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 (A549N3).

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 cytolysis.

Conclusion The current data support the generation of a neuroendocrine phenotype from A549 cell line (A549NE), stable treated with IBMX treatment.

By performing western blot we evaluated the expression of some cell surface proteins. 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.

Abstracts

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

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 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.

PO-281 AUTOMATED MULTIPARAMETRIC TISSUE IMAGING PLATFORM USING EXISTING MICROSCOPE HARDWARE FOR THE DETECTION OF SPATIALLY RESOLVED SINGLE-CELL RESOLUTION DATA

Introduction Characterising complexities of the tumour microenvironment is elemental to understanding disease mechanisms. The spatial relationships between infiltrating immune cells and the remodelling of the cellular matrix is widely recognised as a key component to defining tumour heterogeneity. Current methodologies for analysing the spatial dimension in tissues, like traditional immunofluorescence (IF) and immunohistochemistry (IHC), are limited to a few parameters at a time, restricting the scope of identifiable cells. Conversely, single-cell technologies like mass cytometry and NGS-based tools provide multiplexing capabilities, but at the expense of the associated spatial information. Here we present a novel multiplexed imaging technology, termed CODEX, (CO-Detection by indEXing) that combines the high-parameter space of single-cell methodologies with the spatial analysis.

Material and methods The CODEX technology involves labelling antibodies with oligonucleotide-based tags followed by a single staining step. Over 50 parameters are simultaneously measured in a single specimen in Akoya’s novel, fully automated process. Unlike other cyclic IF approaches involving multiple antibody staining and stripping steps, the CODEX platform involves a single initial staining step and subsequent gentle manipulation of the tissue thereafter. This provides a faster workflow and prevents tissue degradation due to the harsh conditions used to remove adhered antibody. Other multiplexed imaging technologies, including imaging cytometry and MBI, require expensive equipment precluding their routine use across various labs. The CODEX technology developed by Akoya is comprised of a fluids instrument that interfaces with existing microscope hardware as well as a suite of associated specialised reagent offerings.

Results and discussions Here we present the results of an analysis of human tissue samples, including both fresh-frozen (FF) and formalin-fixed paraffin embedded (FFPE) specimens using the CODEX platform. Panels of antibodies were validated against a variety of markers and used to stain human tonsil, lymph node and tumour tissues. These data were processed