Material and Methods: We used the EpCAM expressing squamous cell carcinoma cell lines Kyse 30 and 520 as in vitro model. To measure the potential effects of loss of EpCAM expression, we used the lentiviral pGIPZ shRNAmir system with two different sh-RNAs directed against EpCAM and one control shRNA vector. The EpCAM-suppression of the transduced cells was tested by quantitative RT-PCR and immunoblotting. Those cell lines with at least 80% reduction of EpCAM expression were further analysed. We used the "Fence-assay"® to investigate the migration. The tumour cell invasion was assessed with a commercially available Matrigel-coated Transwell system®. Transcriptome profiling of shRNA-transduced cell lines and control-vector transduced cell lines, respectively, was done with Agilents "*whole genome Array*".
Results: The migration of EpCAM-shRNA-transduced squamous cell carcinoma cells was reduced by 30−50% compared to tumour cells transduced with the control-vector. A 3−4% reduction of the invasion was observed. Both, the reduction in migration and invasion were statistically significant. Changes in the transcriptome expression were noted in shRNA-transduced cell lines compared to control-vector transduced cell lines. The differentially expressed genes fell in the categories cell structure, cell movement and developmental processes.
Conclusion: Our data indicate an active biological role of EpCAM in oesophageal squamous cell carcinoma progression and makes it a promising therapeutic target for this entity. However, the exact mechanisms of action warrant further investigation.

519 Impact of ECM on phenotype and EGFR inhibition in colorectal cancer cell lines

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Background: 3D tumour cell cultures grown in extracellular matrix (ECM) are considered to reflect human tumours more realistic than monolayers grown on plastic. ECM has not only drastic effects on phenotype but also on response to targeted therapies, as recently demonstrated in breast cancer cell lines. Here, we systematically investigated the impact of ECM on phenotype and on EGFR inhibition in commonly used colorectal cancer (CRC) cell lines.
Material and Methods: On-top matrigel assays were performed with SW480, HT29, DLD-1, LOVO, CACO, Colo 205 and Colo 206F cell lines. The phenotype of the 3D culture was assessed and compared to conventional 2D cell culture. Expression of genes involved in proliferation and cell adhesion was analysed on the transcriptional (quantitative RT-PCR) and on the protein level (immunoblotting and confocal imaging). Invasive capacity of the cell lines was assessed via Matrigel®-Boyden chamber assay. EGRF inhibition was achieved using tyrphostin AG 1478.
Results: A specific spheroid growth pattern was observed for all investigated CRC-cell lines. DLD-1 and CACO showed a clear solid tumour cell formation, HT29, SW480 and LOVO exhibited budding structures, while Colo 205 and Colo 206F showed grape-like structures. The 3D culture phenotype of the cell lines was not correlated to their invasive/migratory capacity. A significant reduction of the gene expression was noted for most investigated genes in 3D culture. In contrast, E-cadherin was up-regulated in several cell lines. Effects of EGFR inhibition was noted in 2D and 3D culture of sensitive cell lines.
Conclusion: The observed differences between the cell culture models corroborate the influence of ECM for cancer growth. Compared to conventional 2D cell culture, the 3D cell culture model (Matrigel® on-top assay) offers the opportunity to investigate potential molecular targets under more realistic conditions.

