Stem cells and oral squamous cell carcinoma

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ABSTRACT-
Cancer stem cells(CSCs) are a small population of cells in a tumor. They have the ability to self-renew and maintain the tumor. This review focuses on this concept of cancer stem cells, serving their purpose and leading to the development of tumor. There are many cell biomarkers which have been described for the identification and characterization of cancer stem cells. Also, the role of CSCs in cancer is newer arena of research these days.

KEY WORDS- Cancer Stem cells, metastasis, progeny, prognosis, self-renewal, signaling, therapeutics

INTRODUCTION-
Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common malignancy in the world. It occurs more commonly in men with history of tobacco and alcohol use.[1] HNSCCs accounts for only 4% of new cancer cases diagnosed annually. The morbidity rate is higher in this disease thereby ravaging the life of its sufferers.[2] The development of metastasis both local and distant added to the phenomenon of primary or acquired resistance dictating the poor prognosis of the disease process.[3] A number of key questions remained to be unanswered in this area demanding newer and effective therapeutic modalities in this area. Evidence is accumulating so as to demonstrate that the tumour growth spread is often dependent on new subclass of population known as Cancer Stem Cells (CSCs). The American Association for Cancer Research Workshop on Cancer Stem Cells defined them as a cell within a tumour which possesses the capacity to self-renewal and to generate heterogeneous lineages of cancer cells that comprise the tumour.[4] The unique property of these cells lies in their ability of self-renewal, anti-apoptotic pathways, metastases, tumour growth and development rendering tumour cells immortal.[5] The exact mechanism by which the formation of CSCs is triggered is not
Stem cells and oral squamous cell carcinoma

yet elucidated. However, CSCs are known to grow in a niche composed of fibroblasts, endothelial cells and cellular matrix remarkable properties of developing into a variety of cell types in the human body. Although, there has been a strident increase in CSCs recently, we remain a long way from understanding the molecular mechanisms that fortify the transformation of normal cell into CSC. 

This review article aims at the highlighting the utility and significance of stem cells in diagnosis and therapeutic management of cancer.

**Historical Prospective Of CSCS**–
Julius Cohnheim was the first researcher who stated that tumours are derived from ‘embryonic cell rests’ which are the residual cells ‘left behind’ in the individual. [8] Beard hypothesized that tumour arises from the ‘activated cells’ in adult tissues. Stell was of the opinion that tissue stem cells are modern day equivalents of embryonal rests and most tumours are derived from stem cells. [8] Bonnet et al isolated the cancer stem cells from myeloid leukemia. The terminology of CSC is based on the fact similar to body’s cell in their capacity of self-renewal, differentiation into mature but aberrant progeny. [8] In past, pluripotent stem cells were recognized by their ability to home in to various target specific tissues thereby making them suitable candidates for studies in animal models. CSCs and normal stem cells share similar characteristics as under- [9,10,11]

1. Self renewal.
2. Differentiation capacity to mature progeny.
3. Ability to survive after long time.
4. Resistance to damaging agents.
5. Anchorage independent growth and metastasizing capacity.
6. Active telomerase expansion.
7. Active membrane transporter activity.

**Origin Of Cancer Stem Cell**– The question arises from where do these cancer stem cells originate?? Various hypotheses have been proposed to determine their origin however exact mechanism remains to be unknown. Hahn and Weinberg proposed that three to six genetic events are required for a stem cell to convert into CSCs. Also, some researchers stated these cells are the potential residents of normal epithelia which takes upon 14-28 days upon activation by cell signaling molecules and mutation by chemical carcinogens transform into cancer stem cells.

Gat et al 1998; Zhu and Watt, 1999 in their vivo experiments on keratinocytes suggested that oncogenic events occurring on these cells lead to their development. Furthermore, Gat et al in 1998 and Zhu et al in 1999 studied the origin of stem cells in vitro and concluded that these senescent cells escape the immunosurveillance and divide subsequently forming CSCs.

