Results: The first cohort consisted of 52 invasive breast cancers (M), 36 non-malignant (NM) and 10 normal (N) breast tissue samples. Median age of the patients was 60 years. Median size of breast cancer was 25 mm (IQR 20−40 mm). ER status was not significantly different between tissue types (p = 0.50). Every SKFM expression was quantified in all tissue samples. Fyn was the most expressed SKFM in normal tissue and Lyn in the NM breast tissue. Blk was the least expressed SKFM in all breast tissues. In malignant breast tissue Src and Lyn were most expressed. SKFMs Lck and Lyn were higher expressed in ER negative compared to ER positive tumours. c-Src (p = 0.01) and Fyn (p = 0.03) were expressed at higher levels in lobular compared to ductal carcinomas, whereas Yes (p = 0.006) was only expressed in ductal carcinomas.
Cohort two consisted of 320 patients with median follow-up of 6.3 years. Median age was 58 years (IQR 24−90). Median tumour size was 20 mm (IQR 15−30 mm). In both cohorts majority of the cancer specimens were pathologically graded as G2 and G3. 49% of the patients were axillary lymph node positive. High cytoplasmic Src and membrane Y419Src kinase expression levels were significantly associated with decreased disease specific survival (p = 0.03, p = 0.02). Lyn was not associated with survival at any cellular location. High membrane Lck expression was significantly associated with improved survival (p = 0.03).
Conclusions: All eight SKFM are expressed in different breast tissues. In invasive breast cancer Src kinase is highest expressed and seems to have a negative impact on disease specific survival. Whereas, high membrane expression of Lck provides better clinical outcome in those breast cancer patients. Further investigations are needed to determine underlying mechanisms for this observation.

1024 POSTER DISCUSSION

EGFR single nucleotid polymorphism R521K is a predictor for the occurrence of skin rash

K. Klinghammer[1], M. Knödler[1], V. Budach[2], U. Keilholz[1], I. Tinhofer[2]. *[1]Charité-Universitätsmedizin Berlin Germany, Department of Hematology and Oncology, Berlin, Germany; [2]Charité-Universitätsmedizin Berlin Germany, Department of Radiation Oncology, Berlin, Germany*

Background: Cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) is the first molecular targeting approach for the treatment of head and neck squamous-cell cancer (HNSCC) that demonstrated clinical efficacy with prolonged progression-free and overall survival. The most common side effect of cetuximab is moderate to severe skin rash. In the current study we analyzed whether cetuximab-induced skin rash is correlated with distinct genetic variations within the EGFR gene and focused our analyses on gene polymorphisms known to modulate EGFR expression levels, its capacity of ligand binding or its mitogenic signaling activity. Furthermore the intensity of skin rash and gene polymorphisms were correlated with progression free survival (PFS) and overall survival (OS).
Materials and Methods: 50 patients enrolled in a single-arm phase II multicenter study for second-line treatment of stage III/IV metastatic or recurrent SCCHN with cetuximab/docetaxel were genotyped for EGFR intron 1 CA-single sequence repeat (CA-SSR) polymorphism and the single nucleotide polymorphism R521K within EGFR exon 13. Association between genotypes and incidence/grade of skin rash classified by Common Toxicity Criteria (CTC) was assessed by Pearson's chi-square test. Survival analysis were performed by Kaplan Meier.
Results: The relative genotype distribution within our patient cohort was comparable to that reported by the HAPMAP consortium for a European reference population. Overall, thirty-eight patients (76%) developed skin rash within 6 weeks of treatment. For the CA repeat polymorphism (minor allelic sum 27−33 CA-SSR, major allelic sum 34−40 CA-SSR) we failed to observe an association with skin toxicity (p = 0.17), PFS (p = 0.18) and OS (p = 0.055) In contrast, the R521K variant (Lys allele) was significantly associated with reduced skin toxicity (p = 0.012). In fact, skin rash of grade >1 developed in only 7/27 (25%) of patients with homozygous Lys/Lys or heterozygous Lys/Arg genotypes but in 14/23 (60%) of patients with homozygous Arg/Arg genotype. PFS (p = 0.14) and OS (p = 0.10) were not associated with the SNP R521K. PFS (p = 0.015) and OS (p = 0.031) were, however, significantly associated with the occurrence of skin rash.
Conclusion: Our study suggests that the EGFR R521K but not the CA repeat polymorphism is a useful predictive marker for skin toxicity in HNSCC. Furthermore the occurrence of skin rash is positively associated with PFS and OS. The evaluation of its correlation with EGFR expression, ligand binding and signaling activity is currently ongoing.

