844 MRI tissue characterization in patients with glioblastoma multiforme

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Background: Glioblastoma multiforme (GB) is the most common and aggressive grade IV primary brain tumour in humans. Magnetic resonance imaging (MRI) is the method of choice for diagnosis, evaluation and follow-up of brain tumours. Currently the accepted radiographic criteria for brain therapy response is based on Macdonald criteria. Positive response is defined as more than 50% reduction in tumour size measured on the contrast enhanced area in T1 weighted post contrast images. However this criterion is not sensitive enough, is often questionable and can sometimes be misleading. It is based solely on blood brain barrier impairment which in not specific and can be seen on varied pathologic conditions. Combinations of several MRI sequences can give indications for other tissue classes, including infiltrating peri-tumoural edema, non-enhancing tumour and necrotic area, and therefore may assess therapy response more reliably. The vast amount of data resulting from multi-modal MRI data calls for automated analysis. Various methods for automated segmentation were suggested, many of them focused on the healthy tissue. Studies in patients mainly used unsupervised classification. In this study, we will show segmentation of brain tissues of patients with GB, based on multi-modal MRI data sets, using a supervised algorithm.
Material and Methods: 14 Patients with recurrent GB were scanned several times (1−8 MR scans each). Each scan included several sequences: T1 weighted (W), T2W, FLAIR, 3D SPGR after contrast agent administration and gradient echo. K-nearest neighbor (KNN) algorithm was used, and nine classes were defined: gray/white matter, CSF, skull, arteries, necrosis, edema, peri-tumoural edema and tumour.
Results: The algorithm was successfully applied to all patients, demonstrating longitudinal changes that were generally correlated with the radiology and clinical assessments. The algorithm provided quantitative information about the volumetric changes in each tissue class. Although during the first 4−8 weeks a general reduction of tumour volume was detected in all patients, further changes revealed different response patterns between the patients.
Conclusions: Combination of several MRI sequences can give more specific information regarding tumour assessment and therapy response. This can be obtained more efficiently with automated classification. The extensive analysis, with full volumetric measurements of the different tissue classes based on multi-modal data reflecting several physical and biological parameters may be able to predict therapy response and help with therapy decision making.

845 The RYBP apoptosis pathway is deactivated in cervical cancer patients with 3p-loss

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Background: Genetic gains and losses, i.e. gene dosage alterations, influence gene expression levels and thereby promote tumour growth and progression. The purpose of this work was to identify driver genes in cervical cancer progression, and to explore their role in the development of chemoradioresistance.
Materials and Methods: Totally 188 cervical cancer patients that received chemoradiotherapy with curative intention were included. Gene dosage and expression profiling was performed on 94 of these patients by array comparative genomic hybridization and Illumina gene expression beadarrays respectively, based on pretreatment tumour biopsies. Protein expression was measured by immunohistochemistry.
Results: We identified three deleted regions on 3p11.2-p14.2, 13q13−21, and 21q22, which were associated with poor progression free survival independent of existing clinical markers. The region on 3p is a frequently deleted chromosomal region in cervical cancer and is therefore thought to be important for carcinogenesis. Integrative analysis of gene dosage and gene expression identified 6 genes for which reduced gene expression was highly associated with a lower gene dosage ($p < 10^{-5}$), indicating that these genes were primarily regulated by the 3p loss. Immunohistochemical nuclear staining of one of these genes, RYBP, revealed significant associations between protein expression, gene dosage and gene expression, showing that also the protein level of RYBP was reduced in tumours with loss on 3p. RYBP is known to interact with FADD and DEDD to facilitate death-receptor mediated apoptosis. Gene Set Enrichment Analysis showed that this apoptosis-signaling pathway was significantly deactivated in tumours with 3p-loss.
Conclusions: This work indicates that loss on 3p in cervical cancers leads to apoptosis evasion through loss of RYBP, and that this may lead to poor survival of patients with 3p-loss. Targeting the RYBP apoptosis pathway may be a fruitful strategy to improve the outcome of chemoradiotherapy for patients with cervical cancer.