520 Effect of selenium on rat liver cell proliferation after partial hepatectomy

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Background: Studies on tumour cell lines, animal studies and human trials have demonstrated a tumour preventive effect of selenium (Se). Selenium-treatment in tumour preventive doses inhibit liver cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. The selenium dependent redox-enzyme thioredoxin reductase (TxR1) was over expressed in proliferating nodular liver lesions in the model. In this work we have studied the effect of selenium on regenerative cell proliferation and on the expression of TrxR1 in rats after 2/3 partial hepatectomy (PH).
Material and Methods: Fischer344 male rats were given 5ppm sodium selenite in the drinking water one week prior to PH, and until sacrificed. Non-treated hepatectomised and non-treated sham operated rats were used as controls. Bodyweights and relative liver weight were monitored. Cell proliferation, mitotic figures and occurrence of TrxR1 were determined immunohistochemically (IHC). TrxR1 enzyme activity, mRNA expression, and protein levels were analysed using TrxR1-assay, real-time PCR and western blot.
Results: No differences in bodyweights, relative liver weights and regeneration of liver mass were shown between groups. The peak of the S-phase marker Mib5 coincided while the peak of the mitotic figures was slightly delayed in treated rats. IHC staining for TrxR1 revealed a zonal, periportal increase of enzyme expression at 24h post PH, corresponding to the zone of Mib 5 positive cells and mitotic figures. After PH the TrxR1 enzyme activity increased from 8 hours with a peak at 48 hours post PH in Se-treated animals. In non-treated animals a similar but lower induction of the activity was shown between 8−72 h post PH. The TrxR1 activity was not changed over time after sham-surgery. TrxR1 mRNA increase at 4 hours post PH was seen in all groups.
Conclusions: We have concluded that, although a slight delay of cell division was shown, the gain of liver mass and regeneration of the liver function after PH is not affected by selenite. The increase of thioredoxin reductase correlated with cell proliferation and was further induced by selenium.

521 Enhanced pulmonary tumourigenesis by N-nitrosobis (2-hydroxypropyl) amine after thoracic irradiation with X-rays in new born, juvenile and adult Wistar rats

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Background: The possibility that combined exposures to environmental pollutants and ionizing radiation could increase the risk of lung cancer of the general public is a matter of great concern. We investigated the combined effects of radiation and a chemical carcinogen on pulmonary tumourigenesis in rats exposed at different age in well-defined exposure conditions.
Material and Methods: Female 1-, 5- and 22-week-old Wistar rats were irradiated locally on the thorax with X-rays (3.18 Gy), and/or were given *N*-nitrosobis (2-hydroxypropyl) amine (BHP; 1 g/kg body weight) intraperitoneally 1 week after thoracic irradiation.
Results: Non-irradiated and non-BHP-injected control rats survived to 90 weeks of age when all rats were sacrificed, but administration of BHP with or without irradiation resulted in survival reduction due to kidney, brain, liver and ovarian tumours. The incidences of lung tumours including adenomas and adenocarcinomas in rats irradiated alone at 1, 5 and 22 weeks were 8.7, 15.0 and 20.0%, respectively. On the other hand, the incidences in the rats administered with BHP alone at 2, 6 and 23 weeks were 60.9, 25.0 and 30.0%, respectively. When a combination of irradiation and BHP was used, the incidences in the rats treated at 1−2, 5−6 and 22−23 weeks were 61.9, 65.0 and 55.0%, respectively. The incidence of adenocarcinomas in the rats treated at 5−6 weeks was significantly increased compared to rats exposed to either X-rays or BHP alone.
Conclusion: The combined effects are age-dependent and administration of BHP after X-ray irradiation synergistically enhances induction of lung adenocarcinomas in juvenile rats. These results indicate that Wistar rats exposed to X-rays and BHP are a suitable animal model to study the risk and the mechanisms of the combined effects of radiation and chemicals on pulmonary tumourigenesis.

522 Selenium homeostasis and induction of thioredoxin reductase upon long term selenium supplementation in the rat

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Background: Selenium is an essential micronutrient for human and animals. Selenium treatment in supranutritional but subtoxic doses of 1 ppm and 5 ppm have shown to inhibit cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. Selenium tumour prevention in chronic liver diseases requires long-term selenium supplementation and there is still quiet limited knowledge on selenium long term effects. Thioredoxin reductase (TrxR1) is a selenoenzyme essential for maintaining intracellular redox status and avoid oxidative stress. TrxR1 is overexpressed in proliferating liver nodules in the rat liver model. In this work we have studied selenium homeostasis in serum and liver as well as TrxR1 induction after long term selenium supplementation in the rat.
Materials and Methods: The kinetics of selenium uptake and accumulation and TrxR1 induction after treatment with sodium selenite in the drinking water in doses of 1 ppm and 5 ppm for 10 weeks have been studied in male Fisher rats. After withdrawal of selenium treatment the selenium status and TrxR1 induction were studied at 3 and 6 months of the experiment.
Results: Long term selenite exposure via the drinking water cause a dose dependent increase of blood and liver levels of selenium. This increase levels out at 6 weeks at the same level of selenium regardless of treatment and dose. Thus, there is no accumulation of selenium in blood and liver over time at chronic exposure. The same effect was seen on the induction of TrxR1 activity, while the induction of TrxR1 mRNA was only seen during the first two days of treatment. Discontinuation of selenite exposure did not result in

