Stem Cell Based Treatment of the Radiation-Induced Xerostomia

Final Report

Radiotherapy is currently the method of choice for the treatment of the ~500,000 patients newly diagnosed worldwide each year with head and neck cancer. While radiotherapy is substantially efficacious, the quality of life for these patients is severely compromised due to damage caused by the treatment to the salivary glands resulting in Xerostomia. Attenuation of Xerostomia would significantly improve the quality of life in these patients, while potentially improving the beneficial effects of radiotherapy by permitting higher radiation doses. The purpose of this project is to determine whether intervention through the anti-oxidative stress and cell survival signaling potential of IL-6 through the proprietary fusion protein, Hyper-IL-6 (HIL-6), can prevent the development of radiation-induced Xerostomia. The project has started and the close collaboration between the two groups has been solidified.

In the initial phase of the project we started to optimize the production of the recombinant IL-6 and Hyper-IL-6 proteins. Specifically, we added a hexa-histidine sequence to the COOH-terminus of the Hyper-IL-6 protein, which greatly facilitates the purification procedure. Testing of the biologic activity of this altered protein showed an even increased biologic activity.

Furthermore, we started to expand our mouse colony of sgp130Fc transgenic mice, which overexpress the specific IL-6 *trans-signaling* inhibitor sgp130Fc (see proposal). This protein exclusively inhibits signals via the IL-6/sIL-6R complex, whereas responses mediated via IL-6 alone remain unaffected. These mice will be needed for the proof of principle studies, aiming to demonstrate that IL-6 *trans-signaling* is the relevant mechanism for the gp130-mediated improvement of Xerostomia.
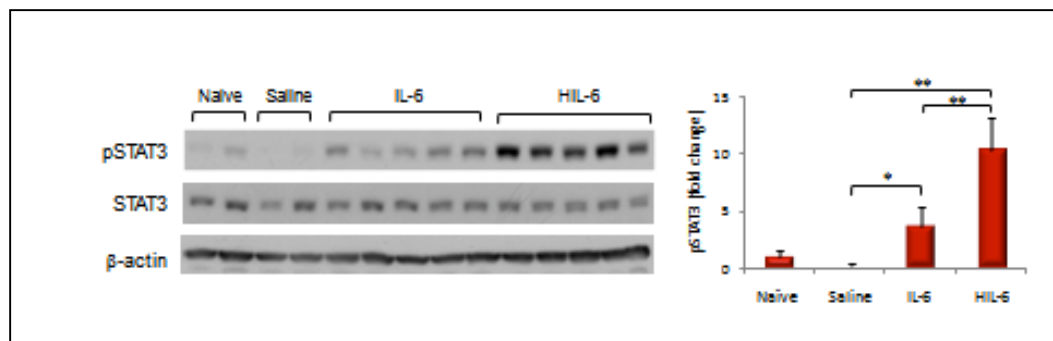
Fig. 1. IL-6 and HIL-6 infusion activates STAT3 in the submandibular gland. Western blot analysis and quantification of p-STAT3 (Tyr-705) and STAT3 in protein extracts of submandibular glands one hour following infusion of IL-6 (15 ng) or HIL-6 (50 ng). Data are means ± SD (n = 2-5). *P<0.01, **P < 0.001.

Our efforts first concentrated on *Specific Aims 1& 2* (see proposal), which were to determine and optimize the effect of local administration of HIL-6 on IR-induced Xerostomia, and to characterize the in vivo activation of IL-6 signaling pathways in cells of the salivary gland following local administration of HIL-6 or IL-6. Western blot analysis of p-STAT3 (Tyr705) levels of salivary gland protein extracts following infusion of either IL-6 or HIL-6 was used to determine the consistency of the protein infusion technique and to determine the strength of the protein activity. Fig. 1 demonstrates that the effect of HIL-6 is clearly superior to that of IL-6 in activating STAT3 in the salivary gland. However, it also appears that significant levels of STAT3 activation are induced following infusion of IL-6 as well. Thus, while our original observations indicated that the expression of the IL-6R in the salivary gland was relatively low and similar to that of the kidney, the present data suggest that certain cells in the salivary gland express IL-6R and respond to IL-6 infused into the gland ducts. Moreover, this analysis also indicates that the technique of infusion of the cytokine induces a relatively consistent response.

In order to understand the implications of this IL-6 signaling we compared the effect of IL-6 and HIL-6 infusion on protection of the salivary gland function following irradiation. Fig. 2 demonstrates that both IL-6 and HIL-6 have substantial efficacy in ameliorating radiation-induced Xerostomia. This effect of both IL-6 and HIL-6 appeared to be consistently reproducible in many experiments.
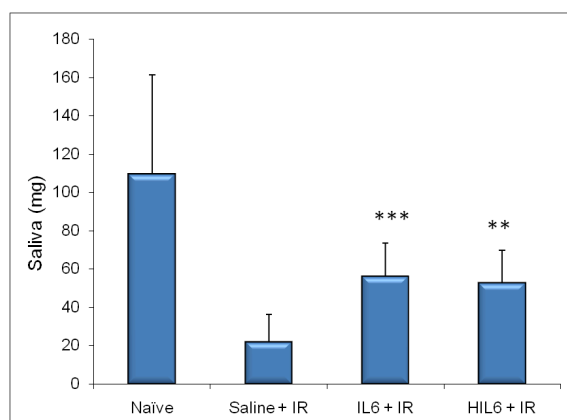
Fig. 2. IL-6 and HIL-6 preserve salivary gland function following irradiation. Saliva secretion in saline treated mice or mice treated with equimolar amounts of either HIL-6 (50 ng/gland), IL-6 (15ng/gland). **P< 0.01, ***P<0.01 *versus* Saline controls, (n=6) .

