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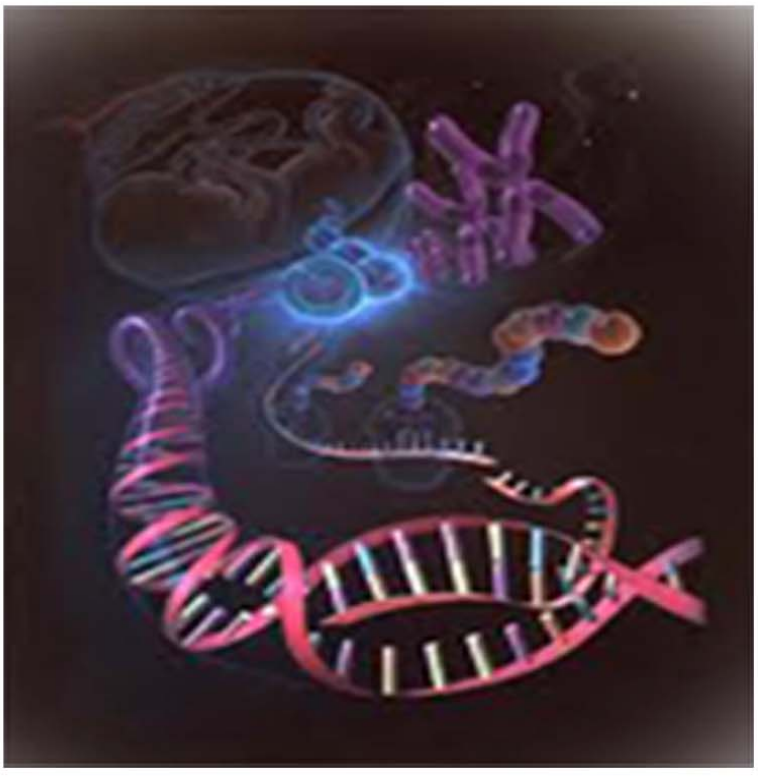

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Review Article

REVIEW ON CANCER STEM CELLS

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Cancer is one of the leading causes of death in the developed, developing as well as undeveloped nations in the world. Cancer is caused due to loss of function of cell cycle check point factors that leads to uncontrolled proliferation of cells. Often cancer originates in a particular tissue but cancer cells migrate to distant tissues by a phenomenon known as metastasis. The invasiveness properties of cancerous cells are often exhibited by the Cancer Stem Cells (CSCs), which possess similar properties as that of normal stem cells such as self renewal ability and differentiation capacity depending upon the tissue-specific occurrence. CSCs are often not destroyed with most of the cancer preventing therapeutics and thus they metastasize to different tissues and cause cancer recurrence. Currently available therapeutics for cancer is targeting only cancerous cells and failed to kill the CSCs which plays a major role in tumorigenicity there by leading to recurrence of cancer.

Keywords: Cancer, Cancer Stem Cells, Anti-Cancer Drug

INTRODUCTION

In recent decades, biomedical research has revealed extraordinary diversity within the broad category of diseases known as cancers. This astounding variety confounds attempts to find common features among the many forms of cancer. However, all clinically significant cancers share at least one common characteristic: excessive proliferation of affected cells. As highly differentiated cells rarely divide, and rapidly proliferating cells have poorly differentiated phenotypes, two basic therapeutic approaches for combating cancers have developed: Differentiation therapy to induce differentiation and Destruction therapy to thwart malignant proliferation Momna Hejmadi (2010). Apart from limited success in some cases, neither of these approaches completely cures cancer.

In the early 1990s, clinical observations and genetic studies of a variety of cancers led to the hypothesis that six genetic mutations were required to convert a normal somatic cell into a cancer cell. These six mutations included: Self-sufficiency for growth signals, Insensitivity to antigrowth signals, Evasion of apoptosis, Limitless ability to replicate, Sustained angiogenesis and tissue invasion and metastasis.

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However, not all cells in a given tissue are created equally in terms of their stage of development and their potential for proliferation and/or differentiation. Stem cells sit at the top of the developmental hierarchy, having the ability to self-renew and give rise to all the cell lineages in corresponding tissues (Madhuri Kakarala and Max S Wicha, 2008).

