Combining radiotherapy with death ligands in cancer treatment : feasibility and molecular mechanisms

Verbrugge, I.

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Summary

The success of radio- and/or chemotherapy in cancer treatment is limited by the small window of differential sensitivity between normal and tumor tissue, and the intrinsic or acquired radio- and/or chemoresistance of tumor cells. Both radio- and chemotherapy induce DNA-damage and impair clonogenicity of tumor cells in various ways, including the induction of an irreversible cell-cycle arrest, death due to mitotic catastrophe, or the induction of programmed cell death (apoptosis). To improve the therapeutic outcome and limit normal tissue toxicity, a need exists for rationally designed drugs that induce cell death by different molecular mechanisms than those used by radio- and/or chemotherapy. Of interest for combined treatment are death receptor ligands such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and CD95/Fas ligand. They induce apoptosis in a great variety of tumor cell types independent of the p53 status of the cell and by partially distinct routes (i.e. independent of the mitochondria) as compared to radio- and chemotherapy. In addition, TRAIL and CD95 death receptors can be upregulated in response to radio- and chemotherapy. Due to their partially different routes to cell death induction, it can be envisioned that combining radiotherapy with death ligands increases its therapeutic effect.

In this thesis, we studied the therapeutic potential and underlying molecular mechanisms of combined modality treatment with conventional anti-cancer regimens (in particular radiotherapy) and death receptor ligands TRAIL and APO010 (MegaFas Ligand).

In **Chapter 2** we investigated the feasibility of combined treatment with radiotherapy and a novel recombinant (isoleucine-zippered (IZ)) form of TRAIL in the p53-mutant human T-leukemic cell line Jurkat that overexpresses Bcl-2 (Jurkat-Bcl-2). We found *in vitro* both using short-term apoptosis assays and long-term clonogenic survival assays that radiotherapy and IZ-TRAIL had a clear combined cytotoxic effect. A combined therapeutic effect was also observed when Jurkat-Bcl-2 cells were grafted in immunodeficient mice. The doses of radiotherapy and IZ-TRAIL used in this study did not result in any local (liver) or systemic toxicity.

In **Chapter 3** we described the molecular mechanisms underlying combined (apoptotic) responses to radiotherapy and IZ-TRAIL in Jurkat-Bcl-2 cells, which are Type II cells and largely reliant on a mitochondrial contribution to apoptosis induction by death ligands. We showed that after irradiation, Jurkat-Bcl-2 cells no longer required the mitochondrial pathway for apoptotic execution. This was not due to modulation of gene or protein expression of known apoptosis regulators. Rather, we found that pretreatment of cell with ionizing radiation strongly improved the capacity of ligand-bound receptor complexes to recruit FADD and activate inducer caspases in the TRAIL death-inducing signaling complex (DISC).

We next tested the feasibility of combination treatment with radiotherapy and APO010 in Jurkat-Bcl-2 cells and variety of solid tumor cell lines (**Chapter 4**). We found that although APO010 and radiation had a clear combined cytotoxic effect on Jurkat-Bcl-2 cells and solid tumor cells *in vitro*, a combined therapeutic effect was not observed when these cells were grafted in mice. APO010 doses in this setting were approximating maximal tolerable levels and reversible systemic and liver toxicity was observed.

To investigate whether a common molecular mechanism was underlying combined responses to conventional anti-cancer regimens and death receptor stimulation in Jurkat-Bcl-2 cells, we studied the mechanism underlying enhanced responses to APO010 in **Chapter 5**. Similar to the results described in Chapter 3, we found that, in response to a variety of stimuli, Jurkat-Bcl-2 cells were conditioned in such a way that APO010 stimulation allowed for the activation of effector caspases in the absence of a mitochondrial contribution. In contrast to unchanged c-FLIP levels that we observed in Chapter 3, we here found that APO010 sensitivity was strongly correlated with the ability of all sensitizers tested to downregulate c-FLIP protein levels. We found that preventing c-FLIP protein downregulation restored resistance to APO010 and that by deliberate downregulation of c-FLIP by RNA interference cells were similarly sensitized to APO010. Consequently, the capability of the sensitizers to increment apoptotic execution in

c-FLIP 'knock-down' cells was largely overruled.
In **Chapter 6** we show that internalization of TRAIL death receptors was not required for TRAIL-induced apoptosis. In addition, we found that cell surface expression of the two TRAIL death receptors, TRAIL-R1 and TRAIL-R2 are differentially regulated; whereas TRAIL-R1 membrane levels were affected by interfering with dynamin-dependent internalization, TRAIL-R2 levels were not affected. We found that members of the MARCH protein family, which are E3 ubiquitin ligases, promoted downregulation of TRAIL-R1 cell surface levels by targeting a distinct lysine residue in its cytoplasmic tail.
The current status and future prospects of the results described in this thesis are discussed in **Chapter 7**.