salispheres contained cells that can be grown in vitro into all salivary gland lineages, showing the capability to differentiate. These observations mirror those reported in the mouse, and suggest that the human salivary gland may contain a population of stem cells with therapeutic potential, in terms of rescuing function of xerostomic salivary glands.
In order to test this hypothesis, we transplanted cells from dispersed human salispheres into irradiated glands of immunedeficient mice. C-Kit-expressing cells from human salispheres transplanted into the gland developed into cells expressing proteins associated with both acinar cell lineages. Considering that c-Kit+ cells reside in their naúve state as ductal cells, we thus demonstrate the multipotentiality of c-Kit+ cells from the human salivary gland. Such transplanted c-Kit+ cells were also capable of rescuing saliva production in locally-irradiated murine salivary glands, indicating the existence of human salivary gland stem cells and a possible clinical applicability.
Of the three major pairs of salivary glands in the human, dysfunction of the parotid gland plays a critical role in the development of post-radiation xerostomia. Therefore, for a possible clinical translation of our findings, we focus more specifically on the parotid gland and its stem cell populations. We observed that salivary gland stem cells are not homogeneously distributed over the rat parotid gland. Indeed, significantly more salispheres could be cultured from a central part of the parotid gland when compared to peripheral parts. Moreover, high-precision proton-irradiation of this central part induced a disproportionally large and detrimental late reduction in salivary flow of the rats, whereas the opposite was seen when the dose was administered to the periphery of the gland. Therefore, we hypothesise further that sparing these stem cell-rich areas of human salivary glands from radiation may reduce parotid gland dysfunction and xerostomia in patients.
Altogether, these data indicate that specific sparing of stem cell-rich regions and, when this is not possible, transplantation of salivary gland stem cells, may prevent or repair radiation-induced salivary gland damage and. This is expected to reduce complications related to parotid gland dysfunction and improve the quality of life of head-and-neck cancer patients treated with radiotherapy.
1. Lombaert, I. M., Brunsting, J. F., Wierenga, P. K., Faber, H., Stokman, M. A., Kok, T., Visser, W. H., Kampinga, H. H., de,Haan G., Coppes, R. P.. (2008). Rescue of salivary gland function after stem cell transplantation in irradiated glands.

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HYPOXIC MODIFICATION OF RADIOTHERAPY WITH NIMORAZOLE IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS: IMPORTANCE OF COMBINED HYPOXIA AND HPV

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Purpose: Hypoxic tumours are associated with increased resistance to radiotherapy. In head and neck squamous cell carcinomas (HNSCC) this resistance can be modified and reduced by use of hypoxia modifying therapy. Both the hypoxic-status as categorized with a recently developed 15-gene hypoxia classifier and the HPV-status has independently proved predictive impact for identification of responders to hypoxic modification of radiotherapy with Nimorazole.
The aim of the study was to evaluate the relevance of a combined approach with both HPV- and hypoxia gene expression classification in HNSCC's treated with radiotherapy +/- the hypoxic modifier Nimorazole (DAHANCA 5 trial).
Materials and Methods: HPV-status (immunohisto-chemical p16-status) has previously been assessed on archival FFPE biopsies of 323 HNSCC from the randomized, double blinded DAHANCA 5 trial. With a recently developed 15-gene hypoxia classifier the same patients were classified as having "more" or "less" hypoxic tumours, respectively. The importance of both classifications was compared in terms of loco-regional tumor control (LRC) and disease specific survival (DSS) at 5 years, and in relation to predictive impact for hypoxia modification of radiotherapy with Nimorazole.
Results: Of the 323 tumour biopsies from the DAHANCA 5 study 114 (35%) were classified as "more" hypoxic with the 15-gene classifier. These patients had a significant benefit of hypoxic modification with Nimorazole compared with placebo in terms of LRC (5-year actuarial values 49% vs 18%; $p = 0.001$) and DSS (48% vs 30%; $p = 0.04$). "Less" hypoxic tumours had no significant effect of hypoxic modification (LRC: 50% vs 44%; $p = 0.39$, DSS: 57% vs 51%; $p = 0.49$) and generally an outcome, which was similar to "more" hypoxic tumours treated with Nimorazole. There was a comparable distribution of "more" and "less" hypoxic tumours among the 84 HPV-positive tumours (38% vs 62%) and the 239 HPV-negative tumours (34% vs 66%), respectively. The HPV-positive tumours had in general a better outcome in response to radiotherapy, which was irrespective of hypoxic modification and hypoxic status. The HPV-negative/"more" hypoxic tumours benefitted substantially from Nimorazole vs placebo (LRC: 43% vs 9%; $p = 0.002$) and reached a response compared to HPV-negative/"less" hypoxic tumours (LRC: 44% vs 38%; $p = 0.48$), where no additive effect from Nimorazole was observed.
Conclusions: A combined approach with both hypoxia-classification and HPV-classification could distinguish between patients who responded to hypoxic modification of radiotherapy and non-responders to such therapy. Responders included patients with HPV-negative and "more" hypoxic tumours only. Thus, the importance of both methods for classification is highlighted.

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FIRST STEPS TOWARDS 4D OFFLINE PET-BASED TREATMENT VERIFICATION AT THE HEIDELBERG ION BEAM THERAPY CENTER

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The application of protons and heavy ions to external beam radiotherapy enables a highly conformal dose