Conclusion These data prompt the need to further investigate the redundancy and hierarchy of immune checkpoints in the tumour microenvironment to effectively harness the anti-tumour immune response and efficiently implement immune checkpoint blockade therapy.

Tumour Heterogeneity – Evolution

PO-323 *IN VIVO* MONITORING OF TUMOUR RESPONSES TO CHEMOTHERAPY USING MULTIPARAMETER FLUORESCENCE IMAGING

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Introduction An increasing number of recent studies suggest that tumour response to chemotherapy cannot be fully described in terms of only interaction of the drug with the main cellular target (e.g. DNA or microtubules) but can include multiple physiological and physicochemical changes. Insight into drug-induced functional alterations in cancer cells and tissues is crucially important for understanding of mechanisms of a drug action and for development new approaches to enable monitoring of the early-treatment response.
Material and methods In our studies, we focus on multiparameteric analysis of tumour responses to chemotherapy using advanced fluorescence imaging techniques. Previously, we developed methodologies for *in vivo* probing several parameters, including cytosolic pH, metabolic status, and viscosity of plasma membrane, in mouse tumour models. Mapping of cytosolic pH in tumours is performed using ratiometric genetically encoded sensor SypHer2 and fluorescence whole-body imaging [Shirmanova et al. BBA-GS 2015]. Imaging of cellular metabolism is based on the visualisation of fluorescence intensities and lifetimes of intrinsic metabolic cofactors NAD(P)H and FAD [Shirmanova et al. Sci.Rep. 2017]. Viscosity is measured using molecular BODIPY-based rotor and fluorescence lifetime imaging microscopy (FLIM) [Shimolina et al. Sci.Rep. 2017].
Results and discussions We showed that acidification of cytosolic pH occurs after therapy with cisplatin *in vivo*, and this, likely, favours metabolic reorganisation of cells. Treated tumours exhibited a decreased relative contribution from free (cytosolic, protein-unbound) NAD(P)H, indicating a metabolic shift from glycolysis towards oxidative metabolism. It is interesting that optical metabolic imaging could allow early detection of tumour response to chemotherapy, before there are changes in tumour size, and metabolic changes were the same for therapeutic agents with different mechanisms of action (cisplatin, paclitaxel). Moreover, tumours treated with cisplatin displayed decreased plasma membrane viscosity. Presumably, this alteration of viscosity participates in cisplatin-induced apoptosis. Similar changes of pH, energy metabolism and viscosity were previously reported for cisplatin-treated cultured cancer cells.
Conclusion These results, therefore, suggest that all investigated parameters play a role in the cytotoxicity of the drugs and may provide a useful approach for monitoring tumour responses to chemotherapy. The study was supported by the Russian Science Foundation (#14-25-00129).

PO-324 INTERFERON REGULATORY FACTOR 1 (IRF1) REGULATES INFLAMMATORY AND METABOLIC PHENOTYPES IN PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction Pancreatic Ductal Adenocarcinoma (PDAC) is the most frequent neoplasia of the exocrine pancreas. This tumour is and is characterised by a pervasive heterogeneity, with the coexistence of a range of histological grades, from epithelial-like to mesenchymal-like features. We previously dissected the transcriptional and epigenetic networks underlying PDAC grading. We identified the association of low grade phenotypes with a cell-autonomous interferon-related signature. Therefore, we set out to investigate the sustainment of inflammatory and interferon-related signatures in well-differentiated pancreatic cancer cells, and to determine the role of this network in PDAC biology.
Material and methods We used cell-line based models of cancer differentiation, xenografts and human samples. We used CRISPR-Cas9 mediated genome editing to delete the transcription factor IRF1 (Interferon Regulatory Factor 1) in low-grade PDAC cells. RNA-seq, metabolic assays (oxygraphy, steady state metabolomics, fluxomics) and cell biology assays were carried out in IRF1 wt and knock-out cell lines. Data validation in human PDAC samples was carried out by immunohistochemistry.
Results and discussions We found that IRF1 is a transcription factor differentially expressed between low- and high-grade PDACs, both in cell lines and in human tumours. IRF1 deletion in low-grade cell lines reduced the expression of genes in the antigen processing and presentation pathways, while its overexpression promoted the expression of the same genes in high-grade cells, where they are normally not expressed. Furthermore, xenografted IRF1-deficient cell lines recruited fewer immune cells *in vivo*. IRF1 deletion also affected epithelial phenotypes, including growth rate, cell shape, motility and collagen remodelling ability. Alongside, we unveiled a role of IRF1 in the control of the metabolism of low-grade PDAC cells, consisting in the control of mitochondrial respiration and lipogenesis as well as of the overall lipid profile of these cells.
Conclusion To conclude, our results provide hints on the regulatory networks controlling cell differentiation in human PDACs. We show that IRF1 acts as a pleiotropic regulator in the low grade component of PDACs, with wide effects on immunological and metabolic features of this cancer population. Our work reinforces the body of knowledge needed for the development of those therapeutic strategies aiming at exploiting immunological or metabolic pitfalls.

PO-325 TOPOLOGICAL-PROTEOMICS OF BREAST CANCER INTRA-TUMOUR HETEROGENEITY REVEALS METABOLIC DIVERSITY WITHIN SINGLE TUMOURS

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