**Cancer growth models and cancer stem cells hypothesis in tumorigenesis**- It can be explained on the basis of two models- [13]

1) The *stochastic model* which suggests that every cancer cell is able to initiate new tumor growth equally.
2) The *alternate hypothesis* is that every tumor contains a rare population of cells termed CSCs or cancer initiating cells (CICs)

A tumor can be seen as an “organ” composed of transformed cells that interact with stromal cells within the tumor microenvironment. The process of tumorigenesis requires multistep initiation of cellular and molecular pathways leading to a series of mutations resulting in the acquisition of replication and growth factor independence, resistance to growth-inhibitory signals, tissue invasion, and metastasis. Thus above two proposed models can very well explain the process of tumorigenesis.

**Concept Of CSCs**-
The pioneer work in this field is attributed to VIRCHOW, a German pathologist who found similarities between embryonic and tumor tissues. CSC hypothesis postulates that tumor
heterogeneity with regard to initiation, progression, response to therapy and metastasis is the result of mutations which either render a normal somatic tissue stem cell cancerous or cause a cancer cell to become stem cell-like. This population of tumor cells consists of rapidly dividing cells (similar to the transient amplifying (TA) cell population in normal tissue) as well as additional CSCs and more differentiated tumor cells. Gao in 2008 identified precancerous stem cells (pCSCs) in cancer and suggested that both pCSC and CSC might also serve as precursors of tumor stromal components such as tumor vasculogenic stem/progenitor cells (TVPCs). Thus, he suggested, the developing process of tumor-initiating cells (TIC) is initiated from pCSC leading to CSC and later cancer, a cellular process that parallels the histological process of hyperplasia/metaplasia (TIC) from precancerous lesions (pCSC) into malignant lesions (CSC → cancer).

**Signalling Pathways For Maintenance Of CSCs**

Various signaling pathways participate in maintenance of stem cell proliferation and tumorigenesis. Of which, Oct-4, Notch, Wnt/Catenin, bone morphogenic protein (BMP), Sonic Hedgehog signalling pathway, Mushashi-1 (Msi-1), are seen mostly. Oct-4 is normally expressed in the inner cell mass of the embryo and maintains totipotency. Notch, normally expressed in vasculature, activates endothelial cells and promotes angiogenesis. Wnt/Catenin affects orientation of chromosomes during mitotic divisions and plays a role in proliferation and inhibits apoptosis. Bone morphogenic proteins (BMP) are members of tissue growth factor-β (TGF-β) superfamily and function as oncogenes and tumour suppressors sometimes. Sonic Hedgehog signalling pathway is a major regulator of some of the fundamental processes including stem cell maintenance, cell differentiation, tissue polarity, and cell proliferation.

**Molecular Markers In Identification Of Cancer Stem Cells**

HNSCCs cause significant morbidity and mortality and have a tremendous bearing on patients quality of life. The identification and characterization of CSC by means of specific molecular markers can guide us in developing efficacious targeted therapies and prevent tumour recurrences. Thus, there extends a need to early detection of CSCs in tumour cell population. The origin of stem cells can be determined using variable techniques like- Colony forming cell assay, Microsphere assay, Side population assay, ALDH assay. Furthermore, with the advent of molecular markers it was feasible to determine their origin precisely.

The Table below shows molecular markers studied in cancer stem cells-[Table -1]

