The effect of salivary gland-derived stem cell transplantation on the regeneration of gamma-irradiated rat submandibular salivary glands: an immunohistochemical study

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Background
Hyposalivation could be a sequela of radiation impairment in patients with head and neck cancer. Regenerative approaches based on the reactivation of endogenous stem cells or the transplant of exogenous stem cells hold substantial promise in restoring the structure and function of these organs to improve patient quality of life. Recently, tissue-specific stem cell therapy has attracted public attention as a next-generation therapeutic reagent. The aim of this work is to assess the regenerative potential of salivary gland-derived stem cells transplantation in gamma-irradiated rat submandibular salivary glands (SMSGs).
Materials and methods
Forty-six adult male albino rats were used in this study. Both SMSGs were harvested from five healthy donor rats and used as a source of stem cells. Cells were cultured for 3 and 10 days. Characterization and assessment of stemness after isolation by flow cytometry was carried out using CD24 stem cell marker by fluorescent analysis cell sorting. The rats were grouped as follows: group I (normal untreated control), group II (irradiation group), and group III (irradiation and transplantation group). They were subjected to whole-body gamma radiation with a single dose of 6 Gy.
Results
Stem cells were successfully isolated from rat SMSGs with positively expressing CD24 and C-kit markers. The statistical analysis revealed significant increase in cell proliferation (proliferating cell nuclear antigen) in both groups II and III compared with the control group I (*P*? 0.05). Regarding caspase 3 results, the statistical analysis revealed highly significant increase in the mean values of groups II and subgroups III compared with the control group I (*P*? 0.05). Concerning C-kit expression, group III showed the highest statistically significant mean C-kit expression, followed by group II and group I (*P*? 0.05). At 2 weeks after transplantation, group II showed the highest statistically significant mean C-kit expression followed by III and control group I (*P*? 0.05).
Conclusion
Transplantation of these C-kit+ submandibular salivary gland stem cell (SMSGSCs) could result in amelioration of the severely reduced quality of life of surviving patients with cancer.

Keywords:
immunohistochemistry, irradiation, isolation, submandibular salivary gland stem cells

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Introduction

Ionizing radiation is a crucial constituent of therapy for nearly all patients with head and neck cancers. Radiation-induced xerostomia has been detected in more than 60% of patients with head and neck cancer receiving radiotherapy (RT). Inside salivary Gland (SGs), mainly the acinar cells in the ionizing radiation field go through brutal damage [1,2]. As these acini are the principal source of fluid secretion in SGs, this brings about severe SG hypofunction resulting in a broad range of complications.

Treatment modalities for salivary gland dysfunction such as xerostomia were saliva substitutes or stimulants [3]. Saliva substitutes may recover some, but not all, problems associated with SG dysfunction. Still stimulants are only helpful for people with some residual SG function. Many approaches to restore SG function have been applied, for example, the assembly of bioengineered glands [4].

As a trial to repossess the function of the SG, predominantly in patients with head and neck

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irradiation, bone marrow-derived stem cells were formerly planned as an easy available source for multipotent stem cells. Still, the usage of bone marrow-derived stem cells in solid tissue regeneration is bounded by controversies and restricted effects [5].

Transplantation of salivary gland-derived stem cells (SGSCs) was then confirmed to be a more sufficient and a graceful way for therapy after successful isolation of stem cells from both human parotid and submandibular salivary glands (SMSGs) [6,7]. Salivary glands have been anticipated as a source of SCs in mice and rats subsequent to tissue breakdown [8,9], and there is experimental evidence that SCs can be isolated from integral, nondamaged rat SMSGs [10].

It has been postulated that the loss of salivary function after irradiation leads to destruction of the SGSCs necessary for maintaining a healthy gland [11]. Derived from this hypothesis, several studies have exposed that SGSC transplantation after irradiation could retrieve glandular function [12]. Preclinical studies have revealed that stem cell transplantation not only rescues hyposalivation [6] but also leads to regain of tissue homeostasis of the irradiated gland, essential for long-term preservation of adult tissue [4,13].

Stem cells might be harvested from SGs before the start of RT and returned to the salivary complex after RT has been finished. These salivary stem cells could then repopulate the damaged SG [14]. In addition, SMSGs can be an expedient source of autologous cells for cellular therapy [15].

Transplantation of human salivary gland stem cells (hSGSCs) to radiation-damaged rat salivary glands retrieved hyposalivation and body weight loss, repaired acinar and duct cell structure, and decreased the number of apoptotic cells. These data propose that the isolated hSGSCs, which have characters of mesenchymal-like stem cells, might be used as a cell therapy mediator to treat the damaged salivary glands [16].

Proliferating cell nuclear antigen (PCNA) was initially known as an antigen that is detected in the nuclei of cells through the DNA synthesis phase of the cell cycle [17]. Maintenance of tissue homeostasis requires the balance of cell death and cell growth. The complex relationship among cell proliferation, differentiation, and apoptosis is a fundamental characteristic in the preservation of normal structure and function of submandibular gland [18].

Caspases are a family of genes important for preserving homeostasis during apoptosis and inflammation. Caspases have been generally classified depending on their roles in apoptosis (caspase-3, caspase-6, caspase-7, caspase-8, and caspase-9 in mammals). Caspase 3 is an effector caspase participating in extrinsic and intrinsic pathways of apoptosis [19]. Some studies have found that apoptosis is produced in the salivary glands within the first 24 h after exposure to a single dose of ionizing radiation [20]. According to a study conducted by Muhvik-Urek *et al.* [21], the inequity between apoptosis and proliferation caused by irradiation may be the motive for gland dysfunction through postirradiation phase.

Since 2004, numerous studies were done in which submandibular and parotid gland SCs were transplanted in animal models after RT [22]. The greatest marker, however, to select SCs for transplantation is still undecided. The most commonly used marker is C-kit [23]. C-kit is a cytokine receptor detected on the surface of hematopoietic stem cells plus other primitive stem cell types, which acts a necessary function in the early stages of hematopoiesis.

