Macrophages Come To The Rescue

Coppes, Robert P.

Published in:
Cancer Research

DOI:
10.1158/0008-5472.CAN-20-3499

Publication date:
2020

Macrophages Come To The Rescue

Robert P. Coppes[1,2]

The study by Zhao and colleagues, in this issue of *Cancer Research*, builds on previous work where they showed that transient activation of Hedgehog signaling within the murine submandibular gland rescued radiation-induced salivary gland dysfunction. The current study provides mechanistic insight into this phenomenon by showing that restoration of radiation-depleted resident macrophages through prorepair paracrine interactions with endothelial cells and epithelial progenitors rescued salivary function.

See related article by Zhao et al., p. 5531

In 2020, 4% of all cancers in the United States are expected to be diagnosed as head and neck cancer (HNC), with an estimated 65,630 cases. The majority of these patients will receive radiotherapy, resulting in a 5-year survival rate, depending on the tumor site, between 45% and 92% (1). A problem with radiotherapy is that it often results in unavoidable coirradiation of normal tissues, seriously compromising tissues such as the salivary glands. Although protocols have been developed to minimize the dose to the salivary glands, still 40% of patients with HNC receiving intensity modulated radiotherapy will experience moderate to severe xerostomia ("dry mouth syndrome"). When unstimulated salivary flow rate is reduced by about 50%, a sensation of oral dryness may occur (2). Salivary gland dysfunction and consequential xerostomia may lead to hampered speech, increased risk of oral infections and dental caries, difficulties with swallowing, food mastication, impaired taste, and nocturnal oral discomfort. These symptoms are extremely difficult to manage and can lead to a dramatic decrease in quality of life.

All cell types of the salivary glands have been shown to be affected by irradiation. Early after irradiation, apoptosis of acinar cells, membrane damage, impairment of microvessels, and reduced parasympathetic signaling have been shown to contribute to the resulting hyposalivation. Long-term, persistent chronic inflammation and fibrosis, and consequential consistent tissue dysfunction and atrophy coincide with a lack of regenerative potential of salivary gland stem/progenitor cells (3). Interestingly, especially in the ductal region where salivary gland stem/progenitor cells seem to reside (4), senescent cells accumulate after irradiation (5, 6). Indeed, the persistence of senescent cells is associated with reduced tissue regenerative capacities, whereas their repression can result in tissue rejuvenation (7), also of irradiated salivary glands (5, 6, 8). Interestingly, IL6 (5) and Sonic Hedgehog (Shh; ref. 8), as well as other prorepair factors, such as KGF, IGF1, HGF, C-CSF, and cytokine-producing mesenchymal, and salivary gland stem cells, have been shown to ameliorate salivary gland dysfunction postirradiation (2). These prorepair factors may turn the tissue balance from a chronic inflammatory and fibrosis-prone environment to a more regenerative favorable situation.

Now, Zhao and colleagues (9) show in a well-designed study that such interactions may be mediated through resident salivary gland macrophages that are in paracrine communication with epithelial progenitors and endothelial cells, rescuing radiation-affected murine submandibular gland (SMG) function.

They first showed that retrograde ductal installation of adenoviral gene transfer of *Gli1* and *Shh*, to locally and transiently activate the Hedgehog–Gli pathway, rescued salivary gland function in 15 Gy locally irradiated mice. This finding was substantiated by the observation that expression of Hedgehog target genes was accompanied with improved salivary flow and increased saliva secretory acinar cell marker, AQP5, expression 90 days after irradiation. Subsequent elegant lineage tracing experiments showed that after transient Hedgehog activation, $Gli1^+$ Hedgehog-responsive cells were comprised of an expanding small subset of macrophages, basal/myoepithelial, and endothelial cells. Gene expression analysis revealed that irradiation inhibited multiple macrophage-related pathways, HGF signaling, and angiogenesis, which were restored by transient Hedgehog activation. However, other cytokines often released by macrophages were not shown, but they could be relevant and may be interesting for further studies. Using flow cytometry, they showed that resident, but not infiltrating, macrophages were depleted after irradiation, but were restored upon transient Hedgehog activation. Depletion of resident macrophages using clodronate liposomes confirmed the suggested role of macrophages in the recovery of SMG from irradiation damage. Clodronate liposomes, however, are not very selective in removing only macrophages and may also affect other phagocytotic cells. Nevertheless, clodronate liposome treatment impaired the transient Hedgehog-activated recovery of irradiation-affected SMG function and acinar cell markers, indicating the importance of resident macrophages, but not excluding a potential role of other phagocytotic cells.

