consists of rapidly proliferating cells as well as post-mitotic, differentiated cells.

Though, stem cells has many implications including developmental biology, fibrosis and epithelial mesenchymal transition the aim of this article is to give a short and comprehensive review of recent advances concerning CSC hypothesis and to describe its impact on modern molecular physiology and pharmacology research.

HISTORY

Rudolf Virchow, the father of modern cellular pathology, was a proponent of the cell theory, which stated that all cells come from other cells and all organisms are made up of cells. He provided the scientific basis for cancer by correlating clinical outcomes with microscopic findings (Virchow 1858).[1] However it still took 100 years until Makino introduced the term "tumor stem cell" for a small subpopulation of cells that were insensitive to chemotherapy and had chromosomal features different from the bulk of cells.[17,21] In the 1970s *in vivo* transplantation experiments and *in vitro* colony forming assays supported Makino's observation that tumors could arise from rare cells with self-renewal capacities. Experiments indicated that these cells are able to recapitulate all cell types within an individual tumor and establish immortal cell lines.[18-21]

Stem cell concepts and their application to cancer are many decades old.[11] Since the 19th century, tumors have been known to show explicit histological heterogeneity. In an article in 1937, Furth and Kahn established that a single cell from a mouse tumor could initiate a new tumor in a recipient mouse.[22,23] CSC was firstly formulated by Nordling in 1953.[24] Beginning in the 1960s, investigators noticed that a small minority of cancer cells initiate a tumor.[25] The theory that accumulation of deoxyribonucleic acid (DNA) mutations gives rise to cancer was further suggested by Ashley, Knudson and Nowell in 1969, 1971 and 1976 respectively.[26-29] Ashley postulated that a cancer initiating cell must survive long enough to accumulate at least three to seven genetic mutations necessary to generate cancer.[26] Nowell hypothesized that the inherent longevity and extensive proliferative capacity of a stem cell make it an ideal candidate cancer-initiating cell.[28] Fialkow *et al*.,[29,30] demonstrated clonal hematopoiesis for both erythroid and myeloid series in patients with chronic myeloid leukemia in 1967 and this was accepted as the first evidence of the CSC concept.

The focus of cancer research shifted in the 1970s, when mutations in oncogenes and tumor suppressor genes were found to cause most human cancers. This let Nowell to formulate the clonal evolution concept,[28,30] stating "it is proposed that most neoplasms arise from a single cell of origin and tumor progression results from acquired genetic variability within the original clone allowing sequential selection of more aggressive sublines. Fearon and Vogelstein formulated a clonal evolution model for colon cancer, in which the progression from early adenoma to invasive carcinoma reflects the stepwise acquisition of mutations in specific cancer genes.[31] Scientists who discovered different stem cells in various tumors are listed in Table 1.

SELF-RENEWAL OF HAEMATOPOIETIC STEM CELLS

One of the most important issues in stem cell biology is to understand the mechanisms of self-renewal. Self-renewal is

Table 1: Cancer stem cell detection by various scientists

References	Tumors
Furth and Kahn (1937)	*In vivo* initiation of tumor in a recipient mouse
Ashley (1969)	DNA mutation in cancer
Knudson and Nowell (1971 and 1976)	DNA mutation in cancer
Lapidot *et al.* (1994)	Acute myeloid leukemia
Bonnet and Dick (1997)	Acute myeloid leukemia
Al-Hajj *et al*. and Michael Clarke (2003)	Breast cancers
Parker Gibbs of the University of Florida (2003)	Bone cancer
Singh *et al*. and Peter Dirks (2004)	Brain tumor
Bao *et al*. (2006) and Piccirillo *et al*. (2006)	Brain tumor
Dalerba *et al*. (2007), O'Brien *et al*. (2007), Ricci-Vitiani *et al*. (2007)	Colon cancer
Li *et al*. (2007)	Pancreatic cancer
Prince *et al*. (2007)	Head and neck carcinomas
Zang *et al*. (2008b)	Ovarian cancer
Alvero *et al*. (2009), Curley *et al*. (2009)	Ovarian cancer
Stewart *et al*. (2011)	Ovarian cancer

DNA: Deoxyribonucleic acid

crucial to stem cell function, because it is required by many types of stem cells to persist for the lifetime of the animal. It is also fundamental to understanding the regulation of cancer cell proliferation, because cancer can be considered to be a disease of unregulated self-renewal.

