1020 POSTER DISCUSSION

Modulation of the susceptibility to T cell lymphoblastic lymphoma by Fas/FasL system: functional polymorphisms at Fas, FasL and Fadd

M. Villa-Morales[1], M.N. Shahbazi[2], H. Gonzalez-Gugel[1], J. Santos[1], J. Fernandez-Piqueras[1]. *[1]CBMSO (CSIC-UAM)-CIBERER, Biologia Celular e Inmunologia, Madrid, Spain; [2]CNIO, Programa de Biologia Celular del Cancer, Madrid, Spain*

Background: In a previous study we reported functional polymorphisms in the coding sequences of *Fas* and *Fas*-Ligand, that contribute to the different effectiveness of the Fas-dependent apoptosis pathway between SEG/Pas (resistant to gamma radiation-induced T cell lymphoma) and C57BL/6J (susceptible) mice. In this study we report new polymorphisms in the coding sequence of another key gene (*Fadd*), and provide new data of interest to understand the molecular mechanisms yielding the different abilities for Fas-induced apoptosis.
Material and Methods: Chimeric Fas and FasL proteins combining intra- and extra-cellular regions from C57BL/6J and SEG/Pas have been constructed, and the levels of induced apoptosis *in vitro* investigated, through TUNEL and Caspases cleavage.
C57BL/6J- and SEG/Pas-derived *Fadd* cDNA have been genotyped. Co-immunoprecipitation assays between Fas-HA and Fadd-FLAG have been achieved to establish their interaction.
Results: The chimeras allowed us to assay the effect of polymorphisms located on the extracellular and intracellular regions of both proteins. The shift from SEG/Pas-derived protein regions to C57BL/6J, results in chimeric systems which drive cell apoptosis to a lower extent than the canonical SEG/Pas-derived system.
Also, the SEG/Pas-derived *Fadd* cDNA exhibits several changes of amino acid compared to C57BL/6J. These, together with the polymorphisms located on the intracellular region of Fas, through which it interacts with Fadd, might have functional consequences. Co-immunoprecipitation assays indicate that the interaction between Fas and Fadd is significantly stronger in SEG/Pas mice than in C57BL/6J.
Three *Fadd* polymorphisms (E51G, H59R, N189D) could not only affect its capacity to recruit Procaspase-8, but also its interaction with Fas, given that at least one of the polymorphic residues (E51G) allows the structural superposition of the Death Effector Domain and the Death Domain of Fadd. On the other hand, the polymorphism at N189 might affect the phosphorylation of Fadd at S191, which has been proposed as a key factor in the functionality of Fadd.
Conclusions: Altogether, these results suggest a model in which a compendium of functional polymorphisms of Fas, FasL and Fadd constitutes a compound haplotype that clearly influences the general activity of the system.

1021 POSTER DISCUSSION

A dysregulated pathway underlying a novel molecular subtype of ovarian cancer

A. Helland[1], M. Anglesio[2], C.N. Johnstone[2], J. George[2], P. Cowin[2], D.D. Bowtell[3]. *[1]The Norwegian Radium Hospital Oslo University Hospital and Peter MacCallum Cancer Centre, Oncology/Genetics, Oslo, Norway; [2]Peter MacCallum Cancer Centre, Research, Melbourne, Australia; [3]Peter MacCallum Cancer Centre and University of Melbourne, Research, Melbourne, Australia*

Background: From a clinical perspective, invasive serous ovarian carcinomas are considered to be a single entity. There is, however, considerable heterogeneity in the clinical course of the disease. Recently, six novel subtypes among serous ovarian carcinomas with impact on survival have been identified by expression profiling [1]. One of these subtypes (cluster5, C5) accounts for approximately 20% of the tumours, and is characterized by high expression of a group of oncofetal genes, like *HMGA2*, *IMP1*, *IMP2* and *LIN28B*. Interestingly this pattern of expression is characteristic of genes recently described to be regulated through the let-7 family of miRNAs [2]. This study aims to shed light on the mechanisms underlying this unique expression signature.
Material and Methods: Advanced serous ovarian carcinomas from the Australian Ovarian Cancer Study that were previously profiled by expression microarray [1] were used in the analyses, along with equivalent "subtype-classed" ovarian cancer cell lines. RNA-expression-levels of proposed let-7 target genes and expression of mature let-7 family miRNAs (*let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, MiR98*) were analysed by TaqMan qPCR-assays. Survival analyses were performed by Kaplan-Meier-methodology.
Results: As suggested by array profiling, C5 tumours displayed high expression of *HMGA2*, *IMP1*, *IMP2* and *LIN28B*, each known to be regulated by the let7 family of microRNAs. This signature was associated with worse prognosis as compared to the whole group of tumours.
The ovarian cancer cell lines A2780 and CH1 share a similar gene expression pattern as the C5 tumours, and express high levels of *HMGA2* and *LIN28B* and a consistently low expression of the let-7 family members. Quantitative PCR of the let-7 family members in tumours in the C5 subgroup revealed lower expression-levels as compared to other subgroups, significantly for let-7i ($P = 0.005$, Kruskal-Wallis test).
Conclusions: A distinct subtype of serous ovarian carcinomas (C5) are characterised by an RNA-expression-pattern indicating let-7 dysregulation. The expression levels of the let-7s confirm this, suggesting that C5-tumours harbour an event that perturbs the Lin28B/Let-7/HMGA2 pathway. Revealing aberrations underlying molecular subgroups could be a prerequisite for development of improved therapy.