Next, the authors performed single-cell RNA sequencing of adult mouse SMGs. Seven-day transient Hedgehog-activated gland cells expressing $Ptch1^+$, as proof of responsiveness to Hedgehog-Gli signaling, were strongly increased in endothelial cells and ductal progenitors, albeit without increasing proliferation marker expression. The authors suggested that the latter was due to indirect induction of proliferation and indicated that an enrichment of putative resident Hgf^+ macrophages occurred upon Hedgehog activation. Similarly, Cd93 expression was strikingly increased in putative endothelial cells, as were the percentages of $Csf1^+$ and $IL34^+$ cells in all SMG cells resulting from direct and indirect Hedgehog activation as indicated by PTCH1 expression. $F4/80^+$ macrophages, $Epcam^{high}$ epithelial progenitor cells, and $Cd31^+$ endothelial cells, selected by flow cytometry, expressed higher levels of the corresponding factors. This indicates that resident macrophages may trigger a positive feedback loop with epithelial progenitors and endothelial cells through Csf1/IL34, Hgf,

[1]Department of Biomedical Sciences of Cells & Systems, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands. [2]Department of Radiation Oncology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.

Corresponding Author: Robert P. Coppes, University of Groningen, University Medical Center Groningen, Ant. Deusinglaan 1, Groningen 9713 AV, the Netherlands. Phone: 315-0361-6124; E-mail: r.p.coppes@umcg.nl

Cancer Res 2020;80:5462–3

doi: 10.1158/0008-5472.CAN-20-3499

and C1q signaling pathways. Analysis of mRNA expression of these genes in resident macrophages after irradiation and Hedgehog activation showed recovery of expression. To show a causal relation, however, gene-knockdown or -knockout experiments are needed.

Previously, this group reported (8) preservation of epithelial progenitors and promotion of angiogenesis upon transient Hedgehog activation after irradiation, and loss of this after macrophage depletion, supporting the idea that SMG macrophages, through paracrine action, take part in the rescue of SMG function. It has already been established that paracrine factors (3, 8) influence the response of the salivary gland to irradiation by improving the regeneration of parenchyma and supportive cells in the tissue. The study by Zhao and colleagues for the first time shows the involvement of macrophages in this process (9). Moreover, the macrophages seem essential for the rescue of salivary gland function after irradiation in a similar way homeostasis of the intestine is maintained (10). Some important questions, however, remain. For instance, what is the contribution of other phagocytotic cells and immune cells, such as natural killer cells and T cells, which are known to support the removal of senescent cells (7). A major characteristic of radiation damage to salivary glands is the accumulation of chronic inflammation and fibrosis with consequential tissue dysfunction and atrophy (3). C57Bl/6J mice although having a pronounced fibrotic response, do not show an extensive inflammatory response to irradiation, as seen in other murine models and humans. This may have led to an underestimation of not only the role of macrophages, but also that of other cells and factors involved in the immunogenic response to irradiation (2, 3). The 15 Gy single-dose irradiation used in this study is indeed relevant, as it is often used in experimental studies as animals are able to maintain their body weight as this dose, and it is equivalent to a clinically relevant fractionation dose of 32 Gy in daily 2 Gy fractions (3). It has, however, also been shown that salivary glands that receive up to 40 Gy fractionated radiation may partially maintain regenerative capability (2). Interestingly, previous experiments have shown that after 10 Gy single-dose irradiation, stimulation of regenerative processes do not add to the endogenous potential of the gland to regenerate; 15 Gy stimulation has a beneficial effect, whereas at 20 Gy there is no regeneration upon stimulation, showing a rather small window of opportunity (3). To substantiate the suggested potential applicability of macrophages and related paracrine interactions, it would, therefore, be relevant to study the phenomenon at different radiation doses. However, to circumvent the potential inability of progenitors to regenerate the tissue at higher irradiation doses, it may be beneficial to combine resident macrophage activation with transplantation of unirradiated autologous tissue-derived stem/progenitor cells that have been shown to rescue up to 80% of the salivary function (11).

