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RADIATION RESPONSES OF STEM CELLS: TARGETED AND NON-TARGETED EFFECTS

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Stem cells are fundamental to the development of any tissue or organism via their ability to self-renew, which is aided by their unlimited proliferative capacity and their ability to produce fully differentiated offspring, often from multiple lineages. Stems cells are long lived and have the potential to accumulate mutations, including in response to radiation exposure. It is thought that stem cells have the potential to be induced into a cancer stem cell phenotype and that these may play an important role in resistance to radiotherapy. For radiation-induced carcinogenesis, the role of targeted and non-targeted effects is unclear with tissue or origin being important. Studies of genomic instability and bystander responses have shown consistent effects in haematopoietic models. Several models of radiation have predicted that stem cells play an important role in tumour initiation and that bystander responses could play a role in proliferation and self-renewal.

STEM CELLS

The human body originates from a single cell that replicates into around 3.72×10^{13} cells⁽¹⁾. In addition to this cells are constantly needed to replace those lost, while also maintaining the proper stratification of organs. Stem cells allow this to be achieved. They possess two key features intrinsic to their role; the first is their ability to self-renew, which is aided by their unlimited proliferative capacity and the second is their ability to produce fully differentiated offspring, often from multiple lineages⁽²⁾ (see figure 1).

Stem cells can be categorised either by their potency (differential capabilities), or at which stage of

development they are derived, although these are linked. For example, early embryonic stem cells (ESC) derived from the zygote are totipotent and are able to differentiate into any cell required for the development of a whole organism⁽³⁾. In comparison, ESCs derived from the inner mass of the blastocyst⁽³⁾ are able to differentiate into all 3 germ layers of the embryo (ectoderm, mesoderm, endoderm⁽²⁾), but are unable to form placental cells (which are derived from the trophoblast- the outer cell mass of the blastocyst⁽⁴⁾), and as such are termed pluripotent⁽³⁾. Foetal and adult stem cells are either multipotent (capable of multiple differentiated progeny) or unipotent (giving rise to only one type of differentiated progeny). Traditionally both foetal and adult stem cells are thought to have differential capabilities limited to the tissue they reside in⁽⁵⁾.

The conventional view of stem cell differentiation can be seen as a hierarchy, with the stem cells at the top, followed by progenitor cells (committed to differentiation with finite proliferative capacity), then transit amplifying cells, and finally differentiated cells⁽⁶⁾. Adult stem cells are usually slowly proliferating as the number of progeny is expanded in the progenitor and transit amplifying divisions⁽⁷⁾.

Stem cells can show considerable plasticity, with some cells capable of giving rise to unexpected progeny e.g. adipose stem cells producing osteocytes (although it is not yet clear whether this occurs *in vivo*). Another challenge to a traditionalist view are satellite cells. Previously categorised as progenitor cells, a fraction of their population have been shown to exhibit stem like properties and replenish the population of satellite cells upon division⁽⁸⁾. It has also been recently reported that

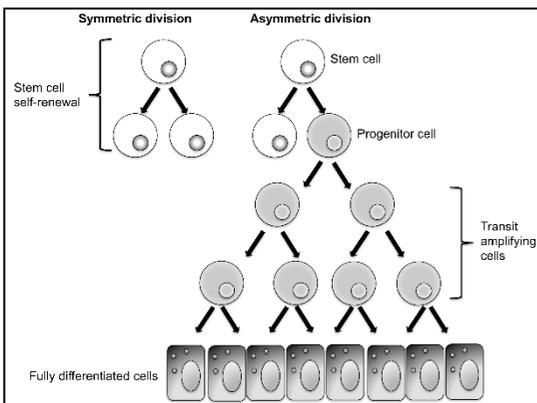


Figure 1. Schematic representation of stem cell self-renewal by symmetric division and provision of progenitor cells by asymmetrical division that ultimately differentiate to yield the mature cells that make the bulk of the tissue.

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epithelial cells can de-differentiate back to stem cells *in vivo* ⁽⁹⁾. A summary of stem cells, their location and progeny can be found in Table 1

Table 1. Main types of stem cells and lineages of their progeny.