In order to establish whether the both cytokines display a similar efficacy in protective effect, we performed a dose response analysis on each protein. A comparison of the dose-response of the salivary gland to either IL-6 or HIL-6 established that both proteins appear to be effective

in ameliorating IR-induced Xerostomia over a range of concentrations (Fig. 3). However, while IL-6 appeared to have maximal efficacy at a dose of about 15ng/gland and began to diminish at higher concentrations, HIL-6 appeared to be effective over a broad range of concentrations. An optimal dose of HIL-6 seemed to be 50 ng/ml.
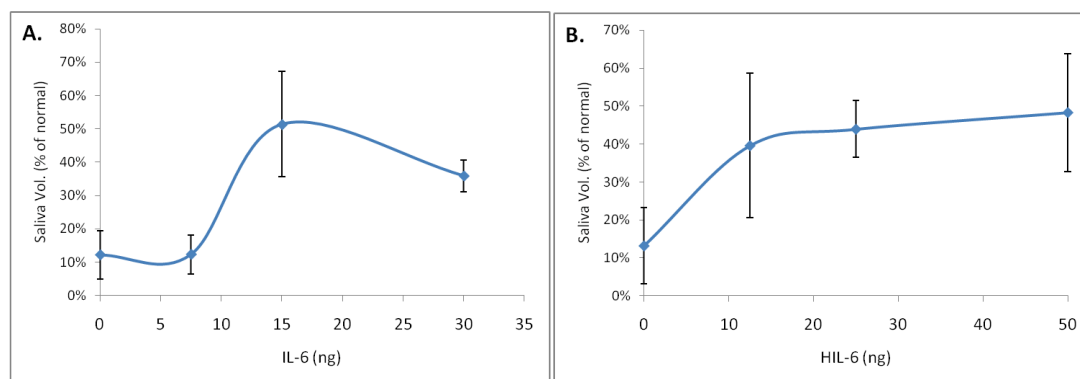
Fig. 3. Dose-Response analysis of IL-6 and HIL-6 in amelioration of radiation-induced xerostomia. Infusion of either IL-6 (A) or HIL-6 (B) to the saliver glands of female mice (C57BL/6) was performed over a range of doses prior to exposure to radiation (13 Gy) to the head and neck. Salivary gland function was determined by pilocarpine induced salivation 2 months following radiation exposure.

These observations indicated that both IL-6 and HIL-6 had significant potential for protecting the salivary gland from radiation-induced damage, but that HIL-6 appeared to be more effective over a broader range of dosages.

Our efforts then centered on the aims outlined in *Specific Aims 2 and Specific Aim 3* in determining the location and types of the cells affected by IL-6 and HIL-6 infusion. In order to approach aims, we carried out immunostaining of salivary glands for p-STAT3 and p-AKT following infusion of IL-6 and HIL-6 (Fig. 4). Initially, our immunostaining protocol for p-STAT3 was experiencing technical difficulties which had to be overcome, and immunostaining for p-AKT which had been performed in our lab in mouse liver had to be established in salivary glands. Since we had established that certain cell populations in the salivary gland appeared to express IL-6R and responded to IL-6 infusion by amelioration of IR-induced damage, we also aimed to identify IL-6R positive cells by immunostaining of frozen sections. Similarly, we established immunostaining for c-Kit in the salivary gland as a marker for salivary gland stem cell populations. Following establishment of these procedures, we intended to perform co-staining experiments in order to determine whether the IL-6R positive cells are also the putative c-Kit positive stem cell populations. In addition, we used these procedures in order to assay the effect of IL-6 and HIL-6 treatment on the ability of these cells to survive following radiation.
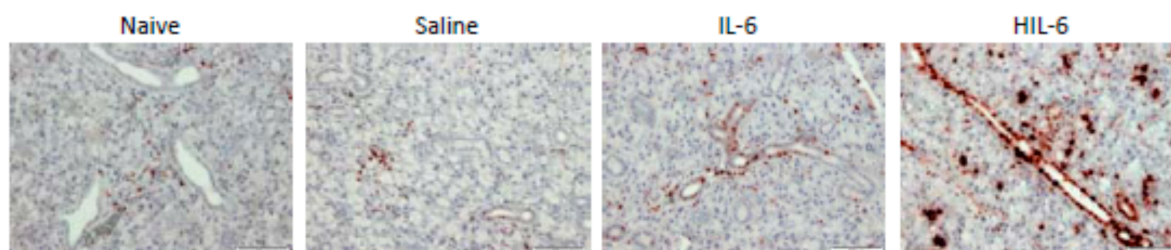
Fig. 4. STAT3 activation in salivary glands following infusion of IL-6 and HIL-6. Representative images of thin sections of submandibular salivary glands infused with IL-6 (15 ng), HIL-6 (50 ng) or normal saline for 45 minutes and immunostained for pSTAT3 (red nuclear staining). Bars are 100μm).

Preliminary results (see proposal *Preliminary Results*) suggested that the salivary glands expressed very low levels the IL-6 receptor (IL-6R), at levels similar to that expressed in the kidney, and as such were not expected to respond to IL-6 in a classical signaling mechanism, but may respond to IL-6/sIL-6R complex (i.e. HIL-6) in a *trans*-signaling mechanism. Thus, our primary hypothesis was that local administration of HIL-6 to the salivary gland prior to radiation would prevent or reduce radiation-induced ROS-mediated injury to the salivary gland and the subsequent development of salivary hypofunction. Indeed, data demonstrated that local administration of HIL-6 to the salivary gland induces a significant radio-protective potential in treated animals (see *Proposal*). However, subsequent Western blot analysis of p-STAT3 (a primary signal transduction mediator of IL-6 signaling) in salivary gland following infusion of either IL-6 or HIL-6 demonstrated that both proteins could activate STAT3 in the salivary gland, although the strength of HIL-6 was clearly superior, indicating the presence of IL-6R at least on some cell populations. Immunofluorescence analysis further suggested that IL-6R may be expressed in ductal cells, but also in minor unidentified cell populations of the paranchyma (Fig 5). We are presently attempting to confirm these observations by immunostaining for pSTAT3 following infusion with IL-6. However, due to problems with our pSTAT3 staining protocol we had to confirm this observation in these cell populations.
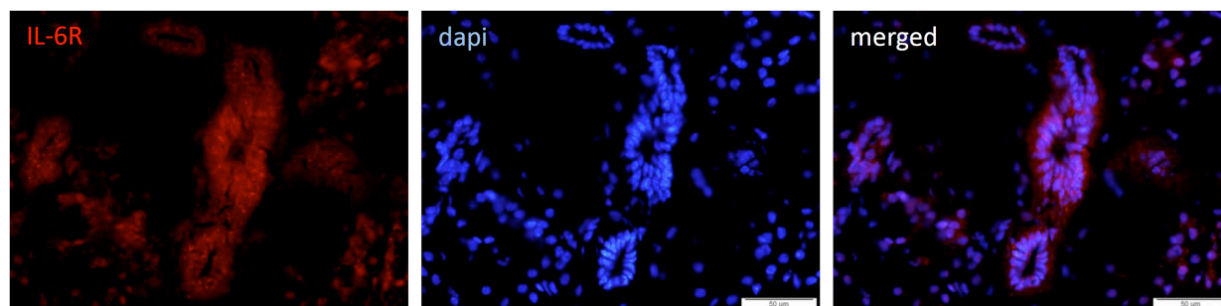
Fig. 5. Immunofluorescence staining for IL-6R in the murine submandibular salivary gland indicates membranal expression in ductal cells. Bars are 50μm.

