Poster Discussions: Oral

Radiobiology

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Modulation of radiation-induced tumor necrosis factor alpha expression in the lung tissue by pentoxifylline

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Purpose: The pathophysiological tissue response after lung irradiation implies the induction of numerous cytokines. Pentoxifylline (PTX) downregulates the production of proinflammatory cytokines, particularly TNF-alpha, in response to noxious stimuli and may, therefore, provide protection against radiation-induced, cytokine-mediated cellular damage. The purpose of this study was to investigate the effects of PTX on the radiation-induced TNF-alpha expression in an animal model of thoracic irradiation.

Methods and materials: C57BL/6J mice underwent thoracic irradiation (12 Gy) without PTX (XRT group) or received both PTX (500 mg/L in drinking water) and irradiation (PTX/XRT group). The mice were sacrified corresponding to the latent period (1h, 24h, 72h, 1w), the pneumonic phase (2w, 4w, 8w, 16w) and the beginning of the fibrotic phase (24w postirradiation). Real-time RT-PCR and immunohistochemistry were used for quantification of TNF-alpha mRNA and protein expression.

Results: Following thoracic irradiation, TNF-alpha mRNA release in the lung tissue (XRT group) was significantly upregulated and reached maximal values during the pneumonic phase. The elevated levels of TNF-alpha correlated with a pronounced increase of positive inflammatory cells, predominantly macrophages. In contrast to the radiation-only group, the lung tissue of the PTX-treated mice (PTX/XRT group) revealed no significant radiation-mediated TNF-alpha response.

Conclusion: We observed a significant reduction of the TNF-alpha mRNA and protein production in the study group that received both PTX and radiation (PTX/XRT group) as compared to the radiation-only group (XRT group). Therefore our results indicate that PTX downregulates the TNF-alpha production in the lung tissue in response to radiation.

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The transcriptional inhibition of DNA repair protein Rad51 enhances radiosensitivity in prostate cancer cells

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Purpose: Although mammalian cells have developed two distinct pathways to repair the DSBs, a key component of homologous recombinational repair pathway is Rad51 protein. The purposes of the present study are to examine the contribution of DNA repair Rad51 protein to the genotoxic effects of ionizing radiation (IR), and to investigate a novel strategy that a transcriptional inhibition using Rad51 antisense oligodeoxynucleotides (ODNs) enhances radiosensitivity in human prostate cancer cells. Materials and Methods: Human prostate cancer DU145 cells were irradiated ranging from 0 to 15 Gy. Two 15-bp antisense and two 15-bp sense ODNs for human Rad51 gene were synthesized, and tranfected by Lipofectamine. Inductions of DSBs by IR were quantitatively evaluated by comet assay calculating the average of tail/head ratio of 200 counted cells. Cytotoxicities were determined by cotonogenic assay at 7days after irradiation. Rad51 foci was immunohistochemically visualized by a Rad51 monoclonal antibody comparing Histon 2A (a sensor of DSBs), Brca1, and Nbs1 (other DNA repair proteins) foci formations by confocal laser microscopy. The transcriptional inhibition of Rad51 gene by antisense ODNs was evaluated by Northern blotting and competitive RT-PCR. Immunoreactivity of the Rad51 protein was assessed by Western blotting. Results: (1) Total Rad51 protein levels of DU145 cells did not change in before and after 10Gy irradiation. However, both number of cells which expressed Rad51 foci and number of Rad51 foci within these cells reached maximum at 4hr after IR and decreased to the control level within 24 h. (2) Approximately 15% of Rad51 foci colocalized with Histon 2A foci, while 16% and 28% of Rad51 foci did with Brca1 and NBS1 foci, respectively. (3) A volume of 100 nM of Rad51 antisense ODNs inhibited the level of Rad51 mRNA expression by more than 70% and reduced the Rad51 protein by about 50%. Combination of the RAD51 antisense and IR showed a greater synergistic cytotoxicity than cells treated with IR alone (control) or cells treated with sense ODNs and IR (SF2 of antisense:0.28- 0.42, control:0.77-0.85, sense:0.45-0.72). Interestingly, the combination resulted in the decrease of Rad51 foci formation. Conclusion: These experiments demonstrate that the transcriptional inhibition of Rad51 can be expected to be a powerful potentiator for radiation therapy by blocking the DNA repair pathway in human tumor cells in a gene therapy context.

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VEGFR tyrosine kinase inhibition by ZK 222584/PTK 787 (ZK) combined with fractionated radiotherapy (RT) in human squamous cell carcinoma (hSCC) in nude mice

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Purpose: To investigate the effect of the antiangiogenetic substance ZK, a specific inhibitor of VEGFR tyrosine kinases, on the growth rate of different hSCC and on the growth delay after fractionated RT of FaDu hSCC.

Materials and methods: Five hSCC lines (FaDu, UT-SCC-14, UT-SCC-15, UT-SCC-33, MKG7) were transplanted s.c. in NMRI nu/nu mice. At a mean tumour diameter of 4 mm animals were treated daily by oral gavaging with ZK (50 mg/kg bodyweight) or with carrier (control). The specific growth delay (SGD) was determined. In a second set of experiments FaDu tumours were irradiated with 15 fractions of 2 Gy and ZK was given either before, during, or after RT.

Results: ZK was well tolerated. A clear-cut decrease of growth rate in tumours treated with ZK was observed in 3 of the 5 investigated hSCC. The SGD to reach 10 times of the starting volume was 0.1 for FaDu, 0.6 for UT-SCC-14, 0.5 for UT-SCC-33, 0.6 for UT-SCC-15, and 0.1 for MGK 7. The application of ZK before and during fractionated irradiation did not significantly change the SGD of FaDu tumours. FaDu tumours treated with ZK after fractionated RT showed a significant increased growth delay compared with irradiated controls.

Conclusions: Inhibition of VEGFR-TK by ZK reduced the growth rate of a panel of hSCC. This effect showed considerable intertumoral heterogeneity. Neoadjuvant or simultaneous application of ZK did not decrease the efficiency of fractionated RT in FaDu tumors, adjuvant application improved the effect of RT. Explanatory studies and experiments testing the effect of ZK on a further hSCC line are under way.

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Oxygenation of cervical cancers during radiotherapy and the impact of hypoxia on microvessel-density

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Objective: The oxygenation status in tumors prior to radiotherapy is a useful predictive parameter The main subject of our investigation was to determine if there is a change of the oxygenation status of cervical cancers during definitive radiotherapy with regard to the prognosis and if there is an association between the pO2 and the microvessel density in primary tumors.