The correlation was only seen for lymph node negative tumours and was independent of clinical variables like tumour volume and stage.
Conclusions: In conclusion, our results indicate that the short EGFR isoforms can be used as a marker of response to chemoradiotherapy in lymph node negative cervical tumours. The absence of prognostic significance of the phosphorylation status indicates that EGFR mediates disease progression through kinase-independent mechanisms.

848 Identification of unknown regulators of radiation-induced checkpoints by siRNA-based large scale screening

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Background: Ionizing radiation-induced DNA damage activates the G1, S and G2/M cell cycle checkpoints. These checkpoints help prevent proliferation of potentially genetically unstable cells and support repair of the damage.
Materials and Methods: To search for unknown regulators of the radiation-induced G2 checkpoint, siRNA-based screening was performed with a siRNA library to human phosphatases. U2OS osteosarcoma cells were seeded and transfected with the siRNAs, treated with IR (6 Gy) and nocodazole at 2 days after transfection, and stained with an antibody to a mitotic marker (phospho-H3) at 10 hours after IR, followed by imaging and analysis of each well.
Results: The siRNA screen for the G2 checkpoint was run successfully. The human phosphatome siRNA library identified two phosphatases, PTPN7 and SSH3, which have been validated as positive hits. The molecular mechanisms involved are being explored.
Conclusion: Here we identified phosphatases which, when depleted, abrogate the G2/M checkpoint, and may therefore contribute to protect against carcinogenesis.

849 Cetuximab penetration and EGFR expression in tumour spheroids: prerequisite for testing a new radiotherapeutic approach

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Background and Aim: Multicellular tumour spheroids are a well-established 3-D *in vitro* culture model for sophisticated experimental therapy testing. We intend to adapt our *Spheroid-Based Drug Screen* to a new antibody-based radiotherapeutic approach. The epidermal growth factor receptor (EGFR) is over-expressed in many epithelial tumours. Blockade of the EGFR signaling through the therapeutic antibody Cetuximab is a target-specific strategy for the treatment of such tumours combined with radiotherapy. Spheroid cultures of different human squamous cell carcinoma cell lines were established and characterized for EGFR expression and antibody penetration, for further examination of radionuclide-conjugated Cetuximab treatment combined with external radiation.
Materials and Methods: Spheroids were cultured using a standardized semi-automated set-up. Spheroid treatment included single dose irradiation (0−20 Gy) and incubation with Cetuximab at different concentrations and time intervals. Spheroids were imaged by phase contrast microscopy after irradiation for analyzing spheroid integrity and regrowth. In a representative spheroid type (FaDu), penetrated Cetuximab was detected in 10 μm median cryo sections by immunofluorescence. EGFR expression was verified by immunostaining and western blot analysis.
Results: Seven out of ten squamous cell carcinoma cells forme spheroids, three of these can be implemented in a routine therapy test platform. Spheroid volume growth, regrowth and growth delay can be easily analyzed after irradiation using the *Spheroid-Based Screen* set-up. Our data further verify that target molecule (EGFR) and penetrated therapeutic antibody (Cetuximab) can be visualized in the same spheroid section. The Cetuximab penetration kinetics reveals increasing numbers of cell layers to bind Cetuximab up to 16 h of exposure. After 24 h Cetuximab has penetrated entire spheroids with a mean diameter of 370–400 μm. The expression of EGFR seems relatively uniform in untreated FaDu spheroids but appears higher in spheroids after Cetuximab exposure. This phenomenon will be studied in further detail.
Conclusions: Spheroids are well suitable to monitor the penetration and impact of antibody (Cetuximab)-based therapeutic strategies. The model can now be applied to test an innovative treatment regime using radionuclide-conjugated Cetuximab combined with external irradiation.
Supported by the German Federal Ministry of Education and Research (BMBF) through grants 01ZZ0502 and 03NUK006.

