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Cancer Stem Cells: Concepts and Therapeutic Implications

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ABSTRACT

The cancer stem cell concept is currently seen as an important breakthrough in cancer research and relies upon the availability of the cancer stem cells. The cells have capability to produce whole tumor mass through self-renewal and differentiation besides, the ability to metastasize possibly through homing. Since such properties are also expressed by the normal stem cells, more research is needed in this field, especially in isolation and characterization. As the conventional therapies are effective only against the bulk of the tumor without any effect on cancer stem cells, it normally relapses. It seems logical to target the cancer stem cells through direct or indirect means along with the conventional therapies, in order to devise effectual therapeutics for cancer. The current review briefly throws light upon the development of the cancer stem cell theory and its concepts besides the possible therapeutic implications.

Key words: Cancer stem cells, therapeutic implications

INTRODUCTION

Cancer is a major health problem both in humans and animals and its percentage of mortality shows inequality being more in developing countries (poor people) compared to developed countries. The percentage of deaths due to cancer have shown an increasing trend in developing countries and by 2025 almost 80% deaths will be due to different type of cancers (Stewart and Wild, 2014). The cancer is also a leading cause of deaths in America being second only to cardiac diseases in human (Anderson and Smith, 2005). Advances in the biomedical research along with the dietary modifications have significantly reduced deaths (>60%) due to cardiac disease during last 50 years of the 20th century but during the same period only 5% reduction in mortality rate due to cancer was witnessed (<http://www.cancer.org>). Presently, annually about 4.2 million people die prematurely (aged 30-69 years) across the world (World Global Health Observatory). Among the animals, pets (canine) that receive more attention have an annual incidence rate of 99.3/100000 in males and 272.1/100000 in female dogs. The higher female to male incidence has been attributed to the higher risk of the mammary tumors in the latter (Merlo *et al.*, 2008).

Cancer though caused by a wide variety of causes but its higher rate in developing countries seems to arise from infections such as Papillomavirus. Besides, adoption of the western lifestyle like smoking, drinking and lack of physical activity makes people more prone to the cancer (Stewart and Wild, 2014). Cancer in humans and animals are highly comparable but the preferences in two fields vary. In the former, main focus is to treat or expand the life span by employing either a single or the combinations of therapy viz. radiation, chemotherapy or surgery.

In veterinary field the cancer therapy normally ends up in euthanasia (Breen and Modiano, 2008). However, since American college of veterinary internal medicine/oncology certified radiation oncology as a separate discipline two decades ago, there has been an increased awareness about the role of veterinary oncology in understanding the cancer further besides changing the therapeutic perspective in animal cancer (Mack, 2006; Khanna *et al.*, 2006).

The conventional therapeutic options for cancer treatment include radiotherapy, chemotherapy, surgery and/or the combinations thereof. Radiotherapy is used based upon the principle that cells replicating faster have more chances of DNA damage upon exposure to radiations. Though the tumor cells are affected primarily but normal cells do also get affected. A number of constraints are associated with the radiation therapy including localized tumor efficacy, suppression of normal stem cells besides the inflammatory effects, damage to the nerve and other tissue upon prolonged use (Hall, 2000). Chemotherapy kills cancer cells by interfering with DNA synthesis or cell functioning. The added advantage over the radiation therapy is its widespread efficacy for tumors at multiple locations and whether superficial or deep. However, the drawbacks in the form of toxic effects, development of drug resistance and need for supplementary treatment options demand research at molecular level in order to achieve the desired results (Ross, 2000). Surgery is used both as a diagnostic as well as the therapeutic procedure and works by removing the tumor mass as a whole. Its disadvantages like usefulness only against the localized tumors, impossibility of removal of the whole tumor without any spillage and inefficiency against the tumors involving the critical organs like heart, brain, etc. demand further research in order to hold complete control over cancer treatment (Gilson and Stone, 1990; Enneking and Maale, 1988).

The main reason behind the failure of all the above mentioned therapies is actually not encountering the source of tumor, the cancer stem cells. Tumors in general are composed of different kinds of cells including neoplastic cells, inflammatory cells, supporting vascular cells and fibroblasts. Barring the neoplastic cells and fibroblasts, all cells have restricted self-renewal capacity and are mostly differentiated and short lived (Rich and Bao, 2007; Han *et al.*, 2013). In contrast neoplastic cells are long lived have self renewal potential and are able to differentiate and form tumor. Such cells are popularly known as Cancer Stem Cells (CSCs) or Cancer Initiating Cells (CICs) or Tumor Initiating Cells (TICs) (Wu *et al.*, 2012; Dou and Gu, 2010; Han *et al.*, 2013). By employing or developing the therapies that could control or destroy the tumor initiating cells, the pathological features of tumors can be controlled and thus, tumor treatment can be achieved. The current review thrusts upon the cancer stem cell model, concepts and its possible therapeutic implications.

