Review Article

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Clinical and Therapeutic Implications of Cancer Stem Cells: A Review

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Abstract

Cancer is a disease of genes. Although a number of pathogenesis have been proposed for cancer, a unifying model of tumorigenesis is yet to be established. Furthermore, the mortality rate associated with cancer has not decreased in spite of the advances in therapy. Many questions still remain unanswered regarding the pathogenesis, therapy, and recurrence. Researchers have now proposed that cancer initiation and progression is driven by a small subpopulation of cancer cells called the cancer stem cells (CSCs). These cells have also been implicated in recurrence, metastasis and therapy of cancer. This review highlights the clinical and therapeutic implications of CSCs.

Key words: Cancer, Cancer stem cells, Cancer stem cell hypothesis, Head and neck squamous cell carcinoma

INTRODUCTION

Tumors were once thought to be composed of a homogeneous mass of proliferating cells. However, with increased understanding of tumor pathogenesis, it is accepted to be heterogeneous aberrant tissue arising from single cancer stem cell (CSC).[1] Inherited and somatic mutations enable a normal cell to ignore all the growth-inhibitory signals, proliferate disproportionately, invade tissue and finally undergo metastasis. The mechanisms underlying these mutations is not completely understood and a unifying "model of tumorigenesis" is yet to be established. During the few years, many advances have been made in the diagnosis, early detection and management of such patients. Despite this, the increase in survival rate is just nominal and recurrence and treatment failures continue to occur in a large number of patients.[2] A number of questions remain unanswered: Why the survival rate has not increased? Why tumors do not respond to treatment? Why tumors recur? Why cancer cells develop resistance to treatment?[3]

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Researchers across the globe are working to find answers to all the above mentions questions and better understand the nature of cancer. One such concept that seems to partly answer some of the questions is the CSC hypothesis. The CSC hypothesis suggests that not all the cells in the tumor have the ability to proliferate and maintain growth of the tumor, but only a subpopulation of cells in the tumor called the CSCs are able to proliferate and self-renew.[1,4]

The concept that cancer might arise from a rare cell population of cells with stem cell properties was proposed about 150 years ago.[5] The first experimental evidence of transplantable hematopoietic stem cells was given in 1955.[6] Cells with stem-like characteristics were described in bone marrow in 1961 by Till and McCulloch.[7,8] CSC theory was first proposed by Hamburger and Salmon in 1977.[5] Hematopoietic CSC was first described by Bonnet and Dick in 1997[8] and in breast cancer CSC was isolated by Clarke *et al*. in 2003.[8,9]

American Academy of Cancer Research Workshop on CSC in 2006 defined the CSC as a cell within the tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor.[10] The CSC theory was first proposed by Hamburger and Salmon who demonstrated that only a small percentage of tumor cells were able to form colonies in soft agar.[7] The first conclusive evidence for CSCs was published

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in 1997 in Nature Medicine. Bonnet and Dick isolated a subpopulation of leukemic cells that expressed a specific surface marker of CD34, but lacked CD38 marker. They concluded that $CD34^+/CD38^-$ subpopulation is capable of initiating tumors in immunodeficient mouse that is histologically similar to the donor.[3,8,11-14]

CSC hypothesis gains support through two features:[1]

1. Similarity between CSCs and normal stem cells in terms of self –renewal, differentiation, drug resistance and migration capacity
2. Genetic and epigenetic damages best accumulated in stem cells because of their long life span.

Current CSC research is focusing on the identification of CSC in solid tumors since stem cells in hematopoietic malignancies such as leukemia have been well characterized.[15] However, many difficulties are encountered when exploring the existence of CSCs in solid tumors, due to the inaccessibility of tumor cells and the lack of appropriate functional assays. An important breakthrough in the study of solid tumor CSCs was the identification of breast cancer CSCs and their biomarkers by Clarke and his colleagues in 2003. They reported that cells expressing CD44+/CD24− cell surface phenotype were able to initiate tumors when they were transplanted into immune-deficient mice.[4] Since then, CSCs have been reported in neoplasms of brain, prostrate, lung, colon, pancreas, liver, melanoma and skin.

