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Radioprotection of normal tissue cells by lentivirus-mediated overproduction of aldehyde dehydrogenase or glutathione

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In the past 20 years, the cure rate of varied types of cancers has improved significantly, owing to the application of comprehensive multimodal treatment (surgery, chemotherapy, and radiotherapy) and novel (neo)adjuvant therapy modalities like thermo-, immune- and biological therapies. Still, radiotherapy (RT) is often a key element in these treatments. The complications and damage to the normal tissues surrounding the tumors drastically limit RT therapeutic capacity, whereas the biggest challenge is damage to the following radiosensitive tissues: the hematopoietic system, the gastrointestinal (GI) tract and the skin. Therefore, efforts have been made to increase the therapeutic width. Image-guided radiotherapy and intensity-modulated radiotherapy provide more precise target coverage and reduce radiation dose to the surrounding tissue. Although these current methods can limit this damage to adjacent normal tissues, additional improvements are still imperative.

The transfer of radioprotective genes using viral vectors is another promising option to further increase the therapeutic width. Delivery of the transgene for the antioxidant enzyme manganese superoxide dismutase and multidrug resistance 1 have been investigated for protecting specific target organs such as lung, esophagus or the hematopoietic system. In this project, the radiomodulating potential of aldehyde dehydrogenase (ALDH) or glutathione (GSH) was determined in a cell model (TK6 cells) by lentiviral (HIV-based) overexpression. GSH, a critical cellular reducing agent and antioxidant, is plenty in some radioresistant cells such as stem cells. In addition, cells deficient in GSH synthesis show a lack of DNA single-strand break repair. However, both oral and cellular uptake of GSH remains problematic. ALDH, encode a group of enzymes which are enriched in (cancer) stem cells and play a critical role in the formation of molecules involved in life processes, catalyzing the oxidation (dehydrogenation) of endogenous and exogenous aldehydes. ALDH has been accepted as a marker for cancer stem cells in varied organs, showing a capacity to resist radio- and chemo- therapy.

In this work, lentiviral vectors encoding GCLM, GSS-GCLM (both limiting and essential for GSH production), ALDH1a1 or ADLH3a1 were successfully produced via transient transfection. The overexpression of ADLH or GSH in TK6-ALDH1a1 (325 ± 58-fold; P=0.042), TK6-ALDH3a1 (12216 ± 1387-fold; P=0.018) or TK6-GCLM (32 ± 2-fold; P=0.004) cells were detected by qRT-PCR and confirmed by ALDH/GSH activity assays, whereas the TK6-GSS-GCLM did not express either gene probably due to vector-size limitations. Interestingly, the overproduction of either ALDH or GSH in TK6 cells did not change the proliferation and clonogenic survival of the radiated cells compared to controls. However, apoptosis of any of the overproducing TK6 cell lines 24h-48h after irradiation was significantly reduced compared to the control groups (P=0.001-0.037), which corresponded with a 20.3-53.0% reduction in apoptosis rates of the ALDH and GSH-overexpressing TK6 cells compared to wild-type TK6 cells after irradiation, whereas this was a maximum of 4.7% for TK6-Mni cells.

These data suggest that some cells may survive but are not able to proliferate and form colonies. Using cell labelling and tracking of irradiated cells, this could be shown for wild-type TK6 cells. After irradiation, culture (72h) and subsequent sorting, TK6 cells with high (no/few divisions) and low (multiple divisions) signal of the label were sorted. The TK6 cells with a high label concentration were not able to form colonies and the intensity of the label remained unchanged after mass culture, whereas cells with low label concentration divided and formed colonies comparable to unirradiated cells. Although not yet shown for the ALDH or GSH-overexpressing cell lines (additional investigations required), there may still be differences in the fraction of cells that survive yet are not dividing after irradiation that may explain the observed differences in apoptosis rates after gene transfer of GSH or ALDH. The observed clonogenic inactivation, yet survival of cells may be a cellular mechanism ensuring that damaged cells cannot harm the integrity of the organism, but still enables them to perform (some of) their normal functions without having them to be remove from the population.

These observations may reduce the utilization of radioprotective gene transfer generally in target cells reacting similarly to irradiation as TK6 cells and in addition relying on clonogenic growth of the transduced cells for repopulation. If this is not the case, like in the normally radiosensitive differentiated progeny of hematopoietic stem cells (themselves radioresistant) for overcoming RT-induced neutropenia, radioprotection may still a good option to increase the therapeutic width of radiotherapy.