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Stem Cell Therapy to Reduce Radiation-Induced Normal Tissue Damage

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Normal tissue damage after radiotherapy is still a major problem in cancer treatment. Stem cell therapy may provide a means to reduce radiation-induced side effects and improve the quality of life of patients. This review discusses the current status in stem cell research with respect to their potential to reduce radiation toxicity. A number of different types of stem cells are being investigated for their potential to treat a variety of disorders. Their current status, localization, characterization, isolation, and potential in stem cell-based therapies are addressed. Although clinical adult stem cell research is still at an early stage, preclinical experiments show the potential these therapies may have. Based on the major advances made in this field, stem cell–based therapy has great potential to allow prevention or treatment of normal tissue damage after radiotherapy.
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The reduction of the damage inflicted to organs at risk during radiotherapy will not only increase the quality of life after the treatment but may also allow dose escalation to the tumor improving tumor control. Stem cell therapy provides a potential prevention or treatment of radiation-induced normal tissue damage. At present, it is relatively well known how most tissues respond to irradiation in terms of tissue degeneration, cell loss, and regeneration potential. Many factors play a role in the response of tissue to irradiation,[1] but, ultimately, the (in)ability of stem cells to reconstitute functional cells determines the onset and the severity of the radiation effects (Fig 1). Already in 1956, Dutch radiobiologists[2] showed that bone marrow transplantation could rescue the hematopoietic system in animals that were exposed to lethal doses of total body irradiation. After that, the first stem cell transplantations were developed; treatments that are common clinical practice nowadays. Because of new scientific knowledge and biotechnological developments, only recently has it become apparent, that a similar strategy may rescue other organs. Currently, a wide variety of stem cell therapies are being investigated for their potential to treat a vast array of clinical disorders.

What is a stem cell? Stem cells are defined by their capacity to self-renew and to produce more differentiated cells. For this purpose, stem cells have to go through an asymmetric cell division, generating 1 daughter that remains a stem cell and 1 progenitor cell (Fig 2). Further transition of these progenitor cells or transit-amplifying cells toward mature cell lineages may involve amplification of progeny (restrictive division). Basically, 3 stem cell types are being investigated for their potential use in stem cell therapy: embryonic stem cells (ESCs), induced pluripotent stem cells (iPS), and adult stem cells.

Embryonic Stem and Induced Pluripotent Stem Cells

Embryonic stem cells, derived from the inner cell mass of the blastocyst, differentiate into all cell lineages of a living organism (ie, these cells are truly pluripotent). Potentially, they could form a virtually unlimited amount of cells for stem cell–based therapy.[3] Host rejection of ESCs could be circumvented by somatic cell nuclear transfer,[4] or the use of parthenote ESCs. In practice, embryonic stem cells have not been used in clinical and preclinical trials successfully yet. This is in part because of ethical problems related to their use and a high probability of teratoma formation.

In 2006, Takahashi and Yamanaka[5] realized a breakthrough in stem cell research by generating pluripotent stem cells from fibroblast cultures by the addition of 4 genes, po-

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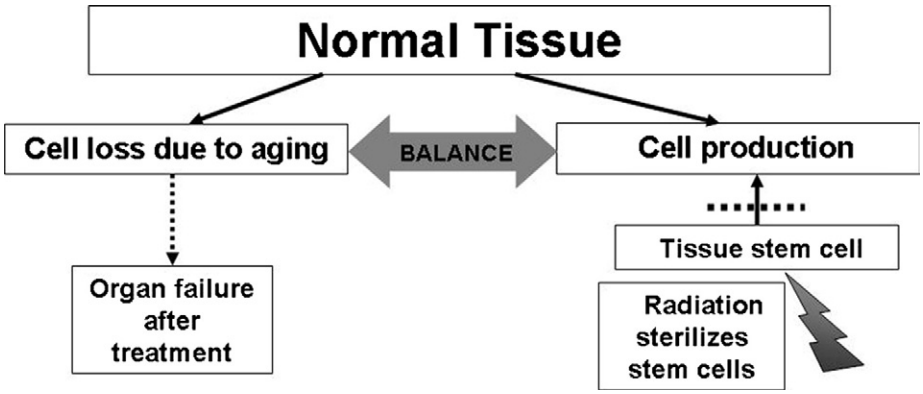

Figure 1 Tissue homeostasis as influenced by irradiation.

tentially solving the ethical issues. Adding *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4* (or Nanog and Lin28)[6,7] enabled dedifferentiation of adult cells into an embryonic-like primitive stem cell state. Importantly, without the oncogene *c-Myc*, iPS cells could be generated from mouse and human fibroblasts[8] albeit with lower efficiency, potentially reducing hazardous effects. iPS cells closely resemble ESCs and have now been derived from fibroblast, stomach, liver, and pancreas cells[9] (reference 9 or references therein). Therefore, it is foreseen that in the not too far away future, these embryonic-like stem cells could provide therapy to the ultimate cure for many disorders, including those induced by radiation. However, before the potential use of iPS cells, it is essential to gain control over cell differentiation and development into tissue-specific pathways, which is certainly not the case at the moment.