850 The role of DNA repair gene polymorphisms in the development of radiation-induced late toxicity in prostate cancer patients

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Background: Intrinsic radiosensitivity is determined in particular by the cells' capacity to repair radiation induced DNA damage. Modulation of repair capacity by single nucleotide polymorphisms (SNPs) in genes responsible for DNA damage signaling and repair might affect cell and tissue response to radiation and therefore influence individual radiosensitivity and the risk of radiation-induced toxicities. The aim of the present study was to evaluate the role of SNPs in genes involved in DNA repair for the development of radiation-induced late side effects in prostate cancer patients treated with radiotherapy.
Patients and Methods: To analyze the role of polymorphisms in DNA repair genes for late toxicity 603 participants from the Austrian PROCAGENE study were included in the present investigation. All patients underwent three-dimensional conformal radiotherapy. Late genitourinary and gastrointestinal toxicity was graded according to standard RTOG criteria. Six functional candidate polymorphisms in XRCC1 (Arg194Trp, Arg280His, Arg399Gln), XRCC3 (Thr241Met) and ERCC2 (Asp312Asn, Lys751Gln) were selected for analysis and determined by 5′-nuclease (TaqMan) assays. Statistic analysis was done using SPSS 16.0 for Windows.
Results: Within a median follow-up time of 35 months, 91 patients (15.7%) developed genitourinary and/or gastrointestinal late toxicity RTOG $\geqslant$ 2. In a Kaplan–Meier analysis, carriers of a XRCC1 280His allele were at decreased risk of late toxicity grade $\geqslant$2 ($p = 0.022$). In a univariate Cox regression model, the relative risk of carriers of a XRCC1 280His allele for late toxicity $\geqslant$2 was 0.28 (95% CI 0.09–0.90; $p = 0.032$), in a multivariate Cox regression model carriage of a XRCC1 280His allele was associated with a relative risk of 0.27 (95% CI 0.09–0.86; $p = 0.026$). No significant associations were found for the remaining polymorphisms.
Conclusion: We conclude that the XRCC1 Arg280His polymorphism may be protective against the development of high-grade late toxicity after radiotherapy in prostate cancer patients. If confirmed in future studies our findings could contribute to the construction of predictive risk models for the occurrence of late radiation-induced toxicity in prostate cancer patients. The increasing knowledge of the influence of polymorphisms on individual radiosensitivity could lead to an individualization of radiotherapy, thereby minimizing radiation-induced toxicity and improving efficacy of radiation therapy.

851 Genetic variation in relation to adverse side effects of radiotherapy – focus on the metabolism of reactive oxygen species

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Background: Improved detection and early diagnosis of cancer are likely to increase the importance of loco-regional control and hence the significance of radiotherapy (RT) in the treatment of this disease. Like most treatments RT has the power to heal but also to harm and is associated with a wide-range of long-term complications depending on the properties of the administered therapy and the tissue affected by the malignancy. In this study we investigate the association between genetic variation in proteins involved in metabolising reactive oxygen species and the level of radiation induced adverse side effects in breast cancer patients as well as the expression level in irradiated fibroblasts. The aim of the study is to identify genetic markers of radiosensitivity and investigate the possible link between expression profiles associated with radiosensitivity and the genetic background.
Materials: BC I: 92 Norwegian breast cancer patients treated with hypofractionated RT (4.3 Gray $\times$ 10) administered to the breast wall and/or regional lymph nodes. Adverse effects evaluated: athrophy, subcutaneous fibrosis, costal fractures, telangiectasias and pleural thickening.
BC II: 302 samples from BC patients treated with RT (2.0 Gy*20, 2 treatments per week) after curatively intended surgery for BC Stage II/III. Adverse effects studied: fatigue, hypothyroidism, telangiectasias and subcutaneous fibrosis.
Fibroblast cell lines from 33 Danish breast cancer patients already analysed with whole genome expression profiling.
Results: For all clinical end-points studied, we identified SNPs significantly associated with the level of adverse effects in samples from the BC I series by two different statistical methods (Mutual information score and Chi-square/ the Cochrane Armitage trend test). For subcutaneous fibrosis the identified

SNPs were: rs744751 and rs731465 both in the 5′UTR region of *TGFBR2*, rs1139793 a missense mutation in *TXNRD2* resulting in an Ile/Thr amino acid change, rs945222 an intronic SNP in *MGMT*, rs1934951 an intronic SNP in *CYP2C8* and lastly rs4073 in the 5′UTR region and the intronic rs2227306 both in *IL8*. In the BC II set only rs1139793 in *TXNRD2* were found significantly associated (after Bonferroni corrections) with the level of fibrosis (p-value ranging from 0.0001 to 0.005 under different genetic models). TXNRD2 is a mitochondrial enzyme central in the regulation of the intracellular redox environment and one of three known enzymes reducing thioredoxin, a potent antioxidant molecule that exerts both anti-apoptotic and anti-inflammatory functions.
With regards to the fibroblast cell lines, a link between mRNA expression after RT and genetic variation in genes involved in among other IL8 signaling were identified. Genetic variation in IL8 was also found associated with level of fibrosis in breast cancer patient receiving RT in the BC I material but this could not be validated in BC II.

