UNDER THE AUSPICES OF:

AFRCP
ASSOCIATION FRANÇAISE POUR LA RECHERCHE SUR LE CANCER DU PANCRÉAS

OCTOBER 16 > 18, 2023

PANCREATIC CANCER SYMPOSIUM

DOMAINE DU HAUT-CARRE, UNIVERSITY OF BORDEAUX

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EDITORIAL

Although pancreatic ductal adenocarcinoma (PDAC) represents less than 2% of new cancers, it is constantly increasing (14,000 new cases/year in France) and is one of the main causes of cancer-related mortality. Indeed, the vast majority of pancreatic cancers are locally advanced or metastatic at diagnosis. The incidence almost equal to the mortality rate proves the incurable nature of this cancer. Despite the progress made in understanding the pathogenesis of PDAC, and the improvement of treatment regimens, the survival rate after treatment remains poor. Thus, basic and clinical research is still the best allies of patients and medical teams to make significant breakthroughs and improve the prognosis of this dreaded disease. The "Pancreatic Cancer symposium 2023" will be the third edition of an international meeting that brings together researchers and clinicians at the forefront of knowledge of pancreatic cancer. It is organized by a non-profit group of French researchers (AFRCP). Considering recent state-of-the-art, this event will focus on current and future noteworthy themes in the field. In particular, the following interdisciplinary sessions will be proposed to the international research community: (I) new modalities for PDAC clinical management, (II) identification and targeting of new tumor vulnerabilities, (III) exploration of the primary and the secondary tumor microenvironment, (IV) new modalities and technologies for pancreatic cancer research, with a special round table on PDAC organoïds, (V) PDAC prevention and early disease. A final session (VI) will be centered on the involvement of the patients in research design.

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Introduction to Bordeaux

Bordeaux: A City of Excellence for Medicine

Bordeaux, located in the southwest of France, is a city with a rich history, elegant architecture, and a renowned wine heritage. As a dynamic medical center, Bordeaux strikes a perfect balance between tradition and innovation in healthcare.

The City of Wine: Bordeaux is globally celebrated for its wine, with the "City of Wine" that delves into the science of wine and health.
Elegant Architecture: The city center is an architectural treasure, ideal for relaxation after medical conferences.
History and Culture: Roman history, historic sites, and a regional museum await you.
Medical Innovation: Bordeaux is a hub for medical innovation in France.
Gastronomy and Leisure: Savor French cuisine and enjoy a vibrant nightlife.
Nature at Your Doorstep: The Gironde estuary and the beaches of the Atlantic coast are within reach.

Bordeaux offers a unique backdrop where medicine meets culture, history mingles with innovation, and well-being is complemented by an unforgettable culinary experience.

PANCREATIC CANCER SYMPOSIUM

DOMAINE DU HAUT-CARRE, UNIVERSITY OF BORDEAUX

SPEAKERS **SUMMARY**

Molecular mechanisms controlling cellular heterogeneity of pancreatic cancer

Axel Behrens

Pancreatic ductal adenocarcinoma (PDAC) shows pronounced epithelial and mesenchymal cancer cell populations. PDAC cellular heterogeneity is an important feature in disease subtype specification, but how distinct PDAC subpopulations interact, and the molecular mechanisms underlying PDAC cell fate decisions, are incompletely understood. Novel findings elucidating the molecular regulation of PDAC cellular heterogeneity will be discussed.

Please find above the required information.

Influence of the tumour microenvironment in pancreatic cancer liver metastasis

Prof Michael Schmid

Presentation summary
Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic disease for which better therapies are urgently needed. Fewer than 1 in 5 patients are diagnosed with local disease, eligible for curative surgical resection with adjuvant chemotherapy, with a poor 5-year survival rate of 38%. For the remaining majority of PDAC patients, diagnosed with distant metastatic disease, a lack of effective treatments inhibiting progression results in a devastating 5-year survival rate of 3%. A better understanding of the molecular pathology of metastatic PDAC is essential for the development of novel therapeutic strategies targeting this lethal disease.