Type	Location	Potency	Differentiated Progeny
Early Embryonic Stem Cells ^(3, 10)	Zygote	Totipotent	All cells
Embryonic Stem Cells (ESC) ^(3, 11)	Inner cell mass of Blastocyst	Pluripotent	All 3 germ layers of an embryo
Mesenchymal Stem Cells ⁽¹²⁻¹⁵⁾	Bone Marrow, teeth, peripheral blood and blood from umbilical cord, breast tissue	Multipotent	Tenocyte, osteocyte, myocyte, adipocyte, chondrocyte, endothelial cells.
Haemopoietic Stem Cells ^(12, 13)	Bone Marrow and peripheral blood	Multipotent	All blood cell lineages, myocyte, hepatocyte, neural stem cell
Neural Stem Cells ⁽²⁾	Central nervous system	Multipotent	Neurons, astrocytes and oligodendrocytes
Mammary Stem Cells ⁽¹⁶⁾	Breast tissue	Multipotent	Myoepithelial cells, ductal epithelial cells, alveolar epithelial cells
Intrahepatic ^(6, 17)	Liver	Multipotent	Hepatocytes, cholangiocytes and epithelial cells.
Adipose ⁽¹⁸⁾	Adipose Tissue	Multipotent	Adipocyte, osteocyte, chondrocyte, neurone
Limbal epithelial ⁽¹⁹⁾	Eye (limbal crypts)	Unipotent	Corneal epithelium
Lung basal ^(6, 20)	Lung	Multipotent	Mucosal cells, basal cells ciliated epithelial cells, pneumocytes
Clara cells ⁽²⁰⁾	Lung	Multipotent	Clara cells, pneumocytes, mucous cells, ciliated epithelial cells, AEC I and II
Mucous Cells ⁽²⁰⁾	Lung	Multipotent	Basal cell, mucous cells, ciliated epithelial cells
Alveolar Epithelial Type II ⁽²⁰⁾	Lung	Multipotent	AEC I and II
Gut epithelial ⁽⁶⁾	Gut	Multipotent	Brush border enterocytes, paneth cells, goblet cells, enteroendocrine cells
Keratinocyte ^(5, 7)	Basal layer of the epidermis, Base of the hair follicle	Multipotent	Keratinocytes (both skin and hair)
Oogonial ⁽²¹⁾	Ovaries	Unipotent	Oocyte
Spermatagonial ⁽²²⁾	Testes	Unipotent	Spermatozoa
Satellite Cells ^(8, 23, 24)	Skeletal Muscle	Multipotent	Myocytes, Adipocytes, Osteocytes

Cardiac ⁽²⁵⁾	Heart	Multipotent	Cardiomyocytes, smooth muscle cells, endothelial cells
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STEM CELLS IN RESEARCH

Efforts to understand the origins of specialised tissue cells, the role of stem cells in tissue homeostasis and more recently the role of stem cells in cancer have led to the development of sophisticated *in vivo* model systems and stem cell isolation techniques for *ex vivo* research. These vary in complexity depending on the stem cell type in question.

One of the most common and useful systems are those that provide information on stem cells function. In other words injection of putative stem cells into the relevant cleared organ results in recapitulation of that functional organ. *Ex vivo* mouse model examples of this are found for the bone marrow and mammary gland^(20, 26). Mouse models have also been developed to study lung stem populations. The lung is a highly plastic organ, with >50% of epithelial lineages exhibiting the stem characteristics of renewal and differentiation, but no single cell capable of differentiating into all lineages (refer to table 1)^(20, 27). As such, mouse lung models often use lineage tracing methods, where cells progeny are marked. This in conjunction with lung injury, to promote proliferation, has been used to identify lung 'stem' populations⁽²⁰⁾.

Lineage tracing in conjunction with inducible transgenic mouse models have been used to study other stem population⁽²⁸⁾. This technique benefits from the ability to use it in a developing mouse, in comparison to the less valid cleared organ model.

Another strategy is to examine endogenous cells or tissues either in single cell isolates or slices of paraffin embedded tissues, for stem cell markers. Frequently used stem cell markers include bromodeoxyuridine incorporation, Ki67, aldehyde dehydrogenase expression, CD49f, CD133 and CD34. These markers (and others) may also be used to estimate CSC frequencies in tumour biopsies⁽²⁹⁻³²⁾.

