Implications of Cancer Stem Cells in Radiotherapy: Current Understanding and Future Perspectives

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Many theories were put forward about origin of cancer and immense research was carried out to understand the mechanisms involved in the origin and progression of the disease. The gain in scientific knowledge about cancer biology and technical advancement in treatment modalities resulted in improvement of clinical outcome in cancer therapy. However, recurrence and metastasis after therapy poses a major concern to clinicians. Resistant nature against therapeutic modalities and metastatic potential of cancer stem cells (CSCs), which even though form a fraction of tumor mass, result in failure of existing modalities of cancer treatment. The current article reviews the historical background of CSCs, involvement of various signaling pathways in the mechanism(s) of radioresistance and potential targets to be exploited in radiotherapy.

INTRODUCTION

Cancer stem cell research has gained substantial attention by biomedical scientists and clinicians in the recent years due to growing realization of their role in various aspects of cancer biology (progression, metastasis) and therapy (chemo- and radio-resistance). The concept of 'cancer stem cell' (CSC) was proposed in 1863 by Rudolf Virchow, an eminent pathologist, who observed the abnormal mixture of undifferentiated embryonal cell with differentiated adult cells in teratocarcinomas. Later on, Cohnheim, a student of Rudolf Virchow, put forward 'embryonic rest theory' in 1875, which states that during embryonic development, certain cells became isolated and manifested their uncontrolled proliferative potential during adult life (Cohnheim 1875; Sell, 2004; Virchow 1863). Similarities in growth characteristics (embryoid bodies) and histopathological features (undifferentiated cells) exist in embryonic tissues and teratocarcinomas. These features of teratocarcinomas led to postulate that the tumors might have originated from undifferentiated stem-like cells (Bignold et al., 2006; Pierce et al., 1959; 1960). Later in 1930s, Furth et al. (1937) demonstrated that a single malignant white blood cell is capable of producing leukemia, a systemic disease in mice. These noteworthy

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developments in cancer stem cell biology at that stage were not pursued extensively and were overshadowed due to gaining significance of mutation associated with carcinogens in tumor development (Bishop 1985; Rous 1983; Weinberg 1985; Yamagiwa et al., 1977). The area of cancer stem cell biology remained relatively dormant till late twentieth century until Lapidot et al. (1994) demonstrated that a small subset of cancer cells (CD34−CD38+) sorted from the blood of acute myeloid leukemia patient resulted in development of leukemia in SCID mice (Lapidot et al., 1994), whereas the CD34+CD38− cells did not possess the ability to develop leukemia (Bonnet and Dick 1997; Lapidot et al., 1994). In 2003, CSCs were first identified in solid tumors by Al-Hajj and colleagues in breast cancer patients (Al-Hajj et al., 2003), where 100 CD44+/CD24−/low cells injected underneath the mammary pad of non-obese diabetic (NOD) mouse strain, an excellent model of autoimmune disease, or SCID mice resulted in tumor formation. However, cells with CD44−CD24+ phenotype did not form tumors even when injected in thousands. Eventually, other solid tumors like brain, prostrate, lung, gastric, head and neck tumors also demonstrated presence of CSCs (Albers et al., 2012; Collins et al., 2005; Eramo et al., 2008; Singh et al., 2003; Takaishi et al., 2009; Tirino et al., 2013). Recently, it was also shown by lineage tracing experiments in mouse models that CSCs are responsible for tumor formation and are resistant to chemotherapy (Driessens et al., 2012; Schepers et al., 2012). Once treated by chemotherapy, these cells survive the therapy and responsible for the re-growth of tumor (Chen et al., 2012). It was hypothesized that the tumor stem cell divides by asymmetric division resulting in the formation of CSC and differentiated cell. Further, these cells divide and form heterogeneous tumor mass comprising various tumor tissue cells and these cells vary according to tumor type and alter during the course of cancer therapy (Fig. 1). The interactions between these various tumor cells and CSCs are hypothesized to play critical role in mechanism of radioresistance and unraveling these mechanisms may have significant implication in clinical outcome of cancer radiotherapy (Peitzsch et al., 2013).

CSCs in Mechanism of Radioresistance
Radioresistance is a major challenge in therapeutic outcome of cancer, associated with magnitude of CSCs in tumour mass (Chen et al., 2013; Shiozawa et al., 2013). Hence, the cure of cancer may depend upon targeting the resistant CSCs. The idea is supported by studies suggesting lower radiation induced apoptosis in glioblastoma cells expressing CD133 (Bao et al., 2006). In addition, the cells expressing CD133 increased significantly after radiation treatment in xenografts of glioblastoma (Bao et al., 2006). In the breast carcinoma cell line, MCF7, a sub-population
of cells expressing CD44^+CD24^- were radioresistant (Phillips et al., 2006). The mechanism of radioresistance of CSCs was known to be associated with lower DNA double stranded breaks after radiation involving preferential activation of DNA damage check point kinases (CHK1, CHK2, ATM and Rad 17) leading to arrest of cell cycle for facilitated DNA repair (Bao et al., 2006, Rich et al., 2007; Yin and Glass 2011). Recently, in glioblastoma samples, the role of self-renewal gene (BMI-1) in mechanism of radioresistance was reported (Facchino et al., 2010). The authors demonstrated that overexpression of BMI-1 resulted in radioresistance of CSCs and silencing of BMI-1 resulted in increased double strand breaks after irradiation (Facchino et al., 2010). In breast cancer, Wnt/beta-catenin signalling has a role in radioresistance and cell survival, resulting in tolerance of DNA damage (Woodward et al., 2007). A higher activity of free radical scavenging pathways in CSCs is another mechanism, resulting in lowering DNA damage after irradiation. This question was addressed in breast CSCs and these results showed that even after 10 Gy of gamma radiation, reactive oxygen species (ROS) generation was lower in MCF7-mammospheres compared to MCF7-monolayer cell cultures (Phillips et al., 2006). This line of evidence gets further supported by our recent finding of increased superoxide dismutase (SOD) activity in CSCs of human