Colonisation of a distant organ is a rate limiting step for metastatic progression, critically facilitated by non-cancerous cells that can be both recruited and resident to the distant metastatic organ. Macrophages, which exist as heterogeneous populations, are among the most abundant stromal cell populations found within the metastatic liver tumours. However, the cellular origin of hepatic metastasis associated macrophages (MAMs) and whether and how MAM heterogeneity and function are evolving during metastatic disease development remains poorly understood.

Combining clinical samples, pre-clinical mouse models, primary cell culture assays, and single cell RNA sequencing, we reveal molecular and cellular insights into MAM heterogeneity and identify potential targets for prevention of PDAC liver metastasis.

Identification and characterization of host factors that drive oncolytic virus infection in pancreatic cancer cells.

Guillaume Labrousse [1,2], *Nina Suarez* [1,2], *Adèle Nevot* [1,2] *and Pierre Cordelier* [1,2]

Oncolytic viruses, whether they arise naturally or are genetically engineered, possess the unique ability to selectively replicate within and eliminate cancer cells. Furthermore, they hold the potential to propagate within the cancerous cell population and trigger a potent anti-tumor immune response, capable of overcoming immune tolerance and rendering "cold" tumors receptive to immunotherapy. Oncolytic viruses often thrive in cancer cells due to inherent defects in their antiviral defense mechanisms. Nevertheless, the precise molecular underpinning the selectivity of oncolytic viruses for tumor cells remain incompletely understood. This lecture is dedicated to the exploration and characterization of host factors associated with the rat H-1PV parvovirus, a virus of therapeutic interest in cancer treatment. We will start with a Cas9 loss-of-function screening conducted in patient-derived primary cell cultures to identify host genes that are essential to viral infection. We will then investigate the roles of such host factors within the viral life cycle and how they influence virotherapy efficacy. By establishing a signature that predicts the effectiveness of oncolytic therapy, this research has the potential to significantly enhance the precision in selecting patients who are most likely to benefit the most from virotherapy.

High-resolution spatial proteotranscriptomics to dissect tumor-stroma interactions in pancreatic cancer

William L. Hwang, MD, PhD,
Assistant Professor, Harvard Medical School
Principal Investigator and Physician-Scientist, Center for Systems Biology/Center for Cancer Research/Department of Radiation Oncology, Massachusetts General Hospital
Associate Director, Radiation Biology & Research Program, MGH Cancer Center

Associate Member, Broad Institute of MIT and Harvard

Treatment failure is ubiquitous in pancreatic ductal adenocarcinoma (PDAC), driven by genetic and phenotypic heterogeneity combined with a highly desmoplastic, immunosuppressive, and densely innervated microenvironment. Identifying novel therapeutic targets in the tumor microenvironment (TME) is critical to improving patient outcomes and may be achieved through a deeper understanding of the cell-intrinsic states and cell-extrinsic interactions driving clinically-relevant properties. In this talk, I will discuss the integrated application of single-cell spatial proteomic and transcriptomic technologies to elucidate interactions between cancer cells and cancer-associated fibroblasts, immune cells, and nerves as well as how these interactions are remodeled by therapy. We developed a novel method for inferring multicellular interactions—Spatially Constrained Optimal Transport Interaction Analysis (SCOTIA), an optimal transport model with a cost function that includes both spatial distance and ligand-receptor (LR) gene expression. Overall, our study demonstrates that characterization of the TME using high-plex single-cell spatial proteo-transcriptomics allows for identification of molecular interactions that may drive emergent properties such as chemoresistance and metastasis.

