CANCER STEM CELLS: HAVE WE FOUND THE HOLY GRAIL OF ONCOLOGY?

Dr. Amit Chawla (post graduate)   Dr. Mandana Donoghue (Prof & head)   Dr. Mohan KP (Asst. Professor)

Dept. of Oral Pathology

INTRODUCTION

Cancer is the leading cause for mortality worldwide and lacks a successful therapeutic protocol. Despite the emergence of various therapeutic combinations none of the treatment options available today are fully curative in patients with cancer. Thus, there is an enormous need for better understanding of tumor biology and novel therapeutic approaches to prevent recurrence or relapse of cancer.

Cancers develop from normal cells that gain the ability to proliferate aberrantly and eventually turn malignant. These cancerous cells then grow clonally into tumors and eventually have the potential to metastasize. A central question which remains is which cells are transformed to form tumors?

Recent evidence suggests that a subpopulation of cells called the cancer stem cells (CSCs) are present within the tumor mass which possess tumorigenic capacity and may be responsible for propagation, relapse and metastasis. These cells have certain stem cell-like properties such as quiescence, self-renewal, asymmetric division and multidrug resistance which allows them to continue tumor growth and evade conventional therapies available today.[1]

A brief review of cancer stem cells is presented here.

CANCER STEM CELLS

The cancer stem cell hypothesis states that within a tumor only a subset of cells, the "cancer stem cells" (CSCs), are capable of initiating and propagating the disease. In various cancers such cells have been identified and prospectively isolated.[2]

These cells have characteristics similar to stem cells such as ability for self-renewal without loss of proliferation capacity with each cell division. Furthermore, they are immortal and resistant to conventional treatment and express typical markers of stem cells.

CSCs chemo resistance is attributed to the activity of multiple drug resistance (MDR) transporters like ATP binding cassette (ABC) drug transporters which don't allow the drug to penetrate inside these cells.[3,4]

CSCs may develop by transforming mutations occurring in multi-potential stem cells by genetic and / or epigenetic events leading to gain of the self-renewal activity and loss of some features of differentiation. Since stem cells have a longer life span, their longevity makes them most vulnerable to the accumulation of mutational genetic events.[5]

Studies of normal stem cells and cancer stem cells from the same tissue have unfolded knowledge on the biology of tumors. Common

molecular pathways regulate self-renewal of both populations. Inappropriate activation of such pathways promoting self-renewal of somatic stem cells and defects in asymmetric cell division has been shown to cause neoplastic proliferation and cancer formation.[6]

To summarize, adult stem cells or progenitor cells have the ability to become malignant and generate CSCs, which then form and maintain the tumor mass. Thus treatment should target these CSCs for effective control of cancer growth and recurrence.

METHODS OF IDENTIFICATION OF CANCER STEM CELLS

Various methods that are employed for the identification of CSCs include

(1) Isolation of side population (SP) cells based on Hoechst dye efflux.

(2) Cancer stem cell markers

(3) Sphere culture.

Isolation of side population (SP) cells

Goodell and colleagues observed that there was a small population of cells in bone marrow aspirates that did not accumulate Hoechst 33342 dye (Hoechst dye is a DNA permeable dye that binds specifically to A-T base pairs). They named these populations of cells as side population cells. The use of this dye or such similar methods to identify side population cells is helpful in identification and isolation of CSCs. They further showed that this SP contained cells capable of repopulating the bone marrow. The SPs have been shown to have CSCs like characteristics such as increased tumorigenicity, expression of stem cell like genes, and self-renewal. Strong tumorigenic ability of SP cells following in vivo transplantation in experimental mice has also been seen.[7]

Cancer stem cell markers

CSCs markers can be classified under
- Cell surface markers
- Genes and their products

Cell surface markers

The lineages assumed by cells during different stages of differentiation can be identified by the presence of protein molecules present on their surface. CD (cluster of differentiation) system is commonly used to define cell surface markers.