Adult Stem Cells

Adult stem cells do not have the ethical problems raised by the use of ESCs and do not form teratomas because they are more committed. Adult (somatic or tissue-derived) stem cells are generally organ restricted and only form cell lineages of the organ from which they originate (unipotency). For now, adult stem cells have obvious experimental and ethical advantages and have therefore been extensively investigated for their potential to regenerate injured tissues.

Adult stem cells are undifferentiated cells found within many tissues of the body that contribute to the repair and regeneration of these tissues in response to injury. Adult stem cells possess the capacity to self-renew and to form all the cell types of the organ from which they originate. Although adult stem cell transplantation has been applied to treat bone marrow deficiencies for a number of decades, no other organs have been clinically repaired successfully by stem cells so far. One of the reasons for this is the difficulty underlying the isolation of stem cells from solid tissues when compared with the easy accessibility of the bone marrow stem cells. Bone marrow stem cells can easily be obtained, isolated, purified, and transplanted. They home naturally to the right location and have a tremendous capability to restore the tissue because a single hematopoietic stem cells (HSCs) may reconstitute all hematopoietic lineages.[10,11]

Bone Marrow–Derived Stem Cells

Next to their capacity to reconstitute bone marrow, bone marrow–derived stem cells (BMCs) have been shown to change phenotype and contribute to the recovery of several injured organs different from bone marrow.[12] Studies in which BMCs contributed to the regeneration of injured organs such as the brain,[13-15] liver,[16-19] lung,[20-22] vascular tissue,[23] kidney,[24-26] skin,[27] and heart[28] opened the possibility of using multipotent BMCs for tissue repair. However, the potential of BMCs to regenerate nonhematopoietic tissue has been debated ever since. Experimental evidence supports the hypothesis that BMCs can change their phenotype when present in an injured nonhematopoietic organ.[12] Cell fusion with tissue-specific differentiated cells, however, has been proposed as an alternative mechanism[29] to explain observations of stem cell plasticity. Whatever the mechanism, stem cell plasticity seems extremely interesting for the treatment of

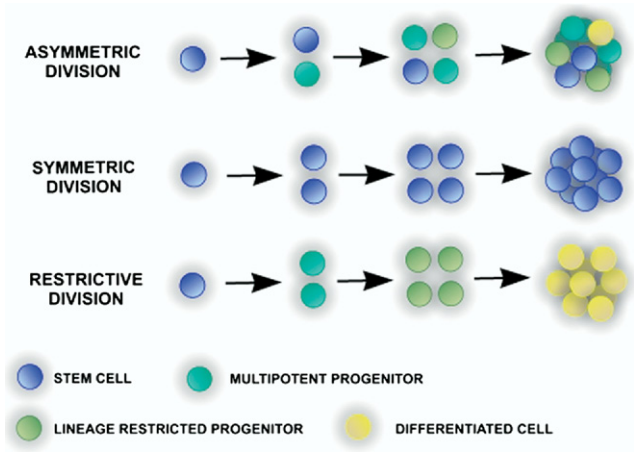

Figure 2 A division pattern of stem cells. Asymmetric division of a stem cell involves the generation of 1 stem cell and a more differentiated progenitor cell. In contrast, via a symmetric division, a stem cell is able to maintain and multiply its own cell number. When 2 more differentiated daughter cells are produced, the process is called a restrictive division. (Adapted with permission.[117]).

radiation-induced tissue damage because stem cells can be obtained from undamaged tissue (eg, outside the radiation field). Therefore, in our laboratory, we tested whether BMCs could reduce radiation damage to the salivary glands.[30] A model was developed that enabled us to assess the potential of BMCs to enhance regeneration of irradiated tissue. To be able to track progeny of transplanted cells, lethally irradiated (9.5 Gy, excluding the salivary gland) female mice were transplanted with bone marrow of male transgenic mice expressing enhanced green fluorescence protein (eGFP). Transdifferentiated cells would then not only show eGFP/Y-chromosome markers but also specific salivary gland markers and lose specific bone marrow markers. After a 4-week recovery period, the salivary glands were irradiated. Thirty days after this irradiation at a time point when a significant level of cell loss is detectable,[31] BMCs were mobilized to the blood by granulocyte colony stimulating factor. Months later, massive engraftment of BMCs (eGFP/Ychrom$^+$) were observed in the salivary glands.[30] Moreover, these glands had a higher number of healthy acinar cells, showed an improved vascularization,[32] and produced more saliva than untreated irradiated glands. However, closer examination of the morphology indicated that although part of the newly formed blood vessels seemed to be bone marrow derived, only very few (<0.1%) acinar cells were derived from BMCs. If BMCs do not provide new salivary gland cells, what could be responsible for the expansion of the number of acinar cells? Most likely, engrafted BMCs, including inflammatory, mesenchymal, and epithelial progenitor cells, secrete growth factors and cytokines that stimulate surviving stem cells to proliferate and form new acinar cells. This mechanism of paracrine stimulation has also been proposed to act in the organ repair by BMC therapy of other tissues.[30,33] Similar results have been obtained for oral mucosa.[34]