Early detection of pancreatic cancer

Michael Goggins MD

Recent studies have shown that pancreatic surveillance of individuals with significant familial/genetic risk can improve the long-term survival of patients diagnosed with pancreatic cancer, particularly if patients are diagnosed with Stage I disease. Pancreatic surveillance relies on pancreatic imaging tests, especially MRI and endoscopic ultrasound. Advanced imaging analysis has the potential to improve the detection of otherwise undetected pancreatic cancers and to infer the presence of pancreatic precursor lesions. Accurate blood tests have the potential to improve the early detection of pancreatic and other cancers. One step towards this goal is improving the diagnostic performance of CA19-9. Knowledge of the gene variants that influence circulating levels of CA19-9 can be used to personalize CA19-9 as a test for early detection. We have begun evaluating a "CA19-9 tumor marker gene test" in the CAPS study. Even the best blood-based face challenges as cancer diagnostics, but may have value when used alongside imaging tests for patients undergoing pancreatic surveillance. There is potential to improve the early detection of pancreatic cancer by investigating the role of emerging multi-cancer detection blood tests, and the identification and refinement of additional risk factors that could be used to better select patients who merit surveillance and to optimize the surveillance intervals for patients with different levels of pancreatic cancer risk. Ongoing clinical trials are needed to evaluate the long-term benefits and harms of pancreatic surveillance

PANCREATIC CANCER SYMPOSIUM
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ABSTRACTS SELECTED ORAL COMMUNICATION

Identification and Targeting of New Tumor Vulnerabilities

New role of protein precursors maturation by the convertases in the malignant phenotype of pancreatic cancer and immune checkpoint inhibitors regulation

Abdel-Majid Khatib [1], Chloé Porcheron [1], Alexia François [1], Siegfried Geraldine [1], Evrard Serge [2], Desolneux Grégoire [2], Bruno Villoutreix [3], Simon Pernot [2]

Therapeutic strategies that improve anti-tumor immunity have changed the natural history of many cancers. However, in pancreatic cancer, immunotherapy and targeted therapies have not brought about the therapeutic revolution that has been observed in other malignancies. Among the reasons to explain this difference is the possibly crucial role played by the pancreatic tumor microenvironment, which has unique features and is different from that of other neoplasms.
Pancreatic cancer evades immunologic elimination by a variety of mechanisms, including induction of an immunosuppressive microenvironment. Using various in vitro and in vivo models, we recently identified the implication of the convertases in the malignant phenotype of pancreatic cancer cells. We found that the repression of the convertases reduced the malignant phenotype of pancreatic cancer cells and enhanced their sensitivity to treatments. We also found that the suppression of the activity of these enzymes in immune cells inhibits PD-1 expression on CD8+ T cells and PBMCs derived from patients. Using bioinformatic screening assay based on the catalytic domain of the convertase Furin and a library of up to 5 106 small molecules we isolated the top best 1000 molecules and assessed their effect on Furin activity using an in vitro enzymatic digestion assay, and selected the top 14 molecule inhibitors (some are FDA approved drugs), based on their enzymatic activity, toxicity profile ...etc, that we found to repress PD-1 expression in PBMCs and CD8+ T cells. Together, these data demonstrate that the convertase inhibitor drugs are a potential novel therapeutic strategy in the field of pancreatic cancer.

Keywords : Converatses, PD-1, Drug development

Identification and Targeting of New Tumor Vulnerabilities

Discovery of the first peptide ligand targeting the EGF domains of MUC4 that rescue accessibility and sensitivity of ErbB2-expressing pancreatic cancer cells to Herceptin targeted therapy

Nicolas Stoup [1], *, *Leila Moum* [2], *Tristan Girgin* [1], *Patricia Melnyk* [2], *Maxime Liberelle* [2], *Nicolas Lebegue* [2], *Isabelle Van Seuningen* [1]