Transplantation of C-kit+ cells in mice submandibular glands can renovate function and morphology and retrieve salivary glands from irradiation damage. Interestingly, these C-kit+ cells are also detected in human salivary glands [12,22]. Whether these C-kit+ cells in humans have the same repairing potential needs to be investigated.

The eventual objective of SC transplantation is regeneration of the function of the salivary gland through differentiation of these transplanted SCs into functional salivary gland cells [12]. So this study aimed to assess the regenerative potential of SGSC transplantation in gamma-irradiated rat SMSGs using immunohistochemical markers C-kit, PCNA, and caspase 3.

Materials and methods

Study design

Forty-six adult male albino rats weighing around 150–200 g were selected as the material of the study from the Animal House of the National Research Center. The rats were given ad-libitum access to food and water. The SMSGs were harvested from five healthy donor rats under aseptic conditions and used as a source of stem cells. The remaining 41 rats were grouped as follows: group I (normal control) (n=5 rats), served as the normal untreated control group; group II (irradiation group 'IR')

(*n*=18 rats); and group III (irradiation and transplantation group 'IR+TR') (*n*=18 rats). They were subdivided into two subgroups: subgroup A (*n*=9 rats), which was injected locally into the SMSGs with SG-derived stem cells at 3 days of stem cells culture, and subgroup B (*n*=9 rats), which was injected locally into the SMSGs with SG-derived stem cells at 10 days of stem cells culture. Group II and group III were subjected to whole-body gamma radiation with a single dose of 6 Gy.

Ethical approval

All experiments were approved by the Ethical Committee on animal testing of the National Research Centre with no. 15062.

Stem cell isolation and culture

Under general anesthesia (ketamin intraperitoneally at a dose of 0.2 ml/100 g), the SMSGs were harvested from five healthy donor rats under aseptic conditions and used as a source of stem cells. Following submandibular gland dissection, salivary gland cells were isolated and cultured. Cell suspensions were prepared by first mechanically disrupting the gland, followed by enzymatic digestion with collagenase type II (Gibco (California, USA), Life Technologies (California, USA)). Then the tissue suspensions were filtered using a strainer (Becton/Dickinson, New Jersy, USA), and collected cells were centrifuged for 10 min to obtain the cell pellets. Then the cell pellets were subjected to magnetic cell sorting of C-kit cells using magnetic-activated cell sorting (MACS) kits (MiniMACS; Miltenyi Biotec, Bergisch Gladbach, Germany).

Flow cytometric analysis

After reaching confluence, the isolated cells were characterized by flow cytometric analysis using CD24 stem cell marker, as primary culture was dissociated into single cells using 0.05% trypsin–EDTA and stained with CD24 antibodies as well as C-kit antibodies to confirm sorting of C-kit+ cell population. Cell analysis was performed using Cytomics FC 500 Flow Cytometer (Beckman Coulter, Miami, Florida, USA), analyzed using CXP Software version 2.2 (Beckman Coulter Inc., Brea California, USA), and evaluated with fluorescent analysis cell sorting scan flow cytometer.

Labeling C-kit+ stem cells with green fluorescent protein

Living colors pAcGFP1-N1 vector (www.clontech.com) was obtained from Clontech Laboratories Inc. (Mountain view, California, USA) Clontech is a Takara Bio Company (catalog no. 632469; Mountain View, California, USA).

Transplantation

The remaining 41 rats were grouped as follows: group I (normal control; *n*=5 rats) served as the normal untreated control group. Group II (IR group): (*n*=18 rats) was subjected to whole-body gamma radiation with a single dose of 6 Gy. Group III (IR +TR group; *n*=18 rats) was subjected to whole-body gamma radiation with a single dose of 6 Gy and was subdivided into two subgroups: subgroup A (*n*=9 rats) was injected locally into the SMSGs with SG-derived stem cells at 3 days of stem cell separation and propagation culture, and subgroup B (*n*=9 rats) was injected locally into the SMSGs with SG-derived stem cells at 10 days of stem cell separation and propagation culture.

Irradiation of the rats

The rats in group II (IR) and group III (IR+TR) were subjected to whole-body irradiation with a single dose of 6 Gy at National Center of Radiation Research and Technology, Cairo, Egypt, using gamma cell 40 (cesium 137 irradiation unit which provides a dose rate of 0.48 Gy/min. It is manufactured by Atomic Energy of Canada Limited, Chalkriver, Ontario, Canada) sublethal dose [24].

Intraglandular injection of cultured cells

After counting the cells, the cells were suspended in equal volumes of Roswell Park Memorial Institute culture media with fetal bovine serum ready for injection of 50 000 cells/100 µl/rat, that is, 25 000 cells/50 µl/gland 24 h after irradiation.

Specimen collection and preparation

After 3 days, 1 week, and 2 weeks of post-transplantation procedure, six rats from group II and group III were killed by anesthetic overdose at each time point, as well as the rats in the normal control group. The skin was removed, and both SMSGs were carefully excised and immediately fixed in 10% formalin for further investigations.

Immunohistochemical analysis of salivary gland

Consecutive slides of 4 µm from paraffin-embedded tissue blocks were cut and mounted on positively charged glass slides (Opti-Plus; BioGenex Laboratory, Fremont, California, USA). Then the sections were dewaxed and labeled for the following commercially available markers: PCNA primary PPA (catalog #PA5-27214), caspase 3 primary PPA (catalog #MA1-91637), and C-kit PPA (catalog #PA5-16770). All of which were ready-to-use using automated stainer (Thermo Scientific (Massachusetts, USA), Lab Vision Corporation, Fremont, California,

USA). The immunostained sections were examined using Ordinary Light Microscope to assess the prevalence of immunopositivity of PCNA staining, caspase 3, and C-kit in the studied cases, and Image Analysis Computer System (model #LC20; Olympus Soft Imaging Solutions GMBH, Johann-Krane-Weg, Munster, Germany) was used to assess area percentage of the positive cells. All the steps were done in the Basic Dental Science Department, Oral and Dental Research Division, National Research Center.

