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

K. Järvinen[1], A. Hotti[1], E. Hölttä[1]. [1]*Haartman Institute, Pathology, Helsinki, Finland*

**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)

L. Dizdar[1], M. Schmelzle[1], J. Wolters[1], N. Lindenlauf[1], S.E. Baldus[2], S.A. Topp[1], J. Schulte am Esch II[1], C.F. Eisenberger[1], N.H. Stoecklein[1], W.T. Knoefel[1]. [1]*Heinrich-Heine University, Department of General Visceral and Paediatric Surgery, Düsseldorf, Germany*, [2]*Heinrich-Heine University, Institute of Pathology, Düsseldorf, Germany*

**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

S. Stahl[1], A. Vaculova[2], V. Kaminskyy[2], S. Rodriguez-Nieto[2], A. Moshfegh[1], R. Lewensohn[1], K. Viktorsson[1], B. Zhivotovsky[2]. [1]*Karolinska Institutet, Department Oncology/Pathology, Stockholm, Sweden,* [2]*Karolinska Institutet, Department Institute of Environmental Medicine, Stockholm, Sweden*

**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

A. Johansson[1], A. Ansell[2], A. Östman[3], K. Roberg[4]. [1]*Linköping University Hospital, Division of Oto-Rhino-Laryngology, Linköping, Sweden,* [2]*Linköping University, Division of Oto-Rhino-Laryngology Department of Clinical and Experimental Medicine, Linköping, Sweden,* [3]*Karolinska Insitute, Department of Oncology-Pathology, Stockholm, Sweden,* [4]*Linköping University Hospital, Division of Oto-Rhino-Laryngology, Linköping, Sweden*

**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

K. Viktorsson[1], G. Efazat[1], P. Haag[1], D. Zong[1], L. Stenke[2], P. Sunnerhagen[3], R. Lewensohn[4]. [1]*Karolinska Institutet, Department Oncology/Pathology Karolinska Biomics Center, Stockholm, Sweden,* [2]*Karolinska Institutet and Karolinska University Hospital, Department of Hematology and Karolinska Biomics Center, Stockholm, Sweden,* [3]*Gothenburg University, Department of Cell and Molecular Biology, Gothenburg, Sweden,* [4]*Karolinska Institutet and Karolinska University Hospital, Department Oncology/Pathology Karolinska Biomics Center, Stockholm, Sweden*

**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,

UV as well as to chemotherapeutics. In the present study, we examined the role of Myh1 in cisplatin-treated lung carcinoma cells.
**Methods:** A panel of small cell lung carcinoma (SCLC: H82, H69, U1285 and U1906) and non small cell lung carcinoma (NSCLC: A549, U1810, H23, H125 and H661) cell lines with different cisplatin sensitivity were analyzed for their basal level of Myh1 using western blotting. Myh1 localization and expression after cisplatin treatment in NSCLC cells was analyzed in cytosolic and nuclear fractions using western blotting, and was confirmed by immunofluorescence analysis. To assess the role of Myh1 in cisplatin-induced apoptotic signaling, siRNA to Myh1 was used and caspase-3 activity examined prior and post cisplatin treatment using flow cytometry.
**Results:** Western blot analysis of Myh1 in the lung cancer cell line panel revealed heterogenous Myh1 expression. In the NSCLC cell line U1810, a relatively cisplatin resistant NSCLC, cisplatin treatment was found to cause an increase in Myh1 both in cytosol and nucleus. This was evident already at 30 min post cisplatin addition and still evident at 2h. Moreover, immunofluorescence analysis of Myh1 after cisplatin treatment of U1810 cells revealed relocalization of Myh1 into nuclear foci. Finally, siRNA to Myh1 was found to increase cisplatin-induced caspase-3 activity in NSCLC U1810 cells.
**Conclusion:** Our data suggest that Myh1 is stabilized by cisplatin treatment in NSCLC cells and can act as a negative regulator of cisplatin-induced apoptotic signaling in this tumour type.