In conclusion, Shh-activated SMG resident macrophages play an essential role in rescuing irradiation-induced hyposalivation through a paracrine interaction with endothelial and epithelial progenitors. In many cases it is still not possible to expose the salivary gland to only a low dose where endogenous repair is possible, even using the most modern radiation techniques. Therefore, activation of resident macrophages and related signaling should be further explored to develop novel approaches to treat irradiation-induced hyposalivation and consequential xerostomia. The use of such prorepair factors, however, remains a delicate issue as several approaches so far have failed because of interference with tumor response. However, the combination of locally applied regenerative therapies, including macrophage activation, could allow a sufficient rescuing of salivary function without affecting tumor control.

Authors' Disclosures

No disclosures were reported.

Received October 15, 2020; accepted October 19, 2020; published first December 15, 2020.

References

1. Cohen E. Head and Neck: Statistics. ASCO.org. Available at https://www.cancer.net/cancer-types/head-and-neck-cancer/statistics.
2. Vissink A, Mitchell JB, Baum BJ, Limesand KH, Jensen SB, Fox PC, et al. Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys 2010;78:983–91.
3. Barazzuol L, Coppes RP, van Luijk P. Prevention and treatment of radiotherapy-induced side effects. Mol Oncol 2020;14:1538–54.
4. van Luijk P, Pringle S, Deasy JO, Moiseenko VV, Faber H, Hovan A, et al. Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer. Sci Transl Med 2015;7:305ra147.
5. Marmary Y, Adar R, Gaska S, Wygoda A, Maly A, Cohen J, et al. Radiation-induced loss of salivary gland function is driven by cellular senescence and prevented by IL6 modulation. Cancer Res 2016;76:1170–80.
6. Peng X, Wu Y, Brouwer U, van Vliet T, Wang B, Demaria M, et al. Cellular senescence contributes to radiation-induced hyposalivation by affecting the stem/progenitor cell niche. Cell Death Dis 2020;11:854.
7. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. Cellular senescence: defining a path forward. Cell 2019;179:813–27.
8. Hai B, Zhao Q, Deveau MA, Liu F. Delivery of Sonic Hedgehog gene repressed irradiation-induced cellular senescence in salivary glands by promoting DNA repair and reducing oxidative stress. Theranostics 2018;8:1159–67.
9. Zhao Q, Zhang L, Hai B, Wang J, Baetge CL, Deveau MA, et al. Transient activation of the Hedgehog-Gli pathway rescues radiotherapy-induced dry mouth via recovering salivary gland resident macrophages. Cancer Res 2020;80:5531–42.
10. De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, et al. Self-maintaining gut macrophages are essential for intestinal homeostasis. Cell 2018;175:400–15.
11. Maimets M, Rocchi C, Bron R, Pringle S, Kuipers J, Giepmans BN, et al. Long-term *in vitro* expansion of salivary gland stem cells driven by Wnt signals. Stem Cell Reports 2016;6:150–62.

Macrophages Come To The Rescue

Robert P. Coppes

Cancer Res 2020;80:5462-5463.