HIF-1a pathway. During the presentation, the analysis and conclusion will be updated to include over 100 samples taken from mCRC patients.

**328** POSTER

**Targeting FGF19 as a therapeutic for hepatocellular carcinoma**

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**Background:** FGF19 is a member of the fibroblast growth factor family which is comprised of 22 members that play important roles in development, angiogenesis, and cancer. Ectopic expression of FGF19 in transgenic mice results in development of hepatocellular carcinomas (HCC) by 10 months of age. FGF19 binds uniquely to FGFR4. FGF19 and FGFR4 are known to play a role in bile acid metabolism in human liver but their role in tumorigenesis is not well characterized.
**Results:** Analysis of FGF19 and FGFR4 expression in human hepatocellular carcinomas confirmed their association with HCC. In this study we show that FGFR4 is required for tumor formation in FGF19 transgenic mice and that FGF19 transgenic mice treated with a tumor initiator (diethylnitrosamine) have accelerated progression of HCC confirming FGF19 acts as a tumor promoter. Exogenous administration of FGF19 to mice with the human liver cell line HepG2 xenografts markedly enhanced tumor growth. Moreover, treatment with an anti-FGF19 antibody effectively intervened with development of liver tumors in FGF19 transgenic mice.
**Conclusions:** These findings suggest that inactivation of FGF19 could be beneficial for treatment of hepatocellular carcinoma.

**329** POSTER

**Siah1 ubiquitin ligase enhances radiation response of breast cancer cells**

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**Background:** Siah proteins are ubiquitin-protein isopeptide ligases (E3) that have been implicated in a variety of cellular actions including cell cycle, proliferation and regulation of cellular response to hypoxia and apoptosis. Many studies have suggested that inactivation of Siah1 plays an important role in cancer progression. We hypothesized that Siah1 may act not only as a tumor suppressor but also as a radiosensitizer.
**Materials:** Siah1 mRNA expression was studied in MCF12A, T47D, SKBR3, MBA231, ZR751 and BT20 breast cancer cells lines using RT-PCR. SKBR3 cells were transfected with Siah1, Siah1L, Siah1dR and a control vector. Radiation-induced apoptosis of transfected SKBR3 cells was searched using flow cytometry while a WST-1 assay was made to study their proliferation. Their invasion ability was investigated by a transwell invasion chamber. A luciferase reporter assay was performed to analyse the effect of Siah1 overexpression on beta-catenin degradation.
**Results:** No expression of Siah1mRNA was found in different breast cancer cell lines. Siah1 and Siah1L transfection enhanced radiation-induced apoptosis in SKBR3 cells. In addition, Siah1 and Siah1L potentiated radiation-induced cellular growth arrest in SKBR3 cells. Moreover, overexpression of Siah1 or Siah1L significantly reduced invasion ability of SKBR3. Interestingly, Siah1 mediated ubiquitination and subsequent proteasomal degradation of beta-catenin in SKBR3 cells.
**Conclusion:** In this study, we demonstrate the biological significance of Siah1 in SKBR3 cells. Furthermore, we confirm that Siah1 participates in degradation of beta-catenin, a potent oncogenic protein. Our results reveal for the first time how overexpression of Siah1, a mediator of cellular growth arrest, can enhance radiosensitivity of breast cancer cells. These findings suggest that development of drugs augmenting Siah1 activity could be a novel approach for the treatment of breast cancer.

**330** POSTER

**Dihydroartemisinin induces Bak-dependent mitochondrial apoptosis in tumour cells and increases efficacy of ionizing radiation**

R. Handrick, K.D. Bauer, F. Freier, C. Belka, V. Jendrossek. *University of Tuebingen, Dept. of Radiation Oncology, Tübingen, Germany*