References

[1] Tothill R et al, Clin Cancer Res, 2008.
[2] Boyerinas B et al, Cancer Res, 2008.

1022 POSTER DISCUSSION

Profiling of microRNA and mRNA expression in lung adenocarcinoma from never-smokers

J.S. Au[1], A.S. Chow[1], W.C. Cho[1], S.C. Law[1]. *[1]Queen Elizabeth Hospital, Clinical Oncology, Hong Kong, China*

Background: MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression by targeting mRNAs for degradation or translational inhibition and have been shown to be important in major cellular processes. Non-smoking-related adenocarcinoma (ADC) of lung is a distinct biological entity of increasing importance globally. Correlation of the miRNA and mRNA expression profiles will throw light on the gene control mechanisms.
Methods: Lung ADC tissue and normal lung parenchyma from the same individual were collected from 10 never-smokers undergoing curative surgery at Queen Elizabeth Hospital, Hong Kong. MiRNA microarray profiling was performed with Agilent Technologies Human MiRNA Array v1.0 (470 unique human miRNAs and 64 human-related viral miRNAs) and mRNA profiling with Roche Nimblegen Inc. Human. HG18 expr 385K Array (47633 genes). Significant up- or down-regulated expression levels were validated by qRT-PCR. Implicated miRNAs were further studied by transfection on cell lines. Computationally predicted mRNA targets for the specific miRNA were correlated with the actual mRNA expression levels measured in microarray.
Results: Fifty-five miRNAs showed significantly differential expression between cancer and normal lung parenchyma (false discovery rate <0.05 and >2-fold change). Thirty-seven miRNAs were downregulated in cancer whereas 18 miRNAs were upregulated.
Hsa-miR-145 was one of the most downregulated miRNAs and was chosen for the present study. The transfection of *hsa-pre-miR-145* significantly inhibited 45% of cancer cell growth in lung ADC cell lines. Quantitative RT-PCR assays showed that the relative expressions of *hsa-miR-145* were effectively increased in all the lung tissue cell lines following transfection with *hsa-pre-miR-145*. Cell morphology examination revealed that *hsa-miR-145* obviously induced apoptosis.
The expression of twelve mRNA targets (*CSTF3, FAM50A, GGCT, GYLTL1B, MMP11, MSI2, NPM3, NUDT1, PC, PMM2, ROD1, and SYNCRIP*) was significantly negatively-correlated with the *Hsa-miR-145* levels after multiple comparison correction by the Bonferroni method.
Conclusions: Down-regulation of *hsa-pre-miR-145* appeared to be an important gene regulation mechanism for the survival of ADC cells and correlated strongly with the upregulation of a number of mRNAs. Further confirmation and elucidation of the functions of these mRNAs is warranted.

1023 POSTER DISCUSSION

Are expression levels of Src kinase family members in human breast tissue related to clinical outcome of breast cancer patients?

B. Elsberger[1], S. Zino[1], R. Fullerton[1], T.J. Mitchell[1], P. Shiels[1], J. Edwards[1]. *[1]University of Glasgow, Department of Surgery, Glasgow, United Kingdom*

Background: There is a paucity in the literature about expression levels of Src kinase family members (SKFMs) in human breast tissue. The aim of this study was to assess m-RNA SKFM expression levels in different human breast specimens and to assess protein expression of the most significant SKFM in invasive breast cancer to establish their association to clinical outcome.
Methods: m-RNA expression of eight SKFMs (Src, Lck, Lyn, Fgr, Fyn, Hck, Blk, Yes) was assessed by quantitative real time PCR. Immunohistochemistry was performed using antibodies to c-Src, Y419Src, Lck and Lyn. Expression was assessed using the weighted histoscore method.

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