Statistical analysis

Data were represented as the mean±SD values. One-way analysis of variance test was used to compare between different groups. Post-hoc test was performed for multiple comparisons using Bonferroni's method. The significance level was set at level of *P* value less than 0.05.

Results

Characterization and assessment of stemness after isolation by flow cytometry

After reaching 70–80% confluence, the isolated cells were characterized by flow cytometric analysis for CD24 stem cell marker. The analysis revealed that 65% of the population was CD24 positive (submandibular salivary gland stem cell (SMSGSCs): 65%) (Fig. 1a). The fluorescent analysis cell sorting analysis, which confirms sorting of C-kit+ cell population after reaching confluence, revealed that in group I, the cells were positive for C-kit (SMSGSCs: 87%), as shown in Fig. 1b, and 97% in group II (Fig. 1c).

Phenotypical analysis

Stem cells were successfully isolated judging by their ability to adhere to plastic plates, and the C-kit+ cells continued to proliferate and propagate reaching 30–40% confluence by day 3 in group-I and 85–95% in group II. The cells appeared with variable morphologies; the initial culture of the isolated cells contained a crowded cell population with a majority of small spherical cells. However, the number of the fibroblast (spindle)-like cells showed apparent increase over time and upon subculture. Some appeared in aggregates, then by time, others appeared stellate shaped (Fig. 2a–c).

Assessment of C-kit+ STC labeling with green fluorescent protein

The green fluorescent protein (GFP) was detected in the unstained sections using inverted fluorescent microscope (Fig. 3a and b).

Immunohistochemical results

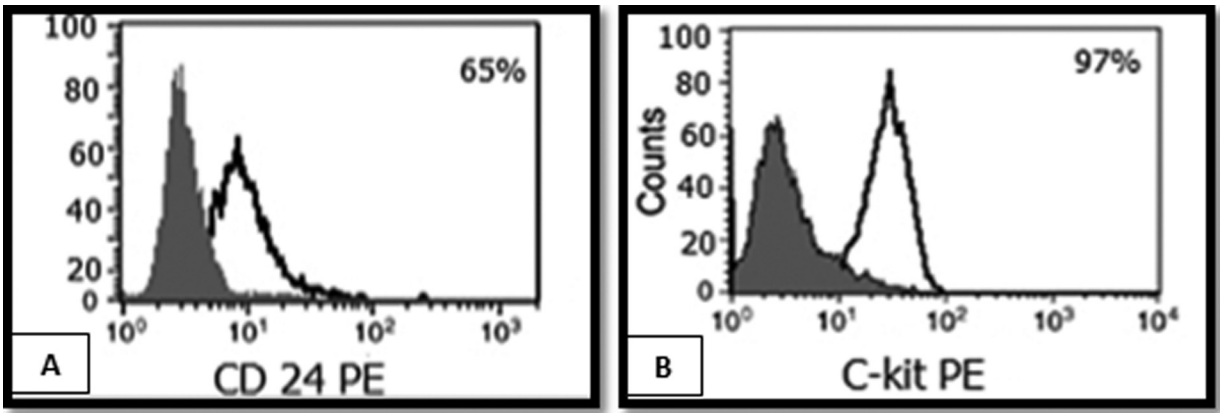
Proliferating cell nuclear antigen staining

The normal control rat SMSGs (group I) showed positive PCNA immunoreaction, seen as brown granular or homogenous stain in the nuclei of acinar and ductal cells (Fig. 4a). In group II, the examined SMSGs revealed positive expression of PCNA immunoreaction in the nuclei and cytoplasm of acinar and ductal cells, with higher expression in ductal cells (Fig. 4b). However, in group III, the examined SMSGs revealed increasing positive expression of PCNA immunoreaction in the nuclei and cytoplasm of acinar and ductal cells, with higher expression in ductal cells (Fig. 4c and Table 1).

Caspase 3 staining

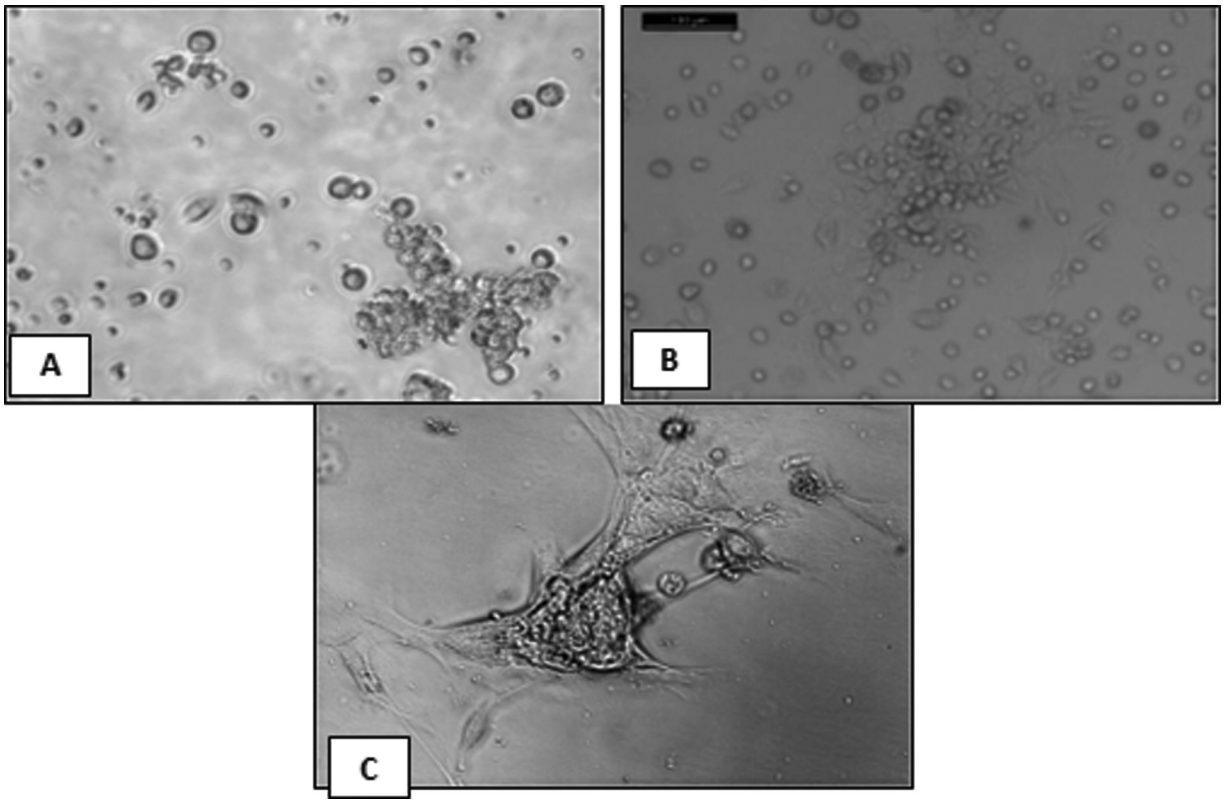
In group I, caspase 3 activity was detected as brown granular cytoplasmic immunoexpression in acinar cells, whereas ductal cells showed either faint positive or negative immunoreactions (Fig. 5a). In group II, the