Stem cells divide to produce two daughter cells. One daughter remains a stem cell (self-renewal). The other daughter becomes a progenitor cell that undergoes expansion and further differentiation into mature cells. Stem cells have the highest potential for proliferation and a much longer life span compared with their progeny and therefore has a greater opportunity to accumulate genetic mutations. The realization that the adult body harbors small numbers of stem cells offered an alternative possibility for the origin of cancer (Reya T *et al.*, 2001).

CANCER DIAGNOSIS

Tumours are usually diagnosed when they produce some effects in the body. Tumours of the skin or of certain exterior organs which can be easily examined such as breast, often present as a noticeable lump. Many cells in tumours die and these dead cells release enzymes which damage the overlying tissues so that a non-healing ulcer may form. A growing number of tests are usually performed to identify the presence of abnormal cells or an abnormal structure. These diagnostic tests can simply confirm or eliminate a primary cancer, or they can help determine the spread of malignancy Bissell (1971).

PRESENT STRATEGIES OF CANCER TREATMENT

Recently many therapies are available for cancer treatment and natural approach on making body healthier can also treat cancer. They include several strategies such as chemotherapy and radiation therapy to kill cancerous cells, increasing oxygen levels kills cancerous cells, normalizing pH levels as acidic levels leads to cancer, stimulating methylglyoxal that prevent cancer cell growth, a strong immune system against cancer cells and fungal infections, reducing toxins intake, preventing free radicals as it causes DNA damage, using effective biochemical enzyme's to kill cancerous cells and resolving stressful issues vital for success against cancer (Peter J Selby *et al.*, 2005).

CANCER STEM CELLS

Cancer Stem Cells (CSCs) are cancer cells (found within tumors or hematological cancers) that possess characteristics associated with normal stem cells, especially the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic (tumor-forming), perhaps in contrast to other non-tumorigenic cancer cells Lawson *et al.* (2009). CSCs may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease (Verheul *et al.,* 2000).

CANCER STEM CELL PROPERTIES

Self-renewal is the process by which stem cells regenerate themselves. Normal adult stem cells help the body repair itself by replenishing

differentiated cells in bone marrow, brain, muscle and other tissues. In contrast, CSCs use the process of self-renewal to generate new cancer cells and cause cancerous growth. Learning how CSCs undertake self-renewal is the first step toward developing therapies that can target the cancer propagation machine. For example, program researchers are investigating which proteins go awry in the adult stem cells that underlie cancers of the blood, breast, ovaries, lung, brain and bladder, among others. Through this work, researchers hope to develop new drugs that shut down these abnormally active proteins.The design of new drugs for the treatment of CSCs will likely require an understanding of the cellular mechanisms that regulate cell proliferation. The first advances in this area were made with hematopoietic stem cells (HSCs) and their transformed counterparts in leukemia, the disease for which the origin of CSCs is best understood. It is now becoming increasingly clear that stem cells of many organs share the same cellular pathways as leukemia-derived HSCs (Bissell, 1971; and Ponti, 2005).

IMPLICATION OF THE CANCER STEM CELL HYPOTHESIS FOR BREAST CANCER PREVENTION

The cancer stem cell hypothesis suggest that, unlike most cancer stem cells within a tumor, cancer stem cells are resistant to chemotherapeutic drugs and can regenerate the various cell types in the tumor, thereby causing relapse of the disease, thus, drugs that selectively target CSCs offer great promise for cancer treatment, particularly in combination with chemotherapy. This combination therapy reduces tumor mass and prevents relapse much more effectively than either drug alone in a xenograft mouse model. Heather A Hirsch *et al*. (2009) provide a rational and experimental basis for using the combination of metformin and chemotherapeutic drugs to improve treatment of patients with breast (and possibly other cancer) cancers (Madhuri Kakarala and Max S Wicha, 2008).

DIFFERENT APPROACHES ON CANCER STEM CELLS

In 1994, John Dick's lab showed that leukemia-initiating stem cells present in the peripheral blood of Acute Myelogenous Leukemia (AML) patients (1 of 250,000 cells) could induce AML when transplanted into Severe Combined Immuno-Deficient (SCID) mice. Demonstration of tumorigenesis in SCID mice became convincing proof of the role of stem cells in perpetuating cancer in various organs (Theocharides *et al.,* 2012).