Relative ease of access and depth of study has meant that hematopoietic stem cells are now very well characterised and can be purified relatively quickly using magnetic bead or fluorescent activated cell sorting (FACS) strategies. These strategies have been adapted for analysis and sorting of stem cells from other tissues, although there is much debate over the correct surface antigens to use as markers of stem and committed progenitors, particularly in the breast. As a result of these discrepancies, populations isolated for *in vitro* experimentation should be checked first for stem cell function using an *in vivo* model system such as

described above. However *in vitro* models can be very informative as well allowing considerably higher throughput and lower costs than *in vivo* experiments. Many *in vitro* systems have been developed to mimic stem cell niche environments that enable these cells to be maintained in culture. These often employ spheroid culture in serum free, non-adherent culture conditions such as those developed by Dontu and co-workers for growth of mammary stem cells⁽¹⁶⁾.

STEM CELLS AND CANCER

The importance of stem cells and their interrelationships with cancer stem cells (CSCs) in cancer initiation has been an important topic in recent years^(33, 34). The long lifetime of a stem cell creates the potential for mutations to be acquired, including from ionising radiation (IR), which may lead to the transition into a CSC⁽³⁵⁾. In addition to this, dedifferentiation of cancer and cancer progenitor cells can produce CSCs^(36, 37).

The existence of CSCs was inferred from the phenotypic heterogeneity observed within solid tumours⁽³⁸⁾. As solid tumours are thought to arise from a single cell, this suggests the cell-of-origin may have stem-like qualities. Further evidence came from comparing studies where human CSCs are able to form tumours in immunodeficient mice^(30, 39, 40), but large numbers of non-stem like cancer cells are required for tumour formation⁽³⁸⁾.

CSCs have been highlighted as critical target populations in cancer therapy, including radiotherapy. Traditional cancer therapies may target the non-stem like bulk of a tumour but leave the small population of more resistant CSCs unaffected⁽⁴¹⁻⁴³⁾. Residual, treatment-resistant CSCs are the likely cause of tumour recurrence.

RADIATION AND CANCER

IR is a well-known carcinogen. Epidemiology studies have investigated the effects of population exposures such as the Japanese atomic bomb exposures and nuclear accidents. An increased risk of most types of solid cancer has been reported following these events^(44, 45). Data from many of these studies supports the linear no-threshold model (LNT) (where risk of cancer is proportional to dose and there is no safe exposure range)^(46, 47) although there have been reports of a saturation of response at high doses^(44, 48). Important epidemiological data has also been revealed, for

example, the role of radioactive contaminated products (such as milk) inducing thyroid cancer in children ⁽⁴⁹⁾.

Radiation doses can be divided into high (above 5 Gy), moderate (between 0.5 and 5 Gy) and low doses (below 0.5 Gy) ⁽⁵⁰⁾. Traditionally radiation was thought to cause carcinogenesis through direct DNA damage, in particular the formation of DNA double-strand breaks (DSB). However, other factors such as non-targeted effects (where direct DNA damage is not the main route) ⁽⁵¹⁾ such as bystander effects (the influence of irradiated cells on non-irradiated cells) ⁽⁵²⁾ are also likely to affect carcinogenesis, especially at low doses ⁽⁵⁰⁾. Research into these phenomena has shown for example low dose hypersensitivity, suggesting that the LNT model may not be applicable to low doses.

RADIATION EFFECTS ON STEM CELLS

A number of groups have attempted to understand the impact of IR directly on early events that may be precursors to carcinogenesis such as DNA damage; proliferation and cell death. The role of IR in transformation of stem cells into CSCs however, is not yet fully understood. Stem cells show modified responses to IR exposure in comparison to differentiated cells, possibly as they have modified DNA repair responses. Tichy et al. (2010) found that murine ESCs favour the homologous recombination (HR) form of DSB repair, over non-homologous end joining (NHEJ) favoured murine embryonic fibroblasts ⁽⁵³⁾. This is likely to be because HR is less error prone, as it requires a template for synthesis of the repaired DNA ⁽⁵³⁾. Despite this, murine ESCs exposed to high doses of IR (5 and 10 Gy) show up to a 100-fold increase in mutation rates (measured by loss of heterozygosity of Aprt gene) in comparison to adult fibroblasts, due to an increase in mitotic recombination ⁽⁵⁴⁾. High doses of IR are known to decrease cell survival, although there are conflicting opinions on whether this is due to apoptosis. Increased senescence (but not apoptosis) has been reported in murine haemopoietic stem cells (HSC) *in vivo* ^(55, 56), while a study using human mesenchymal stem cells (MSCs) *in vitro* found 5 Gy γ -irradiation induced apoptosis ⁽⁵⁷⁾.