The surface markers expressed by normal stem cells when found in particular tumor population indicate the presence of CSCs within that tumor.

These markers can be of different lineages such as
- Hematopoietic stem cell markers
- Mesenchymal stem cell markers
- Embryonic stem cell markers
- Neural stem cell markers

These markers when expressed in particular tumor identify tumor initiating population of cells.[7]

- CD133 - Also known as Prominin- 1, it is a transmembrane glycoprotein localized to plasma membrane protrusions and micro domains. It is expressed in hematopoietic, endothelial and neuronal stem cells.[7]

- CD44 - It is a glycoprotein that is the receptor for hyaluronan (HA), a major component of the extracellular matrix. As a result of binding HA, CD44 activates many receptor mediated tyrosine kinases responsible for tumor initiation in many cancer types.[7]

- CD166 - It is a member of the immunoglobulin super family and a type 1 transmembrane glycoprotein, is involved in cell adhesion and cytoskeleton anchoring. It has been detected in a subset of cells in a variety of human tissues (thought to be pluripotent stem cells) including epithelia, lymphoid

and myeloid cells, fibroblasts, neurons, hepatocytes and pancreas acinar and islet cells.[9]

> Nestin - First identified in the cytoplasm of neuroepithelial stem cells, is also expressed in migrating and proliferating cells during embryogenesis and in various adult tissues undergoing regeneration, such as the central nervous system, liver, pancreas and gastrointestinal tract. Its expression seems to correlate with the high proliferative and migrational activity of the tumors.[9] serve as a potential molecular marker for further characterization of CSC.[10]

> CK (cytokeratin) 19 - Immunofluorescence revealed that cytokeratin 19 was highly expressed on SP cells. It was found that the cell marker, CK 19, may serve as a potential molecular marker for further characterization of CSC.[10]

> Oct4 (Octamer binding transcription factor) and Nanog - These are transcription factors required to maintain the pluripotency and self-renewal of embryonic stem (ES) cells. It was also demonstrated that these factors can activate or suppress transcription and control a cascade of pathways that are intricately connected to govern pluripotency, self-renewal, genome surveillance and cell fate determination.[11]

The two most commonly used surface markers used to identify CSCs are CD133 and CD44

Genes and their products

> BMI-1(B lymphoma Mo-MLV Insertion region), Tie-2(Tyrosine kinase with Immunoglobulin and Epidermal growth factor), Shh (Sonic Hedgehog), Notch and Wnt/ß-catenin(Wingless Integrated site) - These genes and signaling pathways are shared by normal stem cells and CSCs. They have been shown to have important regulatory functions for cancer stem cells.[8]

Sphere culture

Normal cells when cultured in vitro form cell to cell contacts, which stimulates cell cycle arrest causing these cells to stop dividing (contact inhibition). This property is used to distinguish between normal and cancerous cells. CSCs have been isolated using this property since they grow in uncontrolled manner to form spheres in culture. In 1992 Reynolds and Weiss demonstrated that cells isolated from the striatum of adult mouse brain could be clonally expanded by culturing spheres and that these cells could generate both astrocytes and neurons. In humans, CD133+ (positive) CSCs cells isolated from human fetal brain were shown to form spheres in vitro.[7]

EVIDENCE OF CANCER STEM CELLS IN ORAL SQUAMOUS CELL CARCINOMA

Past decade has shown significant increase in the number of studies showing the presence of cancer stem cells in oral squamous cell carcinoma.

While so far a specific CSCs marker for OSCC has not been identified, each cancer cell line represents its own specific set of cancer stem cells as demonstrated by a number of studies. Few of them are mentioned here.

