# ABSTRACT

Resistance of various cancers to conventional therapies including radio- and chemo-therapy is one of the most investigated phenomena in the molecular and clinical oncology. Recurrent disease is characterized by the presence of metastases, which are responsible for 90% of cancer-related mortality. Fractionated ionizing radiation (fIR) combined with surgery or hormone therapy represent the first-choice treatment for medium to high risk localized prostate carcinoma (PCa). In PCa, the failure of radiotherapy (RT) is often caused by radioresistance and further dissemination of escaping (surviving) cells.

To investigate the radioresistance-associated phenotype, we exposed four metastasis-derived human PCa cell lines (DU145, PC-3, LNCaP, and 22RV1) to clinically relevant daily fractions of ionizing radiation (fIR; 35 doses of 2 Gy) resulting in generation of two surviving populations: adherent senescent-like cells expressing common senescence-associated markers and non-adherent anoikis-resistant stem cell-like cells with active Notch signaling and expression of stem cell markers CD133, Oct-4, Sox2, and Nanog. While the radioresistant adherent cells were capable to resume proliferation shortly after the end of irradiation, the non-adherent cells started to proliferate only after their reattachment occurring several days after the irradiation-driven loss of adhesion. Like the parental non-irradiated cells, radioresistant readherent DU145 cells retained tumorigenic potential after injection to immunocompromised mice. We showed that fIR-induced phenotypic plasticity in PCa cells was accompanied with the epithelial-to-mesenchymal transition (EMT) as well as its reverse process mesenchymal-to-epithelial transition (MET). The radiation-induced loss of adhesion was dependent on expression of EMT-driver Snail (SNAI1), as transient siRNA or permanent shRNA-mediated knockdown of Snail prevented loss of protein of adherent junctions E-cadherin (CDH1) and cell detachment. On the other hand, survival of the non-adherent cells required active Erk signaling, as chemical inhibition of Erk1/2 by a Mek selective inhibitor or Erk1/2 downregulation by siRNAs resulted in anoikis-mediated death in the non-adherent cell fraction. Notably, whereas combined inhibition of Erk and PI3K/Akt signaling triggered cell death in the non-adherent cell fraction and blocked proliferation of the adherent population of the prostate cancer cells, such combined treatment had only marginal if any impact on growth of control normal human diploid cells. Importantly, irradiated re-adherent cells exhibited less senescent-like colonies in clonogenic cell

survival assay and enhanced anoikis-resistant survival upon reirradiation pointing to the acquired radioresistance.

Since dormant fIR-surviving non-adherent PCa cells shared common features with metastases-related disseminated tumor cells (DTCs) such as low proliferation potential, expression of stemness markers and capability to resume adherent growth after the end of genotoxic stress and as these characteristics are known to contribute to therapy resistance, development of metastases and tumor recurrence, we investigated the stress-induced 'floatation' phenomenon in more detail. We observed the same phenotypic plasticity in both breast adenocarcinoma cells (MCF-7) treated with fIR (10 x 2 Gy) and cervical cancer cells (HeLa) treated with chemotherapeutic drug 5-azacytidine (4 μM / 24 hours for 7 days), showing that occurrence of viable non-adherent cells is not restricted to cancer cell origin or to the type of genotoxic insult. As a next step, we performed high-throughput whole genome transcriptional profiling of radio- and/or chemoresistant cancer cell populations. Data analysis revealed the exclusive expression pattern in radio- and chemo-therapy-surviving non-adherent cancer cells including active cytokine signaling and induction of interferon-responsive genes.

Taking together, these results contribute to better understanding of radiation-induced heterogeneous molecular response of human metastatic PCa cells, document treatment-induced phenotypic plasticity of stress-surviving cells, decipher a key molecular mechanisms of radio- and chemo-resistance, and finally, provide options to overcome the therapy resistance.