Single-cell analysis reveals distinct responses of salivary gland stem cells to photon and proton irradiation

D. Cinat[1,2], L. Kracht[1], Y. Wu[1,2], I.R. Holtman[1], L. Barazzuol[1,2], R.P. Coppes[1,2]

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

Abstract

An increasing number of head and neck cancer patients is being treated with proton therapy. Besides its physical advantages over conventional photon-based radiotherapy, little is known about the biological response to protons on healthy salivary gland tissue. To investigate and compare the potential differences of these radiation types, we used a murine salivary gland organoid (SGO) model and performed single-cell RNA sequencing to identify transcriptomic changes at the single cell level. Analysis of non-irradiated SGOs showed for the first time the presence of a population of salivary gland (SG) progenitor cells defined by Trp63 and Itgb1; epithelial-mesenchymal stem/progenitor cells defined by Cd44, Itgb4 and Sox4; SG stem/progenitor cells defined by Cd24a, Sox9 and Krt10 and SG luminal duct cells defined by Krt8/18 and Krt7/17. Interestingly, photon and proton irradiation (IR) showed a clear and similar reduction of these stem/progenitor cell populations and enrichment of more differentiated SG duct cells. Nevertheless, differential expression analysis showed a higher downregulation of development-related processes after photon IR compared to proton IR. On the other hand, proton IR exhibited upregulation of survival and differentiation-related processes such us Notch, Tgf-B signaling pathways and aldehyde metabolic process. Furthermore, proton-irradiated samples maintained a higher expression of several Wnt-associated genes such as Wnt4, Wnt10a, Wls (or Wntless) and Ctnnb1 (or Catenin Beta 1) essential for stem cell niche maintenance and self-renewal ability. In line with this, proton IR samples maintained a higher self-renewal capacity compared to photon IR, measured as secondary organoid forming efficiency, and a higher number of more differentiated lobular organoids. In conclusion, our study identifies new potential SG stem and progenitor cell populations, and suggests that photons lead to a higher stem/progenitor functional decline compared to protons thus providing new possibilities for future therapy improvements.

This work was supported by the Dutch Cancer Society KWF Grant nr: 12092