whereas S12 cells, which derived from the W12 line, contain HPV DNA as integrated copies. E2 gene region was screened by using PCR with three separate primers covering the whole genome. Cells where irradiated with singles doses of 0 Gy, 1 Gy, 2 Gy, 3 Gy, 4 Gy, 5 Gy and 7 Gy. Clonogenic survival was analyzed by using the 96-well in vitro test. Survival fraction and survival curves where calculated using Sigma Plot 8.0. At least three experiments where performed for each dose point.
Results: The E2 gene of the S12 cells (passage 88–103) was disrupted in the E2C region. The W12 cells (passage 8−14) with an intact E2 gene showed a higher radiosensitivity with a radiation enhancement factor of 1.5 (4 Gy).
Conclusion: HPV 16 positive W12 cells with an intact E2 gene showed a higher intrinsic radiosensitivity compared to those with an disrupted E2 gene. About 30% of patients with cervical cancer have tumors with an intact E2 gene. Our experiments indicate a better response to radiation treatment might be factor for their better prognoses.

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Gossypol activates the SAPK/JNK pathway and enhances radiation-induced apoptosis

M. Verheij[1], S.F. Zerp[2], T.R. Stoter[3], G. Kuipers[3], D. Yang[4], M. Lippman[5], B. Slotman[6], W.J. Van Blitterswijk[2], H. Bartelink[7], M.V.M. Lafleur[3]. *[1]The Netherlands Cancer Institute, Radiation Oncology – Division of Cellular Biochemistry, Amsterdam, The Netherlands; [2]The Netherlands Cancer Institute, Division of Cellular Biochemistry, Amsterdam, The Netherlands; [3]VU University Medical Center, Department of Radiobiology, Amsterdam, The Netherlands; [4]Ascenta Therapeutics, Drug Development, San Diego, USA; [5]University of Michigan, Department of Internal Medicine, Ann Arbor, USA; [6]VU University Medical Center, Department of Radiation Oncology, Amsterdam, The Netherlands; [7]The Netherlands Cancer Institute, Department of Radiation Oncology, Amsterdam, The Netherlands*

Background: Overexpression of anti-apoptotic members of the Bcl-2 family has been associated with treatment resistance and poor outcome. Gossypol (GP) is a small molecule inhibitor of Bcl-XL and Bcl-2 and induces apoptosis in a wide range of tumor cell lines. Here, we tested the effect of GP on radiation-induced cell death in a panel of human head and neck cancer (HNSCC) and leukemic cell lines Because activation of the SAPK/JNK pathway is important for apoptosis induction by other stimuli, we also investigated the role of this signaling cascade in GP-induced apoptosis.
Material and Methods: Four types of human HNSCC (UM-SCC-11B, UM-SCC-22A, UM-SCC-14C, VU-SCC-OE) and 2 leukemic cell lines (Jurkat T, U937) were treated with increasing doses of GP, radiation and the combination. Apoptosis was quantified by FACS analysis; SAPK/JNK activity was measured by Western blot; isobolographic analysis was performed to characterize the interaction between radiation and GP.
Results: In all cell lines tested, GP induces apoptosis in a time- and dose-dependent fashion, with ED50 values in the uM range. Like radiation, GP rapidly activates SAPK/JNK which can be blocked by the kinase inhibitor SP600125. To demonstrate the critical role of SAPK/JNK activation in GP-induced apoptosis, U937 cells stably expressing the dominant negative mutant of c-Jun, TAM-67, were used. In these U937-TAM-67 cells both radiation- and GP-induced apoptosis was significantly reduced as compared to vector-only controls. By combining radiation and GP, in particular radiation given 24 hours before GP, apoptosis was strongly enhanced. Isobolographic analysis revealed a synergistic interaction between both stimuli.
Conclusion: GP strongly enhances radiation-induced apoptosis in human HNSCC and leukemic cells. Our studies also indicate a requirement of the SAPK/JNK pathway in this response. This type of apoptosis modulation may lead to the development of new effective combination therapies.

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HER2 polymorphism is associated with gastric cancer risk

D. Pinto[1], C. Faustino[2], P. Ferreira[2], A.L. Teixeira[1], A.L. Pinto-Correia[1], H. Sousa[1], M. Dinis-Ribeiro[3], M. Fragoso[2], R. Medeiros[1]. *[1]Instituto Português de Oncologia do Porto FG EPE, Grupo Oncologia Molecular – CI Lab 4º Piso, Porto, Portugal; [2]Instituto Português de Oncologia do Porto FG EPE, Medical Oncology Department, Porto, Portugal; [3]Instituto Português de Oncologia do Porto FG EPE, Gastroenterology Service, Porto, Portugal*

