Poster Discussions: Oral

Radiobiology

526 POSTER DISCUSSION

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

C.E. Ruebe[1], F. Wilfert[1], D. Uthe[2], A. Schuck[2], N. Willich[2], C. Ruebe[1].
[1] University of Saarland, Radiotherapy/Radiooncology, Homburg/Saar, Germany; [2] University of Muenster, Radiotherapy/Radiooncology, Muenster, Germany

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

H. Nishimura[1], R. Sasaki[1], T. Soejima[1], Y. Ejima[1], E. Yoden[1], T. Shirakawa[2], Y. Ota[1], A. Matsumoto[3], K. Sugimura[1]. *[1] Kobe University, Radiology, Kobe, Japan; [2] Kobe University, Urology, Kobe, Japan; [3] Kobe University, Radiation Biophysics and Genetics, Kobe, Japan*

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

D. Zips[1], M. Krause[1], J. Westphal[1], K. Brehner[1], W. Eicheler[1], C. Hoinkis[1], R. Grenman[3], C. Petersen[1], A. Dörfler[1], M. Baumann[1,2]. *[1] University Hospital, Radiation Oncology, Dresden, Germany; [2] University Hospital, Experimental Center, Dresden, Germany; [3] Turku University, Otorhinolaryngology, Head and Neck Surgery, Turku, Finland*

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

G. Haensgen[1], U. Krause[2], F.W. Rath[2], J. Dunst[1]. *[1] Radiotherapy, Martin-Luther-University, Halle, Germany; [2] Pathology, Martin-Luther-University, Halle, Germany*

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.

Methods: Over the last 5 years we investigated 87 patients for the pO2-status of cervical cancer (age 34-80 yrs). All patients were treated with curative intent and the same treatment schedule. The pO2-measurements were done using polarographic needle probes and the Eppendorf-device.The microvessel density was determined in pretreatment biopsies immunhistologically using a CD31-antibody.

Results:. The median- pO2 for all 87 patients was 15 mmHg pooled over 4 measured tracks, so the threshold for classification was set to 15 mmHg. We found only a marginal effect of the pretreatment pO2 in the 3-year-survival (52 +9% vs.69 + 8% p =0,15) Measurements of oxygenation after 11 fractions revealed no changes of pO2 if the group of patients was analyzed as a whole group. The analysis of the pO2-changing after 11 fractions can be classified in 4 groups of pO2-modification: 1. a level >15mmHg at both measured points; 2. a level <15mmHg at both measured points 3. an increased or a decreased pO2 after 11 fractions. In a Cox-model - adjusted to stage -the best clinical results were obtained for patients with a well-oxygenated tumor independent of the time of high pO2-level, the worst results are shown in patients with persistent hypoxic cancers. In 46/87 patients the vasculary density prior to therapy was evaluated. Tumors with a persisting low pO2 showed a significantly higher vessel count in comparison to the 3 other groups (p<0,001).

Conclusiones: Our investigation demonstrated that tumor hypoxia is linked to angiogenesis. This supports the use of hypoxic-modifying strategies.

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Erythropoietin enhances radiation treatment efficacy in patients with pelvic malignancies.final results of a randomized phase III study

D. Antonadou[1], E. Cardamakis[2], M. Puglisi[1], N. Malamos[3], N. Throuvalas[1]. [1] *Metaxas cancer Hospital, Radiation Oncology, Piraeus, Greece;* [2] *University of Patras, Gynecology, Patras, Greece;* [3] *Helenas Maternity Hospital, Medical Oncology, Athens, Greece*

Purpose: To determine the efficacy and safety of the subcutaneous administration of recombinant human erythropoietin (EPO) to patients with pelvic malignancies receiving radiotherapy(RT).

Patients and Methods: 385 patients underwent conventional RT with 2 Gy daily fraction/5days/week to a total dose of 50-60 Gy+/- EPO 10000U daily 5 times per week. All patients received iron supplements. Primary endpoints were weekly haemoglobin increase and local tumor control. Secondary endpoints were safety, disease free survival and overall survival.

Results: There were no significant differences between the two groups for age, Hb levels before RT, site and stage of disease(P>0.1). The mean Hb levels during RT in the EPO group were 12.9±2.6g/dL versus 10.6±2.5 g/dL in the control group. The mean weekly increase of Hb was 0.54g/dL in the EPO group versus 0.17g/dL in the control group.The 4 year disease free survival was 85.3% in the EPO group versus 67.2% in the control group(p=0.0008).No EPO related side effects were observed.

Conclusion: EPO can be safely administered and improves significantly the local tumor control in patients with pelvic malignancies undergoing RT.

Genitourinary cancer

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The importance of implant dose on biochemical outcome following I-125 prostate brachytherapy

R. Stock[1], N. Stone[2], S. Hong[1]. [1] *Mount Sinai School of Medicine, Radiation Oncology, New York, USA;* [2] *Mount Sinai School of Medicine, Urology, New York, USA*

Purpose: To determine the effect of implant dose as assessed by CT based post-implant dosimetry on biochemical outcomes following I-125 brachytherapy for prostate cancer.

