PO-270 SIGNALLING PATHWAYS WNT, HEDGEHOG AND NOTCH IN BREAST CANCER WITH PRESENCE AND ABSENCE OF CANCER STEM CELLS

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Introduction WNT, Hedgehog and NOTCH are typical signalling pathways for breast cancer cells. Activation of these pathways leads to increase of tumour aggressiveness and resistance to chemotherapy. One of the most interesting and perspective for studying type of cancer cells is cancer stem cells. This kind of cells possesses unlimited proliferative activity and high tumour potential. The aim of the research was to study mentioned above signalling pathways in immunohistochemical subtypes of breast cancer with high and low maintenance of cancer stem cells.

Material and methods The material of 219 cases of invasive breast cancer was used. The immunohistochemical method was applied to identify the expression of ALDH1A1 – markers for cancer stem cells, activation of WNT, NOTCH, Hedgehog signalling pathways in cancer cells as well as Oestrogen receptor, progesterone receptor, HER-2/neu receptor and Ki-67 protein expression for detection of immunohistochemical subtype of breast cancer.

Results and discussions In cases with high maintenance of cancer stem cells (ALDH1A1-positive cells) activation of WNT and NOTCH signalling pathways was found in cancer cells of triple negative breast cancer both in 11% of all the cases and in cancer cells of HER-2 overexpression subtype in 25% and 36% respectively. In cases of Luminal B subtype only expression of NOTCH in cancer cells was found. Activation of Hedgehog signalling pathway was not found in cases with high maintenance of cancer stem cells. In cases with low maintenance or absence of cancer stem cells activation of WNT, NOTCH and Hedgehog signalling pathways was detected in cancer cells of Triple negative subtype in 33%, 25% and 14% respectively. Cancer cells of Luminal B subtype expressed WNT in 43%, NOTCH in 50% and Hedgehog in 19% of all the cases. In cases of HER-2 overexpression subtype Hedgehog expression in 7% was additionally found in comparison with cases of the same subtype with presence of cancer stem cells. Reliable results was not found for Luminal A subtype due to small samples.

Conclusion WNT, NOTCH and Hedgehog signalling pathways occur in ALDH1A1-negative breast cancer cells of different immunohistochemical subtypes. Activation of Notch signalling pathway is found in cancer stem cells independently from immunohistochemical subtype. WNT signal pathway can be found in cancer cells of ALDH1A1-positive cases of Triple negative and HER-2 overexpression subtypes. Hedgehog signalling pathway is not typical for cases of breast cancer with high maintenance of cancer stem cells.

PO-271 USING HUMAN LUNG ORGANOIDS TO STUDY PULMONARY NEUROENDOCRINE CELLS

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Introduction Introduction: Lung neuroendocrine tumours (LNETs) arise from a rare lung cell population, called pulmonary neuroendocrine cells (PNECs), and account for 20%–25% of all lung cancers. Despite the involvement of PNECs in LNETs and a number of other respiratory diseases, relatively little is known about their normal function or contribution to cancer development and progression. To date, the study of PNECs has been limited by difficulties in isolating and culturing this cell population.

Material and methods Building on methods developed in our lab for the isolation and long-term expansion of healthy and diseased human airway cells, we have generated lung organoids derived from embryonic lung tissue. We have identified cultures conditions that allow for the enrichment of PNECs in embryonic lung organoids. In parallel, we have begun to generate a biobank of both low- and high-grade LNET organoids.

Results PNEC-enriched embryonic lung organoids can be expanded indefinitely over multiple passages. Preliminary analysis by qPCR and whole mount immunofluorescence shows that PNEC-enriched organoids express a number of PNEC lineage markers including ASCL1, NEUROD1, and UCHL1. PNEC-enriched lung organoids also express PNEC-associated bioactive compounds such as SST and CGRP.

Conclusions We have generated human embryonic lung organoids that contain significant numbers of PNECs. Applying the same principles, we have established organoid cultures of human LNETs. PNEC-enriched organoids and LNET organoids are currently being used for further molecular and genetic analyses.

PO-272 LEUKEMIA-ASSOCIATED NPM MUTATIONS PROMOTE QUIESCENCE OF HEMATOPOIETIC STEM CELLS AND PREVENT THEIR FUNCTIONAL EXHAUSTION UPON ONCOGENE-INDUCED HYPER-PROLIFERATION

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Introduction Acute Myeloid Leukaemia (AML) is a heterogeneous and multi-step disease. The serial acquisition of mutations and the environmental pressure allow one or more clones to expand and contribute to the disease. In particular, 6% of AMLs are characterised by an initial mutation in the DNMT3a gene, followed by mutations in NPM (NPMc) and FLT3 loci (FLT3-ITD). We previously shown that NPMc can drive AML development in mouse model and highly cooperates with FLT3-ITD. Moreover, it has been reported that normal Hematopoietic Stem Cells (HSCs) of elderly people may bear some somatic early AML mutations and this correlate with an increased risk of hematologic diseases suggesting that mutations can shape pre-leukemic HSCs to be more prone to the acquisition of further mutations giving rise to Leukaemia Initiating Cells (LIC).

While the ability of FLT3-ITD to drive HSC compartment exhaustion has been already shown, the impact of NPMc on HSCs remains unclear.

Material and methods Taking advantage of the extended pre-leukemic phase of our inducible NPMc mouse model, we elucidate the role of NPMc in HSCs by functional and transcriptional analysis. Moreover, to investigate the basis of NPMc

and FLT3-ITD cooperation we generate mice carrying both the conditional NPMc transgene and the FLT3-ITD constitutive mutation and, before AML onset, we analyse double mutant HSCs behaviour.