Introduction: Gastric adenocarcinoma (GC) is a major public health problem worldwide. In Portugal, GC represents a sixth of all cancer related deaths, with twice the average mortality of European Union. Several host genetic variations have been regarded as potential risk markers for this neoplasia. The Human Epidermal growth factor Receptor-2 (HER2) plays an important role in cell differentiation and proliferation, being associated with cancer evolution. A single-nucleotide polymorphism in the HER2 gene (Ile – Val) was described. The aim of this study was to evaluate the role of this polymorphism in the development of GC within a southern European population.
Materials and Methods: We conducted a case-control study on 484 individuals, including 162 patients with histological confirmed GC and 322 healthy blood donors from the same geographical area as the cases. DNA extracted from peripheral blood was submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the possible HER2 genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.
Results: We found that the frequency of the Ile/Val genotype was higher in gastric cases (36.4%) than in controls (30.1%), and the same was observed with the Val/Val genotype (4.9% and 2.2%, respectively). A twofold increase in the risk of gastric cancer was found among carriers of Ile/Val and Val/Val genotypes (OR adjusted to age = 2.04; 95% CI: 1.18−3.52; p = 0.011). This risk was even higher when we analyzed only female individuals (adjusted OR = 3.18; 95% CI: 1.44−7.00; p = 0.004). Moreover, we observed that the median time-to-onset of gastric cancer was shorter in the patients carrying the Val allele (63.6 vs 71.6 months for all patients; p = 0.011; and 64.9 vs 78.9 months for women; p = 0.004).
Conclusions: Our results indicate an association between the presence of the Val allele in the HER2 polymorphism and the risk of gastric cancer. Studies hypothesize that the presence of this allele has been implicated in the formation of active HER2 receptors, leading to enhanced signal transduction activation, which may therefore trigger carcinogenesis. In this study, the risk of gastric cancer was even higher in women, and an association between HER2 and estrogen has been extensively studied. Further studies are needed to elucidate this association.

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Expression of intratumoral lactate dehydrogenase 5 (LDH5) and expression of biomarkers for angiogenesis and hypoxia are linked in patients with colorectal cancer (CRC)

M. Koukourakis[1], A. Giatromanolaki[2], E. Sivridis[2], K. Gatter[3], A.L. Harris[4], M. Shi[5], T. Trarbach[6], G. Folprecht[7], G. Meinhardt[8]. *[1]Democritus University of Thrace, Departments of Radiotherapy/Oncology, Alexandroupolis, Greece; [2]Democritus University of Thrace, Departments of Pathology, Alexandroupolis, Greece; [3]John Radcliffe Hospital, Department of Clinical Laboratory Sciences, Oxford, United Kingdom; [4]Cancer Research UK, Molecular Oncology Laboratories, Oxford, United Kingdom; [5]Novartis Pharmaceuticals, Clinical Oncology, East Hanover NJ, USA; [6]Poliklinik Universitätsklinikum, Innere Klinik, Essen, Germany; [7]Universitätsklinikum Carl Gustav Carus, Medizinische Klinik, Dresden, Germany; [8]Bayer Schering Pharma, Clinical Affairs-Oncology, Berlin, Germany*

Background: Results from two Phase III randomized clinical trials in 1st line (CONFIRM 1) and 2nd line (CONFIRM 2) metastatic CRC indicated that PTK787/ZK222584 (PTK/ZK), an oral tyrosine kinase inhibitor (TKI) which blocks all known VEGF receptors (VEGFR), demonstrated greatest efficacy in patients (pts) with high baseline serum lactate dehydrogenase (LDH) levels (>1.5 ULN). To better understand the relationship between angiogenesis, tumor hypoxia and acidosis, we investigated whether CRC pts with high levels of tumor LDH5 would also have increased expression of proteins linked to tumor-cell hypoxia [hypoxia inducible factors: HIF-1 alpha (a), and -2a] dehydrogenase kinase (PDHK) and angiogenesis [VEGFA; phosphorylated VEGFR2 (pKDR)], as well as acidity [carbonic anhydrase 9 (CA9)]. We also assessed vessel density (VD).
Methods: Using a nominal scoring system, we will conduct an immunohistochemical analysis of tissue-sections of primary or metastatic tumor taken from over 100 participants of the CONFIRM 1 and 2 trials. A measure of association between the scores for protein-expression will be estimated by the phi-coefficient (correlation coefficient) and assessed by means of p-values from pairwise Fisher's exact test (two-sided).
Results: An earlier analysis of 42 tissue samples revealed associations between the expression levels of LDH5 and the following proteins: pKDR (Phi = 0.53; p < 0.001), VEGF (Phi = 0.41; p = 0.006), VD (Phi = 0.34; p = 0.052), HIF-1alpha (Phi = 0.56; p < 0.001), and PDHK (Phi = 0.58; p = 0.0014). HIF-1a was associated with pKDR (Phi = 0.38; p = 0.027), VD (Phi = 0.34; p = 0.045), and VEGFA (Phi = 0.33; p = 0.067) expression. VEGFA was associated with PDHK (Phi = 0.52; p = 0.035). These results will be updated using data derived from over 100 samples.
Conclusions: Tumor samples from mCRC patients with high levels of intratumoral protein expression of LDH5 also demonstrated elevated HIF-1a, pKDR, VEGFA, PDHK expression and VD. The results suggested a link between tumor hypoxia and angiogenesis, and demonstrate elevated LDH protein expression may serve as a surrogate marker for an activated

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.

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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.

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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).

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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