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are stromal cells in the bone marrow where they are part of the HSC stem cells niche.[35] MSCs have extensive potential to proliferate and can be selected from the bone marrow based on the expression of multiple non-specific surface markers. MSCs are characterized by their ability to differentiate in vitro into osteocytes, adipocytes, and chondrocytes[36] and have been suggested to be able to form myocytes, cardiomyocytes, and neurons.[37] They have also been found in adipose tissue, peripheral blood, cord blood, liver, and fetal tissues. Contradictory results have been obtained with the transplantation of MSCs in irradiated tissue. The injection of cultured bone marrow–derived MSCs directly into irradiated mice salivary gland induced a strong fibrotic response (unpublished data, Kok and Coppes, April 2005). Similar cells were shown to contribute to the fibrotic response of irradiated lungs[38] but induced repair in the irradiated esophagus.[39] Interestingly, cultured human MSCs were shown to accelerate the repair of skin damage in both immune suppressed mice[40] and in an accidentally irradiated human.[41] These cells were clearly visible in the irradiated skin early after transplantation but in time disappeared without indication of transdifferentiation into skin cells or other tissue. Similar results were obtained in the irradiated gut.[42] Potentially, these MSCs may be the cytokine producing factories responsible for accelerated tissue repair.[43]

Although the different BMCs do not seem to become part of the irradiated tissue, the cytokine-induced mobilization of these cells may yield in some cases improved organ function and may have some clinical potential. Moreover, the formation of bone marrow–derived vasculature, such as that observed in irradiated salivary glands,[32] holds potential for the treatment of vascular damage. Bone marrow–derived endothelial progenitor cells (EPCs) are at least partially responsible for the revascularization effect. The potential of EPCs to treat vascular injuries has recently been discovered. These cells have been identified in bone marrow and peripheral blood and may incorporate into injured vessels to participate in re-endothelialization and neovascularization.[44] Several human clinical trials using bone marrow as a potential source of EPCs in cardiac repair have been initiated.[44,45] Although the ultimate mechanism of endothelial progenitor cell-induced repair is not entirely elucidated, these cells may also stimulate local angiogenesis by secreting growth factors in a paracrine manner. Because these cells can be isolated from the bone marrow and have been shown to reduce vascular damage to the infarcted heart,[46] they may hold promise for tissues where vascular damage is a major factor contributing to radiation toxicity.[47-50]

Tissue-Specific Stem Cells

Although adult tissue-specific stem cells have been identified in the liver,[51] brain,[52] dental pulp, hair follicles, skin,[53] skeletal muscle,[54] adipose tissue,[55] blood,[56] lung[57] and mammary epithelium,[58] and salivary glands,[59] it has been very hard to isolate viable stem cells. Stem cells from solid tissues can only be obtained by exposing the tissue to enzyme digestion procedures necessary to dissociate the cells. However, when isolated from their natural environment (niche), the stem cells can induce rapid cell death or differentiation.

Therefore, to use these stem cells successfully to repair radiation-induced normal tissue damage in the clinic, it is important to understand their nature and qualities, the mechanisms by which they differentiate into mature functional cells, and their capacity to repair damaged tissues in animal models. For the repair of radiation-damage tissue, the appropriate adult tissue stem cells need to be localized and isolated before radiotherapy, grown, probably expanded, and stored at least until the end of therapy or until the most optimal time point for transplantation has been reached. The latter strongly depends on the time course of the radiation response of that particular tissue.