Figure 1



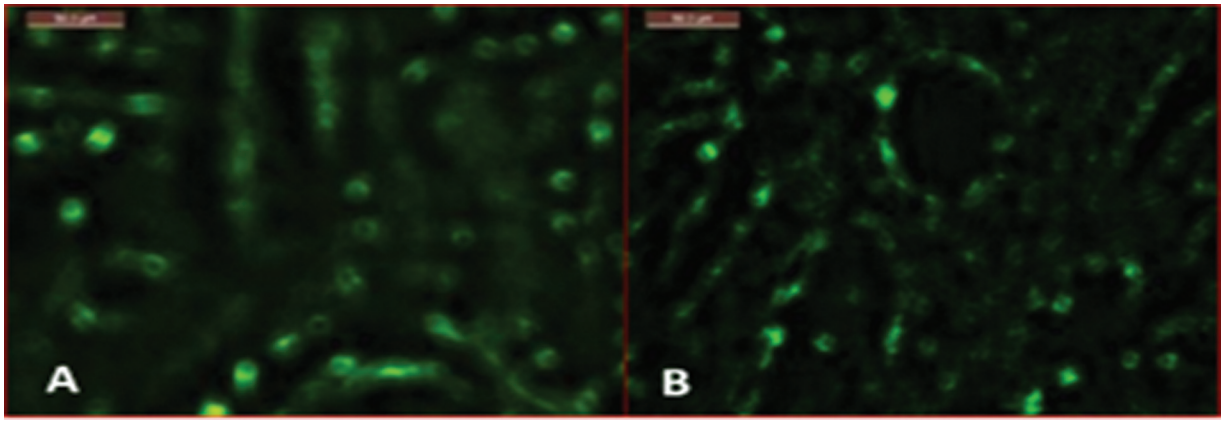
Showing (a) flow cytometric analysis of SMSGSCs for CD24 after stem cell isolation showing 65% positive expression, and (b) flow cytometric analysis of sorted cells for C-kit (97% positivity).

Figure 2



Photomicrograph showing (a) an increase in number of cells, some forming bigger cell aggregates at day 3), (b) some cells exhibit aggregation and others attaining more spindle shapes at day 10) (×200), (c) and higher magnification of an attached cell with a stellate appearance at day 10 (×400).

Figure 3



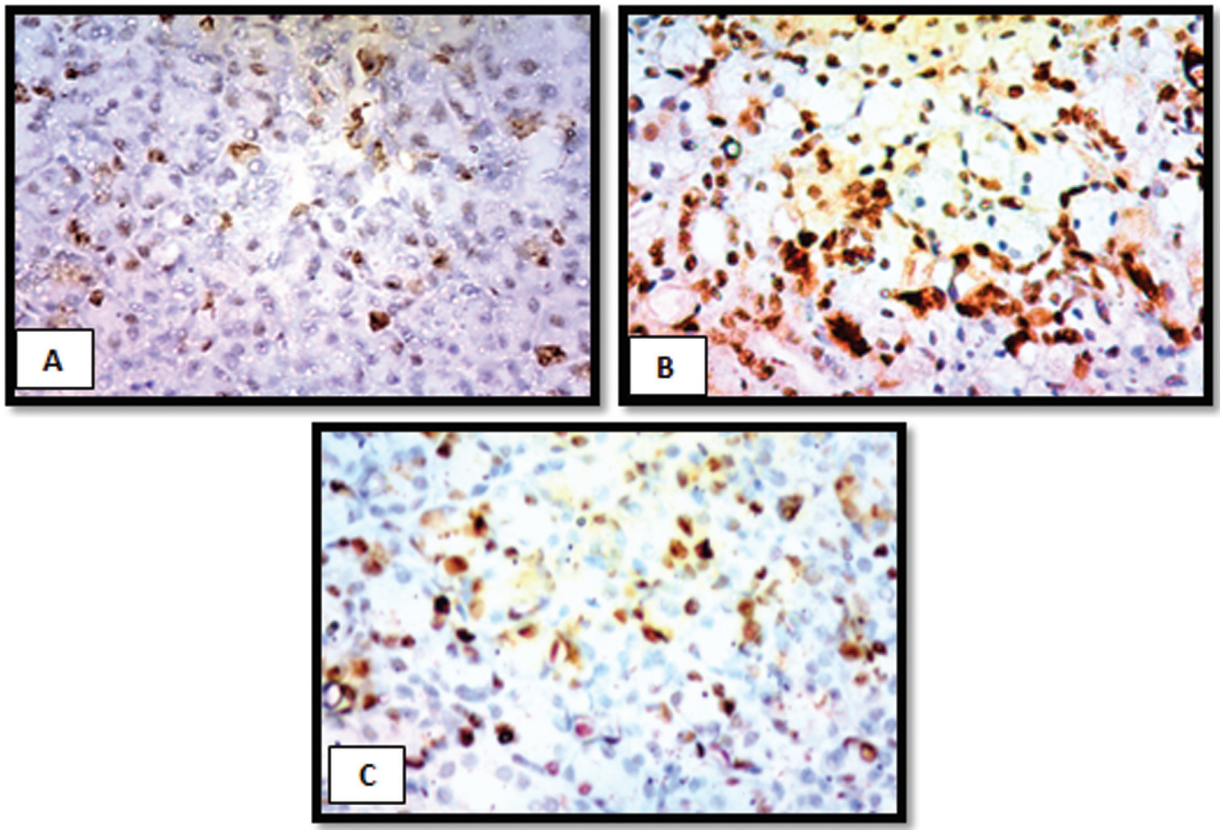
Photomicrograph showing (a, b) green fluorescent protein fluorescence in the unstained sections seen under inverted fluorescent microscope in group III (×200).