Both high and moderate doses appear to cause cell cycle arrest specifically at G2 rather than G1 ^(58, 59). Moderate doses also cause a wide variety of changes to the transcriptome. These include alterations in genes controlling cell cycle, cell death, transcription, cell morphology, molecular transport, amino acid metabolism, growth factors, and oxidative stress, in addition to cytokine, p53, TGF- β , and Wnt signalling ⁽⁶⁰⁻⁶²⁾. Studies of the effect of IR on stem cell differentiation has produced variable effects, with increased differentiation ⁽⁶¹⁾, decreased potency ⁽⁶³⁾ or no effect observed ⁽⁶⁰⁾. There is also debate as to

whether moderate doses induce transcription of apoptosis related genes, with Wilson et al. (2010) reporting an increase in ESCs ⁽⁶⁰⁾, while Rachidi et al. (2007) found a decrease in human epidermal cells ⁽⁶²⁾. Clonogenic experiments support a decrease in apoptosis, and indicate that stem cells are relatively radio-resistant in comparison to progenitor or differentiated cells ^(62, 64). Some inconsistencies may be due to differences in analysis time after irradiation. Sokolov et al. (2011) found that transcriptomes measured 2 hours after 1 Gy showed a pro-apoptotic response, while 16 hours after they showed an increased pro-survival response ⁽⁶⁵⁾.

Low dose IR does not appear to cause an increase in cell death ^(60, 66), but increased proliferation has been observed in several studies. Liang et al. (2011) found a significant increase in proliferation of rat MSC at 50 and 75 mGy ⁽⁶⁷⁾. However, increases in proliferation at 20 mGy and 100 mGy failed to reach significance, indicating a non-linear response ⁽⁶⁷⁾. *In vivo* studies have also found increased proliferation, in both HSCs in (75 mGy) ⁽⁶⁸⁾ and neural stem cells (NSC) (300 mGy) ⁽⁶⁶⁾. Bakinskis et al. (2011) found an increase in proliferation proteins in murine NSC, but no increase in proliferation rate ⁽⁶⁹⁾. Wei et al. (2012) suggested that increased proliferation at low doses (300 mGy) was due to an increase in Wnt/ β -catenin signalling, which was not seen at moderate (3Gy) doses ⁽⁶⁶⁾. This contradicts Wilson et al. (2010) who found increased Wnt/ β -catenin signalling in 2 Gy irradiated cells, but not in 400 mGy irradiated cells.

STEM CELLS AND NON-TARGETED RADIATION EFFECTS

Although there are discrepancies between the reports of

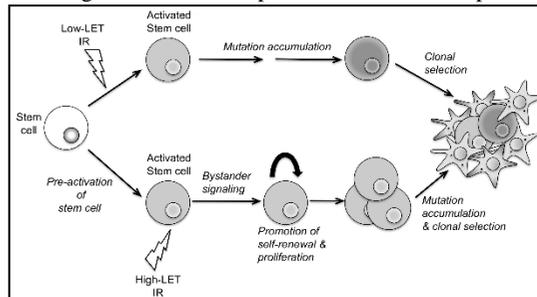


Figure 2. Model of radiation action on stem cells proposed by Shuryak *et al* ⁽⁶¹⁾. Low LET IR is proposed to contribute to carcinogenesis by initiation of normal tissue stem cells to activated stem cells. Some of those may acquire further mutations and eventually become malignant. Conversely, the role of high LET IR is mainly through promotion of already activated stem cells via bystander signals produced by irradiated cells.