In the hematopoietic system, multipotent progenitors constitute 0.05% of mouse bone-marrow cells and can be divided into three different populations: Long-term self-renewing HSCs, short-term self-renewing HSCs and multipotent progenitors. These populations form a lineage in which the long-term HSCs give rise to short-term HSCs, which in turn give rise to multipotent progenitors. As HSCs mature from the long-term self-renewing pool to multipotent progenitors, they progressively lose their potential to self-renew but become more mitotically active. Whereas long-term HSCs give rise to mature hematopoietic cells for the lifetime of the mouse, short-term HSCs and multipotent progenitors reconstitute lethally irradiated mice for < 8 weeks.[10,32]

DEFINITION OF CSC

CSC is defined as a minority cell type within a tumor that has the characteristics of stem cells. Stem cells are determined by three main properties: Differentiation, self-renewal and homeostatic control. Differentiation means the ability to give rise to a heterogeneous progeny of cells, which progressively specialize according to a hierarchical process. Self-renewal is determined as the ability to form new stem cells with a capacity for proliferation and differentiation for maintenance of stem cell pool. Homeostatic control is the ability to balance self-renewal and differentiation according to environmental stimuli and genetic constraints.[33]

ORIGIN OF CSC

CSCs were initially defined by their extensive self-renewal capacity, tumorigenicity and multipotentiality. They are defined experimentally by their ability to regrow tumors hence, referred as tumorigenic cells. It is currently unclear whether CSCs arise from the transformation of stem cells or from the dedifferentiation of mature neoplastic cells.[34-36]

A normal ASC is defined as a somatic cell that can undergo extensive cell division and has the potential to give rise to both stem cells and cells that differentiate into specialized cells. A normal stem cell must possess two qualities to perform its natural function: Self-renewal and differentiation. Self-renewal is a special cell division that enables a stem cell to produce another stem cell with essentially the same development and replication potential. Differentiation is the second function of a stem cell which involves the production of daughter cells that become tissue-specific specialized cells.

The hierarchy in which blood stem cells produce progenitor cells that subsequently produce multiple differentiated cells has been known for many years and numerous *in vitro* and *in vivo* assays have been designed to test the developmental potential of stem and progenitor cells. In the blood system, stem cells first differentiate into transiently amplifying progenitor cells. These cells rapidly proliferate for a short time and produce terminally differentiated cells, such as basophils and macrophages that are quiescent or die during normal tissue maintenance or damage. Because in many organs and tissues the cells with the longest life span are stem cells, they are more likely to accumulate the initial transforming mutations than transiently amplifying progenitor cells or mature cells of an organ with a shorter life span.[35,36]

CSC HYPOTHESIS

The original hypothesis of CSCs was based on the clonal theory of cancer initiation and progression. This hypothesis states that any normal stem cell, upon acquiring a mutation giving it selective growth advantage, gives rise to a neoplastic clone of homogenous neoplastic cells [Figure 1]. The clone, upon expansion and acquisition of additional mutations, expands as a tumor. This model, is called classical or stochastic, assumes that all cells within a given tumor have the same tumorigenic potential.[14,15]

A more-recent hypothesis, the so-called hierarchical model, suggests that any given tumor consists of a heterogeneous population of cells, with only a small proportion of them being CSCs.[16] However, that small, self-renewing population of CSCs is responsible for tumor initiation and growth maintenance. Those CSCs could be tissue stem cells or a more differentiated progeny, which acquired self-renewal capacity.[16,37,38] There are two mechanisms that could mediate the transformation of normal stem cells to CSCs:

- Early progenitor cells can gain mutations which gives them self-renewal capacity[39]

