K Int J Health Sci. December 2022;Vol 5(3): 86-92

REVIEW ARTICLE

Radiobiologic effects of radiotherapy on cancer and stem cells: Radscopal, abscopal and by-stander effects

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Conflict of Interest: There is no any conflict of interest

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Keywords: by-stander, abscopal, radscopal effects, ALC, low dose with SBRT

INTRODUCTION

Cancer stem cell (CSC): There are different stem cells specific to all organs in our body. Totipotent stem cells are cells that can transform into all organs of the body. It takes place in the first 4 days of the embryo. Pluripotent cells are then formed. These cells also have the ability to transform into many different cells. Multipotent cells are then formed. They are cells that can transform into other cells even though they belong to a single germ layer (1).

The clonal evolution model of cancer was first described by Peter Nowell in 1976 (2). According to the clonal evolution model, cancer cells are genetically very unstable. Different cell clones are formed due to this instability. It has been assumed that cancer tissue turns into a heterogeneous structure due to different cell clones. In addition, additional mutations occur that lead to cellular proliferation (2). What is less clear is whether stem cell characteristics change stochastically from one clone to the next. There is evidence that the clonal evolutionary model may be valid for some cancers, but a growing body of scientific data supports the existence of a hierarchical model in most solid tumors (3).

Placenta, amniotic fluid and cord blood stem cells are fetal stem cells. Stem cells in the blood, bone marrow, adipose tissue and organs of adults are called adult stem cells (4). Cancer stem cell (CSC) microenvironment: CSCs are located in a cellular or acellular microenvironment. With the effect of stimuli coming from the microenvironment such as endocrine and neural, they mature when necessary and act as a regenerative agent in the tissue. Non-reproducing stem cells predominate and are subsequently destroyed by apoptosis.

Cancer stem cells are generally located in a quiet state in a niche (nest) within the tumor tissue (5,6). Its microenvironment includes blood vessels, intercellular fluid and matrix, macrophages, cancer and normal cells, fibroblasts, T and B lymphocytes, leukocytes. Oxygenation is increased from time to time due to cells dying by apoptosis or necrosis. As cell mass increases, oxygenation decreases. Unlike other cells in CSC tumor tissue, they are cells with higher pathogenicity and pluripotency (with multiple properties and strength). The rate of CSC was found to be very high in metastatic malignant melanoma, and low in small cell lung, pancreatic, and head and neck cancer (6).

Difference from cancer cells: Management programs are different. In HR, the signals

are controlled and prevent excessive increase. Therefore, while cancer cells (CS) can easily go to apoptosis, CSC is very resistant to apoptosis with the effect of uncontrolled signals, reproduces continuously and is almost immortal (7). Although CSCs are also resistant to cancer treatments and seem to have received a complete response in treatment, some of them can remain silent in their homes for years and can be reactivated by some stimuli and effects such as stress and weakening of the immune system (7). Therefore, it is necessary to choose a treatment that will completely destroy CSCs in cancer treatments. It has been reported that some molecules that play a role in embryogenesis are involved in the reproduction of CSCs. The most well-known of these molecules is the NOTCH molecule. It shows its effectiveness together with WNT, Hh, Fgf and BMP from other molecules. This reproduction can be stopped by the control of CSC signaling pathways. Without this control mechanism, cancer cells reproduce easily (8).

In asymmetric division, one mature, that is, differentiated cell, 1 CSC is formed. In symmetrical division, both are stem cells. Since there are both types of division in cancerous cells, a heterogeneous structure is formed. Asymmetric division during reproduction in CSC is shown in Figure 1.

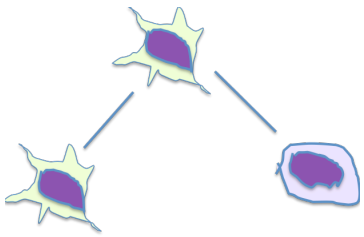

Figure 1. Asymmetric division in cancer stem cells

Reproduction with symmetric division in CSC is shown in figure 2.