852 Prediction of response of locally advanced rectal adenocarcinomas to neoadjuvant chemoradiotherapy by microRNA profiling

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Background: Fluoropyrimidine-based chemoradiotherapy before total meso-rectal excision (TME) is currently the gold standard treatment for stage II and III rectal cancer patients. Pathological complete response (pCR/TRG1) is related with a longer relaps and overall survival. On the other hand, patients with a primary resistant tumours could be spared exposure to chemotherapy and radiation that are associated with substantial adverse effects and costs, and surgery could be scheduled without delay.
We have used microRNA profilling to identify in our series responders and non responders to preoperative treatment.
Material and Methods: Twelve patients (pts) with locally advanced rectal adenocarcinomas who underwent neoadjuvant chemoradiotherapy (capecitabine 825 mg/m2 twice a day for a period of 38 days and 45 Gy/PTV1 + 5.4 Gy/PTV2) were included. Response to therapy was classified by TRG score (TRG – histological tumour regression grade according Mandard; Cancer 1994;73:2680–2686) and patients were divided into two groups: “responders” (“R group”, TRG1/pCR and TRG2) and “non-responders” (“NR group”, TRG4 and TRG5). “R” group (7 pts): 4 pts achieved TRG1 and 3 patient TRG2; “NR” group (5 pts): 4 patients achieved TRG4 and 1 patient TRG5. Sequential tumour biopsies were done before and 14 days after initiation of the therapy. MicroRNAs were extracted from each frozen tumour specimen and expression levels of 667 microRNA genes known to be involved in cancer biology were obtained by Real-Time PCR using 7900HT Fast Real-Time PCR system and TaqMan® MicroRNA Array v 2.0 (667 miRNA, according to Sanger miRBase v10).
Results: MicroRNA gene expression data analysis based on SAM (Significance Analysis of Microarrays) and t-test methods identified 3 microRNA genes (miR-215, miR-378 a miR-451) with significantly up-regulated expression in primary tumours of “NR” ($p = 0.01$). In subsequent cluster analysis this group of microRNA genes was able to discriminate good from poor responding tumours.
Conclusion: Our preliminary data suggest the ability of microRNA expression profiles to predict response/resistance to selected treatment in rectal cancer patients. Supported by grants: Internal Grant Agency of Czech Ministry of Health (IGA MZ CR) NR/9076 and NS/9814.

853 Selective internal radiation therapy of hepatocellular carcinomas using Yttrium-90 microspheres – initial clinical experiences