examined SMSGs revealed positive cytoplasmic and nuclear immunoexpression of caspase 3 in some acinar cells. Most of ductal cells were immunonegative, whereas few showed faint cytoplasmic immunoreactions (Fig. 5b). In group III, the examined SMSGs revealed positive cytoplasmic and nuclear immunoexpression of caspase 3 in ductal cells, whereas few acinar cells showed cytoplasmic immunopositivity (Fig. 5c and Table 2).

C-kit staining

In group I, the site of positive immunoexpression of C-kit was mainly in the ducts (Fig. 6a). In group II, the examined SMSGs revealed positive immunoexpression of C-kit in the luminal surface of excretory and striated ducts (Fig. 6b). In group III, a positive reaction for C-kit was noted in the small intercalated, excretory, and striated ducts (Fig. 6c and Table 3).

Figure 4



Photomicrograph showing (a) normal rat submandibular salivary gland with some proliferating acinar and ductal cells, (b) group II (irradiation) with positive nuclear and cytoplasmic immunoreaction in some acinar and ductal cells (note the immunonegative stromal cells), and (c) group III showing positive nuclear and cytoplasmic immunoreaction in most of acinar and ductal cells. Few acinar cells are immunonegative (antiproliferating cell nuclear antigen ×200).

Table 1 Mean area fraction of proliferating cell nuclear antigen-positive immunoexpression of the studied groups at different intervals

	Group I	Group II	Group IIIA	Group IIIB
Three days	2.22975	14.1513	4.98275	6.1415
One week		2.37875	4.415	3.829833
Two weeks		3.932792	3.526333	2.358917

Statistical results

In PCNA staining, group II (3.93±1.15) showed the highest statistically significant mean PCNA expression, followed by group IIIA (3.53±0.87), IIIB (2.36±1.13), and group I (2.23±0.36). The statistical difference among the groups was highly significant (*P*? 0.05; Fig. 7a).

In caspase 3 staining, group II (42.01±5.49) showed the highest statistically significant mean caspase 3 expression, followed by group IIIB (28.43±6.56), IIIA (17.59±10.03), and group I (8.75±1.78). The statistical difference between the groups was highly significant (*P*? 0.05; Fig. 7b).

In C-kit staining, group II (1.96±1.72) showed the highest statistically significant mean C-kit expression, followed by group IIIA (1.55±1.16), IIIB (1.23±0.76) and group I (0.57±0.55). The statistical difference between the groups was insignificant (NS) (*P*? 0.05; Fig. 7c).