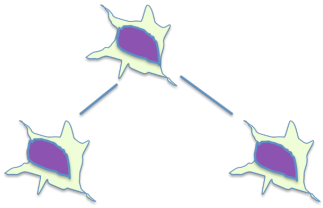

Figure 2. Symmetrical division in cancer stem cells

Reproduction by symmetrical division in cancer cells is shown in figure 3.

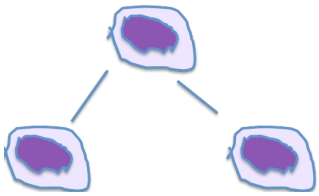

Figure 3. Reproduction by symmetric division in cancer cells

De-differentiation occurs by asymmetrically differentiated cancer cell division (Figure 4).

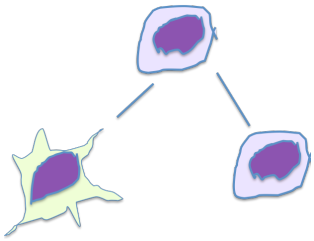

Figure 5. De-differentiation formation by asymmetrically differentiated cancer cell division

Heterogeneous tumor formation is shown in Figure 6.

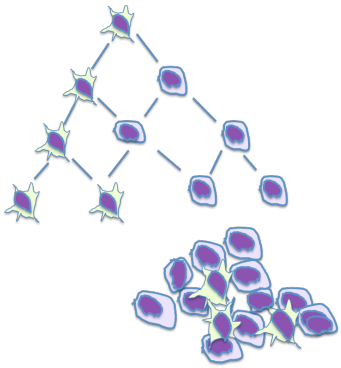

Figure 6. Heterogeneous tumor formation with asymmetric and symmetrical divisions

Initially, only the local radiobiologic effects of radiation were known on CSC. It was later found that the systemic effects were also very important. The most important of these are by-stander, abskopal and radscopal effects (8,9).

By-stander effect: By-stander effect is the effect that occurs in low-dose areas around the radiation field. This effect may occur as undesirable side effects in normal tissues or it may produce a cytotoxic effect in non-target tumor cells in the surrounding area (Figure 7) (9).

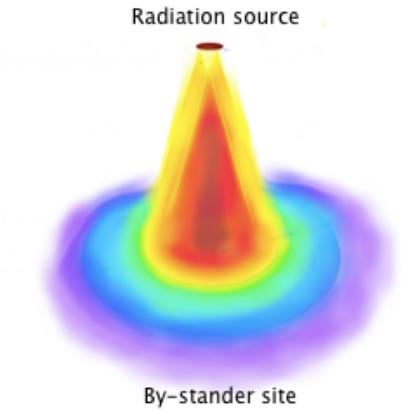

Figure 7. By-stander effect formation with radiation

The most sensitive phase to radiation are the G2 and mitotic phases. It has been reported that by-stander effects, which provide a response even with low-dose radiation, occur in phases that are sensitive to radiation and that the by-stander factor is released. In contrast, this effect is reduced under hypoxic conditions. Therefore, the by-stander effect is reduced in anaerobic and necrotic tumors (9).

Intensity of the by-stander effect in the G2 phase Cells are affected positively or negatively by the induction of oxidative stress and genomic instability caused by the effect of the by-stander environment. In many tumor cell lines in the anaerobic environment, the cytotoxic by-stander effect is very weakened (10). It has been shown that the by-stander cytotoxic effect is significantly reduced in mitochondria pathology and glucose-6-phosphate dehydrogenase deficiency (11).

On the contrary, the cytotoxic effect increases in tumor cells with good oxygenation. Cells can normally live in their own tissue matrix, and die when they move to a different tissue or organ, which is called anoikis. However, when CSC metastasizes, it can live in different matrices (12). In order for radiotherapy to be effective, there must be active cell proliferation in cancer cells. Therefore, aging or dormant cells (S phase, dormant, G0 phase) that have lost their ability to divide are more radioresistant (13). Although a large number of tumor cells are killed by radiotherapy, it is known that quiescent and dormant cells in CSCs can survive and recurrences occur when these cells wake up years later (14). In advanced cancer, since the rate of CSC is high and is still dormant, it has been said that metastasis and recurrence are more frequent with activation in a short time (15). One-third of CSCs in brain glioma and breast cancer cells are dormant. These cell lines sometimes can enter the cell cycle only after radiation (16).

