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

activated in cancer and whether its modulation may have an effect on cancer.
Materials and Methods: LysRS status was analyzed by using protein fractionation assays as described by Yannay-Cohen et al. We used Hela cells and H460 cells overexpressing EGFR generously provided by G. Batist (Mcgill U. Montreal) to study possible relationship to the EGFR pathway. Hint constructs were cotransfected with wildtype or mutant LYSRS encoding constructs to A549 lung cancer cells.
Results: We checked EGFR effects on this pathway − EGF activation of HELA cells resulted in movement of LysRS to smaller protein fractions implying release of LysRS from the multisynthetase comple. Inhibition with Gefitinib of EGFR in a NSCLCA line resulted in the opposite effect. MAPK inhibition resulted in the disappearance of LysRS from smaller protein fractions in a cell line with overactivated MAPK pathway, implying that the presence of LysRS in smaller complexes can be manipulated by MAPK inhibition. Transfection of S207DLys RS pseudophosphorylated mimic with HINT1 prevented HINT1 activity as a tumour suppressor in a colony assay of A549 cells.
Conclusion: Our results suggest that the lysRS-AP4A pathway is activated in cancer. Activation of such a pathway in cancer might have distinct effects as shown in the case of HINT1tumour suppression. This is the first demonstration of the activity of this pathway in cancer. Other signal transduction pathway operating according to the principles of the depot theory may be aberrantly overactivated in cancer. Further research is needed to delineate the precise biological significance of this pathway in different cancers.

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Acquisition of P-glycoprotein Overexpression in Sensitive Tumour Cells

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Background: The overexpression of P-glycoprotein (Pgp/ABCB1), a drug efflux pump, promotes multidrug resistance (MDR) which prevents the successful of clinical cancer treatment. In addition, the overexpression of survivin and XIAP (inhibitors of apoptosis proteins − IAPs) may contribute to MDR phenotype in association with Pgp expression. Recently, it has been reported that Pgp expression can be acquired through transfer of membrane microparticles. Based on this, the aim of this study was establish an *in vitro* model to intercellular transfer of Pgp, and analyze the consequence of this phenomenon on IAPs expression in tumour cells.
Materials and Methods: K562–Lucena cell line (Pgp-positive cells derived from chronic myeloid leukemia) was co-cultivated with MCF7 and A549 cell lines (Pgp- negative cells derived from breast adenocarcinoma and lung carcinoma, respectively) for 24h and 48h. After co-culture, Pgp expression of recipient cells (MCF7 and A549) was assessed by immunostaining and immunofluorescence. Survivin and XIAP expression were analyzed by Western blot and qRT-PCR.
Results: The efficiency of our *in vitro* model to intercellular Pgp transfer was confirmed by flow cytometry, in which we observed high levels of Pgp expression in recipient MCF7 and A549 cell lines after 24h and 48h of co-culture. Furthermore, we observed Pgp expression on plasma membrane of recipient MCF7 and A549 cells after both times by immunofluorescence microscopy, which also revealed clusters of Pgp, possibly suggesting the identification of microparticles. Additionally, we also evaluated survivin and XIAP expression after acquisition of Pgp expression in MCF7 and A549 cell lines. We observed an increase on survivin and XIAP mRNA and protein expression in Pgp-positive A549 cells at 24h and 48h. However, we could not observe changes on survivin and XIAP mRNA and protein expression in Pgp-positive MCF7 cells. These data suggest that Pgp contribution on IAPs expression is cell line-dependent.
Conclusions: Taken together, these data demonstrate that sensitive cell lines acquired expression of MDR proteins when in contact with resistant cells. Besides that, these findings contribute to our knowledge for the emergence of MDR in tumour cells and could be helpful for new treatment approaches.
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Pharmacological Targeting Chaperone Activity and Chaperone Expression Enables to Sensitize Human Tumours to Hyperthermia and Radiotherapy