Nevertheless, we established that infusion of either protein, at a dosage optimized as described above, could significantly preserve salivary gland function following irradiation at a single dose of 13Gy (Fig. 2). This effect has proven to be highly reproducible in our hands.

In human patients, radiotherapy typically involves exposure to radiation doses broken into smaller multiple, or 'fractionated' doses given on a daily basis. This dosing regimen is designed to better target and kill quickly dividing cancer cells and sparing non-dividing normal tissue. In order to approximate the clinical setting, we also tested the efficacy of HIL-6 and IL-6 in preventing Xerostomia induced by radiation administered in a fractionated dosing regimen. Fig. 6 shows that infusion of HIL-6 protein at an optimized in our single-fraction studies, administered 3 hours prior to exposure to 26Gy in 5 fractions (5.2Gy/day for 5 days) also significantly preserved salivary gland function in comparison to saline control treated mice. Interestingly, in the fractionated irradiation dosing regimen, IL-6 treatment appears to be significantly less effective than HIL-6 in preserving salivary gland function and was marginally but not statistically significantly different than Saline control treated mice (*P*=0.089). Thus, we have established that while both HIL-6 and IL-6 can significantly prevent irradiation-induced the loss of salivary gland function, HIL-6 appears to have a substantial advantage in radiotherapy protocols approximating the clinical setting.
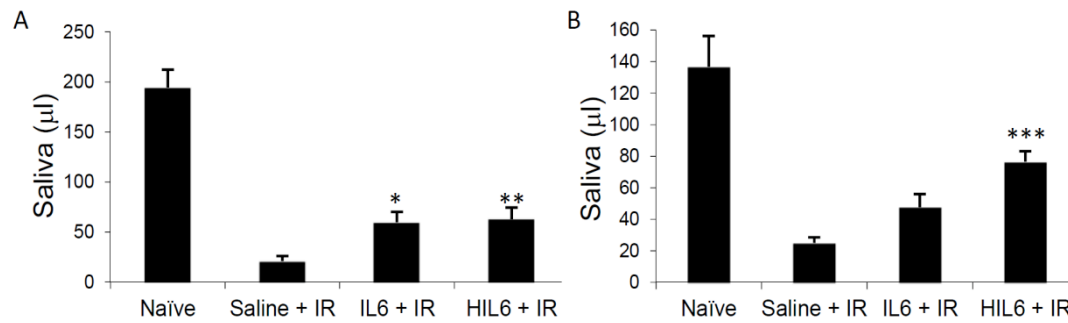
Fig. 6. IL-6 and HIL-6 infusion ameliorate loss of salivary gland function following irradiation. Saliva secretion in saline treated mice or mice treated with either HIL-6 (50 ng/gland), IL-6 (15ng/gland), or normal saline prior to exposure to high dose ionizing radiation in (A) a single fraction (13Gy), or (B) fractionated doses (5 x 5.2Gy). *$P<0.05$, **$P< 0.01$ *versus Saline*, and *** $P<0.001$ *versus* Saline and <0.05 *versus* IL-6; (n=6-7).

In accordance with our 'Plan of Operation' (see *Proposal*), we analyzed the salivary gland tissue samples obtained at early (1-3 days) and late (8 weeks) time points following irradiation in order to identify potential mechanisms through which HIL-6 treatment prevented Xerostomia. Histological analysis of salivary gland samples following irradiation indicated that long-term loss of function was associated primarily with gross morphological changes in acinar cells, but that ductal structures appeared to be largely spared. Our analysis by pSTAT3 immunostaining indicated that ductal cells were the cellular population most directly affected by HIL-6 infusion.

Thus, a paradox existed as to the mechanism of HIL-6 and IL-6 action. Moreover, the mechanism leading to loss of gland function was also unclear, as well as how that mechanism was affected by HIL-6 or IL-6 infusion. Since irradiation-induced tissue injury was thought to mainly occur through a mechanism of oxidative stress and IL-6 induces anti-oxidative stress response, in particular through the up-regulation of genes such as HO-1 and Ref-1, our initial hypothesis has invoked the anti-oxidative stress potential of IL-6 as a potential mechanism through which HIL-6 protects the salivary gland. To evaluate this hypothesis, we have set up protocol to determine oxidative stress levels based on 8-OH-Guanine immunostaining, and applied this method to measure oxidative stress levels following irradiation. Apoptosis has also been proposed as one of the primary mechanisms by which radiation-induced damage leads to loss of salivary gland function. Studies have reported that radiation-induced apoptosis reached peak levels 48 hours post IR and could involve up to approximately 6 percent of the cells. However, our analysis by TUNEL staining of salivary glands taken from mice 24, 48 and 72 hours post-irradiation could not substantiate these reports and revealed only sporadic apoptotic cells following irradiation apoptotic cells even at a dose of 17Gy. We observed substantially less than 1 percent of cells undergoing apoptosis at these times (data not shown).