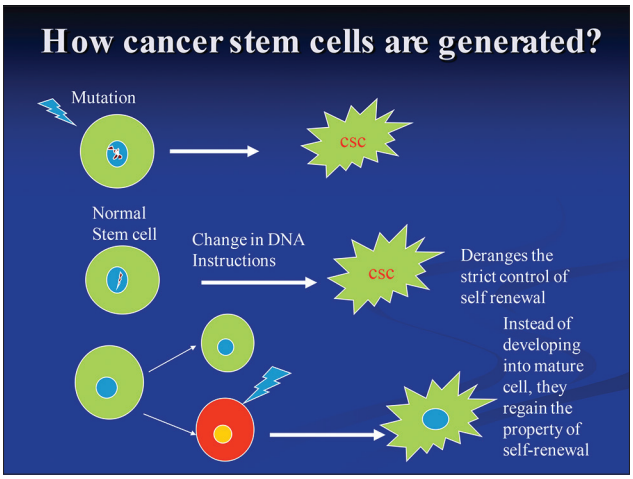

Figure 1: Possible mechanism of cancer stem cell formation

- Fully differentiated cells or cells in the late progenitor stage can become de-differentiated to acquire the properties of stem cells.

It has been clear for decades that some cancers, including some germ lineage cancers, some neuroblastomas and some myeloid leukemias, can differentiate into progeny that have limited proliferative potential despite retaining the oncogenic mutations of their malignant progenitors.[40]

Although the overt differentiation in some germ lineage cancers and some neuroblastomas provided clinical evidence consistent with the CSC model, these rare and unusual malignancies are of uncertain relevance to more prevalent adult cancers. Thus, the CSC model gained increased attention when evidence emerged supporting the model in leukemia and breast cancer. The advent of flow cytometry made it possible to separate phenotypically distinct subpopulations of live cancer cells to compare their tumorigenic potential. Using this approach, some human acute myeloid leukemias and breast cancers were found to follow the CSC model,[11] suggesting that a broad spectrum of cancers might be hierarchically organized into tumorigenic and non-tumorigenic components.[40] Since these studies were published, other studies have taken similar approaches to provide evidence that other human cancers also follow the CSC model, including colon cancer, pancreatic cancer, brain tumors and ovarian cancer.[40]

PATHWAYS REGULATING STEM CELL SELF-RENEWAL AND ONCOGENESIS

Two major types of mutations are involved in the processes: Either activation of oncogenes or inactivation of tumor suppressor genes. In addition, the tumor microenvironment, that is, the CSC niche and cytokine loops play essential roles in the maintenance of CSCs and in tumor growth and development.[1,36,41,42] The tumor microenvironment is composed of diverse immune cells and stromal cells, as well as extracellular components. CSC functional traits might be sustained by this microenvironment, termed "niche."[41] The CSC niche might not only regulate CSC traits but might also directly provide CSC features to non-CSCs. In leukemia and in prostate carcinoma, cancer cells could hijack existing physiological stem cell niches. Although physiological stem cell niches are known to play important roles for quiescence maintenance and resistance to stress-inducing treatments. The influence of the tumor niche on CSC function at the cellular and molecular level still remains to be elucidated.[1,43,44]

Stem cells are minority populations; thus, the inherent difficulty of studying molecular pathways with such a small amount of cells has dramatically slowed progress. Even with the existing drawbacks, multiple crucial pathways have been elucidated in the biology of CSCs and their normal counterparts. The group of protein family responsible for self-renewal of normal and CSCs includes Bmi-1, Wnt (Wingless-Int)/β-catenin, Notch, Hox family, Efflux transporters and Telomerase and the maintenance of CSCs require Shh (sonic hedgehog) and Pten (phosphatase and tensin homolog deleted from chromosome 10). In normal stem cells these proteins helps in self-renewal, regeneration, development, maintenance of hematopoietic stem cells and differentiation of precursor cells. Similarly, these proteins are up regulated and overexpressed by CSCs and/or tumor cell populations.[1]

STEM CELL MARKERS

Tumorigenic populations fulfilling the definition of CSCs have been identified in a number of human cancers, including leukemias,[45-51] bladder cancer,[52] breast cancer,[53] central nervous system cancers,[54] colon carcinoma,[55-57] head and neck cancer,[58] ovarian cancer,[59] pancreatic cancer,[60,61] malignant melanoma,[62] liver cancer[62] and Ewing sarcoma.[63] It is currently not known whether all cancers contain subpopulations of CSCs. Therefore, the current trend is to combine molecular markers to achieve higher specificity. The article briefs about markers that have been used to identify CSCs with an emphasis on markers relevant to head and neck squamous cell carcinoma[64] [Table 2].