**Aims:** Antineoplastic signaling of ionizing radiation involves the intermediate formation of reactive oxygen species (ROS). Consequently, therapeutic outcome of radiation therapy depends on availability of molecular oxygen. We therefore hypothesize that efficacy of ionizing radiation may be increased by a combination with drugs that accelerate the formation of ROS. We and others have shown that the radical forming antimalaria drug artemisinin exerts promising cytotoxic effects on human tumor cells. Aim of the present study was to evaluate the antineoplastic activity of the artemisinin derivative dihydroartemisinin (DHA) alone and in combination with ionizing radiation, and to identify the molecular mechanisms of combined action.
**Methods:** Cell death induction by DHA (0−20 μM), ionizing radiation (0−10 Gy) or the combination was analysed in a Jurkat T-lymphoma cell model (Bax-negative, p53-negative) by fluorescence microscopy, flow cytometry and immunoblotting. In combination experiments cells were irradiated 15 min after DHA treatment. To elucidate the molecular signaling, cell clones with deficiency in the death receptor (caspase-8-, FADD-negative) or the mitochondrial death pathway (deficiency of Bak or overexpression of Bcl-2 or dominant negative caspase-9), respectively, were used.
**Results:** DHA induced apoptosis in Jurkat cells in a time- and concentration-dependent manner yielding 59% apoptotic cells 24h after treatment with 20μM DHA. Characteristic breakdown of the mitochondrial membrane potential, activation of caspases, cleavage of PARP and DNA fragmentation were observed. Absence of FADD or Caspase-8 did not alter apoptosis rates. In contrast, over-expression of antiapoptotic Bcl-2 or expression of a dominant negative caspase-9 decreased DHA-induced mitochondrial alterations and DNA fragmentation. Moreover, DHA-induced apoptosis was completely abrogated in Bak and Bax negative Jurkat cells. Importantly, DHA significantly increased radiation-induced apoptosis in a concentration-dependent manner, exhibiting at least additive effects in the low dose range (5 Gy/2.5−20 μM DHA).
**Conclusions:** Our data implicate that DHA induces apoptosis via a mitochondrial death pathway involving caspase-9 and proapoptotic Bak. The findings that DHA induces apoptosis on its own and increases radiation-induced cell death in Jurkat T-lymphoma cells suggest that DHA may be a promising antitumor agent when used as single drug or in combination with ionizing radiation.

**331** POSTER

**TP73 polymorphism in cervical cancer**

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**Background:** A complex interrelation between viral and cellular genes is necessary for cell cycle control deregulation, affecting the differentiation program and inducing the progressive proliferation and dysplasia of the epithelial cells, followed by progression to malignant conversion towards immortalization.
TP73, a gene structurally similar to TP53, is localized in 1p36.3 region. When overexpressed in cells it could activate the transcription of TP53-responsive genes. Several reports have suggested the importance of TP73 polymorphisms in tumour behaviour. We investigated the role of a TP73 gene polymorphism in the susceptibility to cervical lesions in a southwestern European population.
**Material and Methods:** Peripheral blood samples were obtained from Radiotherapy and Gynaecology Departments, Portuguese Institute of Oncology (Porto, Portugal), from 1998 to 2002. We analyzed the TP73 cytosine thymine polymorphism in peripheral blood DNA of 176 cancer-free control normal donors, 38 high-grade squamous intraepithelial lesions (HSIL) and 141 patients with primary untreated invasive cervical cancers (ICC) by polymerase chain reaction restriction length polymorphism.
**Results:** Our results demonstrate a two-fold increased susceptibility to the development of HSIL in women that are carriers of the AT allele (OR = 2.39; P = 0.022). Furthermore this association seems to be more evident in women with high parity (OR = 12.53; P = 0.007).
**Conclusions:** This is in agreement with the possible role of TP73 in cervical carcinogenesis, namely in HPV infected transition zone subjected to the action of estrogens and in conjunction with disruption of differentiation program of this squamous epithelium that occurs in HSIL phase before the next step to invasiveness and squamous cervical cancer (SCC).