1025 POSTER DISCUSSION

Gene expression profiling identifies Fibronectin 1 and CXCL9 as candidate biomarkers for breast cancer screening

E. Ruiz-Garcia[1], V. Scott[1], C. Machavoine[2], J.M. Bidart[2], L. Lacroix[2], S. Delalogе[3], F. Andre[3]. *[1]Institut Gustave Roussy, Breast Cancer Translational Research Unit, Villejuif, France; [2]Institut Gustave Roussy, Department of Laboratory Medicine, Villejuif, France; [3]Institut Gustave Roussy, Breast Cancer Translational Research Unit Breast Cancer Unit Department of Medicine, Villejuif, France*

Background: There is a need to develop blood-based bioassays for breast cancer screening. In the present study, we have used differential gene expression between breast cancer samples and benign tumors to identify candidate biomarkers for blood-based screening.
Methods: Two candidate proteins (Fibronectin 1, CXCL9) were identified from a gene expression dataset that included 120 breast cancer samples and 45 benign lesions. These candidate proteins were selected as follow: a. differential gene expression between cancer and benign lesion, b. protein released in the extracellular medium (SwissProt) and stable in the serum, c. commercially available ELISA kit, d. Accuracy of the ELISA assay in a feasibility study (n = 23). Concentrations of these two proteins were determined in blood samples by ELISA. Blood samples were from normal volunteers (n = 119) and early breast cancer patients (n = 133). Normal volunteers were blood donors.
Results: Seventy-three percent of the patients presented a cT1-T2 tumour. CA15.3 was within normal range (<30 IU/ml) in 114 patients (86%). Blood concentrations of CXCL9 and Fibronectin 1 were higher in cancer patients as compared to normal volunteers. Mean concentration for CXCL9 was 851 pg/ml (range: 121–3941) and 635 pg/ml (range: 12–4327) in cancer patients and normal volunteers respectively (p = 0.013). CXCL9 concentration was significantly higher in patients with ER-negative breast cancer (mean: 999 pg/ml) as compared to normal volunteers (p = 0.003), a data consistent with gene expression profile. Meanwhile, Fibronectin 1 mean concentration was 190 μg/ml (range) for cancer patients and 125 μg/ml (range) for normal volunteers (p < 0.001). AUC for breast cancer diagnosis were 0.78 and 0.62 for Fibronectin 1 and CXCL9 respectively. A combined score including Fibronectin 1 and CXCL9 dosages presented a sensitivity of 53% and a 98% specificity. Similar performances were observed for ER-negative tumors.
Conclusion: This study suggests that Fibronectin 1/CXCL9 dosage in serum could screen a significant rate of breast cancer, including ER-negative breast cancer. These data suggest that analysis of differential gene expression is a good approach to select candidate biomarker to set-up blood assays cancer screening.

Poster presentations (Mon, 21 Sep, 14:00−17:00)

Basic science

1026 POSTER

Mechanisms involved in increased sensitivity of cisplatin resistant human laryngeal carcinoma cells to lovastatin

M. Osmak[1], G. Fritz[2], T. Cimbora-Zovko[1]. *[1]Rudjer Boskovic Institute, Department of Molecular Biology, Zagreb, Croatia; [2]University of Mainz, Institute of Toxicology, Mainz, Germany*

Background: Cisplatin (cDDP) is a widely used anticancer agent in the treatment of many solid tumors, but development of cDDP resistance limits its efficacy. In comparison to parental human laryngeal carcinoma HEp-2 cells, sublines resistant to cDDP, $CA3_{ST}$ and CK2, have altered cell morphology, adhesion and cytoskeleton organization, suggesting alterations in Rho GTPases activity. Isoprenylation of Rho GTPases is crucial for their targeting to cell membrane, the process which is inhibited with HMG-CoA reductase inhibitor lovastatin. We have found that cDDP-resistant cells are sensitive to lovastatin.
The aim of the present study was to examine possible mechanisms involved in this phenomenon.
Material and Methods: To examine the mechanisms involved in sensitivity of $CA3_{ST}$ and CK2 cells to lovastatin, we used cytotoxicity assay, semiquantitative RT-PCR, Western blot analysis and transient transfection.
Results: Lovastatin treatment increased the expression of RhoB in all cell lines tested, and reduced the expression of Rac1 and Cdc42 (more in cDDP-resistant sublines). The toxicity of lovastatin and its effect on Rho GTPases was inhibited by addition of geranylgeranyl pyrophosphate, and to less extent farnesyl pyrophosphate. We found recently that RhoB downregulation confers resistance to cDDP and hypothesized that decreased RhoB expression could cause sensitivity to lovastatin. However, silencing of RhoB in HEp-2 cells with specific siRNA did not

confirm our hypothesis. We examined several additional mechanisms as possible causes of increased sensitivity to lovastatin, but found no correlation between expression of HMG CoA-reductase, Bcl-2, survivin or P-glycoprotein with the level of sensitivity. Similar sensitivity pattern of $CA3_{ST}$ and CK2 cells to lovastatin and statins with different lipophility and metabolic pathways suggests that altered intracellular accumulation of active drugs was not the cause of altered sensitivity.
Conclusions: Increased sensitivity of cDDP-resistant cells to lovastatin involves several geranylgeranylated proteins, among others Rac1 and Cdc42. Including lovastatin in the treatment of cancer patients could improve the success of chemotherapy for patients with cDDP-resistant tumors.

