PO-277 SINGLE-CELL RNA-SEQ ANALYSIS OF HUMAN PANCREATIC DUCTAL ADENOCARCINOMA

M Rao, M Gao, AP Delgado, A Nemajerova, I Li, R Moffitt, J Kim, J Powers.
Stony Brook University, Pathology, Stony Brook, USA; Stony Brook University, Biomedical Informatics and Pathology, Stony Brook, USA; Stony Brook University, Surgery, Stony Brook, USA

Introduction Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most lethal forms of cancer with a five-year survival rate of 8%. Genetic alterations such as activating mutations in KRAS and inactivating mutations in CDKN2A and TP53, accompanied with changes in surrounding stroma, are involved in progression of pancreatic cancer. However, since these alterations are not well understood at the single cell level, we have been performing single-cell RNA-sequencing (scRNA-seq) analysis of PDAC.

Material and methods We performed scRNA-seq and histological analysis of a resected human PDAC tumour specimen using the 10X platform and single cell cDNA library construction kit followed by sequencing on a HiSeq4000 (3,640 cells; over 10^5 reads per cell). Bioinformatic analysis included both standard pipelines and custom analysis of TCGA PDAC signatures as first described in (Raphael BJ, 2017; Cancer Cell 32, 2185).

Results and discussions Nine distinct cell types were identified by K-means clustering and differential expression analysis. In agreement with histological evidence, slightly less than 10% of the cells were actual ductal carcinoma cells, and the rest were either normal epithelial cells or stromal cells. The TCGA signature identified these ductal carcinoma cells as belonging to the basal subtype of PDAC. Individual clusters included normal pancreatic islet cells, B-lymphocytes, macrophages, mast cells, cells that resemble schwann cells, cancer-associated fibroblasts (CAFs), and normal fibroblasts. Bioinformatic analysis suggested two subtypes of CAFs with different functions: extracellular matrix production, and immunomodulatory, in agreement with a prior report (Ohlund D, Journal of Experimental Medicine, 2017;214(3):579). Histological analysis confirmed presence of the major cell types. Additionally, an epithelial-like cell cluster was observed and further histological analysis is underway to confirm the identity of the same.

Conclusion sc-RNAseq of a resected tumour specimen from a PDAC patient revealed the presence of diverse cell types in addition to tumour cells such as different immune cells, different types of fibroblasts, and a previously undescribed pancreatic cell type. Further application of this technology should yield additional insights and lead to the discovery of novel biomarkers for analysis of pancreatic cancer.

PO-278 ASSESSMENT OF THE INFLUENCE OF STELLATE CELL ON PRIMARYPancreatic Ductal Cancer Cell GROWTH AND DRUG RESISTANCE IN A SPHEROID MODEL: MET INHIBITORS TO THE RESCUE

OF the IN VITRO A549 Neuroendocrine Differentiation on the Activity Cytotoxic T Lymphocytes.

PO-279 EFFECT OF THE IN VITRO A549 Neuroendocrine Differentiation on the Activity Cytotoxic T Lymphocytes.

1Mendietta*, 2RE Nuñez-Anita, 3G García-Acero, 1LC Berumen-Segura. 1Universidad Autónoma de Querétaro, Facultad de Química, Centro de Investigación Genética, Santiago de Querétaro- Querétaro, Mexico; 2Universidad Michoacana de San Nicolás Hidalgo, Facultad de Medicina Veterinaria y Zootecnia, Morelia- Michoacán, Mexico

Introduction Lung cancer is one of the leading causes of deaths by cancer in both sexes worldwide. A primary concern of this disease is the presence of neuroendocrine (NE) phenotype which has been correlated to a decreased survival, an increased number of peripheral tumour cells and an increased percentage of metastasis. A key aspect of NE phenotype is the formation of secretory granules with the fundamental property of secreting a great variety of factors such as hormones and neurotransmitters. In recent years, there has been an increasing interest in the role of this factors as immunomodulators, a primary concern is their role in the bidirectional communication within the tumour microenvironment.