ORIGIN OF CSC

Depending on the tumor type and the phenotype the tumor presents, the sources of origin of CSCs is different. Four main hypotheses were suggested by Costea *et al*. regarding the origin of CSCs in oral squamous cell carcinoma.[16]

1. CSC may originate from normal somatic stem cells.
2. Normal somatic stem cells are the only cells that reside long enough within the epithelium to acquire the number of genetic changes necessary for transformation and cancer development to occur. Multiple genetic changes transform normal stem cell into a CSC.
3. Fusion between hematopoietic stem cell and a mutated oral keratinocyte occasionally occurs producing a cell with stem-like properties, the CSC. Fusion may occur between a mutated hematopoietic stem cell and oral keratinocyte giving the same end result.
4. De-differentiation-oncogenic events could occur initially in an amplifying cell delaying its differentiation and permitting acquisition of additional oncogenic events leading to cancer.
5. Neosis: Exposure of cells to genotoxic agents can yield multinucleated giant cells. Their cell division by cytoplasmic cleavage (neosis) may result in the formation of multiple small CSCs. However, neosis has been described so far only for *in vitro* settings.

CLINICAL IMPLICATIONS OF CSC

The clonogenicity and heterogeneity of tumors can be explained by CSC hypothesis. CSC model may also be involved in initiation and progression, metastasis, and relapse of tumors. CSC is also known to confer chemo and radioresistance to tumors.

Initiation and Progression of Tumors

Tumorigenesis is a multistep process in which an accumulation of genetic and epigenetic alterations form the basis for the progression of a normal cell to a cancerous cell. Only long-time residents of the epithelium/mucosa, most likely stem cells, have the ability to accumulate the number of necessary genetic hits that will result in cancer development.[17,18]

It has been proposed that a stem cell acquires one or more genetic alterations and forms a patch in the mucosal epithelium with genetically altered daughter cells. As a result of this process, CSCs are formed, which can escape the cytotoxic action of immune system killer cells.[1] The patch starts to expand, and a tumor develops. Normal epithelium in certain areas then gets replaced by genetically aberrant cell population.[17]

METASTASIS

It has been suggested that CSCs may be involved in metastasis of a tumor, but the exact mechanism still remains unclear. Several hypotheses have been put forth to explain the mechanism of metastasis by CSCs. The hypothesis proposed by is that the original CSCs that caused the primary tumor might do so, resulting in primary and metastatic tumor that evolve in parallel rather than sequentially.[19] The mechanism suggested by Brabletz *et al.* states that there are two forms of CSCs in tumor progression namely stationary CSC (sCSC) and mobile or migrating CSC (mCSC). According to them, sCSCs are embedded in epithelial tissues or epithelial based tumors and cannot disseminate. mCSCs are derived from sCSC by acquiring transient epithelial-mesenchymal transition (EMT). They are located at the tumor-host interface and mediate metastasis. Finally, the authors hypothesized that sCSC was responsible for the formation of primary tumors while mCSC mediates metastasis.[15,18]

It has also been noted that CSCs acquire the properties of invasion and metasis through EMT induction. Recently, EMT is also known to confer drug resistance property to CSCs.[14]

Resistance to Chemotherapy

Normal stem cells are conferred with an ability to protect themselves from the toxic environment and, therefore, resistant to drugs used to treat cancer. Similarly, CSC may also have an ability to resist chemotherapy and radiotherapy. Several studies have confirmed that CSCs are indeed resistant to current cancer therapies. The mechanisms involved in making CSC resistant to chemotherapy are as follows:

1. Many CSCs are in G0 phase of cell cycle.
2. Resistant to DNA damaging agents and enhanced DNA repair mechanism.
3. Expression of higher levels of bcl-2, an anti-apoptotic protein.
4. Increased expression of ATP-binding cassette (ABC) transporter that can actively efflux cytotoxic drugs.[5]

Resistance to Radiotherapy

1. Enhanced DNA repair via ChK1 and ChK2 kinases (1)
2. Enhanced cell longevity via the histone deacetylase Sir T1(1)
3. Brain CSC can repair double-stranded DNA breaks caused by ϒ-radiation probably through increased activity of the ataxia telangiectasia mutated DNA repair pathway.[8]

CSC in Relapse

Relapse of a tumor may involve the tumorigenic properties of CSCs and their resistance to conventional therapies. A large number of studies indicate that CSC escape the traditional therapies, persist within the tumor mass and cause recurrence. CSC from breast, pancreas and colon are all resistant to chemotherapeutic drugs.[18]

IDENTIFICATION OF CSC

There are various methods employed for identification of CSC which include *in vivo* label retention, *in vitro* clonal assays, flow cytometry using cell surface markers and Hoechst dye exclusion.[2,7,10] Nucleotide analogs such as bromodeoxyuridine and tritiated thymine are used to label slow dividing cells, which is a property of all stem cells. This label is incorporated into newly synthesized DNA and will remain within the cells. *In vitro* clonal assays provide a reliable method for the identification and isolation of cells with stem cell properties from both normal and neoplastic tissue.[19]