We therefore believe that other processes may underlie the loss of gland function, and investigated other possible mechanisms, including that of radiation-induced senescence. To this end we have examined the presence of senescence-associated markers in the salivary gland post-IR that correlate with the protective effect of HIL-6 or IL-6. One molecular marker associated with senescence is the cell cycle regulator, p21. Immunostaining revealed that p21 expression was upregulated in a variety of cell types in irradiated salivary glands (Fig. 7A). Positively staining was observed mainly in acinar cells that presented strong morphological changes following irradiation. However, some less intense sporadic staining in ductal cells were also evident. Positively stained cells were evident even 8 weeks following irradiation. Interestingly, animals administered HIL-6 or IL-6 prior to irradiation exposure displayed significantly fewer p21-positive cells, in direct correlation with the salivary gland function in

these mice (Fig 7B). We therefore believe that p21 is directly associated with the processes involved in loss of salivary gland function. Thus, our immediate operational plan is to strengthen our understanding of p21 related processes by determining the behavior of other senescence and cell cycle related markers at early and late times post-irradiation.
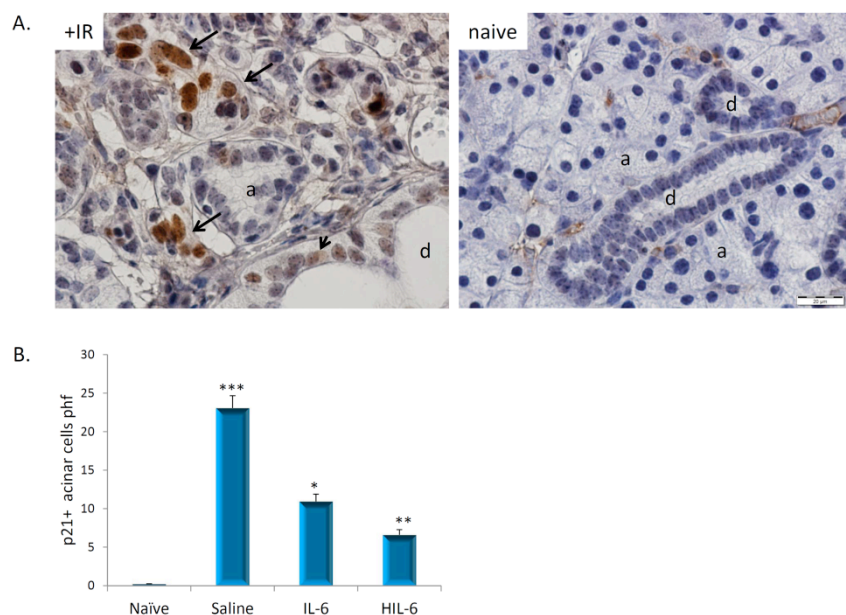

Fig. 7. p21 expression in salivary glands is upregulated following irradiation. A) p21 immunostaining (brown nuclear staining) in submandibular glands 8 weeks following irradiation. (a-acini, d–duct). Bars are 20µm. B) Quantification of p21+ acinar cells in irradiated mice pretreated with HIL-6, IL-6 or Saline. (n=6-7)   ***P<0.001 *versus* Naïve, **P<0.01 *versus* Saline and <0.05 *versus* IL-6; *P<0.05 *versus* Saline.