**332** POSTER

**Solid-phase multiple displacement amplification for multi-loci genotyping of single chromosome molecules**

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**Background:** Despite recent innovations in high throughput shotgun sequencing technologies, complex rearrangements in addition to the

original dizygotic existence of homologous chromosomes still make cancer genome sequencing to be a difficult subject. It has been demanded to establish a convenient method to manipulate long stretch of individual chromosomes for analyzing their sequence information respectively.
**Materials and Methods:** A novel methodology has been developed to amplify single chromosomes for genotyping. A key feature of this methodology is a solid-phase multiple displacement amplification, that is an enzymatic reaction of Phi29 DNA polymerase, within a solidified agarose gel. It consists of following seven steps. (I) Lysis of limited number of cultured cells within a heated agarose gel solution to release chromosome molecules. (II) Careful aliquoting of small volume gel solutions containing limited number of chromosome molecules. (III) Solidification of the gel on ice. (IV) Solid-phase multiple displacement amplification of the gel-immobilized individual chromosome molecules. (V) Recovery of the amplified materials by heating. (VI) Screening of target chromosomes by real-time QPCR. (VII) Multi-loci SNP typing using newly developed on-plastic chip allele-specific primer extension method (Michikawa et al., Anal Sci 2006; 22: 1537–1545).
**Results:** Utilization of agarose gel as a reaction matrix enabled reliable amplification-ready limited dilution of DNA to the level that homologous chromosomes hardly locate together. Aggregation of chromosomes while diluting process was reduced by incubating the gel solution at alkaline pH and at high temperature. Separation of chromosomes thus achieved provided reliable determination of multi-loci genotypes on each amplified homologous chromosome. Using this methodology, we could have successfully determined haplotypes of multiple SNPs in human ATM region that spans 240 kilobase pairs.
**Conclusions:** The methodology developed in this study is effective for genotyping long stretch of individual homologous chromosomes. Since amplified materials are easily recovered in a solution as PCR-ready form, this methodology can be used for various purposes. Further application as of demanding chromosome-wide sequencing is considerable.

**333** POSTER

**High expression of FGF19 in hepatocellular carcinoma (HCC) is associated with poor prognosis**

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**Background:** Hepatocellular carcinoma (HCC) is one of the most aggressive solid tumors associated with poor prognosis. Fibroblast growth factor (FGF) signaling mediates cell-to-cell communication in development and organ homeostasis in adults. Of the four FGF receptor (FGFR) tyrosine kinases, only FGFR4 is expressed in mature hepatocytes. There have been numerous reports correlating up regulation or amplification of FGFR4 and a variety of human cancers. FGF19, a member of FGF family, has unique specificity for FGFR4, but its role in human cancer is not known.
**Materials and Methods:** We investigated mRNA of human FGF19 and FGFR4 expression in 40 HCC specimens using quantitative reverse transcription polymerase chain reaction analysis. Further, we investigated FGF19 and FGFR4 expression by immohistochemistly in 40 patients with HCC. We analyzed the correlation between patients clinicopathological characteristics and FGF19 mRNA expressions by non-parametric analysis and Kaplan-Meier method.
**Results:** Compared with corresponding noncancerous liver tissues, FGF19 was remarkably expressed in HCCs ($P < 0.05$). Immunohistochemical staining also showed increased FGF19 protein in HCCs. Meanwhile FGFR4 was not significantly overexpressed in HCCs. FGF19 expression was not associated with any of the general clinicopathological parameters, including age, tumor size, histological grade, and histological type. With regard to prognosis, both of the disease free survival and overall survival time for patients in the high FGF19 mRNA ratio group ($n = 20$) was significantly poorer when compared with low FGF19 mRNA ratio group ($n = 20$, $P = 0.021$).
**Conclusion:** These results suggest that FGF19 mRNA expression has a prognostic significance for the survival of postoperative patients with HCC. FGF19 may be critically involved in the development of HCCs.