DEVELOPMENT OF CANCER STEM CELL HYPOTHESIS

The concept of cancer stem cells may be ascribed to the research of Cohnheim and Durante, who observed comparable proliferation and differentiation potential in embryonic tissues and cancers, about 150 year back. The unique research made the basis for the possibility of resident stem cells present in adult tissues which upon activation may lead to cancer (Gugjoo *et al.*, 2013). Further, impetus to the concept came from the work of Furth *et al.* (1937) who reported AML in mouse upon single undifferentiated leukemia cell transplantation (Al-Hajj *et al.*, 2003). It was for the first time that self-renewal properties of stem cells were shown. Three decades later, *in vitro* colony assay showed that AML can self-renew, proliferate and can give rise to new tumors (Sell, 2004). Later the concept of blocked ontogeny, maturation arrest of stem cells was formally tested under *in vitro* conditions in 1990's by John Dick and colleagues (Castor *et al.*, 2005) and was also

supported by others (Caussinus and Gonzalez, 2005; Chen and McKearin, 2003). However, all the above studies were conducted on leukemia or liquid phase tumors. The first report of cancer stem cell in solid tumors came from Al-Hajj and colleagues in 2003 who reported it from breast cancer (Al-Hajj *et al.*, 2003). Subsequently CSCs were also observed in a wide variety of solid tumors viz. lung cancer (Kim *et al.*, 2005), colon cancer (O'Brien *et al.*, 2006), prostate cancer (Collins *et al.*, 2005), ovarian cancer (Szotek *et al.*, 2006), brain cancer (Piccirillo *et al.*, 2006), melanoma (Fang *et al.*, 2005) and others. The stem cell hypothesis is thus, different from earlier stochastic model, wherein all the tumor cells are considered to have potential for tumor development (Gugjoo *et al.*, 2013).

TUMOR GROWTH AND METASTASIS

The CSCs are being recognised as distinct cell population in cancers that are capable of developing the whole tumor and thus, represent its characteristic features viz. tumor growth (size), lymph node involvement and metastasis (Han *et al.*, 2013; Gugjoo *et al.*, 2013). Stem cells under normal circumstances are regulated by the microenvironment/niche. The niche is composed of; group of cells at a special location, an anchoring site for stem cells via adhesion molecules, growth factors and signalling molecules like fibroblast growth factors, Wnt, Hedgehog, etc. that regulate stem cell self-renewal property, multiplication and differentiation (Li and Neaves, 2006; Gugjoo *et al.*, 2015). The microenvironment/niche facilitates either proliferation or inhibition of the stem cell properties depending upon the need (Potten *et al.*, 2009; Fuchs *et al.*, 2004). The notion was based upon the observation that stem cells are lost upon loss in niche (Xie and Spradling, 2000). The other question that arises is how does tumor develops from CSCs. The mechanisms that may be involved include the mutations leading to self-sufficient cell proliferation or alteration in niche leading to dominance in proliferation-differentiation signals over the inhibition signals. The finding was supported by a study in which it was found that neuroblastoma requires the coordination in mutations involving both the Schwann cells and supporting cells (Zhu *et al.*, 2012). The long life span of stem cell makes it more prone to mutations. Thus, cancer stem cells like other stem cells have the potential to differentiate into different lineages and accounts for the heterogeneous cell population in tumors (Mannelli and Gallo, 2012). Continuous proliferation following mutation or altered niche thus, may account for the tumor growth (increase in size). Besides, the normal stem cells have characteristic homing (arrest of stem cells within the vasculature of a tissue followed by transmigration across the endothelium) property, which may still be intact in CSCs leading to tissue invasion to nearby lymph nodes or to other tissue far or near (metastasis) (Li and Neaves, 2006).

The CSCs may be defined by the following properties:

- Self-renewal through symmetrical cell division producing two identical cells
- Asymmetrical cell division to produce heterogenous tumour cell population forming bulk of tumor (Clarke and Fuller, 2006; Clarke *et al.*, 2006)
- Capability to produce tumor upon transplantation under *in vivo* conditions unlike normal stem cells
- Possession of specific surface markers, similar to normal stem cells but different from the non-stem cells (Clark *et al.*, 2012; Mannelli and Gallo, 2012; Wicha *et al.*, 2006)

ISOLATION AND IDENTIFICATION OF CANCER STEM CELLS

Isolation and identification of CSCs from tumor mass can be done easily based upon the properties of stem cells as both the CSCs and normal stem cells have a number of common features.