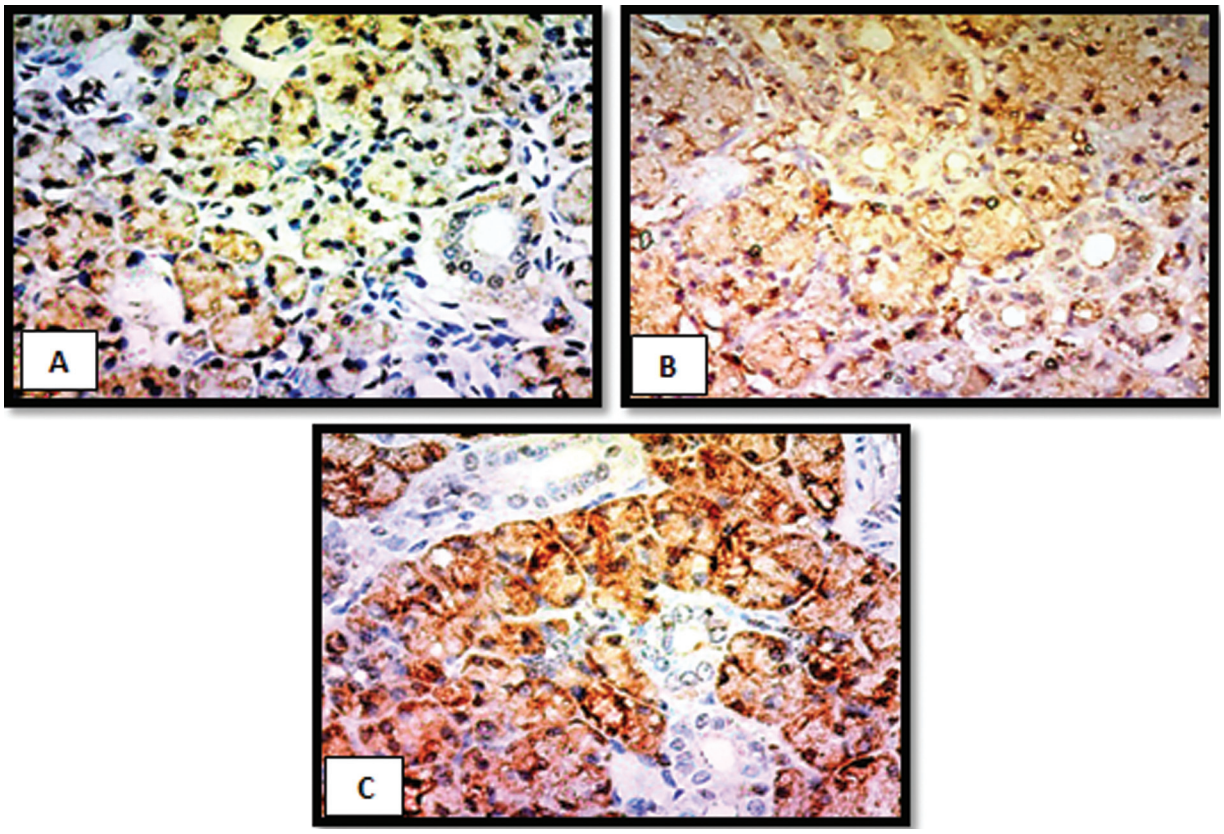
Discussion

Although SGs are radioresistant tissues because of their highly differentiated cellular character, they reveal a frail sensitivity to radiation, which is characterized by a diminution in salivary flow rate, irreversible and progressive loss of glandular weight and acinar cells, as well as morphological changes in gland structure [25,26].

This study was conducted aiming to isolate and characterize and culture the SMSG C-kit+ SCs of albino rats, and then transplant them in irradiated rats and to carry a series of investigations to highlight their regenerative potential aiding in the research of regenerative therapy.

Consequently, adult SGSCs are capable candidates for autologous transplantation treatment in the circumstance of tissue-engineered reproductive salivary glands or

Figure 5



Photomicrograph of group I (control) showing positive cytoplasmic immunoreaction in all acinar cells. Ductal cells and stromal cells are immunonegative. (b) Group II (irradiation) showing positive cytoplasmic immunoreaction in some acinar cells as well as few ductal cells, and (c) group III showing positive cytoplasmic and nuclear immunoreaction in all acinar cells and ductal cells. The reaction in some ductal cells is faint and membranous (anticaspase 3, ×200).

Table 2 Mean area fraction of caspase 3 positive immunoexpression of the studied groups at different intervals

	Group I	Group II	Group IIIA	Group IIIB
Three days	8.75325	40.383	44.1811667	39.35287
One week		44.24529	30.82608	28.1335
Two weeks		42.00892	17.5925	28.42975

direct cell therapy [6,27]. However, the main limitations in handling such cells are their restricted lifespan throughout in-vitro cultivation, leading to a narrow time-window for implantation and a hazard of tumorigenic changes during culture [28,29].

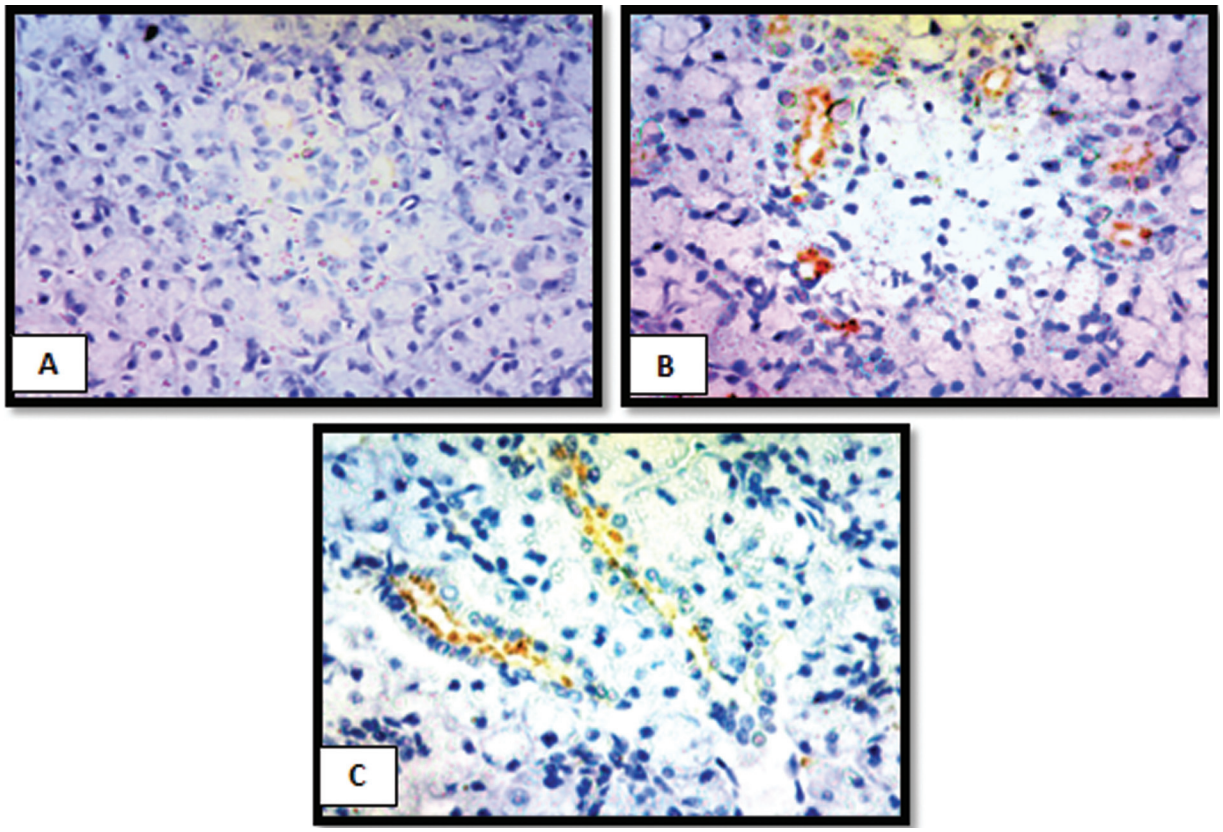
Therefore, the choice of using C-kit+ SCs in the present study was based on the previous studies [22,30] that explored the potentials of this SGSC population. Using a rat experimental in-vivo model in this study, the morphological changes of SMSG, as well as PCNA, caspase 3 and C-kit distribution, following exposure to IR and SGSC transplantation were investigated.

