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Perspectives

A closer look at strategies for preserving salivary gland function after radiotherapy in the head and neck region

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ABSTRACT

Radiation-induced damage to the salivary glands remains a major complication of using radiation therapy to treat head and neck cancer, and it has led a wide range of research attempting to resolve the problem. From this perspective, we sought to briefly discuss relevant and timely approaches aimed at protecting or regenerating irradiated salivary glands, thereby preventing the development of salivary hypofunction or rescuing the functional properties of damaged glands. Such findings include molecular, cell, tissue, organ, and drug-based therapies.

Introduction

The major salivary glands (SG) – parotid (PG), submandibular (SMG), and sublingual – produce up to 90% of all saliva secretion, which is important for the lubrication of the mouth and oropharynx, mastication, teeth remineralization, and microenvironment regulation [1]. Radiation-induced hyposalivation (RIH) and xerostomia (RIX) are the most frequent complications during or after head and neck cancer (HNC) radiotherapy, and they modify salivary flow and composition [2,3]. Both conditions are associated with burning pain; mucosal soreness or ulcerations; impaired taste sensation, chewing, swallowing, wearing dentures, talking or sleeping; and increased oral infections [3].

Importantly, approximately half a million patients are diagnosed each year with HNC and subsequently treated with radiation therapy, 40% of whom will develop some SG impairment as a result [4,5]. Given the considerable controversy regarding the efficacy of chemoradiotherapy in reducing HNC mortality and despite all advances in clinical oncology, radiotherapy either with or without surgery remains the primary option for HNC patients [6,7]. Consequently, growing evidence has addressed the protection or regeneration of SG and yielded promising outcomes.

Therapies have been designed to protect the SG from radiation, provide temporary relief of symptoms, or restore damaged functions. Additionally, advances in cell- and tissue-based therapies have received increased attention for the regeneration of injured tissues. Nevertheless, whether most of these approaches would benefit HNC patients who are affected by RIH/RIX over the long term remains unknown [1,8]. Thus, we sought to present and briefly discuss timely findings that are likely to drive future research in this field.

Molecular candidates

Growth factors, including insulin-like growth factor (IGF-1), keratinocyte growth factor (KGF) and fibroblast growth factor (bFGF), and Wnt signaling have been investigated as potential radio-protectants. The activation of cellular pathways that promote cell survival, DNA repair and growth underlies this protective effect [8]. IGF-1 stimulates endogenous protein kinase B activation in mice acinar cells, which then preserves the salivary flow rate

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(SFR), promotes murine double minute clone 2 phosphorylation, and inhibits p53 transcriptional activation and DNA damage-induced apoptosis [9]. Mice that received IGF-1 for five consecutive days after 5 Gy of irradiation presented with a higher SFR and a larger regenerated PG area [10].

KGF increases the radio-resistance of epithelial cells by enhancing DNA repair and altering both the expression of mediators or antagonists of apoptosis and the ability of cells to scavenge free radicals [11]. Adenoviral vectors encoding KGF that were administered to the SMG of irradiated mice prevented RIH, which led to comparable levels of proliferating positive cells compared to the non-irradiated group; this result was similar for progenitor/stem cells and endothelial cells [12]. Pre- and post-treatment with KGF preserved saliva production and gland weight by abrogating acinar cell depletion and fibrotic cell deposition after irradiation. The pre-treatment of cultured cells with KGF also increased stem cell survival after irradiation, thereby accelerating their proliferation [13].

The physiological agent bFGF repaired radiation damage by inducing cells to undergo extended G2 arrest after irradiation, thus allowing more time for them to recover from DNA damage prior to mitosis and enhancing clonogenic survival [14]. The use of bFGF prior to and immediately after 15 Gy irradiation partially protected PG acinar cells *in vitro* and reduced apoptosis by 44% [15]. The administration of bFGF in mice SMG for three days after irradiation reduced the apoptotic response and preserved the number of acinar cells, improving SFR eight weeks after irradiation [16]. FGF2 cDNA transfer to minipigs with decreased parotid SFR and blood flow post-irradiation protected PG microvascular endothelial cells and limited SFR decline. FGF2 was encoded using a single pre-administration of AdLTR2EF1a-FGF2, which is a hybrid serotype 5 adenoviral vector [17].

Moreover, intracellular Wnt/β-catenin signaling plays an essential role in the differentiation, proliferation, death, and function of various cell types, with increased activity in progenitor cells [18]. In SG harvested from HNC patients with RIX, Wnt-1 expression in the acinar structures was increased along with the upregulation of membrane β-catenin, suggesting that Wnt activation provides a key radioprotective mechanism in irradiated cells [19]. The exposure of Wnt transgenic mice to a single 15 Gy radiation dose led to the transient expression of Wnt-1 in basal epithelia and activated Wnt/β-catenin in SMG, thereby suppressing apoptosis, preserving salivary stem/progenitor cells, and regulating SG homeostasis via increasing active progenitor cells [18].