846 Ionizing radiation inhibits protein translation by bypassing the protein kinase B/Akt

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Background: The protein kinase B (PKB)/Akt signaling pathway is frequently deregulated in tumour cells and contributes to resistance against common anti-neoplastic therapies. Overexpression of the phospho-inositol-3-kinase subunit p110 or deletion of the antagonistic phosphatase and tensin homolog (PTEN) results in a constitutive activation of PKB/Akt. PKB/Akt activates the serine/threonine kinase mammalian target of rapamycin (mTOR) by phosphorylation. The downstream targets of mTOR are the key translation regulator p70 ribosomal S6 kinase (p70S6K) which becomes activated upon phosphorylation and the translational inhibitor 4E-BP1 which becomes inactivated upon phosphorylation and releases the eukaryontic initiation factor eIF-4E to initiate cap-dependent translation. Inhibition of protein biosynthesis can result in decay of instable proteins with a high turnover rate and affect the vitality of the cell.
To date, the impact of ionizing radiation on protein translation is not well understood.
Matherial and Methods: Jurkat T cells (PTEN negative) were treated with 50–100 μM of the PI3K inhibitor LY294002 or irradiated with 10 Gy. Inhibition of translation was verified by Western blotting analyzing the phosphorylation status of p70S6K and the translational inhibitor 4E-BP1. Expression levels of the instable anti-apoptotic protein Mcl-1 and phosphorylation status of PKB/Akt was also determined by Western blotting. Induction of apoptosis and the breakdown of mitochondrial membrane potential (DYm) was analyzed by flow cytometry. Mcl-1 was downregulated by siRNA which was electroporated into Jurkat T cells.
Results: The PI3K inhibitor LY294002 induced dephosphorylation of PKB/Akt, p70S6K and the translational inhibitor 4E-BP1 as well as the downregulation of the anti-apoptotic protein Mcl-1. Downregulation of Mcl-1 by siRNA was sufficient to induce DYm breakdown and DNA degradation within 6 h after electroporation. Ionizing radiation did not affect the phosphorylation status of PKB/Akt but also reduced phospho-4E-BP1 and phospho-p70S6K levels indicating an inhibition of protein translation. The inhibition of protein translation correlated with a drop of Mcl-1 levels, DYm breakdown and apoptosis induction.
Conclusions: In Jurkat T cells, protein translation is regulated by the PI3K/Akt pathway. Ionizing radiation bypasses this pathway to inhibit protein translation. Reduced protein biosynthesis results in a decline of the anti-apoptotic Mcl-1 which is followed by DYm breakdown and apoptosis induction.

847 Prognostic value of EGFR phosphorylation and short isoforms in cervical cancer patients receiving chemoradiotherapy

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Background: The epidermal growth factor receptor (EGFR) is known to be overexpressed in various tumours. It may contribute to tumour aggressiveness through its tyrosine kinase phosphorylation, or by promoting metabolic homeostasis independent of kinase activity. Investigating phosphorylated EGFR is problematic using immunohistochemistry (IHC), because of the difficulty of designing phosphoamino-specific antibodies. Several naturally occurring EGFR isoforms exist in addition to the full length transmembrane form. Some of them contain only various parts of the extracellular domain, thus lacking the intracellular kinase domain. Little is known about the function of these short isoforms and their role in cancer progression. The aim of this study was to investigate the expression of the different isoforms and the phosphorylation status of EGFR in cervical cancers and to evaluate their prognostic significance.
Material and Methods: A total of 185 cervical carcinoma patients receiving chemoradiotherapy with curative intention was included. Gene dosage was measured by array comparative genomic hybridization based on pretreatment tumour biopsies. Protein levels of EGFR was measured by IHC using two antibodies binding to the extracellular and intracellular domain, respectively. Combining the data from the two antibodies reflected the expression of short isoforms, while the data from the antibody binding intracellularly reflected the full length isoform. A proximity ligation assay with high specificity was used to evaluate EGFR phosphorylation.
Results: EGFR amplification was present in 14.4% of the tumours, and correlated with poor survival. The full length form of EGFR was expressed in 96% of the cervical tumours, and phosphorylation of EGFR was seen in 62% of the cases. Neither the total EGFR protein level nor the phosphorylation status of EGFR were found to be prognostic. Expression of only short isoforms, however, was highly correlated with poor survival independently of end point (progression free survival, overall survival, locoregional control).

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