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**

K.H. Hotzel, R.P. Pai, R.F. Ferrando, L.R. Rodriguez, T.L. Le, J.G. Greve, L.D. Desnoyers, A.A. Ashkenazi, D.F. French. *Genentech, Pathology, South San Francisco, USA*

**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**

H. He, E. Fokas, L. Wang, B. Kleb, R. Engenhart-Cabillic, H. An. *Philipps University, Radiotherapy and Radiation Oncology, Marburg, Germany*

**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**

R. Craveiro[1], D. Pinto[2], I. Bravo[1], R. Catarino[2], D. Pereira[3], A. Matos[4], H. Pereira[1], R. Medeiros[2]. *[1]Portuguese Institute of Oncology, Radioterapia, Porto, Portugal; [2]Portuguese Institute of Oncology, Molecular Oncology Group, Porto, Portugal; [3]Portuguese Institute of Oncology, Medical Oncology, Porto, Portugal; [4]Portuguese Institute of Oncology, Gynaecology, Porto, Portugal*

**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**

Y. Michikawa[1], K. Sugahara[2], T. Suga[1], Y. Otsuka[1], N. Shiomi[1], M. Iwakawa[1], T. Imai[1]. *[1]National Institute of Radiological Sciences, Research Center for Charged Particle Therapy, Chiba, Japan; [2]Tokyo Dental College, Department of Oral and Maxillofacial Surgery, Chiba, Japan*

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