These features include ability to form sphere in non-adherent medium, drug exclusion by efflux transport over activity, presence of different cell surface markers, enzyme activity or signalling molecules (Alison *et al.*, 2011; Ghani *et al.*, 2011; Zhang *et al.*, 2011). However, the above mentioned *in vitro* tests do not differentiate between normal stem cells, progenitor cells and cancer stem cells. The differentiation between normal and cancer stem cells can be done by *in vivo* tests and serial transplantation of such cells in animal models is considered as gold standard (Clarke *et al.*, 2006).

THERAPEUTIC IMPLICATIONS BASED ON CSC MODEL

The conventional therapeutic options for cancer treatment include chemotherapy, radiotherapy or surgical excision. However, these options stand without the desired results besides producing lot of side effects including local or systemic toxicity, drug resistance or loss of function (Ajani *et al.*, 2009; Rich and Bao, 2007). The CSCs being the cardinal reason behind tumor development account for failure of conventional therapies in cancer patients. The main reasons behind the therapy failure due to involvement of CSCs include gradual cell cycle kinetics, better DNA repair capability, over expression of multi-drug resistance membrane transporters and anti-apoptosis proteins and finally micro-environment (acidosis, etc) (Rich and Bao, 2007; Raguz and Yague, 2008; Signore *et al.*, 2013; Gugjoo *et al.*, 2013). Therefore, the need of the hour may be to combine conventional therapies with the options that aim at the CSCs.

CSC BASED THERAPEUTIC OPTIONS

Targeting cancer stem cell marker and metabolic pathways: Cancer stem cell markers, though also possessed by normal stem cells, may be targeted to eliminate tumour source. The cytotoxic drugs specific to CSCs markers and also the inhibitors targeting drug detoxifying enzymes, efflux pumps and or transcription factors may yield desired results.

Different tumors express different surface markers like CD44+, CD24+ and ESA+ in pancreatic CSCs and CD133 in hepatocellular and gastric CSCs (Smith *et al.*, 2008), which may be targeted to destroy CSCs. The CD133 is considered as an important marker and upon its down regulation, cell growth, motility, ability to form spheroids and above all capacity to metastasize would reduce (Rappa *et al.*, 2008). Such a down regulation may be achieved either by direct monoclonal antibody application (Rappa *et al.*, 2008) or by antibody drug conjugate like monomethyl auristatin F (Smith *et al.*, 2008).

Drug detoxifying enzymes like aldehyde dehydrogenase act as a marker for CSCs and normal stem cells. It plays a significant functional role in self-protection, differentiation and expansion of stem cells (Marcato *et al.*, 2011). In human breast cancer cell lines positive for ALDHhiCD99+ standard cancer therapy was ineffective but cells were sensitive upon inhibition of ALDH activity (Crocker and Allan, 2012). This explains the role of ALDH towards resistance against the conventional cancer therapies and offers a therapeutic option against CSCs.

Drug efflux pumps are normally over expressed in CSCs or normal stem cells and give protection against the noxious agents/drugs. Among the efflux pumps ABCG2 is a potent CSC marker. Besides it also is expressed by normal stem cells and the blood brain barrier. Phytochemical agent, curcumin has been found to inhibit the drug efflux mechanism of CSCs in rat C6 glioma cell line (Fong and Chan, 2012). However, the negative effect upon the normal stem cell and the blood brain barrier need to be taken care.

Cancer stem cell niche target: Niche form the basis for maintaining the activity of CSCs as well as normal stem cells. Targeting the niche could help target the CSCs by exhausting the nutritional

source and the essential signals required for normal functioning of CSCs (Han *et al.*, 2013). However, the niche targeting may affect the normal stem cells also, demanding further detailed research.

Among the microenvironment/niche factors, microvascularization has been found to be important for CSCs maintenance and thus could be a target for therapeutic approaches (Calabrese *et al.*, 2007). Hypoxia can control CSCs and may be considered for control of tumor progression. Antiangiogenic therapy induces CSCs niche hypoxia and thus, radioresistance to CSCs (Morrison *et al.*, 2011). The anti-angiogenic agents like sunitinib, may be combined with CSCs targeted drugs to achieve better results (Conley *et al.*, 2012).

