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Radiosensitization of tumor by caffeine encapsulated in liposomes

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It has been known that ATM plays a central role in response of cells to ionizing radiation by enhancing DNA repair. We have investigated the possibility of increasing radiosensitivity of tumor cells with the use of ATM inhibitors such as caffeine. Human colorectal cancer RKO.C cells and RKO/ATM cells (RKO cells overexpressing ATM) were used in the present study. The clonogenic cell survival in vitro indicated that RKO/ATM cells were markdely radioresistant as compared to RKO.C cells. Presence of 3 mM of caffeine significantly increased the radiosensitivity of cells, particulary the RKO/ATM cells, so that the radiosensitivity of RKO.C cells and RKO/ATM cells were almost similar. The radiation induced G2/M arrest in RKO/ATM cells was noticeably longer than that in RKO.C cells and caffeine treatment shortened the radiation induced G2/M arrest in both RKO.C and RKO/ATM cells. Pentoxifylline and wortmannin were also less effective than caffeine to radiosensitize RKO.C or RKO/ATM cells. However, wortmannin was more effective than caffeine against human lung adenocarcinoma A549 cells indicating the efficacy of ATM inhibitor to increase radiosensitivity is cell line dependent. For in vivo study, RKO.C cells were injected s.c. into the hind-leg of BALB/c-nuslc nude mice, and allowed to grow to 130mm^3 tumor. The mice were i.p. injected with caffeine solution or saline and the tumors irradiated with 10 Gy of X-rays. The radiation induced tumor growth delay was markedly increased by 1–2 mg/g of caffeine. It was concluded that caffeine increases radiosensitivity of tumor cells by inhibiting ATM kinase function, thereby inhibiting DNA repair that occurs during the G2/M arrest after radiation. We are now investigating the feasibility of using temperature-sensitive liposomes [DPPC:DMPC:DSPC = 4:1:1] for improving the delivery of caffeine to tumor cells in vivo. In our preliminary studies in vitro, we observed that heating the cells with the liposome-encapsulated caffeine at 42ºC significantly increases the radiosensitivity of cells, as determined with clonogenic survival assay. Experiments are in progress in our laboratory to reveal the effectiveness of the liposome-encapsulated caffeine to increase the radiosensitivity of tumors in vivo.

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Optimization of novel antiangiogenic treatment modalities in combination with ionizing radiation

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Backgound: Ionizing radiation targets multiple components in a tumor and activates as part of a stress response the PI3K/Akt survival pathway in different cell types. Here we investigated the receptor tyrosine kinase-dependence of radiation-induced PI3K/Akt-activation in tumor and in endothelial cells and tested the radiosensitizing potential of ErbB- and VEGF-receptor-directed inhibitors in vivo using different animal tumor models.
Materials and methods: Human umbilical vein endothelial and A431 tumor cells were used for mechanistic studies to investigate radiation-induced receptor tyrosine kinase-mediated PI3K/Akt-activation. For in vivo tumor growth delay investigations spontaneously growing ErbB2-overexpressing murine mammary tumors or isogenic but ectopic allograft tumors were irradiated with a minimally fractionated treatment schedule (4×3Gy) alone or in combination with a clinically-relevant VEGF-receptor tyrosine kinase inhibitor and a dual inhibitor of the VEGF- and ErbB-1/2- receptors.
Results: Radiation-induced, VEGF-ligand-independent PKB/Akt-phosphorylation in endothelial cells was strongly downregulated by the specific VEGF receptor tyrosine kinase inhibitor, while the ErbB-receptor tyrosine kinase inhibitor did not affect PKB/Akt-stimulation in response to irradiation. An opposite receptor-dependence for radiation-induced PKB/Akt-phosphorylation was observed in ErbB-receptor overexpressing tumor cells. To optimally overcome this survival network we determined the treatment response in vivo of ionizing radiation in combination with the different antiangiogenic inhibitors. Interestingly the treatment response in the spontaneous tumor model strongly exceeded the response of the isogenic allograft tumors to irradiation and the treatment combination with the different receptor tyrosine kinase inhibitors. More important in comparison to the VEGF-receptor inhibitor the dual receptor tyrosine kinase inhibitor showed an enhanced potency when used in combination with irradiation, indicative for an additional cooperative, non-overlapping antitumoral effect.
Conclusions: Our mechanistic and efficacy-oriented data demonstrate that as part of a combined treatment modality concomitant targeting of an intrinsic treatment threshold in tumor and endothelial cells extensively sensitizes for ionizing radiation and further suggest that multiple preclinical tumor models should be evaluated to test novel combined treatment modalities in radiochemotherapy.

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Gene expression profiling of human glioblastoma. A translational research study to the randomized trial EORTC 26981/NCIC CE.3 testing radiotherapy ± temozolomide

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Background: Glioblastoma (GBM) represent the most common and most malignant form of gliomas (WHO grade IV). We have shown that glioma subtypes, can be differentiated by their gene expression profiles (Godard et al. Cancer Res 2004). Here we determined gene expression profiles of GBM from patients randomized either to temozolomide and radiotherapy (RT) or RT only. The ability to differentiate response to therapy and outcome by means of molecular diagnostics would have great clinical impact since it would allow identification of subgroups of patients who are most likely to benefit from currently available therapies. Further, molecular profiling may define new tumor subclasses and allow identification of novel molecular targets for future therapeutic approaches.
Material and Methods: Frozen GBM biopsies have been collected from patients enrolled in the prospective randomized phase III trial testing radiotherapy alone or in combination with concomitant and adjuvant temozolomide treatment (Stupp et al. Proc Am Soc Clin Oncol 2004 {abstract #2}). A subset of seventy-three frozen GBM samples were collected from 12 of the 82 centers participating in the trial. Gene expression profiles on Affymetrix GeneChip arrays (HG-U133 Plus 2.0) that feature 47,000 transcripts were successfully obtained for 61 GBM. A second set of 11 GBM collected during the respective phase II trial (Stupp et al. J Clin Oncol 2002), establishing the experimental arm of the randomized EORTC trial, will serve as independent validation set. This homogenous group of patients will allow us to link gene expression profiles with clinical endpoints.
Results: First step unsupervised analysis with the Coupled Two-Way Clustering algorithm allow confirmation of some known properties of GBMs, such as the presence of EGFR-overexpression in 50% of the cases. Correlated upregulation of contiguous genes was observed on chromosome 12q13–15, reflecting the known amplicon around CDK4, SAS, and MDM2, respectively, and including some genes from the Ink4a/Rb pathway. Molecular subtypes of GBM were uncovered, characterized by differential expression of homeobox genes. Other clusters of correlated genes provide insight into subgroups of gliomas indicative of variable hypoxic stress.
Conclusions: Gene expression profiles analyzed by CTWC delivered biologically relevant information and provided new insights into molecular pathways implicated in GBM. This information is necessary to understand the molecular bases of response to therapy to offer customized treatments in the future.