In 2003, Michael Clarke's lab conclusively showed the presence of stem cells in breast cancer. The following year, Peter Dirks' lab unequivocally proved stem cell involvement in brain cancer.Within established tumors, the great majority of the cancer cells cannot sustain the lesion nor establish it elsewhere in the body. Only a few cells within the tumor, the cancer stem cells, are tumorigenic and possess the metastatic phenotype. This circumstance has implications for both therapy and research. It explains why treatments that substantially reduce the tumor mass by removing proliferating cells fail to cure patients with some types of cancer, because CSCs are usually slowly cycling cells and thus insensitive to these treatments. It also introduces a note of caution among researchers seeking insight from analysis of a cross-section of the tumor cell population. Gene expression profiles obtained from samples of heterogeneous tumor

tissue probably bear limited resemblance to those of the tumor stem cell population, which forms only a tiny fraction of the whole tumor. The realization that cancers may rely on 'cancer stem cells' that share the self-renewal feature of normal stem cells has changed the perspective with regard to new approaches for treating the disease (Clarke *et al.,* 2003; and Singh *et al.,* 2003).

Epidemiologic studies indicate that diabetes is correlated with increased risk of breast and other cancer, but they showed that metformin with low dose, a standard drug for diabetes, inhibit cellular transformation and selectively kill cancer stem cells. Their study has given a new insight into combinatorial therapies, that the combination of metformin and a well-defined chemo-therapeutic agent (Doxorubicin), kills both cancer stem cells and non-cancer stem cells in culture. The isolation of CSCs and analysis was performed by flow cytometry by sorting the cells and then cells were stained with CD44 antibody and CD24 antibody. Finally, two types of cells one cancer stem cells with $CD44^{high}/CD24^{low}$ and other ones non stem-transformed cell ($CD24^{high}/CD44^{low}$) were isolated, then they were treated cells with metformin and cell growth was assessed *in vitro*. It was observed that mice treated with the combination of metformin and doxorubicin remain in remission for at least 60 days after treatment is ended. These observations provide independent and further support for the cancer stem cell hypothesis (Singh *et al.,* 2007; and Wendy *et al.,* 2009).

Yong Zhong *et al.* (2007) demonstrated that MCF7 and SK-OV3 were composed mainly of cancer stem cells that in serum-containing medium, most viable MCF7 and SK-OV3 cells could generate a self-renewal clone which could give rise to breast and ovarian cancer in vivo, both of above cell lines is comprised of SP and non-SP cells that in the long-term culture, SP (side population) cells may undergo asymmetrical cell division and differentiate into NSP (non-side population) cells. With doing single cell cloning culture of unsorted MCF7 and SK-OV3 cells using routine procedures of limited dilution, we showed that in serum-containing medium, most viable MCF7 and SK-OV3 cells could generate a self renewable clone, which could subsequently give rise to breast and ovarian cancer *in vivo*. Because these clones and sub-clones were generated from single cells, one can conclude that the clone-initiating cells are, indeed, multipotent. Therefore, most viable MCF7 and SK-OV3 are cancer stem cells, according to the above criterion of cancer stem cell (Yong Zhong *et al.,* 2007).

Several stem/progenitor cell-like subpopulation can exist in breast cancer tissues, there is increasing evidence pointing to the existence of breast cancer stem cells and their role in tumorigenesis by Wendy W Hwang-Verslues *et al.* (2009) They found that the expression of stem cell markers varied greatly among breast cancer cell lines. The stem/progenitor cell properties of different marker expressing-cell populations were further assessed by *in vitro* soft agar colony formation assay and the ability to form tumors in NOD/SCID mice (Wright *et al.,* 2008).

Muhammad Al-Hajj *et al.* (2003) used flow cytometry to isolate cells that were positive or negative for each marker to determine whether these markers could distinguish tumorigenic from non-tumorigenic cells. Finally, the ability to prospectively identify tumorigenic cancer cells will facilitate the elucidation of pathways that regulate their growth and survival and follow this we can achieve more effective therapies.