Localization of stem cells. Adult stem cells are very rare; for example, 1 in 100,000 to 150,000 cells in the bone marrow is an HSC in mice,[60] and the frequency in humans is even lower.[61] Moreover, they are dispersed throughout the body and behave very differently, depending on their local environment.[62] One of the oldest techniques to define stem cell localization is the label-retaining cell (LRC) assay. After a

period of continuous administration of nucleotide analogs, such as BrdU or ^{3}H-TdR, these are incorporated in the DNA and label all dividing cells. In a subsequent chase period, during which no nucleotide analog is administered, the label will be diluted with every cell division. The less frequently dividing cells will retain the label; the LRCs[63] are considered to contain the stem cells. By using this technique, the localization of tissue stem cells in the intestinal crypts,[64] the bulge of the hair follicle,[65,66] the bladder,[67] the limbal region of the cornea,[68] and the terminal end bud of mammary glands[69] was established. However, another study indicated that the label-retaining technique is neither a sensitive nor a specific marker of HSCs.[70] Recently, transgenic reporter mice have been developed expressing a stem cell–specific protein fused to GFP or Lacz[71-74] for the imaging and the lineage tracking of stem cells in tissues like skin and intestine. One of these studies[71] clearly showed that the most primitive intestinal stem cells are different from those observed by BrdU label tracking. Therefore, the LRC technique for stem cell detection is debated, and the obtained results need to be validated.

The largest obstacle in stem cell biology today is the absence of a common and unique marker that unambiguously labels stem cells. Consequently, one of the major points of interest in stem cell research is the identification of specific markers of resident tissue stem cells. Such a marker would be of great help to unravel the stem cell niche. Consequently, the identification of novel stem cell–related markers is of major interest. Both histologic and genetic analyses have revealed the existence of stem/progenitor cell–related markers (ie, epitopes on the cell surface [eg, SCA-1, CD24/CD29, CD133, CD49f, and c-Kit] or intracellular proteins [eg, Musashi-1]). Some of these are cell type and organ specific, but others are expressed in several tissues. However, because a single marker that defines a stem cell has not been found yet and may indeed not exist, several markers need to be combined. Nevertheless, in addition to in vitro and in vivo functional assays, morphologic observation of the expression of potential markers might be a helpful tool in the identification of the tissue stem cell and the niche in which it lives.

Stem Cell Isolation

The next step after having successfully determined the location of adult stem cells is to isolate (or enrich) these cells by physically purifying them from other cells. Even in cases in which information about stem cell location is lacking, cells have been successfully sorted, as is the case for the HSCs reviewed by Huh et al.[75] Basically, 3 methods for sorting stem cells can be applied: (1) selective cell culture, (2) fluorescent-activated cell sorting (FACS) or magnetic-activated cell sorting based on expression of cell surface markers, and (3) FACS selection of dye excluding cells (side population). However, in practice, a combination of these methods will be required to achieve optimal enrichment.

Culture of Stem Cells From Solid Tissues

The isolation of stem cells from solid tissues can be obtained by exposing the tissue to (several rounds of) digestion procedures with enzymes that disrupt the extracellular matrix. When cultured in appropriate media conditions, spherical, nonadherent cell clusters (spheres) can be formed that are enriched for stem/progenitor cells. The best known are the spheres obtained from mammary glands (mammospheres)[76] and brains (neurospheres),[77] both of which have been shown to contain progenitor and stem cells. For neurospheres, 4% to 20% of the cells have stem cell potential, whereas the remaining population consists of progenitor cells in various stages of differentiation.[78] Subsequently, the self-renewal capability of stem cells can be shown by the formation of secondary and tertiary spheres from single cells originating from the original sphere.[79] Based on the frequency of cells that are capable of forming these secondary and tertiary spheres, the percentage of stem cells can be estimated.

FACS of Stem Cells

Another possibility to enrich and/or select for stem cells is to use cell-type–specific markers. Using FACS analysis, stem cells can be tagged with fluorescently labeled antibodies and may ultimately be sorted as single cells. For instance, to enrich for HSC, commonly a set of markers is used (lineage^{-}Sca-1^{+}c-Kit^{+}).[80] Similarly, mammary gland stem cells have been isolated based on the expression of Lin^{-}CD24^{med}CD29^{high} markers.[81,82] Subsets of these cells can be further divided based on the expression of CD49f^{high}, SCA-1^{low}, CK14$^{+/-}$, or SMA$^{+/-}$.[76,81] This is not a complete list of markers to purify stem cells. However, clearly the number of markers necessary to select putative stem cells can be very high.

A second approach for direct isolation of stem cells is based on genetic manipulation of stem cells to express a fluorescence label. For example, skin stem cells expressing GFP from a stem-cell specific promoter have been selected by FACS.[73,74] Many knock-in reporter models are being developed and will greatly improve our knowledge of adult tissue stem cells.