We next set out to determine a mechanistic basis of IL-6 and HIL-6 induced protection of the salivary gland to ionizing radiation. Since irradiation-induced tissue injury is thought to mainly occur through a mechanism of oxidative stress induced apoptosis, and IL-6 induces both anti-oxidative stress and anti-apoptotic responses in cells, our initial hypothesis invoked this as a potential mechanism through which IL-6/HIL-6 protects the salivary gland. Apoptosis has previously been cited as one of the primary mechanisms by which radiation-induced damage leads to loss of salivary gland function. Published studies have reported that radiation-induced apoptosis reaches peak levels 48 hours post IR and can involve up to approximately 6 percent of the cells. We therefore analyzed apoptosis in the salivary glands of mice in our experimental model. However, in our hands, and contrary to previously published studies, TUNEL analysis of salivary glands taken from mice 24, 48 and 72 hours post-irradiation revealed only focal populations of cells undergoing apoptosis following irradiation at doses of 13-15Gy (Fig. 8). Only at a dose of 17Gy were TUNEL positive cells observed in significant levels in both duct cells and acini. Interestingly, these were primarily ductal cells. However, this response did not appear to represent activation of apoptosis, because it was not accompanied by either caspase-3 activation (Fig. 9), or by fractured nuclei typical of apoptotic cells in H&E stained thin sections (data not shown). We therefore conclude that apoptosis is probably not a pronounced mechanism underlying IR-induced Xerostomia in these mice.
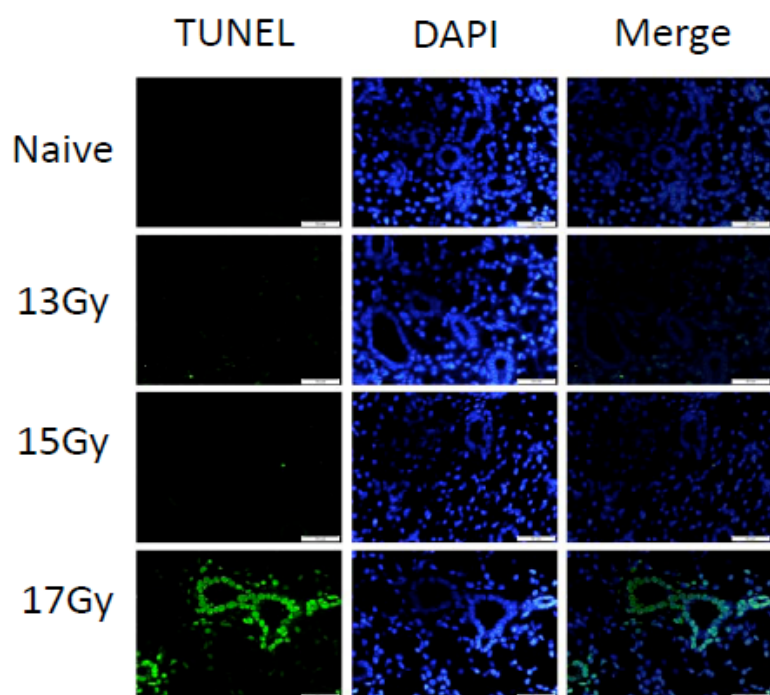
Fig. 8. TUNEL labeling indicates DNA breaks at high-dose irradiation. TUNEL analysis was performed on salivary glands removed 48 hours following exposure to irradiation at increasing doses. Scale bar, 50 μm.
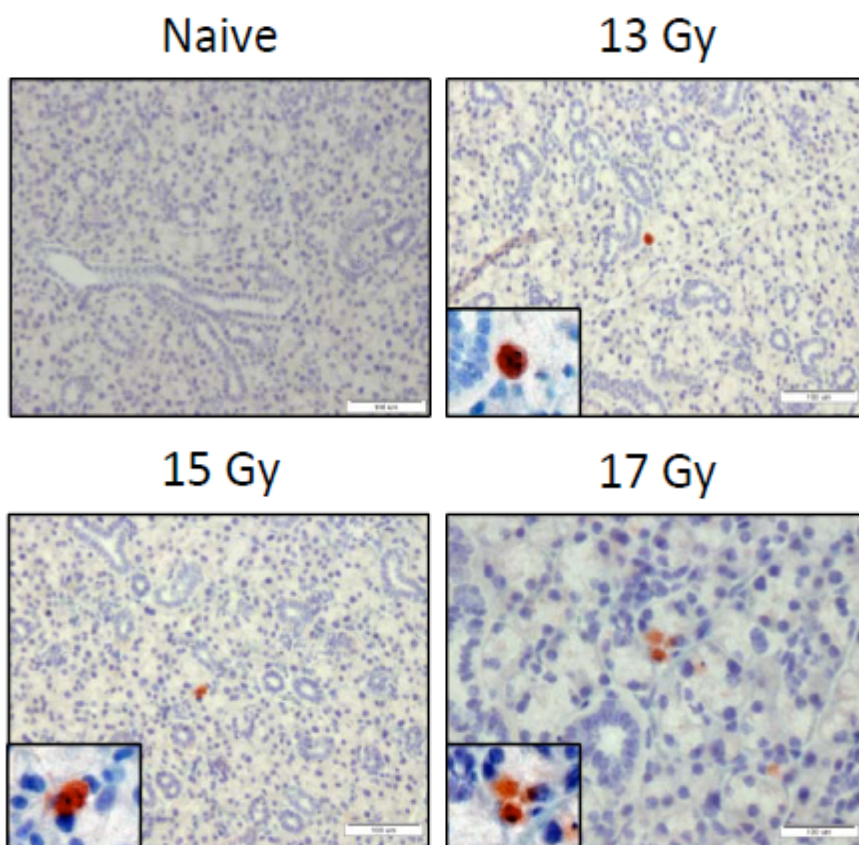
Fig. 9. Caspase 3 immunostaining in submandibular salivary glands suggests limited apoptotic response to high-dose irradiation. Caspase 3 immunostaining (red stain, and magnified image in inset) was performed on salivary glands excised 48 hours following exposure to irradiation in in increasing doses indicated. Scale bar, 100 μm (Naïve, 13Gy, 15 Gy) and 50 μm (17Gy).
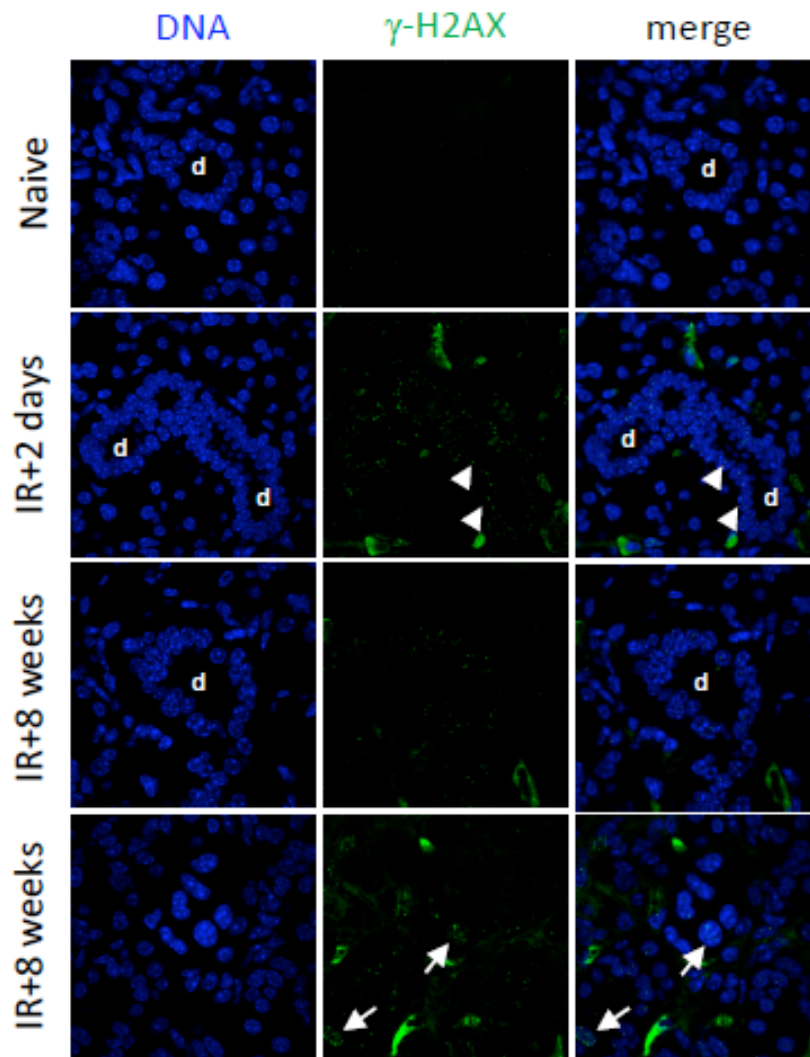
Fig. 10. DNA breaks in cells of the salivary gland persist 2 months following irradiation. γ-H2AX immunofluorescence staining (green punctate staining in nuclei (arrow heads) in submandibular salivary glands counterstained with dapi from naïve and irradiated (13Gy) mice is observed predominantly in duct cells (d). Giant nuclei in acinar cells with multiple γ-H2AX foci (arrows) are evident at 8 weeks post-IR.