significant reduction of neither selenium content nor TrxR1 expression levels during the following weeks and even at later time points. Sodium selenite at the dose levels of 1 and 5 ppm did not affect body weight or relative liver mass.
Conclusion: Long term treatment of selenite doesn't cause accumulation of selenium or permanent changes of TrxR1 expression. Thus selenium at doses up to 5 ppm could be used in long term tumour prevention.

523 Caspases in c-Myc induced apoptosis

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Background: c-Myc is a transcription factor that can promote both cell growth and cell death, apoptosis. Caspases have been found to play an important role in mediating and amplifying the apoptotic signal. The current view is that c-Myc induces mitochondrial permeability changes which then lead to the activation of the caspase cascade. The order of the activation of the caspases is, however, still elusive.
Material and Methods: In this study we used the Rat-1 MycER^{TM} fibroblast cell line expressing the conditionally active c-Myc-mutant oestrogen receptor chimera. c-Myc was activated and apoptosis induced by adding 4-hydroxytamoxifen in low serum conditions. The cells were harvested at the different time points to study the kinetics of the activation of caspases after c-Myc induction.
Results: Studies with pan caspase inhibitors showed that caspases are required for c-Myc-induced apoptosis in Rat-1 MycER^{TM} fibroblasts. Several key cellular proteins, such as protein kinase Cδ, poly(ADP-ribose) polymerase, replication factor C, 70 kDa subunit of U1 snRNP, fodrin, Mdm-2, lamins B1 and B2 and ataxia telangiectasia mutated (ATM)-protein were specifically processed by caspases. We also show the order in which the caspases-3, -7, -8, -9 and c-FLIP (a catalytically inactive homologue of caspase-8) become activated.
Conclusions: Caspases are centrally involved in mediating the apoptotic signal of c-Myc in rat fibroblasts, as judged from the caspase inhibitor studies and specific cleavage of a number of vital cellular proteins. The order of activation of caspases was not consistent with c-Myc primarily inducing mitochondrial permeability change and consequent activation of caspase-9.

524 Oesophageal cancer proliferation is mediated by cytochrome P450 2C9 (CYP2C9)

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Background: Cytochrome P450 epoxygenases (CYP's) have been recently shown to promote malignant progression. Here we investigated the expression and potential clinical relevance of the epoxigenase CYP 2C9 in oesophageal cancer.
Methods: We determined the expression of CYP 2C9 in esophageal adenocarcinoma (EAC; n = 78) and oesophageal squamous-cell carcinoma (ESCC; n = 105) compared to adjacent normal oesophageal mucosa (NEM; n = 79) on the transcriptional and protein level. Results were correlated with histo-pathological data. The proliferation index was analysed by Ki67 immunostaining. To assess its biological role in oesophageal cancer, CYP 2C9 was inhibited with sulfaphenazole in the EAC cell lines OE33 and PT 1590 and the ESCC cell lines KYSE-30 and KYSE-270. Proliferation was measured by MTT assay and Cell-cycle analysis was performed by using BrdU-FACS.
Results: The highest CYP2C9 expression was detected in NEM. The expression level in EAC was comparable to NEM but was significantly lower in ESCC. Interestingly early tumour stages showed a significantly higher CYP 2C9 expression compared to progressed tumours in both histologies. Furthermore we noted a correlation between CYP 2C9 expression and Ki67-positive proliferating tumour cells. Pharmalogical inhibition of CYP2C9 decreased cell proliferation in vitro, which was reversed by application of 11,12-EET. Inhibition of CYP 2C9 led to a G0/G1 cell-cycle arrest.
Conclusion: CYP 2C9 seems to be relevant for early oesophageal cancer development by promoting tumour cell proliferation. Thus pharmacological inhibition of CYP 2C9 might contribute to a more efficient therapy in CYP 2C9 expressing oesophageal cancers.