Side Population

Stem cells are thought to efficiently express ABC transporters, which are membrane pumps able to exclude toxic substances, enabling the protection from potentially harmful chemicals. A cell population can be enriched for stem cells using their ability to exclude fluorescent dyes, such as rhodamine 123 and Hoechst 33342. The extent of efflux activity is related to the maturation state, the more primitive the stem cell the higher the efflux activity.[83] The inhibition of the membrane pump by agents, such as verapamil, serves as a control for specificity. These so-called side population cells have been isolated for HSCs,[83] skeletal muscle, lung, liver, heart, testis, skin, mammary gland, and cardiac muscle.[84] However the mammary gland side population seems to consist more of progenitor cells than stem cells.[85] This emphasizes that each technique by itself may not be selective enough.

Transplantation

Until now, only very few studies have shown successful transplantation of nonhematopoietic stem cells to ameliorate radiation-induced damage. Rezvani et al[86] transplanted immortalized neural stem cells into the irradiated spinal cord and observed a delay in the development of paralysis. Regret-

fully, only paralysis was scored, and no proof of the involvement of the transplanted cells was provided. In addition, genetically modified cells were used with obvious disadvantages. Stem cells preferably should be isolated "freshly" from the tissue as described earlier, whereas stem activity should be ascertained in functional assays. Furthermore, stem cell clonality (ie, the capacity of a single cell to give rise to almost all cell type lineages of the appropriate tissue) should be shown. In vitro, this can be accomplished by the serial generation of clones from 1 single cell and their subsequent differentiation in different progeny cell types. One should show that 1 single purified candidate stem cell is able to regenerate/repopulate a particular organ. In fact, this single stem cell should be able to integrate itself in the host environment, give rise to the appropriate mature cell types, and maintain itself in time. This has been shown only in a very few cases.

Recently, we have developed a method to isolate, culture, characterize, and successfully transplant salivary gland stem cells.[59] After enzymatic digestion, dispersed salivary gland cells were grown in culture to form spherical, nonadherent cell clusters called salispheres. Earlier studies using label-retaining assays indicated that the duct compartment of the salivary gland contains the stem/progenitor cells.[87] Indeed, salispheres contained not only cells that express markers for duct cells (cytokeratin 7, 8, and 14) but also markers for stem cells (SCA-1, c-kit, and Musashi-1). After prolonged culturing in medium or in 3-dimensional collagen gel, acinar cells expressing amylase and mucins were formed, indicating the capability of the isolated cells to differentiate into mature functional secretory cells. Next, stem cells were isolated from male eGFP+ mice submandibular glands and enriched by the floating sphere culture. Subsequently, the culturing method was combined with flow cytometry to select cells that expressed the stem cell marker c-Kit$^+$/CD117 (receptor for stem cell factor) from the salispheres. c-Kit has been found to be expressed on stem cells of many other tissues (eg, heart and HSCs[88,89]). Male salisphere-derived c-Kit$^+$ cells expressing eGFP were injected in irradiated female mice submandibular glands.

Only 300 of these c-Kit$^+$ cells were sufficient to induce a remarkable recovery of the submandibular glands 2 months after transplantation. Glandular weight recovered; an almost normal number of healthy acinar cells and a nearly normal saliva production were observed in recipient mice.[59] In contrast to the mobilization experiments with BMSCs, duct and acinar cells of these transplanted glands did express donor markers (eGFP/Y-Chromosome), indicating that they originated from transplanted cells. Still, this does not show that the transplanted cells contained true stem cells (clonality) because transplantation of progenitor cells could yield similar results, albeit not with a long-lasting restoration of the salivary gland. To elucidate this, we harvested cells from irradiated submandibular glands of responding repopulating mice (first transplant) and grew salispheres from these glands. It was shown that these cells were eGFP and Y-chromosome positive. Next, only 100 c-Kit$^+$ cells selected from these secondary salispheres were transplanted into secondary irradiated female recipients (second transplant). Again, these mice responded to the transplantation with an increase in saliva secretion and normalization of the morphology.[59] Furthermore, all newly formed cells again originated from the first donor mice as they expressed eGFP and the Y-chromosome. This experiment unequivocally proved that the transplanted c-Kit$^+$ cell population contained true stem cells. Furthermore, it shows that stem cell transplantation into solid organs is feasible and may result in complete restoration of irradiated mice submandibular glands.[59] These studies show that tissue stem cells can be isolated from primary recipients and transplanted into a tissue to ameliorate radiation damage. It is also a proof of principle that tissue stem cell transplantation may be used successfully to functionally rescue irradiated solid tissues.

Interestingly, in human salivary glands, similar cells could also be found. c-Kit/CD117$^-$-expressing cells were detected in human parotid and submandibular glands obtained from patients with a squamous cell carcinoma of the oral cavity in whom a neck dissection procedure was performed. Furthermore, after enzymatic digestion, dispersed human parotid and submandibular gland cells could be cultured and formed salispheres. Moreover, from these salispheres c-Kit$^+$ cells could be isolated.[59] Although these results look very promising, further testing of these cells in in vitro assays and in vivo assays, including transplantation in immunodeficient mice, will need to be performed to reveal the true potency of these cells. Currently, we are attempting to further purify the salivary gland stem cells of mouse and man.