1027 POSTER

Sorafenib overcomes TRAIL resistance of hepatocellular carcinoma cells through the inhibition of signal transducers and activators of transcription 3

K.F. Chen[1], T.H. Liu[1], W.T. Tai[2], P.J. Chen[1], A.L. Cheng[3]. *[1]National Taiwan University Hospital, Medical Research, Taipei, Taiwan; [2]National Taiwan University College of Medicine, Institute of Molecular Medicine, Taipei, Taiwan; [3]National Taiwan University Hospital, Oncology, Taipei, Taiwan*

Background: Hepatocellular carcinoma (HCC) is one of the most common and lethal human malignancies. Recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-tumor agent. However, many HCC cells show resistance to TRAIL-induced apoptosis. Sorafenib, a tyrosine kinase inhibitor, was recently approved by FDA for HCC. In this study, we showed that sorafenib sensitizes resistant HCC cells to TRAIL-induced apoptosis.
Material and Methods: HCC cell lines (PLC5, Huh-7, Sk-Hep1, and Hep3B) were treated with sorafenib and/or TRAIL and analyzed in terms of apoptosis, signal transduction.
Results: HCC cells, including PLC5, Huh-7, Hep3B and Sk-Hep1, showed significant resistance to TRAIL-induced apoptosis (up to 1000 ng/ml). The combination of sorafenib (starting at 5 μM) and TRAIL restored the sensitivity of HCC cells to TRAIL-induced apoptosis. Thorough comparisons of the molecular change before and after treatment with these agents, we found signal transducers and activators of transcription 3 (Stat3) played a significant role in mediating TRAIL sensitization of sorafenib. Our data showed that sorafenib down-regulated phospho-Stat3 (Tyr 705) and subsequently reduced the expression levels of two Stat3-related proteins, Mcl-1, and survivin, in a dose- and time-dependent manner in TRAIL-treated HCC cells. Knocking down Stat3 by RNA-interference reversed overcame apoptotic resistance to TRAIL in HCC cells, and ectopic expression of Stat3 in HCC cells abolished the TRAIL sensitizing effect of sorafenib, indicating Stat3 inactivation plays a key role in mediating the combination effect.
Conclusions: Sorafenib sensitizes resistant HCC cells to TRAIL-induced apoptosis at clinical achievable concentrations, and this effect is mediated via the inhibition of Stat3.
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1028 POSTER

Aloe emodin, a natural anthraquinone targeting multiple facets (migration, invasion, angiogenesis) of tumour metastasis

P. Sathiadevan[1], S. Babykutty[1], V. Vijayakurup[1], C.J. Jayakrishnan[1], S. Gopala[1], P. Srinivas[2]. *[1]Sree Chitra Tirunal Institute For Medical Science and Technology, Department of Biochemistry, Trivandrum, India; [2]Rajiv Gandhi Centre for Biotechnology, Molecular Therapeutics Laboratory, Trivandrum, India*