Methods: 234 patients were treated from 1991 to 1999 with I-125 brachytherapy without hormonal therapy or external beam irradiation for T1-T2 prostate cancer. All patients had Gleason scores < 7. Presenting PSA ranged from 1.3 to 189 (median 6.8). Clinical stage was T1b-T2a in 173 patients and T2b-T2c in 61 patients. All patients were implanted using a real-time ultrasound guided technique. One month post-implant, all patients underwent CT based dosimetric analysis. Implant dose was defined as D90 (dose delivered to 90% of the prostate on dose volume histogram). D90 values ranged from 15 to 256 Gy (median 163 Gy). All values conformed to TG43 guidelines. PSA failure rates were calculated with actuarial methods using the ASTRO definition. Follow-up from date of implant to last seen ranged from 24 to 119 months (median 47).

Results: Implant dose had a significant effect on freedom from PSA failure (FFPF) rates. Patients with D90 values of < 140 Gy (71), 140 - < 160 Gy (97), 160 - < 180 (100) and > or equal to 180 Gy (97) had FFPF rates at 7 years of 63%, 85%, 95% and 88%, respectively (p=0.0025). Overall, patients with D90s > or equal to 140 Gy had a FFPF rate at 7 years of 90% versus 63% for those with D90s < 140 Gy (p=0.0003). In addition, pretreatment PSA also significantly affected PSA failure with FFPF rates at 7 years of 82%, 83% and 24% for PSA levels of < or equal to 10, >10-20 and > 20, respectively (p=0.0001). A dose response cutpoint of 140 Gy was seen in both patients with initial PSA < or equal to 10 (190) and those with PSA > 10 (44), p=0.001 and p=0.04, respectively. The median follow-up was 48 months and 46 months for patients with D90 < 140 Gy and > or equal to 140 Gy, respectively. A multivariate analysis testing the effect of dose, PSA, score and stage on FFPF rates found dose to be the most significant predictor of outcome with p values of <0.0001, 0.03, 0.31 and 0.58, respectively.

Conclusions: Implant dose is the most significant predictor of PSA failure following I-125 prostate brachytherapy. Based on this analysis, optimal D90 values from the post-implant CT analysis should be > or equal to 140 Gy. Current data reveal no significant improvements with values > 180 Gy.

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Inhibition of 20S Proteasome results in serum IL-6 and PSA decline in patients (pts) with Androgen-Independent Prostate Cancer (AIPCa) treated with the Proteasome Inhibitor PS-341

C. Papandreou[1], D. Daliani[1], R. Millikan[1], S. Tu[1], L. Pagliaro[1], J. Adams[2], P. Elliott[2], P. Dieringer[1], C. Logothetis[1]. [1] *UT-MDACC, GU Medical Oncology, Houston, USA;* [2] *Millennium Pharmaceuticals, Inc., Boston, USA*

Purpose: Assessing surrogate markers of NFkappaB activity in patients with androgen-independent prostate cancer(AIPCa) treated with proteasome inhibitors is of great importance for drug development. Preclinical studies indicate that PS-341, a specific proteasome inhibitor, inhibits NFkappaB, which is implicated in the progression of PCa in bone and resistance to therapy. Serum IL6 concentration can serve as a surrogate of NFkappaB activity.

Methods: We studied 43 pts (age: 64 (45-78), PS:0/1: 43) with metastatic AI PCa treated on a Phase I trial of PS-341, administered intravenously weekly x 4 every 6 wks over 14 dose levels (0.13-1.6 mg/m2) for evidence of anti-tumor activity, serial serum (s) IL-6 concentration (by ELISA) and 20S proteasome inhibition (20S-PI). 20S-PI is measured ex vivo in peripheral blood (PB) using a fluorogenic substrate.

Results: 4/15 pts with 45-55% 20S-PI (0.75-1.21 mg/m2) and 16/18 pts with > 70% 20S-PI (1.32-1.6 mg/m2) had serum available for analysis. Patients with 45-55% 20S-PI had 40% suppression in median sIL-6 but no change in PSA slope or concentration, while pts with >70% 20S-PI had 80% decline in median sIL-6 with parallel decline in PSA slope (63% of pts) and PSA concentration (19% of pts). We also observed radiographic partial response (PR) in 2 patients.

Conclusions: Our data demonstrate a dose dependent decline in sIL-6 and PSA slope and concentration in pts treated with weekly PS-341. The biologic effect occurs within a tolerable dose range of PS-341. This data suggests that weekly PS-341 may be active in prostate cancer and supports the view that its action may be mediated through the inhibition of NFkappaB.

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Pronounced radiosensitization of estramustine phosphate in the treatment of locally advanced prostate cancer

M.S. Khil[1], A. Sharif[1], L.J. Bricker[2], J.H. Kim[1]. [1] *Department of Radiation Oncology,* [2] *Division of Medical Oncology, Henry Ford Hospital, Detroit, MI, USA*

Purpose: Since the potential of Estramustine phosphate (EMP) as tumor radiosensitizer has been shown extensively by us and others in animal models, a clinical study was designed to test the hypothesis that EMP would preferentially enhance the anti-tumor effects of radiation therapy for the treatment of prostate cancer. A prospective phase II trial was carried out to determine whether the combined EMP and external beam radiotherapy (EBRT) would increase the tumor control rate of locally advanced prostate cancer with no enhanced normal tissue toxicity.

Methods: Between January 1991 and March 2000, 75 patients (pts), stage T2 through stage T4, were entered into the study. Forty-seven (63%)