<table>
<thead>
<tr>
<th>Reference</th>
<th>Stem cell marker studied</th>
<th>Cancer cell lines studied</th>
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<tbody>
<tr>
<td>Barth et al., 2004[17]</td>
<td>CD34, CD117 (receptor of stem cell factor, SCF)</td>
<td>Oral cavity, pharynx, and larynx</td>
</tr>
<tr>
<td>Kojc et al., 2005[18]</td>
<td>CD34, transforming growth factor beta 1 (TGF beta 1)</td>
<td>Larynx</td>
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<tr>
<td>Tan et al., 2006[19]</td>
<td>Stem cell factor</td>
<td>Nasopharynx</td>
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<tr>
<td>Prince et al., 2007[20]</td>
<td>CD44, BMI 1</td>
<td>HNSCC generated in immunodeficient mouse model</td>
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<tr>
<td>Zhou et al., 2007[21]</td>
<td>CD133</td>
<td>HNSCC cell lines</td>
</tr>
<tr>
<td>Pries et al., 2008[22]</td>
<td>CD44</td>
<td>Hypopharynx, larynx, and oropharynx</td>
</tr>
<tr>
<td>Chou et al., 2008[23]</td>
<td>Oct-4, Nanog, and CD133</td>
<td>Oral squamous cell carcinoma stem like cells</td>
</tr>
<tr>
<td>Chen et al., 2009[24]</td>
<td>Bmi-1 1, Pakt (+) CD133, CD44</td>
<td>Tongue</td>
</tr>
</tbody>
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Role of Cancer Stem Cells In Progression Of OSCCs-
It can be studied under following heads-
1) CSCs with oral mucosa- Oral mucosa is composed of different layers of which basal cell layer has capacity to proliferate utmost. The basal cell layer is however attributed to possess the population of Transit Amplifying cells which have capacity to regenerate. The other proposed mechanism suggested the origin of CSCs to be either hypothetical nonepithelial stem cell sources in the oral mucosa include vessel wall-derived cells, blood-derived stem cells, muscle-derived stem cells, and adipose-derived stem cell. After understanding the possible role of CSCs in HNSCC, Oliveira et al. tried to find the possible influences of these CSCs in oral squamous cell carcinoma using CD44 and CD24. They suggested in their result that the absence of immunoexpression of these two investigated markers can be used in combination with other clinicopathologic information to improve the assessment of prognosis in OSCC.

CSCS, OSCC, EMT, AND HYPOXIA.-
Costea et al. in their hypothesis suggested a potential involvement of the stromal microenvironment OSCC progression. As already known, the activated fibroblast (myofibroblasts) inside the tumor stroma stimulate the transformed keratinocytes, thus influencing stem cell division patterns and with further genetic alterations of these keratinocytes leads to evolution of more invasive clones. OSCC is found to rely on hypoxia cellular response system for tumor progression. Focal hypoxia which is found in OSCC also may be due to quantitative and qualitative alterations in tumor vasculature, leading to local reduction of oxygen availability.

Therapeutic Strategies-
CSCs harbors the mechanism of self renewal and are found to play a role in treatment resistance of glioblastoma and pancreatic cancer cases. It has been concluded that stromal environment and CSC habitat play a vital role in the behavior of cancer cells targeting the stem cell niche directly can weaken the source of nutrition and change the essential signals needed by CSCs to proliferate. Therapeutic strategies target at microenvironment of tumour which contributes to self renewal and formation of reactive oxygen species (ROS) at that site. Hypoxia has been understood to play a key role in tumor progression and hypoxic tumor microenvironment in turn has a control over the CSCs. So, when the antiangiogenic agents are administered in combination with CSC-targeted drugs, more effective results are attained in cancer therapy, along with inhibiting hypoxia inducible factors (HIF).

CSCs are less sensitive to chemo- and radiotherapy and also have a lower immunogenicity. They contribute to tumor dormancy by having a slowcell cycle kinetics (quiescent state) which protects CSCs from chemo radiotherapy. So, therapeutic aspects should target the stem cells habitat at tumour microenvironment, HIF and other contributing factors of tumour cell survival.

Future Prospectives-
In our pursuit of understanding the CSCs it is paramount to accurately detect them from entire tumour biological architecture. These cells
Stem cells and oral squamous cell carcinoma

Aakruti Agrawal, et al.

warrants importance in their differences with respect to resistance to radiation or chemotherapy. Significant efforts should be carried out to detect these cells thereby predicting the nature of malignancy. There exists a lacuna in the study of CSCs due to their meagre number present in the tumour microenvironment. Hence, they should be considered as additional tumour cell type and therapeutic treatment strategies should be targeted to these enigmatic cell populations.

CONCLUSIONS-
CSCs continue to be a mystery in the field of oncology. Molecular mechanisms which transform normal cells to CSCs are yet not fully understood and be further strongly evaluated. Role of continuing research in this cell population will help us to guide in understanding this entity and will further improve the therapeutic outcomes in HNSCCs.

Conflicts Of Interest: None declared

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REFERENCES-
19. E.-L. Tan, G. Selvaratnam, R. Kananathan, and C.-K. Sam, “Quantification of Epstein-Barr virus DNA load, interleukin-6, interleukin-10, transforming growth factor-β1 and stem cell factor in plasma of patients with nasopharyngeal carcinoma,” BMC Cancer, vol. 6, article 227, 2006