Adult Stem Cell–Based Therapies

Although the irradiated mouse salivary gland is so far the only solid tissue for which it is shown in mice that stem cell therapy can be used to rescue an organ, potentially it could be used for many other tissues of which the stem cells have been isolated and used to treat a variety of (experimental) disorders. The (pre-)clinical successes of stem cell therapy that potentially can be translated to a radiotherapy setting are discussed later.

The hematopoietic system. The only routinely applied clinical stem cell therapy to date is hematopoietic stem cell transplantation, which is used to restore blood production after treatment of patients suffering from leukemia, as well as various other types of (blood) disorders. HSCs and their regenerative and therapeutic potential have provided a paradigm for other cell-based therapies.

The skin. Some years ago, skin progenitor cells were isolated and expanded[90] and have been suggested to arise in peripheral tissues, such as the skin, during development and maintain multipotency into adulthood.[91] Currently, it is known that epidermal stem cells can be found in 2 distinct locations: namely in the basal layer and in the bulge region of the hair follicle. Bulge stem cells have mainly been associated with a role in the hair cycle, whereas basal layer stem cells have been hypothesized to play a role in wound healing.[92-94] Skin stem cell therapy is basically initiated to assist in skin and hair transplants for patients with burns or skin-related disorders.

Of all adult stem cells, epidermal stem cells are the most accessible ones, and they can be expanded very efficiently in culture.[95,96] Clinically, human skin keratinocytes can be cultured to form epithelial sheets on fibrin matrices, which can be used to treat patients with burns.[97] In mouse studies, progress has been made in selecting and transplanting skin stem cells on the skin of recipient mice, and these cells have been shown to grow hair in nude mice.[98] Interestingly, the engrafted cells contributed to both the formation of hair follicles, epidermis, and the sebaceous glands, indicating that these stem cells spontaneously differentiated into other tissue-specific cells when placed in an appropriate environment. These stem cells could be readily tested in a radiation setup.

The mammary gland. The ultimate demonstration of the mammary gland stem cells was made by Shackleton et al[81] and Stingl et al[82] who showed that a complete organ could be generated from 1 single stem cell. They isolated $Lin^{-}CD29^{high}CD24^{+}$ mammary gland cells and transplanted a single multipotent mammary gland cell into a cleared fat pad of virgin or pregnant mice. This transplantation resulted in a fully developed functional mammary gland with all mammary gland cell types present (alveolar, myoepithelial, and luminal cells). Subsequent serial transplantations showed self-renewal of the transplanted stem cell and proved the principle that organ formation can occur from 1 single stem cell. Although this is scientifically very important because it shows the existence of a single stem cell for a tissue, clinically, for the treatment of radiation damage, this is of less relevance.

The eye. Stem cell therapy in the eye has been aimed at treating corneal limbal stem cell deficiency and retinal degenerations and has become a major forefront in ophthalmology. The first ex vivo culture of human limbal stem cells was reported by Pellegrini et al[99] in 1997. Limbal stem cells were transplanted in the cornea of a patient with alkali burns.[100] Later in time, stromal scarring and neovascularization produced a healthy organized cornea, allowing full recovery of visual acuity. Since then, additional reports have been published on the use of these stem cells to cure limbal stem cells.[101]

The inner ear. Stem cells have been isolated from spheres and express marker genes of the developing inner ear and the nervous system and are capable of differentiating into hair cell-like cells.[102] Potentially, they could ameliorate hearing loss after chemoradiotherapy.

The kidney. A study performed by Bussolati et al[103] reported the successful transplantation of isolated human kidney $CD133^{+}$ stem cells in immunocompromised mice. These cells could form/take part in tubular structures in vitro and in vivo, showing the capability of these human renal (stem) cells. When further enriched, these cells (CD24/CD133)[104] could perhaps take part in the regeneration of damaged tissue and could therefore be of potential interest to repair radiation damage to the kidney.