### 528 Sarcoma cell lines express stem-cell associated features

M. Santarosa[1], A. Caragnano[1], R. Maestro[1]. [1]*CRO-National Cancer Institute, Experimental Oncology 1, Aviano, Italy*

Although soft tissue sarcomas comprise about 1% of human malignant tumours, they are a life-threatening cancer and pose a significant diagnostic and therapeutic challenge. Cancer initiating cells (CIC), that display stem-like features, have recently been identified in several malignancies as the major responsible for tumour growth and chemoresistance. Therefore, clarify the role of CIC in sarcomas might help in the setting of more efficient therapeutic approaches.
To assess whether a "stemness" component exists in sarcomas a series of 18 sarcoma-derived cell lines were investigated for the expression of genes known to be involved in the stem phenotype (OCT3/4-POU5F1, NANOG, SOX2 and the NOTCH1 pathway). The study was carried out by RT-PCR, qRT-PCR and by immunofluorescence.
Stem-like cells are reported to grow as spheroids in medium enriched of EGF and bFGF but devoid of serum. On this ground we compared the expression pattern of cells grown as adherent cells vs cells grown as spheroids in this medium. This analysis was conducted in 5 cell lines (SKUT-1, MG63, RD, RMS13 and RH28).
Preliminary results indicate that all but one (RD) cell lines cultured in stem medium were able to give rise to spheroids, suggesting that sarcoma cell lines might do have a component of CIC.
NOTCH pathway was activated in 10 out of 18 sarcoma cell lines grown in standard conditions, as demonstrated by the expression of the NOTCH targets HES1, HEY1 and HEY2. NOTCH targets was further upregulated in the spheroids of 3 out of 4 cell lines, but was also expressed at high levels in RD floating cells grown in stem medium.
No expression of OCT3/4 and NANOG was observed in any of the cell lines investigated, irrespective of growth conditions. SOX2 was expressed in the leiomyosarcoma cell line SK-LMS-1 in standard condition and was activated in all sarcoma-derived sheroids.
Our results suggest that a CIC component may actually exist in sarcoma cells and that SOX2 could be an important regulator of CIC in this tumour setting.

### 529 The role of aromatase and epidermal growth factor receptor in non-small cell lung cancer

I. Kritikou[1], E. Giannopoulou[1], A. Koutras[1], K. Dimitropoulos[1], H. Kalofonos[1]. [1]*University of Patras, Department of Medicine, Patras – Rio, Greece*

**Background:** Targeted therapy provides an exciting project for treatment of non small cell lung cancer (NSCLC). Aromatase catalyses the final step of estrogen synthesis in several tissues including lung. EGFR signaling is implicated in cell proliferation and metastasis. Cross-talking between these pathways has been reported. The aim of this study is to evaluate the antitumour effect of the combined inhibition of aromatase and EGFR.
**Material and Methods:** *In vitro* experiments were performed on H23, H358 and A549 NSCLC cell lines. Exemestane and erlotinib were applied. Cell proliferation was measured by MTT assay and cell death was detected using annexin V/propidium iodide assay. Cell migration was determined by boyden chamber assay. pEGFR status was estimated using an appropriate ELISA kit and EGFR location was detected by immunofluorescence assay using confocal microscopy.
**Results:** Exemestane and erlotinib, either alone or in combination, inhibited cell proliferation, through an increase in cell apoptosis. However, the combination of the agents had a synergistic effect only on H23 cell lines. The tested agents and their combination inhibited the migration of H23 cells. Exemestane inhibited H358 cell migration whereas erlotinib reversed this effect. No change was found on cell migration of A549. Further, pEGFR levels were increased by exemestane in H23 cells and decreased in A549 cells. These experiments are in progress for H358 cells. Moreover, it was found that EGFR translocated in mitochondria after exemestane application in H23 cells while erlotinib reversed this effect. These experiments are ongoing for H358 and A549 cells.
**Conclusions:** Although each agent alone exerted an antitumour effect on the proliferation of all cell lines, their combination had a synergistic effect on H23 cells. Exemestane activated EGFR pathway in H23 cell line suggesting the treatment of these cells should include an anti-EGFR agent.

