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,