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Background: Ionizing radiation and hyperthermia are therapeutic modalities in fight against cancer. Because multiple heat shock protein 90 (Hsp90)-dependent pathways ensure tumour cell survival, pharmacological inhibitors of the Hsp90 chaperone activity, e.g. 17-N-allilamino-17-demethoxygeldanamycin (17AAG), may be synergistic with antitumour effects of hyperthermia or radiation. The problem is that 17AAG activates the heat shock transcription factor 1 (HSF1) thus inducing cytoprotective chaperones Hsp70 and Hsp27 which enhance thermo- and radioresistance of the 17AAG-treated tumour cells. Here we combined 17AAG with inhibitors of the HSF1-mediated Hsp induction to sensitize human tumour cells to mild hyperthermia and clinically relevant doses of radiation.
Materials and Methods: Hyperthermia (42–43 C, 60 min) or gamma-irradiation (2−6 Gy) were used as mono-treatments or with 17AAG and inhibitors of the Hsp induction such as quercetin, triptolide or NZ28 to kill MCF-7 and HeLa cells derived from human breast or cervical tumours. The cytotoxicity was determined in fluorescence staining and clonogenic assay. The protein levels were analyzed by immunoblotting. The 17AAG-induced inhibition of Hsp90 chaperone activity was assessed on retardation of the chaperone-dependent reactivation of luciferase in the heat-stressed transfectants.
Results: It was found that 20−100 nM 17AAG enhanced apoptosis and impaired clonogenicity in the cancer cells subjected to hyperthermia or low doses (2−4 Gy) of gamma-radiation. This enhancement of cytotoxicity correlated with a degree of the Hsp90 chaperone activity. As biomarkers of the Hsp90 activity inhibition, the specific depletion of Akt and Raf-1 and upregulation of Hsp70 and Hsp27 were revealed in the samples of 17AAG-treated cells. Addition of either 10−30 uM quercetin or 2−5 nM triptolide, or 0.3−1 uM NZ28 fully prevented the Hsp accumulation in the 17AAG-treated cancer cells and rendered them much more sensitive to hyperthermia and radiation; the powerful sensitizing effects of extremely low concentrations of triptolide seemed particularly impressive.
Conclusion: The synergism in cytotoxicity under combining of hyperthermia or gamma-radiation with 17AAG and triptolide appears to be due to (i) blockade of the Hsp90-dependent antiapoptotic pathways, and (ii) blockade of the 17AAG-induced upregulation of cytoprotective Hsp70 and Hsp27, while the target cells undergo cytotoxic treatments.

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Expression of NF-kB Transcription Factor, C-erbB2, Estrogen and Progesterone Receptors in Tumours of Patients With Breast Cancer

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Background: The aim of present study was to study expression of NF-kB (p50/p65), c-erbB2, ER and PR in breast tumours and analyse correlation between these markers.
Methods: We selected 41 patients with locally advanced breast cancer who had not been treated with radio- or chemotherapy. The I stage was diagnosed for 4, II − for 27, III − for 6, IV − for 3 patients, 3 patients had "x" stage at that moment. All tumours belong to invasive ductal carcinoma with different tumour grade.
NF-kB, c-erbB2, ER and PR expression was determined by immunohistochemistry. For staining interpretation the H-score method was evaluated. The score is obtained by the formula:
3×% of strongly staining + 2×% of moderately staining +% of weakly staining. Expression level of markers was graded as weak (H-score 0−49), moderate (50−99) or strong (100−300).
Results: The association between ER and PR expression and grade was defined. G3 tumours had lower expression of receptors (H_{ERmean} = 77, H_{PRmean} = 23) in comparison to G2 (H_{ERmean} = 89, H_{PRmean} = 75) and G1 (H_{ERmean} = 143, H_{PRmean} = 95).
We had formed three groups of patients. Patients from first group had high expression of p50 and p65, second- high expression of p50, third- low expression of subunits. The relation between expression of NF-kB and other markers was evaluated. In the first group ER, PR and c-erbB2 expression was low (H_{ERmean} = 39, H_{PRmean} = 37, $H_{c-erbB2mean}$ = 34), in the second- moderate (H_{ERmean} = 73, H_{PRmean} = 47, $H_{c-erbB2mean}$ = 98), in the third- high (H_{ERmean} = 149, H_{PRmean} = 99, $H_{c-erbB2mean}$ = 123).
Also, NF-kB expression in different molecular types of breast tumours was analyzed. Breast tumours are sub-divided on Luminal (high/moderate ER, moderate/low c-erbB2), HER2 (low ER, high c-erbB2), Luminal-HER2 hybrid (high/moderate ER, high c-erbB2), and basal-like (low ER and c-erbB2) types. Our data show the highest p65 expression in basal-like type ($H_{p65mean}$ = 75), moderate in luminal ($H_{p65mean}$ = 43) and HER2 ($H_{p65mean}$ = 44) type, and low in Luminal-HER2 hybrid ($H_{p65mean}$ = 26) type.
Conclusion: As a result of researches was defined that G3 tumours have lower ER and PR expression in comparison to G2 and G1 tumours. p50 and p65 levels were found to be changed in dependence from expression of each marker (ER, PR, c-erbB2) and also from tumour's type (Lumilal, Her2, hybrid, basal-like). So, our findings are the reason for further study this characteristics, because the results of such investigations may be useful for search of chemotherapeutic scheme, and for prognosis of disease course.