Basic Science/Molecular Predictive Assays

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Basic Science

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Treatment of colorectal tumors with apoptosis inducing TRAIL receptor antibodies HGS-ETR1 and HGS-ETR2 in combination with radiotherapy: additive effects *in vitro* and dose dependent growth delay *in vivo*

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Introduction: The development of agonistic TRAIL receptor antibodies has given rise to the development of TRAIL receptor targeted antibody-based treatment protocols. TRAIL receptor antibodies from HGSI (Rockville, USA) are already the subject of clinical phase I/II trials. Thus far, no data demonstrating combination treatment with ionizing irradiation have been published.
The aim of the present study was to show at least additive effects when a TRAIL receptor-based therapy was combined with ionizing radiation *in vitro* and *in vivo*.
Methods: Apoptosis induction after combination therapy (HGS-ETR1 or HGS-ETR2 at 0.01, 0.1, and 1.0 mg/ml and 2.5 and 10 Gy) was determined by fluorescence microscopy and western blot analysis of caspase-8 and PARP. The colorectal tumor cell lines Colo205, HCT 116 and HCT-15 were used. Growth delay experiments were performed after combination therapy with fractionated irradiation (d1–5, SD 3 Gy) and the TRAIL receptor antibodies (i.p. in 3 concentrations on d1, 4 and 8) with Colo 205 xenograft-bearing NMRI/nu nude mice.
Results: HGS-ETR1 and HGS-ETR2 showed *in vitro* and *in vivo* dose dependent and at least additive effects in combination with ionizing radiation. *In vivo,* a growth delay was observed in tumor volume doubling time from 5.6 days in untreated animals to 71 days and 116 days in animals treated with the combination of radiation+HGS-ETR1 (10 mg/kg body weight) or HGS-ETR2 (10 mg/kg body weight), respectively. HGS-ETR2 displayed increased activity when compared to HGS-ETR1, both *in vitro* and *in vivo*.
Conclusion: These results demonstrate, for the first time, additive effects when the new monoclonal agonistic TRAIL receptor antibodies, HGS-ETR1 and HGS-ETR2, were combined with irradiation in three different colorectal tumor cell lines. Additionally, a significant growth delay was observed in the xenograft tumors *in vivo*. Thus, HGS-ETR1 and HGS-ETR2 are promising new agents for combination treatment with radiation.

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Immunotherapy target identification in lung cancer by comprehensive protein expression profiling

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Global protein expression changes that accompany the conversion of healthy epithelial cells into tumors were tracked with mass spectrometry-based proteomics. Plasma membranes were isolated from tumor tissue and matching normal epithelium in thirty patients, following resection of the lung tumor. The plasma membranes were enriched with a cocktail of four antibodies, coupled with magnetic bead isolation. Proteins were fractionated, digested to tryptic peptides, and analyzed by mass spectrometry. Protein expression was compared quantitatively at the peptide level to identify approximately 9% of the peptides that were reproducibly differentially expressed in the tumor (5 fold up-regulated in at least 9/30 patients). The technology used, QToF (Quadropole Time of Flight) mass spectrometers allowed the accurate comparison of relative protein abundance in each tumor/normal pair. The approach also allowed direct determination of the peptide sequence and protein identity for 80% of the targeted peptides by the analysis of replicate samples on the same instruments. On average, three sequenced peptides showing similar differential abundance behaviour, were found to support each protein identified. Overall, 300 membrane proteins were identified as potential antibody therapy targets. Included among the identified proteins are most of the known immunotherapy targets discovered to date such as the Vitronectin receptor, Transferrin receptor and CD 98, as well as many not previously seen to be up-regulated in cancer.

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Dendritic cell derived-exosomes boost effector CD8+ T cells in the absence of tumor induced-T regulatory cells: synergistic antitumor effects of cyclophosphamide and exosomes based vaccines

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Dendritic cell derived-exosomes (DEX) are nanomeric antigen presenting vesicles harboring immunogenic MHC/peptides complexes when combined to adjuvants. Since CD4+ CD25+ T regulatory cells (Treg) inhibit the induction of antitumor immunity, we addressed the capacity of Treg to restrict primary and secondary CD8+ T cell responses elicited by DEX in tumor bearing mice. Here we show that DEX combined to the alkylating agent cyclophosphamide (CTX) can mediate peptide dependent-tumor regression of pre-established burdens in HLA-A2.1 transgenic mice bearing B16F10 HLA-A2.1 Gp100 melanoma. These anti-tumor effects are superior to what is observed with peptide Gp100 + CTX and the best DEX vaccination approach currently available DEX+ ODN-CpG [1].
NK cell depletion using NK1.1 Ab (PK136), does not abrogate these antitumor effects. However, these antitumor effects were peptide dependant since empty or irrelevant peptide loaded DEX were inefficient, suggesting the crucial role of CD8+ T lymphocytes. Interestingly, CTX abolishes tumor-induced Treg suppressive function and the curative effects of CTX+DEX vaccines are abrogated by the adoptive transfer of Treg. DEX significantly boost expansion and Tc1 differentiation of peptide-induced primary CD8+ T cell responses in the presence of CTX, this effect was not observed using peptide Gp100. The magnitude of secondary T cell immune responses induced by DEX vaccines is subject to control by tumor induced-Treg.
Thus, these results imply that therapeutic vaccines aimed at boosting tumor–primed effector T cells could benefit procedures that minimize the effects of Treg.

References

[1] Chaput et al, JI, 2004.

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The new pro-apoptotic compound MPI-0441138 enhances anti-tumor activity of ionizing radiation

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Background: The combination of radiotherapy and chemotherapy is a standard of care for cancer and improves the survival of patients. The aim of this study was to investigate the anti-tumor effects caused by a novel small molecule chemotherapeutic MPI-0441138 in combination with ionizing radiation in breast, colorectal, lung, head and neck, prostate carcinoma cell lines.
Material and methods: Ten carcinoma cell lines were treated with MPI-0441138 for various periods of time prior to and after exposure to ionizing radiation. Treated cells were then assessed for apoptosis induction, cell viability and cell cycle distribution. Induction of apoptosis was determined by FACS analysis. Cell cycle and apoptosis-related proteins were determined using Western blot analysis.
Results: Despite differential sensitivity to ionizing radiation or MPI-0441138 treatment, all carcinoma cell lines displayed enhanced antiproliferative and apoptotic effects when used in combination. Cell cycle analysis after 96 h of treatment with MPI-0441138 or ionizing radiation demonstrated a consistent block at the G2/M phase for both agents. The combination of irradiation and MPI-0441138 led to the more pronounced cell cycle arrest in G2/M phase in all investigated cell lines. Western blot analysis revealed a significant modulation of cell cycle and apoptosis-related proteins after combination treatment with MPI-0441138 and irradiation.
Conclusion: This is the first study to document the ability of MPI-0441138 to enhance anti-proliferative and apoptotic effects of ionizing radiation against human cancer cells. Understanding the preclinical pharmacology of combination treatment with MPI-0441138 and irradiation in human cancer cells will be instrumental in developing new strategies in the clinic for improving the treatment of patients with solid tumors.