The central nervous system. For brain disorders, such as Parkinson disease (a degeneration of dopaminergic neurons) or Huntington disease (mutation in Huntington gene), most studies focus on the use of embryonic or fetal stem cells. However, adult neuronal stem cells have been isolated from rats and grafted into the brain. Meissner et al[105] showed that mouse neuronal stem cells from cultured neurospheres injected in the subventricular zone of brains of Parkinson-diseased mice could differentiate into astrocytes and neurons and reduce motor defects. The use of neuronal stem cells in an irradiated central nervous system seems to be more complicated because it was indicated that certain progenitor cells may not differentiate into functional neurons after irradiation of the brain.[106]

The heart. After the observation of an enhancement of cardiovascular damage in patients irradiated for breast cancer of the left breast,[107] interest in radiation-induced heart damage revived. Regretfully, the heart has a very low regenerative capacity after injury, suggested to be caused by a small number of cardiac stem cells. Nevertheless, adult cardiac stem cells might still be candidates for stem cell therapy. Several subsets of potential cardiac stem cells have been observed (lin^{-}, $c\text{-}Kit^{+}$,[88] $SCA\text{-}1^{+}$,[108] SP cells,[109] and is/L[110]). Of these, the $lin^{-}c\text{-}Kit^{+}$ cells were shown to improve heart function after ischemic injury when injected into the border zone of ischemic-induced hearts[88] or into the coronary arteries.[111] As described previously, endothelial progenitor cells[46] may also be very beneficial[44] as cardiac problems after irradiation partly arises from coronary artery damage,[112] although a combination of several stem cell types may be necessary to repair myocardial and valve defects.

Muscle. Both bone marrow and muscle-derived stem cells were shown to improve muscle regeneration in an animal model of Duchenne muscular dystrophy being capable of improving both muscle regeneration and bone healing.[113,114] Potentially, such an approach could reduce muscle weakness after radiotherapy.

The lung. Bronchioalveolar stem cells have been identified[115] and have been associated with diseases such as cystic fibrosis and chronic obstructive pulmonary disease.[116] These cells are clearly involved in the regeneration of lung tissue but so far have not been successfully used in a transplantation setting, although bone marrow derived cells could also have some beneficial effects.[57]

Limitations and Future Perspectives

Although studies have reported the successful transplantation of adult stem cells into host tissues, many hurdles remain if this approach is to be used successfully in the treatment of radiation-induced injuries. One of the biggest problems in cell-based therapy (Fig 3) is the lack of sufficient numbers of cells and their inefficient differentiation into specific cell types. Therefore, one of the goals for future research is to