Radiotherapy not only causes dormant CSCs to enter the cell cycle, but can also dose-dependently induce them to develop a carcinogenic metabolism, especially at high doses after 5-10 years (17). In some studies, it has been shown that radiotherapy can sometimes increase CSC, and sometimes decrease it significantly, especially in low-dose treatments, when given with some antibiotics and agents such as doxycycline (18,19). Radiation can transform non-tumorigenic cancer cells into CSCs by increasing the effect of embryonic transcription factors Sox2, Oct4, Klf4 and Nanog in polyploid cells (20). Current research results have shown that ionizing radiation cannot completely kill dormant CSCs due to radioresistance, but can awaken them, causing them to enter the cell cycle, which can lead to malignant behavior. In addition, ionizing radiation can induce reprogramming of differentiated cancer cells, causing them to differentiate into CSCs

and acquire tumorigenic abilities in the process (20).

Fractionated radiotherapy and by-stander effects: It was thought that non-irradiated cells receiving signals from nearby irradiated tissue would respond differently than expected due to the effect of the signal they received in each fraction. As the dose level increases, the by-stander effect decreases on the contrary (21).

Adaptive response: When cells previously exposed to very low doses of ionizing radiation were subsequently exposed to a high dose of radiation, fewer adverse effects and genetic damage were found in these cells than in other cells. This is due to the development of an adaptive response due to the induction of repair mechanisms by low dose exposure. This response is also known as the radioadaptive by-stander response and may cause radioresistance in normal tissues (22,23). Although there was an increase in AP-endonuclease protein levels in by-stander cells exposed to direct radiation, the absence of an increase in this protein level in cells exposed to radiation also supports this view.

There are radiation-related biological phenomena that explain the by-stander effects: With the advent of molecular biology, radiobiology research has focused on cell cycle kinetics, DNA damage and repair processes, and cell death mechanisms. Effects such as genomic instability, adaptive response, by-stander effects and low-dose hyper-radiosensitivity were investigated with low-dose radiation. The late and early effects of radiation have been investigated. The rate and degree of early radiation side effects also increase late side effects.

Radiation and abscopal effect: MHC-1 provides the recognition of cancer cells and increases local and extra-area cytotoxicity with the effect of radiation. This is called the abscopal effect (24). IFN-gamma increases the MHC-1 ratio. Therefore, the abscopal effect may also be strengthened. Abskopal effect was first described in 1953 as a rare RT-related effect. In a study in 2015, GM-CSF treatment with RT in metastatic solid tumors was proven as the first immunoRT (25).

In the PASIFIC study in 2017, striking positive results were obtained regarding that abscopal effect can be increased with durvalumab after CRT in stage 3 lung cancer (26).

Immune check point inhibition by RT: PD-1 is found in peripheral tissues, tumor microenvironment, and T cells. It keeps tumor cells away from immune system surveillance. PD-L1 and PD-L2 are transmembrane protein found in the tumor and its microenvironment. Induced by IFN-gamma ir. In order for PD-1 to inhibit T lymphocytes, it must associate with PD-L1 and PD-L2 bonds. PD-1 activity may increase or decrease by stimulating some signaling pathways in a dose-dependent manner with RT. Depending on the patient, tumor and tumor microenvironment, PD-1 release may increase or decrease. The daily fraction dose of RT and the number of days it is applied also affect this oscillation. At the appropriate dose of SBRT (At least 600 cGy), the release increases during SBRT, but on the contrary decreases at lower daily doses. How can we practically determine the amount of increase and decrease in this oscillation? Generally, if there is an increase in the amount of lymphocytes, acute phase reactants and cytokines, this indicates that PD-1 release is also increased. In the period of increased release, the effect of immunotherapy and RT is also maximized.

