1020 POSTER DISCUSSION

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

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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.

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A dysregulated pathway underlying a novel molecular subtype of ovarian cancer

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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

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1022 POSTER DISCUSSION

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

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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?

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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.