Alda-89, an aldehyde dehydrogenase-3 activator (ALDH3) activator, also improves SMG post-radiation function; it causes no measurable toxicity *in vivo* and does not lead to the accelerated growth of HNC cell lines or to tumor growth. This finding suggests that short-term treatment with ALDH3 agonists relieves RIX without affecting tumor growth [20]. Salivary function loss is accompanied by cellular senescence, persistent DNA damage response (γH2AX, 53BP1), and the expression of senescence-associated markers (SA-βgal, p19ARF, DcR2) and secretory phenotype factors (PAI-1, IL-6) in irradiated mice and human SG. Notably, sustained IL-6 expression in SG after radiation-induced DNA damage was required for senescence and hypofunction, which were prevented by IL-6 pre-treatment through enhanced DNA damage repair. Hence, IL-6 may represent a promising therapeutic strategy for RIH [21].

Although Hedgehog (Hh) signaling is not activated by irradiation in mouse SG, transient Sonic Hh over-expression activated Hh in ductal epithelia. This action repaired salivary function by preserving functional stem/progenitor cells mediated through Bmi1 and Chrm1/HB-EGF activities. Parasympathetic innervation was also rescued via neurotrophic factors (i.e., Bdnf, Nrtn) preservation. Taken together, these findings suggest that transient Hh activation might serve as a target to restore radiation-induced SG dysfunction [22].

Radioprotective drugs

Prophylactic treatments with muscarinic-cholinergic agonists, such as lidocaine and histamine, have been used to stimulate secretion from the remaining salivary cells [23]. The prophylactic use of bethanechol, which is a cholinergic agonist, was shown to prevent SG dysfunction and RIX by increasing saliva secretion in a recent phase III trial [24]. Amifostine, a free radical scavenger, was utilized for RIX prevention; however, it has been associated with severe side effects and the undesirable effect of tumor protection [23].

Additionally, pretreatments with phenylephrine, isoproterenol, pilocarpine, and methacholine reduced the effects of radiation in mice for up to 60 days following irradiation but not after 120–240 days. In contrast, the pre-irradiation stimulation of muscarinic acetylcholine receptors combined with methacholine plus α-adrenoceptors and phenylephrine reduced both early and late SG damage [25]. Data from a recent systematic review of randomized clinical trials indicate that pilocarpine hydrochloride reduces RIX compared with placebo in 42–51% of patients. However, pilocarpine provokes several dose-dependent side effects due to parasympathomimetic stimulation, leading to a withdrawal rate of 6–15%, which limits the evidence to support its use in RIX management [26].

Deferoxamine (DFO), a bacteria-derived siderophore from *Streptomyces pilosus*, restored the secretory function of irradiated SMG when it was administered intraperitoneally in mice for three days before and/or after point-fixed irradiation (18 Gy). DFO enhanced angiogenesis, reduced acinar cell apoptosis, preserved SG stem/progenitor cells (Sca-1+), and restored SFR [27]. Lidocaine, which is a membrane stabilization agent, has a protective effect on the muscarinic agonist-induced water secretion capacity of acini in rabbits and preserves SG secretory function during radiotherapy by protecting carbachol-induced Ca2 + influx in acini [28,29].

A daily injection of histamine in 5 Gy irradiated mice reversed RIH, conserved glandular mass with normal appearance and preserved the structural organization of secretor granules three days post irradiation by suppressing ductal/acinar cells apoptosis, reducing apoptotic cells, and preventing radiation-induced decrease in cell proliferation [30]. In addition, a histamine H4 receptor ligand, JNJ7777120, completely reversed RIH, conserving glandular mass with normal histological appearance and reducing apoptosis and atrophy of SMG in irradiated mice [31].

Irradiation leads to the generation of reactive oxygen species, which are involved in the mechanism of SG damage and the impairment of fluid secretion. The activation of calcium-permeable channel transient potential melastatin-like 2 (TRPM2) channels *in vivo* is associated with increased oxidative microenvironments, resulting in long-term xerostomia. This effect was transitory in knockout mice. Additionally, subsequent recovery was noted after treatment with tempol (a free radical scavenger) or 3-aminobenzamide (a PARP1 inhibitor), suggesting that TRPM2 antagonists are targets for ameliorating RIX [32].

Stem cells

It has been proposed that salivary stem cell transplantation in post-irradiated HNC patients regenerates the function of the SG via the differentiation of these transplanted cells into functional SG cells [1,33]. Stem/progenitor cells can be harvested from the SG before the start of radiotherapy and returned to the salivary complex after radiotherapy has been completed to repopulate