The rats in irradiation group II and irradiation and transplantation group III were subjected to whole-body irradiation with a single dose of 6 Gy sublethal doses in accordance with the irradiation protocol performed by Ahmad *et al.* [24]. This dose was utilized to induce sufficient damage to the SGs without jeopardizing the life of the animals.

Stem cell-based therapy has established growing interest over the past decade, but direct confirmation of the homing and implantation of the transplanted cells is contradictory; therefore, consistent labeling and tracking techniques are necessary. GFP gene transduction using lentiviral vectors is a consistent tagging and tracking method [31]; therefore, it was used in the current study for the purpose of labeling and tracking transplanted SCs.

In this study, the negative GFP expression, which was observed in nontransplanted irradiated glands, and the positive staining for GFP in the irradiation and transplantation group III might indicate the homing and/or differentiation of the transplanted SCs in irradiated SG tissue. These results demonstrated that transplanted cells can restore radiation-damaged SG and outline new ducts that may contribute to

Figure 6



Photomicrograph of (a) group I, with normal rat submandibular salivary gland, showing luminal positive C-kit immunoexpression in the ducts, (b) group II (irradiation) showing positive cytoplasmic immunoexpression in the luminal surface of the cells of striated and excretory ducts, and (c) group III showing positive luminal immunoexpression in the small intercalated and striated ducts (anti-C-kit ×200).

Table 3 Mean area fraction of C-kit-positive immunoexpression for the studied groups at different intervals

	Group I	Group II	Group IIIA	Group IIIB
Three days	0.57125	1.639833	1.411083	2.513167
One week		1.405083	2.22475	2.678083
Two weeks		1.962333	1.550917	1.226167

regeneration. Similar results were reported by Lombaert *et al.* [12] who used anti-GFP bright-field immunolabeling, which confirmed that the expression of GFP was limited to ducts in SC-transplanted SMSGs.

The complex association between cell proliferation, differentiation, and apoptosis is a fundamental characteristic in the continuation of normal structure and function of SMSG [21,32,33]. To evaluate the regenerative process in SG tissue, proliferative and apoptotic activity was immunohistochemically estimated in the different epithelial cell compartments of rat SMSGs.

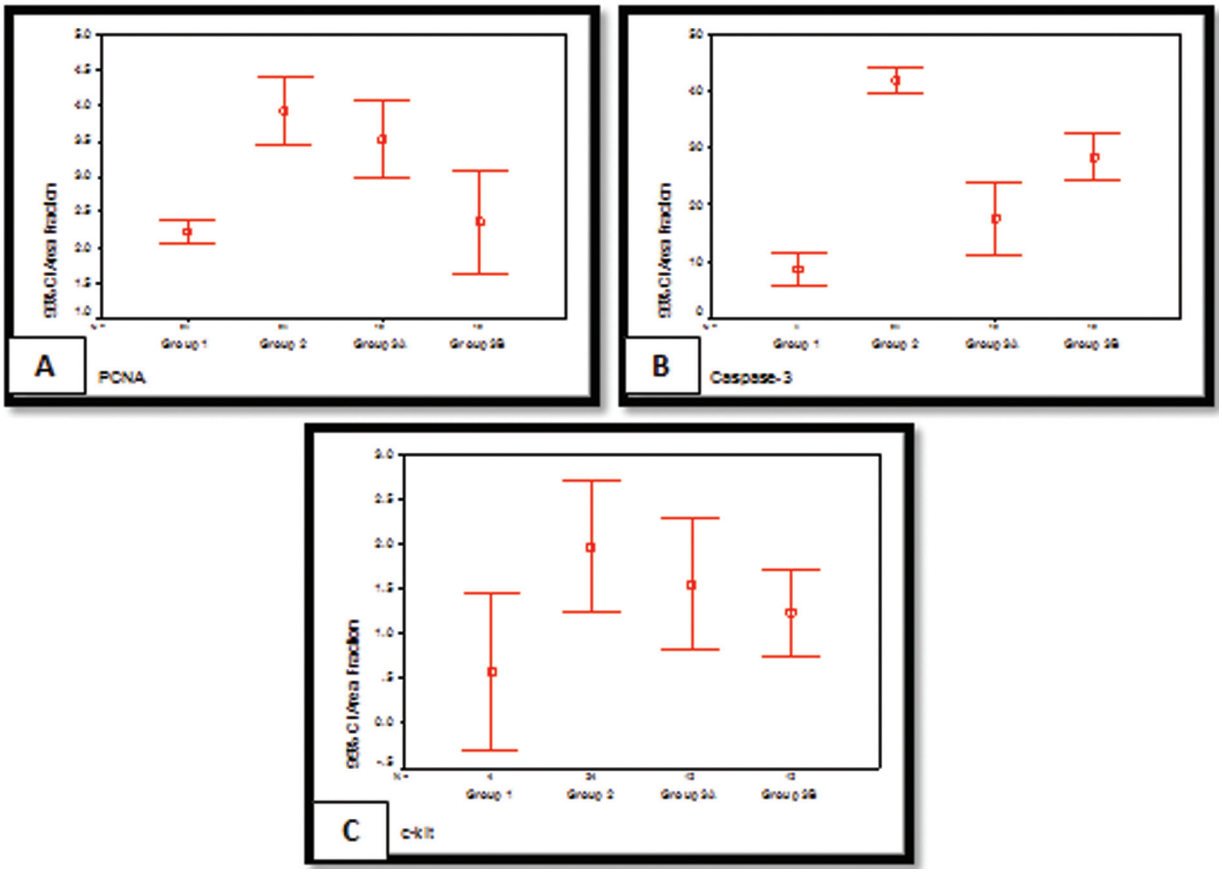
PCNA binding has been reported as identifying the proliferating cell population in irradiated tissues [34]. For this reason, this antibody was used in the present study to assess the proliferative capacity of irradiated glands.