Apoptosis is only one of the possible expected outcomes of DNA damage induced by ionizing radiation. Therefore, in order to determine the role of DNA damage in the salivary glands, we examined DNA damage response (DDR), as indicated by the presence of phosphorylated γ-H2AX protein in salivary gland cells, over time post-irradiation (Fig. 10). This analysis revealed that nuclear γ-H2AX immunostaining was most prominent 48 hours post-IR and appeared to be most strongly present in ductal cells, but also to significant levels in acinar cells. Surprisingly, positive staining for γ-H2AX appeared to persist in the irradiated gland up to 8 weeks post-IR especially in giant acinar cells but also in the duct cells, suggesting the persistence of DNA damage. The persistence of DNA damage is an indication of cell senescence, and is one of the expected outcomes of exposure to ionizing radiation. Senescence is commonly associated with the expression of a variety of markers, including expression of the lysosomal β-galactosidase (SA-β-GAL) as well as cell cycle inhibitors $p16^{INK4}$, p53, $p21^{CIP1}$, DcR2, and also $p19^{ARF}$. Cells undergoing senescence also exhibit profound changes in the cells secretome, called the senescence associated secretory phenotype (SASP). SASP genes included plasminogen activator inhibitor-1 (PAI-1), IL-8 (KC and MIP-2 in mice) and IL-6. But, irradiated salivary glands, 2 weeks post-IR, also display a significantly increased expression of senescence associated genes including p21, DcR2, p19, PAI-1 and IL-6, the increase of which, with the exception of PAI-1, persists at least 2 months following irradiation (Fig. 11A). Of the senescence-associated genes tested, p21mRNA was the most significantly up-regultaed following irradiation. P21 immunostaining revealed dramatic staining in acinar cells with distended morphology, including giant and multiple

nuclei, with notable, although less intense staining in ductal cells evident (Fig. 11B). These types of cells also appeared to be DcR2 positive by immunostaining (Fig. 11B). Following irradiation, the sali-
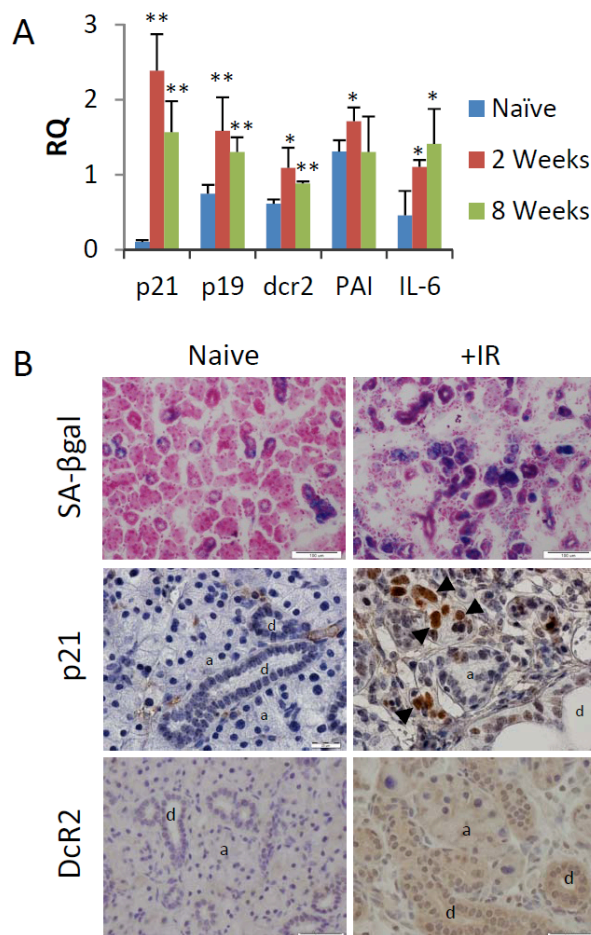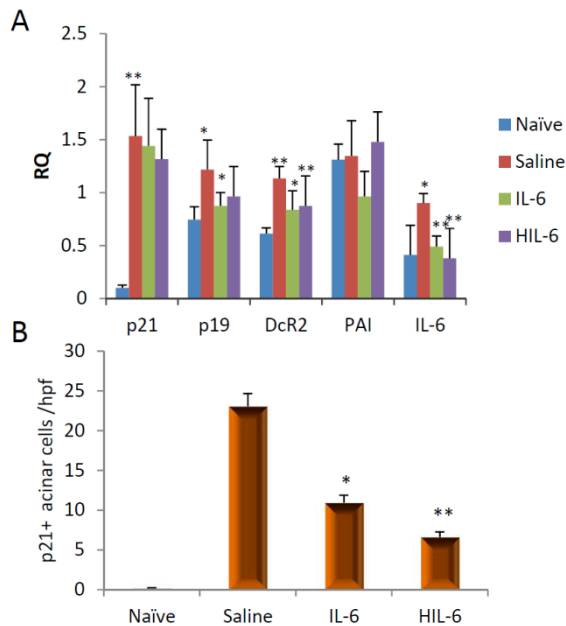

Fig. 11 Persistent expression of senescence markers in submandibular salivary glands 2 months following irradiation. (A) Analysis of mRNA levels of senescence associated genes in mouse submandibular salivary glands 2 and 8 weeks following irradiation (13Gy) by qPCR. (B) *In situ* SA-β-gal staining in frozen sections and immmunostaining of p21 (brown nuclear stain) (arrow heads indicate positively stained acini with giant nuclei) (Scale bars, 100 μM); and DcR2 in paraffin embedded thin sections of salivary glands before and 8 weeks post-irradiation (5.2Gy x 5). a, acini; d, duct. (Scale bars, 50 μM).

vary glands display a strong increase in SA-β-gal activity, particularly in ductal cells, but also in some acini (Fig. 11B). Taken together, these observations indicate that cellular senescence, rather than apoptosis, involving both ductal cells and acinar cells, is a dominant and persistent outcome of irradiation in the salivary gland. This suggests that senescence may be one of the main mechanisms mediating the loss of salivary gland function following irradiation.