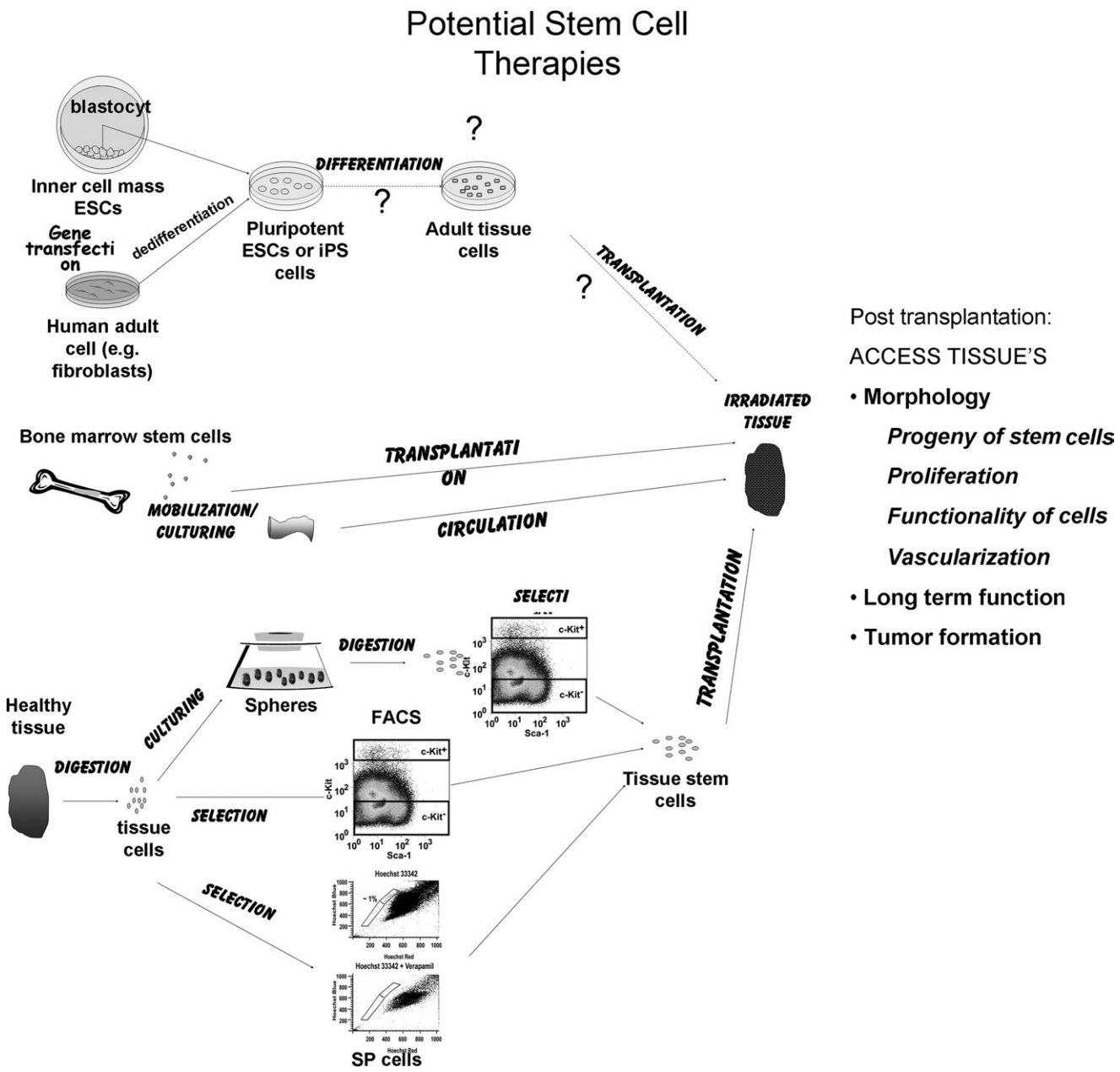

Figure 3 A schematic representation of (potential) regenerative therapies. iPS, induced pluripotent stem cells; SP, side population cells.

generate protocols that allow the expansion and differentiation of stem cells into specific cellular phenotypes. Moreover, it will be crucial to avoid differentiation into other cell types, which could be harmful to the host tissue after transplantation. Furthermore, new methods need to be developed to prove the functionality of stem cell-derived cells and to follow their fate in vivo. Safe delivery, migration, proper integration, contribution to functionality of the host tissue, and longevity of engraftment are all issues that should be fully investigated and optimized before this strategy can be applied in a clinical setting.

Furthermore, before adult stem cell therapy can be applied in the clinic, it has to be translated to meet the demands of good manufacturing practice and good clinical practice. Next, in a first trial, a carefully selected group of patients in whom the development of tissue damage after radiotherapy is expected should be selected to receive stem cell transplantation. It is expected that in humans cells can more easily be injected at the right site. For instance, in the salivary gland, they could be injected retrogradely through the duct orifices (according to a method that is routinely used to apply a contrast liquid to the ductal system for sialography), a method known to be successful and feasible but somewhat impractical in mice.[117] An optimal delivery of stem cells may enhance the success rate of transplantation because it does not further damage the gland. If successful, genetically modified and/or allogenic stem cell transplantation could be considered.

Conclusions

The explosion in stem cell research during the past decade has resulted in the notion that adult stem cells can be used in the near future as a powerful tool in the treatment of radiation-induced tissue damage. Understanding the nature and qualities of stem cells, the mechanisms by which they differentiate into mature, functional cells and their behavior in animal models will bring us closer to using stem cells in the clinic for replacing cells that are damaged by radiation.[118] Bone marrow transplantation has exemplified the power of stem cell therapy. However, there is a long road ahead before other stem cell-based therapies will become common practice. Although BMC survival has been observed in many tissues after transplantation/mobilization in different animal models, functional improvements are often small. One of the reasons is the lack of a sufficient number of stem cells surviving radiation and the lack of differentiation of BMCs into functional cell types after transplantation. In some preclinical studies, successes have been obtained in the treatment of radiation damage and other disorders with adult tissue stem cells, but still a great amount of uncertainty exist about their location, phenotype, and potential. Although much progress has been made in the isolation of putative stem cells, for most tissues no definitive stem cell marker or combination of markers has been found yet. Furthermore, each stem cell population seems to have its own complex set of pathways and genes involved in its regulation. Identifying the molecular mechanisms involved in stem cell regulation will allow scientists to manipulate their characteristics, for instance by inducing their self-renewal or differentiation into specific cell types.

Therefore, future studies have to be aimed at designing methods and protocols to expand and, if necessary, predifferentiate stem cells ex vivo. In addition, issues dealing with immunity and tumor formation should be extensively studied before clinical trials are initiated. Finally, although the loss of stem cells plays an important role in radiation-induced tissue damage, many other factors influence normal tissue response.[1] It is necessary to obtain a thorough knowledge of the radiosensitivity of the tissue's stem cells and their interaction with the changed environment of the stem cell niche both for irradiated and transplanted stem cells. To develop optimal therapies, the stem cell's response to DNA damage, cytokines and growth factors, and interaction with the extracellular matrix to which they will, or can be, exposed to need to be determined. Stem cell research has begun to explore the unique qualities of stem cells as well as their vast clinical potential. Although many questions remain to be answered, significant progress has been made during the last few years. In the near future, cell-based therapies may restore function of radiation-damaged tissues.

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