Brca 1-deficient mouse mammary tumor harbor heterogeneous cancer stem cell populations, and CD^{44+}/CD^{24-} cells also identified as human breast cancer stem cell populations, in addition long-term spheroid-forming assay may allow rapid screening of tumors for enrichment in cancer stem cells, identification of additional stem cell markers, and development of potentially curative therapies that target the putative cancer stem cell.

Mollie H Wright *et al.* (2008) were derived cell lines from individual Brca 1 mouse mammary tumors and non-overlapping populations of putative cancer stem cell markers $CD44^{high}/CD24^{low}$ and $CD133^{+}$. Only cell lines that contained a significant fraction of cells with these markers formed spheroid structures without preliminary sorting, and expansion of these these spheroids structures *in vitro* led to further spontaneous enrichment in cells with stem cell markers (Mollie *et al.,* 2008).

Farhad Vesuna *et al*. (2009) in their investigation demonstrate that the over expression of twist in breast cancer cells promote the generation of a breast cancer stem cell phenotype characterized by the high expression of CD144 little or no expression of CD29 and increased aldehyde expression dehydrogenase 1 activity. In addition, twist-over expressing cells exhibit high efflux of Hoechst 33342 and Rhodamine 123 as a result of increased expression of ABCC1 transporters. Twist over expression was 15% higher in the / subpopulation compared with the the subpopulation. Mammosphere formed by stable twist overexpressing cell lines, they indicate twist transcriptionally regulates CD24 expression in breast cancer cells (twist directly regulate a breast cancer cells (twist directly regulate a breast cancer stem cell phenotype through down-regulation of CD24 expression) (Madhuri Kakarala and Max S Wicha, 2008).

CD133 is widely used as a marker for the identification and isolation of neural precursor cells from normal brain or tumor tissue.Yirui Sun *et al*. (2009) demonstrated that CD133 and a second marker CD15 are expressed heterogeneously in uniformly undifferentiated human neural stem cell culture; they further showed that CD133 is down regulated the mRNA level in the cells lacking CD133 immunoreactivity. Cell cycle profiling reveals that CD133 negative cells largely reside in /, while CD133 positive cells are predominantly in S, or M phase. On the other hand, they observed that the higher cloning efficiency for CD133 negative versus CD133 positive cells might be because/cells are intrinsically more clonogenic, or they may be more resistant to flow sorting, compared cells in S, or M phase (Nubuko *et al.,* 2000; and Yirui *et al.,* 2009).

Since the discovery that neural tissues contain a population of stem cells that form neurosphere in vitro, so sphere-forming assays have been adapted for use with a number of different tissue type for the quantification of stem cell activity and self-renewal. The mammosphere assay has been used to quantify both stem cell/ early progenitor activity and stem cell self-renewal in normal mammary tissue and ductal carcinoma (IDC) *in situ.* Mammosphere forming efficiency (%) is calculated as follows (Frances *et al.,* 2012):

(The number of mammosphere per well / number of cells seeded per well) × 100

Also mammosphere self-renewal can be calculated as follows:

(Total number of 2° mammospheres formed / Total number of 1° mammosphere formed)

It has reported that mammosphere formation increases with each passage, which would suggest that the cancer stem cells are undergoing symmetrical self-renewal.

CONCLUSION

Cancer is one of the leading cause of death worldwide, thus it is very necessary to search for therapies against this disease. The currently available therapies include radiation therapy and chemotherapy which suffer with their own set of disadvantages. The recently emerged concept of CSCs has led to new hypothesis about tumors progression. The CSC paradigm postulates that dysregulated tissue specific stem cells or progenitor cells are precursors for cancer biogenesis. This whole study was undertaken with the objective to understand the , anti-cancer drugs with anti-proliferative properties targeting not only the differentiated tumor cells but also the cancer stem cells will generate an effective therapy against cancer by reducing the relapse and metastasis of cancer cells.

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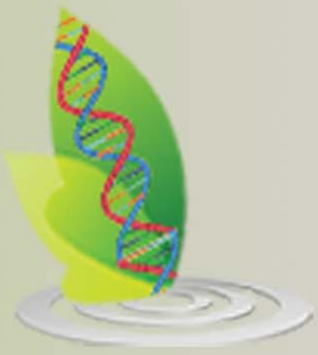

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