We next reasoned that if IR-induced Xerostomia is mediated by senescence, then prophylactic treatment of the salivary glands with IL-6 or HIL-6 may be expected to reduce the expression of senescence associated markers. Analysis the salivary glands 8 weeks post-IR revealed that both IL-6 and HIL-6 treatment significantly reduced the expression of senescence-associated genes, including p19, DcR2, and the SASP associated cytokine, IL-6 (Fig. 12A). Interestingly, there was no clear difference in the levels of these mRNAs between the IL-6 treated and HIL-6 treated mice. However, p21 immunostaining of submandibular salivary glands revealed that HIL-6 treatment significantly reduced the numbers of p21 positive acinar cells, and in this respect was more efficient than IL-6 treatment (Fig. 12B). Thus, the formation of the p21^{+} acinar cells and their reduction by HIL-6 treatment correlated directly with the level of protection of salivary gland function. These observations suggest that preventive effects of IL-6 and HIL-6 pretreatment may be directly related to senescence and the formation morphological changes observed in the acinar cells. In our next steps we hope to determine:

1) the effect IL-6 and HIL-6 treatment on the DDR; and 2) the effect of radiation on salivary gland progenitor cells and how they are affected by IL-6 or HIL-6 treatment.

Fig. 12. HIL-6 and IL-6 pre-treatment reduces persistent expression of senescence markers in submandibular salivary glands 2 months following irradiation. (A) Analysis by qPCR of mRNA levels of senescence associated genes submandibular salivary glands of mice 8 weeks following irradiation (5.2Gy x 5) and pretreated with IL-6 (15 ng), HIL-6 (50 ng), or normal Saline. *P<0.05, **P<0.01, by Student's t Test. Comparisons are for Saline *versus* Naïve, and IL-6 or HIL-6 *versus* Saline. (n=4-7) (B) Quantification of p21 positive acinar cells in submandibular salivary glands from (A) following p21 immunostaining as described in Fig 8B. *P<0.05 and **P<0.01 versus Saline and P<0.05 versus IL-6; (n=4-5), by Student's t Test.

In our proposal, we had hypothesized that radiation-induced processes leading to the loss of salivary gland progenitor cells may be responsible ultimate deterioration of salivary gland function over time. Previous reports have indicated that salivary gland progenitor cells express cell surface markers c-Kit and Sca-1. In order to determine whether c-Kit$^+$ cells diminished 8 weeks following irradiation we have utilized an immunofluorescence staining protocol (Fig. 13) on naïve and irradiated tissue samples. Our determination of c-Kit$^+$ cells by in either paraffin embedded sections or frozen tissue sections did not show significant differences in irradiated versus naïve samples, leading us to suspect the specificity of the staining protocol (data not shown). In order to overcome this obstacle, we used a FACS based protocol for the assessment of freshly isolated c-Kit$^+$/Sca-1$^+$ salivary gland cells.
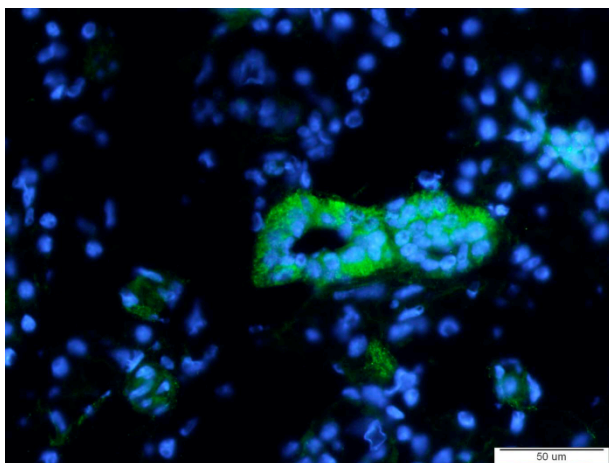
Fig. 13. c-Kit immunofluorescence staining in submandibular salivary gland. cKit postitive staining (green) was observed mainly ductal cells, but also at lower levels in unidentified parenchymal cells. Bar is 50 µm.

In the final aim of our *Proposal* we have utilized our sgp130Fc transgenic mice to determine the effect of blocking IL-6 *trans*-signaling on IR-induced Xerostomia. Sgp130Fc is a recombinant analog of the native sgp130 protein that competitively inhibits gp130 mediated trans-signaling by binding to and sequestering IL-6/sIL-6R protein complexes. Sgp130Fc transgenic mice been described in previous reports by our group. The purpose of this

experiments was to determine whether IL-6 *trans*-signaling plays an inherent role in the maintenance of salivary gland function following IR. If this hypothesis was correct, we would anticipate that in the presence of sgp130Fc the severity of IR-induced Xerostomia would increase. Comparison of the effect of 14Gy radiation on sgp130Fc transgenic mice versus wild type mice suggests that inhibition of IL-6 trans-signaling by sgp130Fc may slightly increase the loss of salivary gland function (Fig. 14). However, the effect was not dramatic and did not reach statistical significance (P=0.054). Nevertheless, it is possible that more significant differences in the effect of sgp130Fc may become apparent at lower doses of radiation, or alternatively after exposure to radiation in a fractionated dosing regimen.
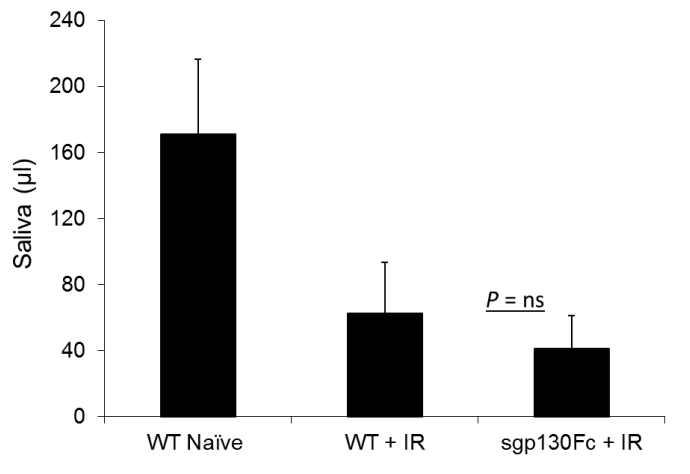
Fig. 14 sgp130Fc Transgenic mice do not exhibit significantly enhanced loss of salivary gland function following irradiation. Saliva secretion in mice 8 weeks following exposure to single fraction irradiation (13Gy) (n=6-12).