**334** POSTER

**IFN-gamma induces transient MHC I expression in neuroblastoma cells – influence of suppressor of cytokine signaling (SOCS) 1**

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**Background:** Major histocompatibility complex (MHC) class I expression is an obligate condition for cells concerning their recognition by the immune system. In differentiated neuronal cells, protein overexpression of the suppressor of cytokine signalling (SOCS) family downregulates MHC I expression constantly. However, little is known on the role of SOCS proteins in MHC class I regulation in neuroendocrine differentiated tumour cells. The aim of this study was to determine the effect of different cytokines on the expression of MHC class I and II molecules as well as SOCS1 and SOCS3 in the human neuroblastoma cell line SH-SY5Y.
**Materials and Methods:** FACS analysis and RT-PCR were used to detect MHC class I/II and SOCS1/3 expression, respectively. MHC expression was measured after 6, 12, 24 and 48 h of treatment with the cytokines IFNγ, IL-1β or TNFα. Cellular levels of SOCS1 and SOCS3 mRNA in SH-SY5Y cells were determined after 0.5, 1, 2, 4, 8, 16 and 24 h treated with IFNγ only. To assess a synergistic effect of cytokines on either MHC or SOCS expression, SH-SY5Y cells were incubated with combinations of the cytokines for 24 h and analyzed by FACS or RT-PCR.
**Results:** Neither MHC class I nor MHC class II expression was detectable in untreated SH-SY5Y cells and they expressed detectable levels of SOCS1 and SOCS3 mRNA. Incubation with IFNγ resulted in an induction of MHC class I molecules with a maximum after 12 h of stimulation and a constant decrease after this time point. SOCS1 expression increased significantly after 4 h when it reached saturation. The SOCS3 mRNA level was not modified by IFNγ treatment. Expression of MHC class II remained unaffected. Combinations of cytokines including IFNγ, showed an effect comparable to a treatment with IFNγ alone indicating no role of IL-1β or TNFα on either MHC or SOCS expression.
**Conclusions:** These data show that MHC class I in neuroblastoma cells is controlled by SOCS1 and, in contrast to differentiated neurons, can be induced by IFNγ treatment. However, IFNγ also induces SOCS1 expression, which might be responsible for the downregulation of MHC class I expression as part of a classical negative feedback loop.

**335** POSTER

**Irradiation-induced side-effects in the lung: establishment of a murine model for analysis of physiological and histological alterations**

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**Background:** Pneumonitis and fibrosis are dose-limiting side effects of radiation therapy. Unfortunately, the underlying mechanisms are still unclear. To study the putative connection between radiation-induced tissue damage and the development of pneumonitis, we recently established a murine model for radiation-induced pneumonitis.
**Materials and Methods:** 4−6 week-old female C57BL6/J mice were adapted to a total-body plethysmograph and subsequently enrolled into the study at a body weight of approximately 20 g. Following anaesthesia, mice were placed in holders and their right hemithorax were irradiated with a single dose of 0/12.5/22.5 Gy using a linear accelerator ($n \geqslant 5$ mice/dose group). Thereafter, pathognomonic alterations of pneumonitis were subsequently analysed at defined time points (d1-d84).
**Results:** Mice developed characteristic histopathological alterations indicative for pneumonitis as judged by alveolar wall thickness, interstitial edema, interstitial and peribronchial inflammation already at day 21. These alterations were paralleled by increased breathing frequency and pulmonary resistance. Moreover, increased leakage of albumin into bronchioalveolar lavage fluid was observed. In addition, the invasion of inflammatory cells was studied histologically as well as by measuring the myeloperoxidase content in the lung.
**Conclusions:** This model can now be used to study the role of specified signalling molecules involved in cell death induction, damage recognition and/or immunoregulation by means of genetically defined mice strains. The detailed knowledge of the underlying mechanisms is a prerequisite for the design of radioprotective treatment.

**336** POSTER

**The aurora kinase inhibitor MK-0457 (VX-680) demonstrates anticancer activity alone or in combination with docetaxel (Dtx)**

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**Background:** MK-0457 (VX-680) reversibly inhibits aurora kinases A, B and C (Ki's of 0.7, 18 and 4.6 nM, respectively), FLT3 (Ki 30 nM), wild type