Results and discussions: Minor allele frequencies for M420del were 0.18 and 0.1 in CML patients and controls; for M408V 0.4 and 0.27 respectively, closely paralleling those reported in western populations.

No significant association between different genotypes of M420del and M408V was observed with either time to achieve complete haematological response (CHR) (p=0.341 for both SNPs), or presence of optimal/sub-optimal molecular responses (p=0.125, 0.629 for M420del and M408V respectively).

To analyse the combined effect of these two SNPs, CML cases were divided into 4 groups: patients with mutant (homo/heterozygous) M420del and wild type homozygous M408V, failed to achieve an optimal molecular response to imatinib, unlike those with mutant genotypes (homo/heterozygous) for both SNPs (p=0.02).

Conclusion: Mutant M420del allele may be linked to poor outcome of imatinib treatment in CML; however simultaneous presence of mutant M408V allele appears to circumvent this effect. These SNPs in hOCT1 gene occur at reasonable frequencies in Indian population, to be of clinical interest as predictors of response to imatinib in CML.

PO-471 EXOSOMES AND TRANSFERRING OF HORMONAL RESISTANCE OF BREAST CANCER CELLS

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Introduction: The main goal of the present work was the study of the molecular mechanism of acquired hormonal resistance of tumour cells, in particular - the study of the intercellular interactions and exosomes and their role in the progression of the resistance.

Material and methods: Hormone responsive MCF-7 and hormone resistant MCF-7/T breast cancer cells were cultured in standard DMEM medium supplemented with 10% fetal calf serum. The transcriptional activities of NF-kB and AP-1 were determined by reporter luciferase assays. Then exosomes from cell supernatant were isolated by differential ultracentrifugation using standard protocol.

Results and discussions: Here, using the estrogen-dependent MCF-7 breast cancer cells and estrogen-independent MCF-7/T cells we have demonstrated the ability of the resistant cells-derived exosomes to initiate the estrogen-independent growth of the parent MCF-7 cells. The subsequent analysis of the key signalling proteins revealed the ability of the exosomes of the resistant cells to inhibit the oestrogen signalling as well as to stimulate the Akt protein kinase and transcription factors AP-1 and NF-kB in the recipient cells. Importantly, the cell treatment with PI3K/Akt inhibitor wortmannin prevented the exosome-induced resistance giving the additional evidence for central role of this pathway in the mediating of exosomal resistance.

The data validation was performed on the resistant subline MCF-7/M which was selected under long-term cultivation of the parent MCF-7 cells with biguanide metformin and demonstrated the cross-resistance to metformin and tamoxifen. We have shown that the exosomes of the resistant MCF-7/M cells lead to the partial resistance of the parent cells to both drugs. Moreover, the acquired resistance was characterised with the analogues signalling rearrangement: inhibition of the oestrogen signalling and activation of the Akt, AP-1 and NF-kB proteins - similar to the described above hormonal resistance. Advanced analysis of the exosomal microRNA revealed 27 microRNA differentially expressed in the exosomes of the resistant cells and associated with the progression of hormonal resistance.

Conclusion: Totally, we demonstrated the new mechanism of horizontal transferring of hormonal resistance by exosomes, identified the possible intercellular targets of exosomes and revealed the main features of the exosomal transcriptome. This study was supported by Russian Science Foundation, grant 14-15-00362 M.K.
PO-473 QUANTIFICATION OF ERCC1-XPF COMPLEXES IN OVARIAN CANCER XENOGRAFTS WITH DIFFERENT SENSITIVITY TO CISPLATIN

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Introduction Epithelial ovarian cancer is the most lethal gynaecological cancer due to the development of resistance to a platinum based therapy. As DNA repair capacity is a key determinant for the cellular response to platinum (DDP) agents, DNA repair functional assays are required to study its relevance in DDP resistance. We set up a proximity ligation assay (PLA) to study the activity of nucleotide excision repair (NER) in patient derived ovarian carcinoma xenografts (PDXs) sensitive (S) and resistant (R) to DDP.

Material and methods Patient derived xenografts from fresh ovarian carcinomas were recently established in our laboratory. DDP antitumour activity was evaluated in most of the PDXs. Mice were sacrificed when tumours reached 1,5 g. Tumours were fixed in formalin and paraffin embedded (FFPE). PLA was performed on tumour slides, using DuolinkII reagents (Sigma-Aldrich) and following the manufacturer instructions. PLA detects the presence of the protein complexes ERCC1-XPF, that are quantified as foci per nucleus and represent a biomarker of NER activity. Images were acquired by Olympus Virtual Slider (Olympus) and analysed with ImageJ software. Statistical analysis was performed with GraphPad Prism7.

Results and discussions Our xenobank comprises PDXs with different response to DDP: MNHOC266 and MNHOC230 are very sensitive to the drug, while MNHOC315 is resistant. We also obtained three sublines resistant to DDP (MNHOC124R, MNHOC124LPR and MNHOC239R) starting from sensitive PDXs (MNHOC124S, MNHOC124LPS and MNHOC239S), after several in vitro drug treatments. Statistically significant higher level of ERCC1-XPF foci could be observed in MNHOC124R and MNHOC124LPR as compared to their sensitive counterparts. No differences were observed between MNHOC239S and R PDXs, even if the number of ERCC1-XPF foci in MNHOC239S was statistically higher than the ones observed in MNHOC124S and in MNHOC124LPS. MNHOC266 and MNHOC230 showed levels of foci comparable to those of MNHOC124S and MNHOC124LPS. mRNA and protein levels of the different isoforms of ERCC1 and of XPF were not different among the PDXs studied.

Conclusion PLA for the detection of ERCC1-XPF complexes was set up in FFPE xenograft tumour slides. These preliminary results highlight a possible link between DDP resistance and higher NER activity that need to be confirmed in a wider panel of PDXs. In addition, these data confirm the importance to develop functional assays to directly evaluate the activity of different DNA repair pathways to predict DDP activity.