525 Global gene analysis reveals ephrin B3 as a potential radio sensitizing target in non small cell lung cancer cells

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Background: The staurosporin analogue PKC 412 but not Ro 31–8220 has previously been found to sensitize resistant U-1810 non small cell lung carcinoma (NSCLC) cells to conventional radiation (IR). Here we use this cell line as a model system to reveal genes of importance for radio resistance.
Material and Methods: Total gene profiling of U-1810 cells was performed after IR alone, or in combination with PKC 412 or Ro 31–8220 using Affymetrix gene array platform.
Results: Co-administration of PKC 412 or Ro-31 8820 with IR was found to cause up regulation of 140 and 179 genes and down regulation of 253 and 425 genes respectively. These genes were annotated to several different processes such as cell proliferation, cell growth, cell death and metabolism. The alteration of some genes (CDH6, TGFB1/4, PPP2R2C, ESR1, RAB33A, and Ephrin B3 (EFNB3)) were verified by real time quantitative PCR. Analysis and interpretation of gene profiling data suggested decreased expression of Ephrin B3 as a possible radio sensitizing mechanism of PKC 412. SiRNA-mediated suppression of Ephrin B3 revealed a decrease in cell proliferation, an increase in cell death and an elongated cell phenotype. Moreover, silencing of Ephrin B3 in combination with IR caused a decrease in IR-induced G2-arrest and induced cellular senescence in G1.
Conclusion: All together, this study suggests Ephrin B3 as a putative gene involved in the mechanisms of radio resistance in NSCLC cells.

526 Cancer-associated fibroblasts desensitizes head and neck squamous cell carcinoma cells to epidermal growth factor receptor-targeted therapy

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Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with 650 000 new cases worldwide every year. Overexpression of the epidermal growth factor receptor (EGFR) is common in HNSCC and associated with poor prognosis; however, EGFR-targeted therapy has shown limited efficacy in the treatment of this malignancy. Cancer-associated fibroblasts (CAF), which are the major component of the stromal compartment, are known to reduce the sensitivity of tumour cells to certain anti-cancer treatments. Thus, their influence on the response to cetuximab, an antagonistic EGFR antibody, was investigated.
Material and Methods: CAF were isolated from 7 HNSCC patients and co-cultured with HNSCC cell lines in a transwell system. Following cetuximab treatment tumour cell proliferation was determined by a crystal violet assay. The expression and activation of EGFR and the downstream signaling molecules Akt and Erk were analysed by western blotting.
Results: In tumour cell monocultures, cetuximab (30 nM) treatment caused a reduction in the tumour cell proliferation rate. In contrast, cetuximab did not affect the growth of CAF cultures. In co-culture with CAF the cetuximab-induced growth inhibition was reduced, and full protection was observed in one of the tumour cell lines investigated. Fibroblast conditioned media gave similar results, confirming that the protective effect is mediated by CAF-derived soluble factors. Furthermore, CAF desensitized tumour cells to treatment with gefitinib, an EGFR tyrosine kinase inhibitor, suggesting that the protective mechanism involve modulation of intracellular signaling rather than interference with cetuximab binding. In order to identify the molecular mechanism conferring resistance to EGFR-targeted therapy we are now studying the influence of CAF on the expression and activation of proteins involved in proliferation- and survival signaling, including EGFR, Akt, and Erk.
Conclusion: These results identify a previously unrecognized CAF-dependent modulation of cetuximab sensitivity, and also present preliminary data on the underlying mechanism. In a longer perspective these results should aid clinicians in the selection of HNSCC patients for cetuximab treatment. Finally, they suggest targeting of CAF-derived factors, yet to be identified, as a novel strategy to improve the effects of cetuximab treatment.

527 The DNA glycosylase Myh1 is stabilized by cisplatin and inhibition of Myh1 expression increases cisplatin-induced apoptotic signaling in lung carcinoma cells

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Background: The base excision repair DNA glycosylase Myh1 is important for repairing endogenous and exogenous induced DNA base damages. Co-deletion of *myh1* and *rad-1* in yeast causes hypersensitivity to hydroxyurea,