1040 POSTER

Explore the Role That DMET Genotyping Platform May Play in Search of Genetic Polymorphism Associated With Severe Toxicity

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Background: We performed the Drug Metabolizing Enzymes and Transporters (DMET) platform analyses in a cohort of colorectal cancer (CCR) patients receiving fluorouracil, folinic acid and oxaliplatin (FOLFOX) chemotherapy in order to determine association with severe toxicity.
Material and Methods: This is a proof of concept in which DNA was purified from peripheral blood of strict selection: 7 patients (pts) by the presence of severe (grade 3) neuropathy and 4pts by cardiotoxicity. DNA processing and genotype identification for each patient sample were performed using the Affymetrix DMET platform, which offers the ability to scan 1936 variants in 225 genes related to drug metabolism and disposition has recently been introduced in experimental medicine. Genotypes were determined for every SNP site, reported as homozygous wild-type, heterozygous, homozygous variant or 'no call'. Information from each gene was obtained through genetic databases in order to make a first screening and reduce the number of variants unrelated to toxicity.
Toxicity was assessed in accordance with NCI CTCAE v3.0. Primary end-point was the identification of polymorphisms associated with development of severe toxicity.
Results: We obtained call rates of between 68 and 99% and information from 60 genes with any level of evidence. Genetic variations in five genes (that is, GSTP1, GSTM1, GSTT1, NQO1 and ATP7A) were identified in all pts with neurotoxicity. 5pts showed null polymorphisms or mutations in the glutathione family and 2pts harbored heterozygous variants for NQO1. These genes are involved in detoxification of platinum. And other 4pts had homozygous variants for ATP7A. This encodes a transporter of copper and has a potential role in platinum efflux. These data revealed that variants in these genes were associated with detoxification process and intracellular accumulation of platinum. In the group of pts with cardiotoxicity, all of them had heterozygous variants for SLCO1B1, this protein is exclusively localized to the basolateral membrane of hepatocytes and is involved in active cellular influx of many endogenous and xenobiotic compounds. 3pts harbored heterozygous variants for ABCB1, that is drug transporters, is expressed in the cardiac endothelium and several studies suggest that mediate QT prolongation and cardiotoxicity. These genes may be related to fluorouracil metabolism directly, and thus could be affected drug action.
Conclusions: DMET identifies detoxification and copper transporter pathways as possible responsible for intracellular accumulation of platinum that could play a role in severe neurotoxicity development.
ABCB1 and SLCO1B1 could have the potential to cause cardiac side effects. It would be interesting to run prospective studies of association to assess predictive value of these polymorphisms.

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Expression of Tumour-promoting Cysteine Rich 61 is Regulated by Tra2-β1 and Acidosis

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Background: The matricellular protein *Cysteine rich 61* (*Cyr61*) displays a remarkable diversity of multiple cellular functions involved in significant physiologic and pathologic processes. Cyr61 is known as an important player in tumour progression, promoting neovascularisation and metastasis. Our prior investigations elucidated an oxygen-dependent *Cyr61* alternative splicing process characterized by retention of its intron 3, regulating its biological function in a hypoxia-driven on/off switch mechanism.
Methods: Gynaecological cancer cell lines were treated with 0.2% lactic acid at a pH of 6.2 for 24 hrs. RNA was isolated followed by RT-PCR. Immunocytochemistry was carried out with the avidin/biotin method. Transfections of *tra2beta*-shRNA-Plasmids were performed in various cell lines.
Results: In this work, we identified extracellular acidosis as a potent inducer for altered Cyr61 alternative splicing pattern regulating *Cyr61* expression. Intriguingly, splicing factor *htra2-beta1* displayed an opposite effect on *Cyr61* expression. Nuclear htra2-beta1 protein expression was found markedly reduced under acidic conditions. In keeping with these conclusions, we show that htra2-beta1 can specifically bind a 'GAAG' motif in *Cyr61* exon 3 RNA, that the splicing factor displays acidosis-dependent protein localization in cellular compartments, and shRNA-mediated *htra2-beta1* knock-down triggers the same effects on *Cyr61* alternative splicing like acidosis or hypoxia, respectively.
Conclusion: According to our recent findings *Cyr61* alternative splicing is influenced by acidosis, a concomitant phenomenon of proliferating, hypoxic cancer cells. The interplay of hypoxia and extracellular acidosis with the microenvironment-dependant binding activity of splicing factor htra2-beta1 regulates Cyr61 expression.

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Expression of the Ribonucleases Drosha, Dicer and Ago2, Major Constituents of the MicroRNa Machinery, in Human Non-small Cell Lung Carcinomas

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Background: MicroRNAs (miRs) are small (16−29 nt), single, non-coding RNA molecules that regulate gene expression via cleavage of targeted mRNA or via translation repression. miRs are implicated in various physiological and pathological processes, including neoplasia. Production and function of miR requires a set of proteins, reffered as the miR machinery. Three ribonucleases, Drosha (in the nucleus) and Dicer and Ago2 (in the cytoplasm) process the primary transcripts to generate mature miR, which is incorporated into the RNA-induced silencing complex (RISC) that binds on target mRNA mediating RNAi functions. Argonaute-2 (Ago2) ribonucleases are major constituents of RISC. Herein, we explored the expression and distribution of Drosha, Dicer and Ago2 in non-small cell lung carcinomas (NSCLC) and investigated their role in lung carcinogenesis.
Materials and Methods: The expression and distribution of Drosha, Dicer and Ago2 were evaluated on 5 human lung cancer cell lines with Western blotting and immunofluorescense at protein level and with RT-PCR at mRNA level. Immunohistochemistry was performed for the assessment of expression/distribution of these enzymes on parafin embedded tissue from 80 NSCLC patients.
Results: In the examined cell lines, Drosha, Dicer and Ago2 were detected at both protein and mRNA levels. Ago2 and Dicer displayed primarily cytoplasmic localization, whereas Drosha expression was mainly nuclear. The immunohistochemical results paralleled our *in vitro* findings. The examined molecules were detected in the vast majority of the well and moderately differentiated carcinomas but only in a small fraction of the poorly differentiated tumours. Ago2 cellular levels were significantly lower in poorly differentiated compared to well/moderately differentiated tumours ($p < 0.001$).
Conclusions: 1) Drosha, Dicer and Ago2 are expressed in lung cancer cells in a well-orchestrated fashion. 2) The significant downregulation of Ago2 in poorly differentiated carcinomas implies that deregulation of this enzyme may be implicated in NSCLC carcinogenesis in humans. 3) Since the RISC complex proteins correlate with RNAi-based gene silencing it is possible that alterations of their expression levels might reflect the response of NSCLC cancer to fucture RNAi-related theraphies.

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Lysyl TRNa Synthethase – Ap4a Pathway − a Possible Role in Cancer

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Background: The Depot theory suggests that proteins released from large complexes may have a totally different role outside these complexes. We have described a new role for Lysyl tRNA synthase (Lys RS) which following MAPK phosphorylation can leave the multisynthetase complex produce Adenosine tetraphosphate (Ap4A regulate transcription, and cause inactivation of HINT1, a known tumour suppressor. (Lee et al Immunity 2004 PMID: 14975237, Yannay Cohen et al Mol Cell 2009 PMID:19524539). We wanted to check whether this pathway is also