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Cooperative *in vivo* cytotoxicity of ionizing radiation and the protein kinase C-inhibitor PKC412 in p53-deficient, radioresistant tumor cells

K. Zaugg, H. Resch, I. Hegyi[1], C. Glanzmann, D. Fabbro[2], S. Bodis, M. Pruschy. *Dept. Radiation Oncology, University Hospital Zurich; [2]Novartis Pharma Inc., 4002 Basel; [1]Dept. Pathology, University Hospital Zurich, Switzerland*

Purpose: To test the radiosensitizer PKC412 (N-Benzoyl-staurospofine) *in vivo*, using different treatment schedules and analysing tumor histology.

Methods: p53−/−, E1A/ras transformed mouse embryo fibroblasts (2–4 × 10^6) were injected subcutaneously into the back of nude mice. Treatment with different regimens during 4 and 10 consecutive days (3 Gy/day locoregional, 100 mg/kg PKC412/day (p.o.) or combined) was started when tumors reached a minimal size of 165 mm^3 ± 10%. Tumor size was measured daily and tumor response to combined treatment was analysed histologically with HE and TUNEL staining.

Results: Combined treatment with PKC412 (100 mg/kg daily) and locoregional irradiation (3 Gy daily) on 4 or 10 consecutive days conferred a strong tumor growth control during treatment and follow-up-period in p53-deficient tumors. Daily treatment of tumors with PKC412 or IR alone resulted only in partial tumor growth delay. In contrast to p53+/+ tumors, histological analysis of p53−/− tumors after combined treatment only showed a minimal amount of apopotic cell death.

Conclusions: These data show that PKC412 is a promising radiosensitizer in vivo. The histological analysis of the treated tumors indicates that the mechanism of induced cell death is different in p53+/+ and p53−/− tumor cells. No signs of apoptosis could be observed in p53−/− tumor cells after combined treatment. Thus, the radiosensitizing effect of PKC412 observed in the p53−/−, radioresistant tumor cells points towards a novel approach how to overcome treatment resistance.

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Changes in tumour microvessels and microcirculation during fractionated radiotherapy of mouse AT17 tumours

P.L. Debbage[1], J. Griebel[2], S. Seidl[1], M. Brandl[5], A. DeVries[2], A. Kreczy[3], P. Hutzler[4], P. Lukas[2]. *[1]University of Innsbruck, Institute of Histology and Embryology, Innsbruck; [2]University of Innsbruck, Clinic for Radiotheraphy and Radiooncolgy, Innsbruck; [3]University of Innsbruck, Institute Pathology, Innsbruck, Austria; [4]GSF-Neuherberg, Department of Pathology, Neuherberg-Munich; [5]GSF-Neuherberg, Institute of Radiology, Neuherberg-Munich, Germany*

Purpose: Radiation dose-dependent changes in tumour blood flow and vascular structures were analysed.

Methods: Fractionated radiotherapy (single 3 Gy doses to totals of 0, 42, 78 Gy) of mouse AT17 mammary adenocarcinomas, then dynamic contrast-enhanced MRI (Magnevist, Schering) to quantify tumour perfusion, then mapping of tumour vascular structures by intravital lectin perfusion. Pathohistology with HE.

Results: In the control group (0 Gy) radially coursing microvessels gave rise to numerous fine branches supplying nests of tumour cells. Tumour perfusion was characterised by Gd-DTPA concentration-time curves with relatively broad peaks. In the 42 Gray group tumour cell densities were attenuated and the microvascular supply consisted predominantly of large-calibre radial vessels lacking fine branches. The Gd-DTPA curves showed smaller half-peak values. In the 78 Gray group sparsely distributed small clusters of tumour cells remained. The topographical organisation of the blood vessels was altered, with fine-calibre radial microvessels and irregular arborisations of wide-calibre vessels centrally. Gd-DTPA concentration-time curves tended to revert to broader half-peak values.

Conclusions: Characteristic topographical changes in microvessel architecture during fractionated radiotherapy were reflected in changes of the time-course of Gd-DTPA concentration in the tumour tissue. This has considerable impact on the interpretation of contrast enhanced MR studies.

