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.
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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