Results and discussions We have found that NPMc expression lead to the expansion of the HSC compartment through the enforcement of a stem-cell transcriptional program that increases self-renewal by promoting quiescence. We then investigated how the NPMc dependent quiescence program is linked to its oncogenic function. The expression of NPMc +in the FLT3-ITD background prevents the HSCs exhaustion imposed by FLT3-ITD and restores their repopulating capacity. Accordingly, gene expression analysis revealed a strong dominance of NPMc +with the restoration of the same transcriptional program observed in the NPMc HSCs. These data strongly suggest that NPMc imposes a HSC-specific program that, in combination with the oncogenic signal provided by FLT3-ITD, allows the selection of the LIC and the occurrence of AML.

Conclusion In conclusion, enforcement of quiescence might be a critical function for the maintenance of the transformed clone during both the pre-leukemic and the leukemic phase. As consequence, interfering with quiescence key determinants may eradicate the reservoir of quiescent cells responsible for disease recurrence.

PO-273 EVALUATION OF THE ROLE OF SOX2 AS CANCER STEM CELL MARKER IN SARCOMAS

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Introduction Sarcomas often show a limited response to cytotoxic drugs which still remain as the most utilised agents for the treatment of these diseases. This lack of response could be explained by the existence of drug-resistant Cancer Stem Cells (CSCs), which are responsible of tumour relapse. It has been established that transformed human mesenchymal stromal/stem cell (MSCs) are the most likely cell-of-origin for many types of sarcomas. Therefore, we previously developed a model of sarcomagenesis based on transformed Bone Marrow Mesenchymal Stromal/Stem Cells (BM-MSCs). This model has proven very useful to explore the evolution of CSCs subpopulations and to search for CSC-specific markers and therapies. Here we use this model to analyse the role of SOX2 as a CSC-specific marker by isolating subpopulations presenting high SOX2-mediated transcriptional activity.

Material and methods We depleted or overexpressed SOX2 in a xenograft-derived cell line (T5H-O) generated by a cell-origin-model of undifferentiated pleomorphic sarcoma developed from transformed BM-MSC. In addition, these cells were transfected with a lentiviral-based reporter system in which a composite SOX2/OCT4 response element (SORE6) coupled to a minimal cytomegalovirus (CMV) promoter controls the expression of fluorescent reporter genes. T5H-O cells expressing this system allowed us to detect and isolate viable cells expressing transcriptionally active SOX2 and/or OCT4 by flow cytometry.

Results and discussions The depletion of SOX2 protein inhibited CSC related-properties of T5H-O cells (e.g. tumorsphere forming potential) whereas T5H-O cells expressing high levels of SOX2 dramatically increased their invasive properties and *in vivo* tumour growth potential. We found that SORE6 activity in our sarcoma model was mainly due to SOX2, rather than OCT4. Comparing to SORE6- population, SORE6 +positive cells showed enhanced tumorsphere-forming potential and increased invasion ability. Importantly, SORE6 +cells were significantly more tumorigenic than the SORE6- population, thus indicating that SOX2 activity marks a subpopulation with increased tumor-promoting ability in sarcomas. Finally, we found that EC-8042 and trabectedin, antitumour drugs previously reported to target CSCs in sarcoma, were able to eliminate the SORE6 +CSC subpopulation in sarcomas.

Conclusion Our results indicate that SOX2 activity is a bona fide CSC-marker in sarcoma, and that SORE6 reporter system is an excellent approach for testing the effectiveness of CSC-specific treatments.

PO-274 TUMOUR SUBTYPE-SPECIFIC CELLS OF ORIGIN OF MALIGNANT MESOTHELIOMA

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Introduction Malignant Mesothelioma (MM) is an aggressive malignancy of the lining of the thoracic and peritoneal cavity. The primary cause is previous asbestos exposure. The pathological diagnosis of MM is rather complex due to a lack of useful biological markers and because multiple cell types are involved in the development of MM. We aimed to investigate to what extent the tumour subtype is determined by the cell of origin rather than the somatically acquired driver mutations.

Material and methods Co-deletion of the conditional tumour suppressors, NF2, p53 and Cdkn2a in freshly isolated mesothelial cells using Cre viruses allowed us to establish clonal cell lines with epithelial, sacomatoid and biphasic morphology as also observed in human MM. Cells were analysed for clinical relevant protein marker profiles, tumorigenic potential and RNA expression. Results were compared to (poly)clonal cell lines obtained from conditional mice injected intra-thoracically with lentiviruses expressing Cre-recombinase driven by tissue-specific promoters.

Results and discussions Using the *ex vivo* approach we were able to obtain clonal cell lines that upon transplantation gave rise to the main three MM tumour epithelial, sarcomatoid and biphasic subtypes. The epithelial and sarcomatoid phenotypes observed *in vitro* retained in the tumours, also after serial transplantation of the cell lines. Clonal biphasic cells co-expressed both epithelial and sarcomatoid markers and external factors could skew these biphasic cells towards a more epithelial or sarcomatoid phenotype. Transplantation of clonal biphasic cells only gave rise to tumours when the cells were grafted in immune-deficient mice, this in contrast to the epithelial and sarcomatoid cell lines that effectively gave tumours in syngeneic immunocompetent recipients. Analysis of tumour cell populations and derived clonal cell lines induced by lentiviral Cre-mediated switching of the same tumour suppressors in the mesothelial lining of conditional mice showed a high