A small subpopulation (1-2%) of CD133^{+} (positive) CSCs was identified in Oral squamous cell carcinoma (OSCC) cell lines and tissues. These CD133^{+} CSCs possess higher clonogenicity, invasiveness, chemoresistance and increased in vivo tumorigenicity as

compared to CD133⁻(negative) counterparts. This suggest that CD133⁺ cells represent a small subpopulation of CSCs that may contribute to chemoresistance in human OSCC.[12]

OSCC cell lines highly expressed the stem/progenitor cell markers and ABC transporter gene (Oct-4, Nanog, Nestin, CD133 and ABCG2). Elevated expression of CD133 was shown in the enriched OSCC cell lines from OSCC patient's tumors. Hence the enriched OSCC cell lines possess the characteristics of both stem cells and malignant tumors. Additionally, expression of CSCs markers (Nanog/Oct-4/CD133) indicates the poor prognosis of OSCC patients.[13]

NEW CANCER TREATMENT STRATEGIES

Treatment strategies for the elimination of cancer need to consider the consequences of the presence of CSCs. Recent studies of cell lines derived from OSCC indicate the presence of subpopulations of cells with phenotypic and behavioral characteristics of CSCs. Hence individual analysis of 'tumor stem cell' markers will be an important tool for innovative therapies and for determining the prognosis of patients with head and neck squamous cell carcinoma (HNSCC).

CHALLENGES

To achieve effective implementation of new therapies, physicians will require methods of determining the types of cancer stem cells present in a given patient's tumor. It is important that agents directed against cancer stem cells discriminate between cancer stem cells and normal stem cells. This will require identification of realistic drug targets unique to cancer stem cells.

Clearly, there is much excitement and momentum in this emerging field. Investigation of cancer stem cells offers the possibility of generating novel targets that could overcome issues of drug resistance, improve therapeutic efficacy, and make cancer treatment more successful and effective.

Whilst numerous studies have been done and many more are still underway their widespread acceptance and application to the practice of medicine is yet to occur. Continued research efforts in this area will surely witness practical applications of cancer stem cells in near future.

REFERENCES

1. Bhattacharyya S, Khanduja KL. New hope in the horizon: Cancer stem cells. Acta Biochim Biophys Sin (Shanghai). 2010 Apr;42(4):237-42.

2. Pries R, Witrkopf N, Trenkle T et al. Potential stem cell marker CD44 is constitutively expressed in permanent cell lines of head and neck cancer. In vivo. 2008 Jan-Feb;22(1):89-92.

3. Dean M, Fojo T, Bates S. Tumor stem cells and drug resistance. Nat Rev Cancer 2005 Apr;5(4):275-84.

4. Donnenberg VS, Donnenberg A. Multiple drug resistance in cancer revisited: The cancer stem cell hypothesis. J Clin Pharmacol.2005 Aug;45(8):872-7

5. Hermann PC, Bhaskar S, Cioffi M et al. Cancer stem cells in solid tumors. Semin Cancer Biol. 2010 Apr;20(2):77-84.

6. Weissman IL. Normal and neoplastic stem cells. Novartis Found Symp. 2005;265:35-50.

7. William L. Farrar. Cancer Stem Cells.Cambridge University Press. http://www.cambridge.org/gb/knowledge/isbn/.

8. Clarke MF, Dick JE, Dirks PB et al. Cancer Stem Cells—Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. Cancer Res 2006 Oct;66(19):9339-44.

9. Klein WM, Wu BP, Zhao S et al. Increased expression of stem cell markers in malignant melanoma. Modern Pathology 2007;20:102–107.

10. Wang J, Guo L, Chen L et al. Identification of Cancer stem cell–Like side population cells in human nasopharyngeal carcinoma cell line. Cancer Res April 2007;67(8):3716-24.

11. Loh YH, Wu Q, Chew JL et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet. 2006 Apr;38(4):431-40.

12. Zhang Q, Shi S, Yen Y et al. A subpopulation of CD133(+) cancer stem-like cells characterized in human oral squamous cell carcinoma confer resistance to chemotherapy. 2010 Mar 28;289(2):151-60.

13. Chiou SH, Yu CC, Huang CY et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res. 2008 Jul;14(13):4085-95.