This study revealed the increased expression of PCNA in ductal cells more than acinar cells in all the studied groups 3 days after transplantation, especially in irradiation group II, which could be explained by the fact that IR produces elevated cellular proliferation between several cells types in percentage to the degree the cells are killed. This was in accordance with the studies done by Muhvic-Urek *et al.* [21] in SMSG and Farid *et al.* [35] in parotid glands, who reported that after the initial decline of the proliferation index at day 1 after irradiation, there was a subsequent increase in the proliferation rate.

In addition, ducts contain stem cell population that may function in the postnatal growth of the acinar and duct components of the SGs and also play a role in tissue regeneration during damage of the SGs [36].

One week after transplantation, the proliferation indices were lower than those found at 3 days in all the studied groups, a datum in contrary to that reported

Figure 7



Showing (a) floating error bar of proliferating cell nuclear antigen expression at 2 weeks after transplantation, (b) floating error bar of caspase 3 expression at 2 weeks after transplantation, and (c) floating error bar of C-kit expression at 2 weeks after transplantation.

by Bralic *et al.* [37] who reported maximum proliferation capacity in all the gland compartments at day 6–7 after irradiation. Moreover, irradiation and transplantation group III showed the highest statistically significant mean PCNA expression, followed by group II, indicative of the regenerative potential of SGSCs.

Two weeks after irradiation, the proliferation indices of all gland compartments in all the studied groups showed declined records as indicated by lower area percent of PCNA. These data are in agreement with that reported by Farid *et al.* [35]. The most rational clarification for the deprived recovery of acinar cells after moderate to high doses of ionizing radiation is that the lifetime proliferative ability of the acinar cells and their progenitors is somewhat exhausted by repetitive mitoses in attempts to restore cells lost to radiation and is reduced further by DNA/chromosomal damage in still-viable cells [33,38].

Enhanced apoptosis of acinar cells is recommended to be one of the main reasons for SG impairment after exposure to ionizing radiation [21]. Many methods for the detection of apoptotic cells have been reported based on early or late events in the apoptotic pathway [39]. Thus, caspase 3 was used in this study to assess the apoptotic activity in the experimental groups. In the current study, regarding the irradiation group II, the apoptotic activity was found to increase with time peaking at 1 week after transplantation. This result was consistent with the data reported by Jeong *et al.* [16].

However, apoptotic activity was seen to decrease with time in the irradiation and transplantation group III, peaking at 3 days after transplantation, and then declined at 1 week after transplantation. These results were consistent with that reported by Jeong *et al.* [16]. On the contrary, these results were not consistent with that reported by Farid *et al.* [35]. This may be related to difference in radiation dose, dose rate, mode of delivery, technique and method of apoptosis detection, and animal species. To this, Belikova *et al.* [40] attributed irradiation-induced apoptosis to activation of caspases 3 and 7.Although subsequent decrease in apoptotic activity was detected 2 weeks after irradiation in all the studied groups, apoptotic activity

was still higher in the irradiation group II than in the irradiation and transplantation group III, indicating better recovery and regeneration potential in these transplantation subgroups.

For long-standing homeostasis, viable stem cells are required. Thus, we investigated the detection of the SC marker C-kit in the tissues in the irradiated and the transplanted groups. The current study showed positive expression of C-kit exclusively in ductal luminal surfaces and sometimes in periductal cells in all the studied groups.

In the control samples, the excretory and striated duct cells showed positive expression of C-kit, confirming that the main ducts of SG include the tissues' endogenous stem/progenitor cells. These data were consistent with that reported by Sumita *et al*. [5] who noticed that the appearance of C-kit was detected mostly in the ductal section of nonirradiated control.

In the irradiation group II, C-kit immunoexpression was observed to be confined to the ductal compartment. At 1 week after transplantation, the immunoexpression was lower than that noted at 3 days after transplantation. This might be attributed to the sterilizing effect of IR on the tissue's endogenous SCs. These data were consistent with that stated by Nanduri *et al*. [41] and Sumita *et al*. [5] who reported a reduction of all SC marker expressing cells.

In the irradiation and transplantation group III, at 3 days after transplantation, a larger number of ducts including striated and intercalated ducts showed positive expression of C-kit, suggesting that C-kit+ cells survived in an irradiated surroundings and are capable to generate pools of differentiated acinar cells.

A higher peak of C-kit expression was noted 1 week after transplantation in irradiation and transplantation subgroup B (group IIIB) followed by irradiation and transplantation subgroup A (group IIIA), with significant difference compared with the control groups. These results are indicative of potential long-term recovery of the glands. Two weeks after transplantation, a statistically insignificant difference between the transplantation group and the control group was noted, indicating a possible differentiation of the putative stem cells and glandular regeneration, as indicated by lower levels of PCNA and caspase 3 in this group compared with the irradiation one, a finding suggestive for the restoration of glandular homeostasis.

Conclusion

This study model may guide in the near future to clinically applicable use of salivary gland stem cells, as our results suppose that transplantation of these cells might result in amelioration of the highly reduced quality of life for surviving patients with cancer.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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