CSC populations are commonly defined by presence or absence of various combinations of cell-surface proteins, such as $CD44^{+}/CD24^{-}$ populations in breast cancer. By staining cells with antibodies against these markers, these cells can be identified and isolated by flow cytometry and/or fluorescence-activated cell sorting (FACS). These isolated CSCs are implanted in soft agar or immunodeficient mice.[19] They form colonies in soft agar or produce tumors in the mice, which are histologically and phenotypically similar to that in the host. When used properly, FACS analysis is the most robust tool in the identification of CSCs. The surface markers employed for isolation are CD44, CD24 and CD133. CD44 molecule is a transmembrane glycoprotein and may play an important role in facilitating adhesion, migration and invasion.[2,19] CD24 is a single chain protein bound to the extracellular membrane of the cells. This marker is expressed in pancreatic cancer but not in breast and prostate cancer.[18] CD133 (Prominin-1) is also a transmembrane glycoprotein and is expressed in tumor cells of the brain. It is recently been found to be expressed in lung, pancreatic, liver, prostate, gastric and head and neck cancer. It is known to increase the survival of cells *in vitro* and confer chemoresistance to tumor cells.[2,19]

Aldehyde dehydrogenase (ALDH) activity in normal and malignant stem cells converts retinol to retinoic acid, which is crucial for differentiation pathways. ALDH expression is elevated in hematopoietic and leukemic stem cells. It enriches other markers such as CD44, CD133, etc. and also confers chemoresistance to the CSC.[2,15,19] Hoechst 33342 is a DNA dye used for flow cytometric analysis of the DNA content of live cells. This dye can penetrate intact cell membranes but are rapidly expelled out of the cells by ATP-dependent ABC transporters. Lack of fluorescence by flow cytometry helps identify this population of cells.[19]

THERAPEUTIC IMPLICATIONS OF CSC

According to the CSC hypothesis, if only a subpopulation of cells drives tumor formation then therapies have to be developed to identify and target these cells.[20] The current therapy targets the bulk of the tumor mass and is unlikely to target CSCs. There are many recent reports of drugs that specifically target CSCs.[21] Until date, the most successful targeted therapy is the development of imatinib (Gleevec) that targets BCR-Abl in patients with chronic myeloid leukemia. It induced complete remission in the majority of the patients.[3,5,20,22] Parthenolide and rapamycin appear to kill CSC of acute myeloid leukemia but not normal hematopoietic stem cells. Temozolomide preferentially eliminates CSC in glioblastomas.[18]

Therapies using properties of miRNA to inhibit CSC markers have shown promising results. For example miRNA -34a inhibits pancreatic CSCs and miRNA-128 inhibits BMI1 in breast cancer and gliomas.[1]

CSC MODEL IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

Only a few studies are reported of CSC in head and neck cancers. Prince *et al.* reported CD44 cells from HNSCC could produce tumors in immunodeficient mice.[2,14,23] Few biomarkers such as Oct-4, Nanog, CD133, CD44 and ALDH have been identified in CSC of HNSCC. $CD133^+$ cells display clonogenicity and tumorigenicity in xenograft models when compared to $CD133^-$.[2] Chen showed that ALDH activity correlated with disease staging in HNSCC and that higher enzymatic activity correlated with expression of EMT genes and enriching cells with CSC properties.[2] Also, $ALDH^+$ HNSCC cells are less sensitive to radiation than $ALDH^-$ cells.[18] Tumors of head and neck contain cancer cells lacking markers associated with non-tumor cell lineages present within the tumor (Lin+ cells) and express CD44 (Lin-$CD44^+$ cells). Lin-$CD44^+$ cells have the capacity to produce tumors in immunocompromised mice. These cells are associated with poor prognosis.[24] CD44 has also been implicated in metastatic spread and disease progression in HNSCC.[2,18] Nanog/Oct-4/CD133 triple positive status predicted a poor prognosis for patients with oral cancer.[15] However, none are conclusive. Currently, there are no consistently well-defined biomarkers or matured technologies to identify CSC in HNSCC.

CONCLUSION

CSCs are proven to exist in hematopoietic tumors, and targeted therapies have also been developed. CSC theory satisfactorily answers some of the questions related to tumor biology. However, many researchers disagree with the concept of CSC as many things remain to be elucidated regarding their role in tumorigenesis. CSC concept had opened a new area of research. It is becoming increasingly clear that cancer is a stem-cell disorder. Studies have to be directed to further our knowledge regarding the new concept. The ability to prospectively identify, isolate and study CSCs will significantly alter the way we think about, study and treat cancer.

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