### 530 Isolation and functional characterization of cancer ctem cell-derived exosomes

L. Brunetto[1], T.L. Haas[1]. [1]*Istituto Superiore di Sanità, Hematology Oncology and Molecular Medicine, Roma, Italy*

Cancer stem cells (CSCs) represent a small subpopulation of highly malignant tumour cells within the mass of solid tumours. CSCs are thought to be responsible for tumour initiation, growth and distant spread. Here we describe the isolation and functional characterization of lung cancer stem cell (LCSC)-derived exosomes. Exosomes are microvesicles of endosomal origin, which are secreted by various cell types. However, the biological significance of exosome secretion by tumour cells and the presence of exosomes in malignant effusions is not entirely clear yet.
We cultivated LCSC lines isolated from different histotypes of primary lung tumours including adenocarcinoma, squamous cell carcinoma and large-cell carcinoma, in a defined serum-free medium. These conditions allow for the propagation of undifferentiated, CD133-positive stem cell-like cells in spheroid cultures. Here we describe the isolation procedure to obtain exosomal particles from the supernatant of these cultures. The isolated exosomes were analysed for the expression of a number of exosomal proteins such as tetraspanins (CD9 and CD81) and transferrin receptor (CD71) by western blot analysis. In addition, we were able to demonstrate that exosomes derived from LCSCs induce migration of several lung cancer cell lines, such as A549 and NCI-H460. Moreover, we found that LCSC-derived microvesicles enhanced the matrix metalloproteinase (MMP) activity of stimulated target cells. Since MMP expression is induced by Wnt signaling, we investigated the presence of Wnt proteins in our exosomal preparations. We found that exosomes obtained from different LCSC lines contained a considerable amount of Wnt3a protein.
The presence of Wnt proteins suggest a tumour enhancing property of LCSC exosomes, which confer an enhanced migration and proliferative potential of target cells. A better knowledge of the exosomal-cellular mode of communication could lay the basis for the development of diagnostic and therapeutic anti-cancer strategies.

### 531 Synergy between HIF1a and LOX is critical for tumour progression

F. Pez[1], F. Dayan[2], J. Durivault[2], B. Kaniewski[1], G. Aimond[1], B. Deux[3], P. Clezardin[3], P. Sommer[1], J. Pouysségur[2], C. Reynaud[1]. [1]*Institute For The Biology and Chemistry of Proteins, Homeostasis and Therapy of Tissue Degeneracy, Lyon, France,* [2]*Institute of Developmental Biology and Cancer, Hypoxia Signaling Metabolism and Cancer, Nice, France,* [3]*Medical University Laennec, Mechanisms and Treatments For Bones Metastasis of Solid Tumours, Lyon, France*

The microenvironment of solid tumours is exposed to hypoxic conditions which lead to the activation of Hypoxia-Inducible Factor 1 (HIF1), a key transcription factor involved in cellular adaptation to changes in oxygen level. HIF1 plays a critical role in various cellular and physiological events, inducing the expression of several transcriptional targets such as Lysyl Oxidase (LOX). LOX is an amine oxidase that catalyzes crosslinking of fibrillar collagens and elastin in the extracellular matrix. Furthermore, LOX expression in tumour cells lines correlates with tumour progression and metastatic potential.
Using three different human colorectal carcinoma cell lines, LOX was stably overexpressed or knocked down by lentiviral transduction. In these models, we pointed out that besides HIF1-dependant regulation of LOX, LOX can also act on the HIF1 pathway under hypoxic conditions. Indeed, LOX enzymatic activity up-regulates HIF1a protein synthesis, and this action is mediated by the PI3K/AKT pathway. Thus, these results emphasize the existence of a mutual regulation between two main actors of tumoural progression: HIF-1a and LOX. To further determine the implication of both proteins in tumour progression, we generated human colorectal carcinoma cell lines modulating LOX and/or HIF1a expression. Our results show that LOX enzymatic activity increase cell proliferation and clonogenic potential *in vitro* and this role is partly dependant of HIF1a. Subcutaneous inoculation into the flank of Balb/c nude mice strongly reinforced these datas. Indeed, the tumours resulting from LOX overexpressing Hct116 cells were notably larger. HIF-1a silencing in LOX overexpressing cells strongly but not fully reduced the tumour development due to LOX forced expression. It suggests that LOX and HIF-1a act in synergy to favor tumour formation.