different groups, likely due to differences in the time of analysis post IR or model system used, an overview of low dose responses could be suggestive of a non-targeted mechanism, both *in vitro* and *in vivo*. In most cases this seems to be a pro-survival affect. These effects have been shown *in vivo* for normal tissue as well as cancer model systems. Reports of a role of bystander effects on stem cells is also both tissue type and species dependent. Murine mesenchymal stem cells of the bone marrow do not display a bystander effect^(70, 71) in contrast to HSCs of both mouse and human origin, in which bystander effects and genomic instability have been described⁽⁷²⁻⁷⁴⁾ and in human NSCs, where radiation induced bystander signals induce differentiation⁽⁷⁵⁾. In the breast, an increase in the CSC population, compared to sham-irradiated mice, has been observed in Blab/c mice when the stroma was irradiated prior to inoculation of Trp 53 null epithelial cells. In these experiments an increase in tumour formation was also induced by low dose IR of the stroma, 100 % in 10 and 100 mGy-irradiated mice compared to 69 % in sham irradiated mice⁽⁷⁶⁾. Suggesting that factors secreted by the stroma activate stem cell self-renewal pathways. An increase in the stem cell population may create a larger target pool from which stem cells carrying mutations may arise. An *in vitro* investigation of bystander effects in normal HSCs employed a grid that allowed either 100% or 50% of cells to be irradiated. These experiments showed that will shielding with the grid resulted in a decrease of cell killing, the frequency of chromosome aberrations was not affected by the grid with 1Gy irradiated cells having 0.135 aberrations per cells compared to 0.132 when 50% of cells were shielded by the grid⁽⁷⁷⁾.

STEM CELLS AND RADIATION CARCINOGENESIS

A model describing the distinct roles of low and high LET radiation on stem cells has been proposed and tested *in vivo* using persistent dysplasia in mouse mammary glands as a positive outcome⁽⁷⁸⁾. Figure 2 is a schematic interpretation of that model. That research suggests that low LET IR directly initiates normal stem cells to an activated cell state, in which they are primed to acquire genetic mutations that ultimately lead to a malignant state. This results in a direct dose rate effect at low doses. In contrast, that group proposed that high LET IR contributes to carcinogenesis mainly through a bystander signalling mechanism. In that case a small number of cells are traversed by one or more tracks of densely IR, resulting in secretion of signal molecules that promote proliferation of nearby unirradiated stem cells that have been activated by an earlier event. The risk of carcinogenesis is then decreased as the dose rate

increased due to a dominance of cell killing over bystander stimulated promotion.

SUMMARY

Stem cells are fundamental to normal embryonic development and tissue homeostasis. As such it is not surprising that studies of the DNA damage response of ESCs has shown a strong preference in these cells for the high fidelity form of DNA DSB repair, homologous recombination. This idea is supported by reports in some types of CSCs that they exhibit lower frequencies of DSBs compared to non-stem like cancer cells which is explained by increased ability to scavenge reactive oxygen species and slower DSB repair rate that favours repair by HR⁽⁷⁹⁾. Whether similar mechanisms exist in normal adult stem cells remains unclear and future research should be directed at understanding mechanisms that stem cells (other than HSCs) use to deal with mutagenic insults. As long-lived adult stem cells are relatively more quiescent they have been proposed to have an increased propensity to accumulate carcinogenic mutations. In recent years, so called cancer-stem cells have been observed and isolated in many types of human cancers including leukaemia and breast cancer. These cells are so named because they share many traits with normal stem cells most importantly the ability to self-renew. Several researchers have proposed normal adult stem and progenitor cells as the origin of CSCs.

The role of IR and non-targeted radiation effects in the activation of stem cells to a malignant state is unclear. However research in this area shows that whether a stem cell is sensitive or resistant to IR is highly dependent on the tissue of origin. Never the less several models of radiation carcinogenesis predict that stem cells play a major role in tumour initiation. In particular radiation induced bystander signals appear to promote proliferation and self-renewal. Future expansion of this area of research should define the mechanism(s) of action. Although *in vitro* and *ex vivo* studies are highly valuable it is clear that in depth *in vivo* analysis of the impact of IR on normal tissue stem cells is required to understand their role in carcinogenesis.

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