Background: The present study evaluated the apoptotic, antimetastatic and antiangiogenic property of a naturally occurring anthraquinone, aloe emodin (AE) in colon cancer cells. This compound is known to induce apoptosis in various other tumour cell types *in vitro*. Studies validating its role in influencing the regulatory molecules involved in metastasis and angiogenesis are rare. Colorectal tumors are one of the rapidly metastasizing tumours and a major cause for cancer deaths world wide.
Methods: Cell viability was assayed by MTT staining for the detection of the antiproliferative activity of drug. Ability of this drug to induce apoptosis was identified by annexin/propidium Iodide staining, loss of mitochondrial membrane potential and TUNEL assay. Inhibition of cancer cell migration was assessed by wound healing and transwell migration/transwell invasion assays. Cell growth inhibition and cell cycle distribution induced by AE was evaluated by FACS. Endothelial cell proliferation and migration assays as well as *in vitro* tube formation assays were used to evaluate the antiangiogenic activity of AE. Effect of AE on the expression of molecular players involved in apoptosis (caspases, PARP, MAPKs) migration (MMP2, MMP9, MAPKs) angiogenesis (VEGF) was assayed using substrate zymograpghy, PCR, western blot and fluorescent tagged peptide assay.
Results: Treatment with AE we observed positive annexin staining, loss of mitochondrial membrane potential and strong TUNEL positivity. The growth inhibitory capacity of AE was through induction of G2/M arrest. We have observed down regulation of phosphoERK1/2, activation of caspase and fragmentation of PARP on treatment with AE. Our results showed that a relatively non toxic level of AE suppressed the phorbol-12-myristyl-13-acetate (PMA) induced migration and expression/activity of MMP2/MMP9. We have also analyzed the involvement of signaling molecules (MAPKs) and specific transcription factors in AE mediated up regulation of MMP2/9. AE also inhibited Human Umbilical Vein Endothelial cells (HUVECs) proliferation migration/invasion and *in vitro* tube formation. We have also analyzed its effects on VEGF expression.
Conclusion: *In vitro* anticancer activity of AE on colon cancer cells depends on the ability to induce apoptosis, inhibit cell migration, *in vitro* tube formation and down regulation of key MMPs involved in metastasis. Thus AE can be projected as a prospective antitumor agent even though further research is warranted.

1029 POSTER

Improved distribution and efficacy of oncolytic virus in solid tumors by apoptosis-inducing pretreatments

S. Nagano[1], K. Setsuro[1], Y. Boucher[2]. *[1]Graduate School of Medical and Dental Sciences Kagoshima University, Department of Orthopaedic Surgery, Kagoshima, Japan; [2]Massachusetts General Hospital/Harvard Medical School, Edwin L. Steele Laboratory Department of Radiation Oncology, Boston, USA*

Background: For successful eradication of the tumor by oncolytic gene therapy, initial widespread distribution of the virus within the tumor is crucial. However, viral distribution is limited by the large size of viral vectors, which limits their penetration through the interstitial matrix and the narrow spaces separating tumor cells. This study tested if the void space resulting from tumor cell apoptosis improves the distribution and efficacy of oncolytic HSV.
Material and Methods: We used two different approaches to induce apoptosis, which are 1) tet-regulated expression of apoptotic gene and 2) cytotoxic agents. For tet-regulated apoptosis system, MDA-MB-435S cells were transfected with tet-inducible CD8/Caspase-8 plasmids. For cytotoxic agents, paclitaxel or recombinant TRAIL was used. *In vivo*, MDA-MB-435S cells were implanted into the SCID mice and apoptosis was induced by doxycycline-regulated expression of CD8/Caspase-8 or cytotoxic agents. To study the effect of pretreatments on the viral distribution, oncolytic HSV expressing GFP was injected intratumorally following to different pretreatments. Finally the effect on tumor growth was assessed in MDA-MB-435S tumors.
Results: *In vitro,* both caspase-8 activaion and cytotoxic treatments induced significant apoptosis on tumor cells. Paclitaxel followed by TRAIL induced significantly more apoptosis than single treatment. In mice with MDA-MB-435S tumors, both the activation of caspase-8 and pretreatment with cytotoxic agents induced 9.0% and 4.0% apoptosis, respectively. In contrast to the limited viral distribution of 13% of the tumor section in control tumors, viral distribution was significantly improved by both caspase-8 activation (42.4%) and paclitaxel-TRAIL (30.3%). In tumor areas with a high density of apoptotic cells, the cellular shrinkage produced interstitial void spaces and channels that facilitated HSV distribution. We also show that the intratumoral injection of oncolytic HSV after caspase-8 activation or the paclitaxel plus TRAIL pre-treatment produces a significantly longer tumor growth delay than the administration of HSV before the induction of cell death, demonstrating the importance of sequence of the treatments.
Conclusions: Cancer cell death improves the intratumoral spread and therapeutic efficacy of oncolytic HSV. Thus the administration of cytotoxic agents before the injection of virus could significantly enhance the efficacy of oncolytic viral therapy.

1030 POSTER

Complementary treatment with (−)-epicatechin enhances the anti-cancer effect of etoposide in the spleen of Brown Norway rats with acute myeloid leukemia

M.A. Papiez[1], M. Piskula[2]. *[1]The Jagiellonian University Collegium Medicum, Department of Cytobiology, Krakow, Poland; [2]Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Department of Food Technology, Olsztyn, Poland*

Background: It has been proven that tea catechins possess antileukemic properties, which might be useful in complementary treatment. The aim of the study, was to examine whether the adjuvant therapy with (−)-epicatechin (EC) can affect the anti-cancer effect of etoposide (Eto) in Brown Norway rats with acute myeloid leukemia (BNML).