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Changes of tumor oxygenation during radiotherapy

P. Stadler, H.J. Feldmann, C. Creighton, M. Molls. *Klinik für Strahlentherapie der Technischen Universität München, Germany*

Purpose: Hypoxia is one of the most important reasons for radioresistance. Recent studies in animals showed fractionation (hyperfractionated versus conventional fractionated) and dose dependent changes in tumor oxygenation during radiotherapy. We recently reported a decrease of the tumor oxygenation after 30 Gy conventional fractionated radiochemotherapy (Radiother Oncol 48: 157–164, 1998) whereas Lartigau et al. (Eur J Cancer 34: 6, 856–861, 1998) found an increase after hyperfractionated radiotherapy. Now we evaluated in which way the changes of the hypoxic fraction corresponded to the changes of the hypoxic subvolume (HSV) of the tumor.

Methods: We investigated 33 patients with locally advanced head and neck cancer pretherapy and after 30 Gy conventional fractionated radio- or radiochemotherapy (5 FU + Mitomycin C) by using the Eppendorf-histograph. In 23 of these patients we determined ultrasonographically the change of volume during this time interval.

Results: We observed a significant increase of the hypoxic fraction (below 5 mm Hg) after 30 Gy ($p < 0.05$). In addition we found a significant volume reduction during therapy ($p < 0.001$). Moreover the calculated "hypoxic subvolume" (hypoxic fraction (%) × tumor volume (ccm)) decreased statistically significant during the treatment ($p < 0.01$).

Conclusion: The significant decrease of the HSV during a radioncological, treatment shows that the hypoxic areas of the tumor can shrink in spite of increasing proportion of hypoxic tumor sites.

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Radiation and a p53-stabilizing agent cooperate in wild type (wt) p53-mediated growth inhibition of human lung cancer cell lines

J. Huober[1], S. Nakamura[2], J.A. Roth[2], R.E. Meyn[3], D. Wallwiener[1], T. Mukhopadhyay[2]. *[1]University of Tuebingen, Department of Gynecology, Tuebingen, Germany; [2]MD Anderson Cancer Center, Department of Thoracic and Cardiovascular Surgery, Houston, TX; [3]MD Anderson Cancer Center, Department of Experimental Radiotherapy, Houston, TX, United States*

2-Methoxyestradiol (2-ME), a metabolic byproduct of estradiol is known to induce wt p53, and inhibit growth of human lung cancer cell lines. We evaluated whether radiation and 2-ME cooperate in inducing apoptosis in wt p53-containing H460 and A549 cells as well as mutated p53-containing H322 cells.

Cells were radiated and/or treated with 2-ME. All 3 treatments inhibited the growth of wt p53-containing cells, with the strongest growth inhibition after combination treatment. H322 cells were less sensitive to either treatment and showed no significant additional growth inhibition with the combination. Wt p53 and p21 protein expression in H460 and A549 cells was higher following radiation and 2-ME than after either single treatment, while p53 and p21 levels remained virtually unchanged in H322 cells. The proportion of apoptotic cells in H460 (43%) and A549 (31%) after combination treatment was above any of the other treatment groups.

In a nu/nu mice model employing H460 cells injected subcutaneously at the hind leg, irradiation and 2-ME as single treatments were barely effecitve. Combination therapy however, led to significant tumor growth suppression.

Our data suggest that irradiation and 2-ME cooperate in stabilizing wt p53, with possible therapeutic implications for the future.

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Endothelial cell injury and cell kinetics in normal rectum after neoadjuvant hyperfractionated radiochemotherapy

K.K. Richter[1], A. Tannapfel[3], M. Schilli-Westermann[1], B. Erdmann[2], A. Brandl[1], M. Thiele[1], Th. Wendt[2], J. Scheele[1]. *[1]Departments of Surgery; [2]Radiation Oncology; [3]Friedrich-Schiller-University Jena, Institute of Pathology; University of Leipzig, Germany*

Purpose: This prospective study assessed pathogenic cellular and molecular mechanisms of radiation enteropathy: endothelial cell function, proliferative activity and apoptosis-regulating factors in irradiated and nonirradiated normal rectum of patients with rectal cancer. The results were correlated with histopathologic injury and also clinical symptoms according to the CTC.

Methods: Irradiated and nonirradiated normal rectal specimens from patients with rectal cancer were excised intraoperatively, six cm proximal to the tumor, and immediately snap-frozen and also fixed in formaldehyde. Specimens were analyzed by immunohistochemistry using antibodies against markers of endothelial cell function (thrombomodulin, TM), proliferative activity (Ki67), and apoptosis regulating proteins (bcl2, bax protein). DNA-sequencing of the p53 gene (exon 5–11) was also performed.

Results: Irradiated rectum showed mucosal denudation, ulceration, and microvascular injury, and exhibited significant less proportions of TM-pos-