Conclusion:

During the execution of this research grant, we have made significant progress in our understanding of the potential of HIL-6 as a therapeutic agent in the prevention of radiation-induced Xerostomia, as well as uncovering a potential molecular and cellular mechanistic basis underlying the loss of salivary gland function following irradiation. Our progress includes: (i) establishment of the principle of amelioration of IR-induced Xerostomia by HIL-6 and IL-6 protein infusion; (ii) establishment of a dosing/treatment regimen; (iii) establishment of the reproducibility of treatment; (iv) verification of treatment efficacy following a fractionated radiation dosing regimen; (v) correlating persistent DDR and senescence as a probable mechanism underlying xerostomia; and (vi) correlating the effect of HIL-6 and IL-6 with determination of the molecular mechanism of HIL-6 efficacy. Future studies will be aimed at continued testing of our primary hypothesis that the effect of HIL-6 on salivary gland progenitor cells as a basis of IL-6/HIL-6 mediated protection, and will also continue to determine a molecular mechanism underlying the protective effect of IL-6 signaling on the background of IR-induced DNA damage.

The current state of knowledge of the activity of HIL-6 on salivary glands let us hypothesize that phase I clinical trials should be initiated to assess the potential of HIL-6 as a therapeutic agent. This project has paved the way of the promising therapeutic protein HIL-6 to the use in the clinic.

1. Deviations from the proposal
None.

2. Changes in prospectives of the goals
None.

3. Results from third party relevant to the project
None.

4. Changes of goals
None.

5. Continuation of development
During the entire period no important changes as compared to the initial proposal.

6. Publications of the research results (outside of the final report)
None.

7. Patent applications
None.

Supporting Literature

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ERFOLGSKONTROLLBERICHT

BIO-DISC5: Stammzell-basierte Behandlung von Strahlungsinduzierter Xerostomie
Förderkennzeichen: 1315797
Prof. Dr. Stefan Rose-John, Universität Kiel

1) Beitrag des Ergebnisses zu den förderpolitischen Zielen des Förderprogramms

Es handelt sich um ein Programm zwischen dem deutschen Ministerium für Erziehung und Forschung (BMBF) und dem israelischen Ministerium für Wissenschaft und Technologie (MOST), das vor über 40 Jahren gegründet wurde. Das Projekt BIO-DISC5 hat im Rahmen dieses Programms zu einer weiteren Vernetzung von deutschen und israelischen Wissenschaftlern geführt und hat die Grundlagen für eine weitere Zusammenarbeit gelegt. Es ist abzusehen, dass die Ergebnisse zu einer gemeinsamen klinischen Studie zur Behandlung der Xerostomie führen werden.

2) Wissenschaftlich-technisches Ergebnis des Vorhabens

Strahlentherapie, wird weltweit bei etwa 500.000 Tumor-Patienten angewendet. Während diese Therapie bei etwa 50% der Patienten erfolgreich ist, ist die Lebensqualität bei 80% der Patienten massiv beeinträchtigt. Dies liegt an der bestrahlungsbedingten Schädigung der Speicheldrüsen. Die resultierende Xerostomie wirkt sich auf Kauen, Schlucken und Sprechen aus und bewirkt Mundhöhlenentzündungen, Zahnverfall und Veränderungen in der Geschmacksempfindung. Falls es möglich wäre, die Xerostomie zu mildern, könnten die positiven Auswirkungen der Strahlentherapie durch höhere Bestrahlungsdosen noch verbessert werden. Wir konnten zeigen, dass die Xerostomie durch eine Schädigung der Speicheldrüsen und durch Schädigung von Nervenzellen und Endothelzellen hervorgerufen wird. Es zeigte sich, dass eine Korrelation zwischen der Schädigung und intrazellulären redox-aktiven Metallionen besteht. Weiter wurde demonstriert, dass die Bestrahlung die Zerstörung von Speichelzellen-Granula bewirkt, wobei sich der Inhalt der Granula in das Zellinnere ergießt wodurch freie Radikalen und reaktive Sauerstoffspezies entstehen. Es konnte gezeigt werden, dass das anti-oxidative und zellschützende Potential von Interleukin-6 die Entwicklung von strahlungsbedingter Xerostomie verhindern kann. Hierbei konnten wir im Tierversuch das therapeutische Potential eines von uns generierten Designerprotein (Hyper-IL-6) bei der strahlungsbedingten Xerostomie klar demonstrieren.

3) Fortschreibung des Verwertungsplans

Im Rahmen des Projektes wurden keine neuen Schutzrechte angemeldet. Wir konnten allerdings auf der Basis einer bereits getätigten Anmeldung weitere Daten erheben. Derzeit sind wir bemüht, Investoren zu finden, die bei einer Translation unserer Ergebnisse in die Klinik weiterhelfen können. Mit einer GMP-Produktion unseres Designerproteins Hyper-IL-6 soll noch in diesem Jahr begonnen werden.

4) *Arbeiten, die zu keiner Lösung geführt haben*

Wie im Abschlussbericht ausführlich dargelegt, sind wir anfänglich der Hypothese nachgegangen, dass der Mechanismus des programmierten Zelltods eine zentrale Rolle in der Xerostomie einnimmt. Es hat sich allerdings während des Fortgangs des Projektes gezeigt, dass zelluläre Seneszenz und die Schädigung durch Sauerstoffradikale ein zentraler Schädigungsmechanismus bei dieser Erkrankung ist.

5) *Präsentationsmöglichkeiten für mögliche Nutzer*

Die erzielten Ergebnisse werden derzeit zur Publikation in einer internationalen biomedizinischen Fachzeitschrift vorbereitet und es ist geplant, die Ergebnisse noch dieses Jahr auf einschlägigen Fachkonferenzen vorzustellen.

6) *Einhaltung der Ausgaben- und Zeitplanung*

Die Ausgaben- und Zeitplanung wurde eingehalten.