It remains unclear the immunomodulator effect of factors secreted in NE phenotype of lung cancer. The objective of this work was to test the effect of factors secreted by the lung adenocarcinoma cell line (A549_neu) with NE phenotype on the activity of cytotoxic T lymphocytes (CTL) in vitro.
**Material and methods** Human lung adenocarcinoma A549 cells were treated with [cAMP] increasing agents up to 120 hours (isobutyl-1-methylxanthine (IBMX, 0.5 mM), forskolin (FSK, 0.5 mM) or both IBMX+FSK) to observe the changes in the phenotype acquisition: morphology and neurite number (LM), Chromogranin A (CgA by FACS).

Co-cultures and fluorescence release were used to evaluate the interaction of cytotoxic activity of T lymphocytes (C: Jurkat) against target cells (T: A549/GFP) and to observe the effect of the neuroendocrine differentiation on the cytotoxic activity, using as target cells original A549 cells and transdifferentiated A549(NE).

**Results and discussions** Changes in the A549 cell morphology were observed (size and presence of neurite-like projections), decreased proliferation rate and the overexpression of neuroendocrine marker chromogranin A were also observed in all treatments since 72 hours (except for CgA overexpression with IBMX treatment).

The fluorescence release of A549/GFP after C: T co-cultures showed an increasing cytolytic time-dependent effect and C/T ratio. The NE phenotype acquisition diminishes the fluorescence release of target cells in 24 hour co-culture suggesting a decreased cytolytic activity.

**Conclusion** The current data support the generation of a neuroendocrine phenotype from A549 cell line (A549 NE), stable for 48 hours after cAMP stimuli withdrawal. In co-cultures with CTL, there is evidence of diminished cytolytic means of the acquisition of the NE phenotype.

**PO-280 MUTANT KRAS MEDIATES FIBROBLAST-INDUCED COLORECTAL CANCER CELL INVASION**

1J Kennedy-Darling*, 1G Dakshinamoorthy, 1S Mistry, 1NN Ikuilina, 2C Streck.
1Akoya Biosciences, Research and Development, Menlo Park, USA; 2Akoya Biosciences, Product Management, Menlo Park, USA

**Introduction** KRAS is the most frequently mutated oncogene in colorectal cancer (CRC), being a potent initiator of tumorigenesis, a strong inducer of malignancy, and a predictive biomarker of non-response to anti-EGFR therapies. As such, extensive research has been done to exploit KRAS and its downstream signalling effectors as therapeutic targets. However, KRAS proved difficult to target, and inhibition of its signalling effectors has never resulted in significant clinical responses, highlighting the need for a better understanding of KRAS-associated signals. Since the tumour microenvironment plays a key role in tumour aggressiveness, research on this area became an attractive alternative as new targets for therapy may arise from the study of cancer cell-microenvironment crosstalk. The aim of this study was to characterise, at the molecular and functional levels, the role of mutant KRAS in mediating CRC cells-fibroblasts crosstalk.

**Material and methods** Using fibroblasts-conditioned media (CM) as a chemoattractant, we performed matrigel invasion assays with KRAS mutant CRC cell lines in which we silenced KRAS using siRNA. Additionally, we performed ELISA assays to quantify the levels of fibroblasts-secreted factors and resorting to western blot we evaluated the expression of some cell surface proteins.

**Results and discussions** By performing in vitro invasion assays we observed that the CM promoted CRC cell invasion in a KRAS-dependent manner. Analysis of the CM for the detection of pro-invasive factors, revealed the presence of high levels of HGF. Accordingly, neutralisation of HGF in the fibroblasts CM abrogated CRC invasion, and supplementation of control CM with HGF induced invasion in a KRAS-dependent manner. Additionally, we have also observed that KRAS regulates the expression of HGF receptor, C-MET, along with other C-MET co-receptors.

**Conclusion** In conclusion, our results show that KRAS may be an important modulator of response to fibroblasts-secreted factors that induce CRC cells invasion. Therefore, this work suggests that targeting of C-MET can be a useful tool to abrogate invasion of KRAS mutant tumours and sets a rational to test C-MET inhibitors in the treatment of KRAS mutant CRC patients, who currently lack effective therapeutic options.