Targeting stem cells signaling pathways: Normal stem cells maintain their characteristic features viz. self-renewal, multiplication or differentiation through signaling pathways. The same signaling pathways when dysregulated, normal stem cells turn into the CSCs. Although, a number of signaling pathways are involved, the well studied pathways are Hedgehog (Hh), Notch, Wnt/ β -catenin and are believed to play an important role in CSCs formation and thus may be effective targets to control CSCs and thereby tumor formation (Han *et al.*, 2013).

Hedgehog (Hh) is an essential signaling pathway for CSCs maintenance in different cancers including pancreatic cancer, colorectal or gastric cancers (Song *et al.*, 2011; Merchant and Matsui, 2010; Chen *et al.*, 2007), besides its significant role in therapeutic resistance (Chen *et al.*, 2007). Thus, inhibiting the Hh pathway would control CSCs and tumor formation. Smoothed signaling molecule (SMO) may be inhibited with inhibitors like cyclopamine, however, tumors that harbour lesions beyond the SMO, cyclopamine remains ineffective (Xia *et al.*, 2012; Singh *et al.*, 2011). To overcome the problem, glioma-associated oncogene homolog (Gli) proteins are blocked with the agents like arsenic trioxide together with the SMO blockade (Ng and Curran, 2011).

Notch pathway has an important role in cell-cell communication and in multiple cell fate decisions both during embryonic as well as adult phase (Soltanian and Matin, 2011; Wang *et al.*, 2008). It is involved in stem cell proliferation, differentiation and also apoptosis and may act as oncogenic in human tissues/organs like cervix, lung, head, prostate, etc and oncosuppressive in skin and hepatocellular carcinoma (Wang *et al.*, 2008). The γ -secretase inhibitors (GSIs) that block Notch pathway in glioblastoma have been found to result in reduced neurosphere growth and clonogenicity *in vitro*, reduced CSCs markers expression and reduced tumor growth *in vivo*. Thus GSI may be useful in glioblastoma by inhibiting notch pathways but may be toxic as they block all the four notch ligands and also γ -secretase substrates (Wang *et al.*, 2011).

Wnt, the other important signalling pathway is involved in multiple biological processes like embryogenesis, development, cell proliferation, survival and differentiation (Klaus and Birchmeier, 2008). It plays an essential role in self-renewal and maintenance of normal stem cells as well as CSCs in tissues (Wend *et al.*, 2010; Katoh and Katoh, 2007). Upon oncogenic mutations, Wnt dysregulation leads to CSCs formation and subsequent tumor development (Reya and Clevers, 2005; Reya *et al.*, 2001). There are two types of Wnt signalling pathway inhibitors viz. small molecule inhibitors (NSAIDs) and biologic inhibitors including Mab SiRNAs (Wend *et al.*, 2010; Katoh and Katoh, 2007). However, the signaling pathway that may be blocked have potential to interfere with the essential pathways of normal stem cells.

Induction of apoptosis: Dysregulation in apoptotic mechanism also contribute to the cancer development, progression as well as resistance to conventional therapy. The mechanism that might

interplay includes increased DNA repair after conventional radiotherapy/chemotherapy, alteration in cell cycle check point and MDR protein upregulation (Signore *et al.*, 2013). Thus, drugs like bicyclic cyclohexamines that induce apoptosis may offer good therapeutic option (Hexum *et al.*, 2012).

Differentiation of CSCs: By differentiation, CSCs population will deplete and thus, will have no self-renewal property (Soltanian and Matin, 2011). The CSCs differentiation may be achieved with retinoic acid and drugs targeting tumor epigenetic changes (Massard *et al.*, 2006). To eliminate the cancer, thus, combined use of CSCs differentiation agents and chemotherapy may prove to be a good therapeutic approach (Han *et al.*, 2013).

CONCLUSION

Cancer stem cells and normal stem cells share a number of features especially the self-renewal and differentiation. Besides, the mechanisms that make normal stem cells resistant to the chemotherapy/radiotherapy are also applicable to the cancer stem cells. The cancer stem cells develop from the mutations in normal stem cells/progenitor cells leading to uncontrolled proliferation. The alterations in microenvironment may also be responsible for the tumor formation by dominance in growth promoting signals over the growth inhibiting signals. Characteristic homing property of normal stem cells may still be intact in cancer stem, thus accounting for the metastasis. Isolation and identification of cancer stem cells may be made based upon the properties of normal stem cells and to differentiate former from latter, *in vivo* transplantation tests are required. Therapeutic options follow the basic characteristic features or related features of the cancer stem cells; however, need to elucidate further for careful clinical applications as most of the features are also shared by normal stem cells.

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