Calretulin increases the affinity of tumor cells with T lymphocytes. This sensitivity forms the basis of the increased efficacy of RT on the tumor. An increase in the absolute lymphocyte count (ALC) increases the immune response. There is a parallelism between lymphocyte increase before RT and disease-free life. If the lymphocyte count in RT is above the median value, the probability of abscopal effect increases.

In one study, it was argued that although the absopal effect seems rare, it can be

increased to 100% by restructuring the tumor microenvironment with appropriate RT techniques (27). If the post-RT ALC is above the median value, the abscopal response is reported as 3.9% if it is below 34.2%. It was predicted that the abscopal response could be strengthened by combining radiation with ICI (Immune check point inhibitors). In the study of Formenti et al., using anti-CTLA-4 immunotherapy and palliative radiation in patients with non-small cell lung cancer (NSCLC), the median overall survival was found to be significantly higher in patients with disease control, probably due to the increased abscopal effect (28). Radiation-induced cell lysis can release existing intracellular neo-antigens, and radiation can induce new mutations by directly damaging DNA. Findings pointing to the systemic effect of RT, independent of its local effect, suggest that the abscopal effect is greater than expected. RT resistance and **Cancer associated fibroblasts (CAF):** CAF, or cancer-associated fibroblasts, constitute the most resistant cell population to RT in the cancerous stroma. RT resistance may persist even with 18 Gy SBRT. The increase of antiangiogenic agents through CAF cells with the effect of RT can also make the tumor radiosensitive (29).

Immunomodulator effect of low-dose radiotherapy and radscopal effect: This radiation strategy, which is applied in combination with high-dose stereotactic RT and low-dose RT, was defined by James Welsh and this strategy was called the “Radscopal” technique (30). Low dose RT together with SBRT and/or IT may play a prominent role in the antitumor immune response. Many studies have confirmed the immunomodulatory effect of low-dose RT, the so-called 'radscopal effect'. The possibility of early and late side effects, and the patients with a higher probability of secondary cancer formation can be determined by molecular studies to be performed by determining genetic characteristics. With genetic analyzes and the development of biomarkers, patients most likely to benefit from radiotherapy can also be identified. Cellular functions are controlled by a spatial structure and by factors secreted by the stroma and endothelium. With three-dimensional tissue culture models, the effects of radiation on cells and tissues can be revealed more clearly. Various tests and markers have been determined to show the level of cancer stem cells in tissues. The most important of these is CD44 and the other is ALDH1. CD133 is also important in some cancer types such as brain glioma (31-33). By performing these tests, the rate of stem cells in a cancerous tissue can be determined, and cancer treatments and RT resistance can be determined in advance. Some antibiotics and agents have been tried to reduce radioresistance. Doxycycline has been tried in early and late stage cancers and when given together, it has been determined that the effectiveness of RT with doxicycline increases by 90% and it increases the effectiveness of RT 4-5 times with cytotoxicity (19). CSCs use the same growth factors and same pathways as normal stem cells. Tumors with high epidermal growth factor (EGFR) receptors have higher radioresistance and a worse prognosis. Therefore, RT with EGFR inhibitors if EGFR receptor positive and with tyrosine kinase inhibitors if tyrosine kinase positive decreases radioresistance and increases cytotoxicity and survival rate. Response rates are higher in patients treated with an agent such as RT, CT, and CSC-targeted EGFR (32-35).

It is not possible to make a good cancer treatment without destroying the cancer stem cells. Although the tumor seems to have disappeared completely, dormant CSC can be awakened and activated even after 10-20 years. Recurrent tumors are always more resistant to treatments. This is because the cancer microenvironment has changed with the old treatments, and the vascular bed and oxygenation have been impaired, and cancer stem cells have gained a more invasive character in this environment. New treatments are needed.

Despite the progress of immunotherapy, it still has a dilemma, such as being too expensive and limited in effectiveness. Researchers should contribute to this issue through joint studies on basic sciences and clinical aspects.

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