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Background: Hepatocellular carcinoma (HCC) is the 5th most common cancer in the world and the 3rd most common cause of cancer related deaths, and is estimated to occur at a global rate of 1 million new cases annually. Often an indolent disease, clinical symptoms often only appear at an advanced stage of disease, and most patients with primary HCC are diagnosed at an intermediate to advanced stage of the disease, for which there are no standard therapies available.
Selective internal radiation therapy (SIRT) via transarterial delivery of Yttrium-90 (Y-90) microspheres is an emerging modality in the therapy of patients with unresectable HCC. We present our initial clinical experiences in regards to Y-90 SIRT in patients with unresectable HCC.
Materials and Methods: Retrospective review of patient’s referred to the Department of Nuclear Medicine and PET for Y-90 SIRT was performed. 104 cases were reviewed. 24 cases did not meet criteria for Y-90 microsphere therapy after initial dosimetric assessments, 35 cases were excluded because they were part of a multicenter therapeutic trial, and 5 cases were excluded because these were for tumours other than HCC.
35 patients (14 female and 21 male, mean age 66 years, range 49−79) with unresectable HCC were reviewed; all of whom underwent Y-90 SIRT, and with 5 patients having repeat treatment. Tumour staging was performed using the Barcelona Cancer Liver Criteria (BCLC), of which there were 2 BCLC stage A, 24 BCLC stage B and 14 BCLC stage C cases. Comprehensive pre-therapeutic evaluation was performed, with Y-90 activity dose (range 1.0−4.0 GBq) depending on dosimetric evaluation findings.
Results: At the 3-month post therapy assessment, there was 1 complete response (2.5%), 8 partial response (20%), 19 stable disease (47.5%), and 3 progressive disease (8%), based on CT RECIST assessment criteria. 9 patients (22%) did not have any follow-up imaging available for assessment.
Median time to tumour progression was approximately 510 days. As of end 2009, median time of survival has not been reached, with 2 recorded deaths that were presumably tumour related.
All patients tolerated the Y-90 SIRT well, with no acute complications encountered. One patient who underwent repeated Y-90 SIRT developed radiation pneumonitis approximately 3 months following therapy, possibly related to the relatively high lung shunting and cumulative activity administered. 7 patients developed transient hepatic transaminitis, but were generally asymptomatic and recovered without further complications. No complications were encountered in the remainder of the patients.
Conclusions: Our clinical experiences show promising clinical results, and with an overall low incidence of complications after Y-90 microsphere therapy if patients are selected appropriately and target delivery is performed meticulously. Y-90 SIRT shows good clinical promise, and appears a viable option in the treatment of patients with unresectable HCC.

854 The Amyloid Beta Precursor Protein-Binding Protein 1 (APP-BP1) encoding gene is involved in the radiosensitivity of the Human Papillomavirus (HPV)-positive SCC90 oropharyngeal cell line

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Background: Human papillomavirus (HPV)-positive oropharyngeal cancers define a head and neck squamous cell carcinoma (HNSCC) distinct clinical sub-population of patients associated with an increased survival. We previously have shown that HPV-positive oropharyngeal lesions display a loss of genetic material in the16q chromomal region, and a decreased expression of the gene that encodes the Amyloid β Precursor Protein-Binding Protein 1 (APP-BP1), located in 16q22. APP-BP1 is required for the repression of p53 transcriptionnal activity by regulating its post-translation conjugation to NEDD8 (NEDDylation). Thus, we postulate that the deregulation of APP-BP1 in p53-positive HPV-related HNSCC could be involved in an increased radiosensitivity.
Material and Methods: We used the SCC90 (oropharynx; HPV16-positive; wild type p53; radiosensitive) and SQ20B (larynx; HPV-negative; mutated p53; radioresistant) cell line models. *APP-BP1* overexpression was achieved by cell transfection. The influence of *APP-BP1* expression levels on cell sensitivity to ionizing radiations was assessed by measuring the clonogenic survival of cells after a 2 Gy irradiation. Cell death rates were evaluated by Propidium Iodide staining and FACS analysis.
Results: In order to validate our cell line model system, we used a qRT-PCR approach to assess the expression of the *HPV16 E6*/*E7* mRNA, of $CDKN2A^{p16}$ (used as a biomarker for an active HPV genome), $WAF1/CIP1^{p21}$ (a p53 target gene), and *APP-BP1* in the SQ20B and SCC90 cell lines. Similarly to what is observed in human tumours, SCC90 show high *HPV16 E6*/*E7* mRNA and $CDKN2A^{p16}$ expression as compared to SQ20B cells. As a consequence of the presence of a wild-type p53, they express higher levels of $WAF1/CIP1^{p21}$. Interestingly, they display a diminished expression of *APP-BP1*. SCC90 cells were transfected with an *APP-BP1* expression vector and irradiated with X rays. We observed an increased radioresistance (Surviving fraction at 2 Gy: 0.89) as compared to mock transfected cells (Surviving fraction at 2 Gy: 0.36). *APP-BP1* overexpression also correlated with the repression of the p53 transcriptional activity and with a slightly diminished cell death rate.
Conclusions: Altogether, our results suggest that increased *APP-BP1* expression levels induce the radioresistance of SCC90 cell line via the inhibition of p53 transcriptionnal activity. Interestingly, the NEDDylation pathway has recently been proposed to be a potential target for cancer therapy.