**Figure 1:** Role of CSCs in tumor radioresistance, recurrence and metastasis with relevance to cancer radiotherapy: Tumor initiating cell undergoes asymmetric cell division resulting in CSC and/or differentiated tumor cell. These cells further divide abnormally to form heterogeneous tumor mass containing mixture of CSCs and non-CSCs at various stages of differentiation. After radiation therapy, radiosensitive cancer cells get killed and radioresistant cancer cells survive. The surviving cancer cells regrow and may result in highly resistant clones. These clones may cause recurrence and metastasis.
lung adenocarcinoma (A549 cells), correlating with higher clonogenic survival of CSCs after radiation treatment of up to 6 Gy (unpublished data, personal communication).

**Targeting CSCs for Radiosensitization during Cancer Radiotherapy**

CSCs are known to play a critical role in radioresistance of tumors and hence, it is imperative to target them for enhanced tumor killing. To radiosensitize CSCs, several groups have targeted self-renewal signalling cascades in different tumors. Cox2 inhibitor (NS398) was used to target Akt signalling, which resulted in radiosensitising of the radioresistant oesophageal cells (Che *et al.*, 2011). By silencing, T-cell factor-4 in Wnt signalling, radiosensitization was achieved in colorectal cancer cell lines (Kendziorra *et al.*, 2011). In glioma CSCs, Notch signalling was targeted by blocking Notch1 or Notch2 ligands to radiosensitize the cells (McGowan *et al.*, 2011; Wang *et al.*, 2010). JAK/STAT signalling was also implicated in radioresistance of head and neck carcinoma CSCs and non-small cell lung carcinoma CSCs. Targeting these cells with STAT3 inhibitor, cucurbitacin, resulted in apoptosis and loss of tumorigenesis in xenograft mouse model (Chen *et al.*, 2010; Hsu *et al.*, 2011). TGF-β signalling gets activated in response to radiation, mediating its effect through Smad family and competes with Notch-ICD (intra cellular domain) protein (Masuda *et al.*, 2005; Tian *et al.*, 2009). Interaction of these pathways is responsible for maintenance of CSCs in tumors. Thus, targeting the molecules associated with the signalling events may offer novel therapeutic intervention for improved radiotherapy.

Some of the signalling molecules like PTEN, mTOR, CD23 and CD44 are

| Table 1: Drugs used to target cancer stem cells to overcome radioresistance |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Cancer Types** | **Targets** | **Drugs** | **References** |
| Glioma and medulloblastoma | Notch Signalling pathway | RO4929097 | http://clinicaltrials.gov: NCT01122901 |
| Small cell lung cancer | PI3-mTOR pathway | VS-5584 | American Association of Cancer Research Annual Meeting: April, 2014 |
| Lung cancer, Head and neck cancer | JAK/STAT signalling | Cucurbitacin I | (Chen *et al.*, 2010; Hsu *et al.*, 2011) |
| Breast cancer | Glutamate-cysteine ligase inhibitor | Buthioninesulfloximine | (Diehn *et al.*, 2010) |
differentially regulated in CSCs and normal stem cells, which can be exploited for targeted therapy of CSCs (Chen et al., 2013). Targeting the tumor suppressor PTEN in CSCs, sparing normal stem cells, was shown to be achieved in leukemia (Yilmaz et al., 2006). Recently, efforts were made to target glioblastoma CSCs using CD133 antibody tagged with gold nanorods for therapy using photoablation (Wang et al., 2011). The self renewal gene BMI-1 expressed in CSCs was also targeted in colon cancer in a mouse model (Kreso et al., 2014). Potential agents to target these signalling cascades of CSCs are currently in clinical trials (Table 1).

The other strategy evaluated to effectively kill CSCs using proton and heavy ion radiation like carbon ions are under consideration (Chang et al., 2010; Schlaff et al., 2014). Protons were observed to induce higher level of ROS and apoptotic death in CSCs in non-small lung carcinoma cell lines (Chang et al., 2010). The effect of carbon ion on CSCs was compared against X-rays, and the conclusion was that at the same dose, CSCs were enriched in xenograft tumors irradiated with X-rays compared to tumors treated with carbon ions (Cui et al., 2011). In the recent study, it was also shown that by targeting check point kinases and blocking ALDH1 activity in combination can radiosensitize CSCs in head and neck cancer by photon or carbon ion radiation (Bertrand et al., 2014).

FUTURE DIRECTIONS

Even though substantial research has been made in the area of cancer stem cell biology, a more focussed research is required to understand the molecular interaction of CSCs with other cancer cells and components of tumor microenvironment. This would provide deeper understanding about role of CSCs in cancer radioresistance (Fig. 2). Characterization of novel biomarkers is very crucial to isolate CSC population. Furthermore, investigation of molecular
signaling mechanisms pertaining to radioresistance in CSC population may have significant implications in cancer therapy. Quantification of CSCs in tumor samples using sensitive, high throughput, reliable and economic techniques would be required to translate the knowledge gained in cancer stem cell biology to clinical level. Evaluation of CSCs in biopsy samples in cancer patients and their clinical correlation with tumor recurrence and metastasis may provide CSC-based prognostic markers in cancer therapy. It may be worth mentioning that better understating about exciting facts of cancer stem cell biology will benefit the cancer patients in coming days, which however, needs bridging the gap between laboratory and clinics.

CONFLICT OF INTEREST
The authors claim no conflict of interest.

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