PROGNOSTIC SIGNIFICANCE OF CSC

Resistance of tumors to the broad armamentarium of cancer treatments may largely be due to the presence of residual CSCs, which are very hard to eradicate. The presence of circulating tumor cells in patients with cancer correlates with poor prognosis.[65-66] In CSC model, the functional differences between tumorigenic and non-tumorigenic cells can have important implications for therapy. Evidence has been presented for radiation resistance in brain tumor-initiating cells and breast cancer initiating cells. Cancer progression and therapy resistance may be influenced by the properties of cancers stem cells in cancers that follow the model, but therapy resistance and disease progression can also arise through genetic changes unrelated to the question of whether a cancer follows the stem cell model. There are several mechanisms that mediate CSC resistance to chemotherapy and radiation.[16] They include the quiescent nature of CSCs, their presence in hypoxic niches, the upregulation of DNA damage response and the increase in CSCs of drug efflux.

Alternative therapeutic methods of selectively targeting CSCs are currently in development. Interruption of key signaling

Table 2: Cancer stem cell markers

Markers	Normal stem cell	Cancer stem cell
Oct 3/4, Sox 2, Nanog	Maintenance of pluripotency and self-renewal of embryonic stem cells	Highly expressed in poorly differentiated tumors than in well differentiated breast cancers, glioblastoma and bladder carcinoma Up regulation of transcriptional factors in stem-like cells
CD133 (prominin-1)	Marker for hematopoietic stem cells Expressed in epithelial cells and in somatic stem cells from neural tissues, prostate and kidney	Higher clonogenicity, invasiveness and tumorigenesis in human oral squamous cell carcinoma
CD44	Capacity of self-renewal and differentiation	Up regulation in solid malignancy (breast cancers) Up regulated in head and neck squamous cell carcinoma
Aldehyde dehydrogenase	Stem like properties including colony formation, self-renewal and tumorigenesis	Marker of normal and malignant human mammary stem cells Prognostic marker for breast cancer and a strong predictor of metastasis and poor patient outcome
Side population	Enriched in hematopoietic stem cells	Identified in normal tissue like skin, lung, brain and liver Identified in solid tumors like hepatocellular carcinoma, glioma, gastrointestinal cancer, ovarian cancer, neuroblastoma and breast cancer

CD:Cluster of differentiation

pathways (e.g., Wnt, hedgehog and Notch pathways) could be one such approach. On the other hand, application of epigenetic manipulations (such as histone deacetylase inhibitors and DNA methyltransferase inhibitors) could lead to re-expression of tumor suppressor genes. Another attractive approach is immunotherapy targeting CSCs.

CSCS–NANOTECHNOLOGY

In cancer various treatment modalities have been implicated since decades but none these have given promising results as a permanent cure. In order to overcome the chemo/radio resistance of CSCs, nanotechnology based novel methods are being explored. In 1978, Widder *et al*.,[67] first proposed the usage of nanoparticle for drug delivery application and stated that in cancer nanotherapeutics, tumor-specific, targeting ligand/receptor based nanoparticle assembly will be employed for active targeted drug delivery. A novel strategy for blocking the ligands responsible for the interactions between the CSCs and the niche for their survival and colonization at the metastatic site had been studied recently.[2] Despite of various hurdles challenged by nanotechnology, it has started blooming into the clinical sector. Although the first generation of nanomedicines, which have reached to the clinics,[44] but still there is a necessity for even effective and precisely targetable drug delivery especially to the CSCs. Apart from active targeting, due to the factors such as high leaky tumor vasculature and hydraulic conductivity of tumor, the nanoparticles were up taken by the tumor by enhanced permeation and retention effect.[23,66,67]

CONCLUSION

CSCs are a specific subset of transformed cells that are able to sustain primary tumor growth according to a hierarchical pattern. Like any model, the CSC concept needs to be constantly adapted to the currently available data and thus is steadily evolving. CSCs may have unstable phenotypes and genotypes, which makes it difficult to identify reliable and robust biomarkers for the development of targeted therapies. Evolving the CSC concept will help to focus research toward developing improved therapies without risk of tumor recurrence and allow for the targeting of fatal late-stage cancers.

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