Introduction and Objectives

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies in the world. PDAC survival rate has remained unchanged since the 1970's, whereas its incidence is constantly increasing. Moreover, this cancer exhibits no efficient treatments, since conventional therapies (FOLFIRINOX, gemcitabine) and/or targeted therapies, including immunotherapies, often remain inefficient or fail. This absence of efficient therapeutics makes this cancer a serious problem of public health, for which new therapeutic targets and/or new therapeutic options are urgently needed1. In this way, we have identified the MUC4 oncomucin as a potent therapeutic target since it is a glycoprotein, highly antigenic, overexpressed at the surface of PDAC cells in the early stages till metastatic disease and forming a complex with oncogenic ErbB2/HER2 receptor. Moreover, MUC4 has also been shown to impair efficiency of anti-ErbB2/HER2 Herceptin targeted therapy via steric hindrance mechanisms2. Our work led us to identify the EGF1 and EGF2 domains of MUC4 extracellular region as the region to target as these domains interact and activate ErbB2 oncoreceptor, participate to ErbB2 oncogenic signaling and PDAC tumor progression3,4. Herein, from these EGF domains depend the interaction and the oncogenicity of the MUC4-ErbB2 complex. These domains could therefore be targeted to modulate ErbB2 oncogenic pathway and decrease pancreatic cancer cell proliferation. Methods/Results: Using molecular dynamic simulation that led to a MUC4-ErbB2 3D model and peptide libraries (160 000 small molecules) virtual screening against our MUC4-ErbB2 model, we have identified a peptide ligand with in vitro anti- proliferative effects and targeting the EGF1 and EGF2 domains of MUC4. In this work, we show that the inhibitory peptide has no significant toxicity on pancreatic cancer cells. Using Microscale Thermophoresis (MST) and GST pull- down approaches, we show that the peptide ligand specifically binds the EGF domains of MUC4 to decrease the interaction with ErbB2 and its activation. By co-immunoprecipitation assays in MUC4-expressing pancreatic cancer cells treated with the inhibitory peptide, we show that (i) the MUC4-ErbB2 complex is disrupted, and (ii) we correlate that to a decrease of cell proliferation and a decrease of activation of ErbB2-dependent oncogenic pathways. Most interestingly, we show for the first time that this inhibitory peptide, after binding to MUC4 and thus freeing ErbB2, leads to increased accessibility of Herceptin to the ErbB2 receptor and thus increase sensitivity of PDAC cells to Herceptin targeted therapy. Discussion/Conclusion: Together, these results pave the way to the design of new therapeutic inhibitory small molecules targeting the EGF domains of MUC4, which could represent a new alternative strategy to overcome ErbB2 therapeutic targeting failure in pancreatic cancer (but also in other cancers overexpressing MUC4-ErbB2 complex such as lung, oesophagus, stomach, ovary, breast...) by proposing new combined therapies made of inhibitory small peptides targeting MUC4 EGF domains and Herceptin.

Keywords : Converatses, PD-1, Drug development

Identification and Targeting of New Tumor Vulnerabilities

Ornithine aminotransferase supports polyamine synsthesis in pancreatic cancer

Nada Kalaany [1*]

1. Endocrinology, Harvard medical school, boston children's hospital, Boston, UNITED STATES
** Auteur correspondant : nada.kalaany@childrens.harvard.edu*

There is a need to develop effective therapies for pancreatic ductal adenocarcinoma (PDA), a highly lethal malignancy with increasing incidence and poor prognosis. Although targeting tumour metabolism has been the focus of intense investigation for more than a decade, tumour metabolic plasticity and high risk of toxicity have limited this anticancer strategy. Here we use genetic and pharmacological approaches in human and mouse in vitro and in vivo models to show that PDA has a distinct dependence on de novo ornithine synthesis from glutamine. We find that this process, which is mediated through ornithine aminotransferase (OAT), supports polyamine synthesis and is required for tumour growth.

This directional OAT activity is usually largely restricted to infancy and contrasts with the reliance of most adult normal tissues and other cancer types on arginine-derived ornithine for polyamine synthesis. This dependency associates with arginine depletion in the PDA tumour microenvironment and is driven by mutant KRAS. Activated KRAS induces the expression of OAT and polyamine synthesis enzymes, leading to alterations in the transcriptome and open chromatinlandscape in PDA tumour cells. The distinct dependence of PDA, but not normal tissue, on OAT-mediated de novo ornithine synthesis provides an attractive therapeutic window for treating patients with pancreatic cancer with minimal toxicity.

Keywords : pancreatic cancer, metabolism, polyamines, de novo ornithine synthesis, glutamine, arginine

Identification and Targeting of New Tumor Vulnerabilities

Ornithine aminotransferase supports polyamine synsthesis in pancreatic cancer

Liaki Vasiliki[1]

1. Spanish national cancer research center (cnio), Madrid, SPAIN

Pancreatic cancer is one of the most lethal cancer types and it has been projected as the second highest in mortality by 2030. Currently, standard treatment only includes cytotoxic chemotherapy, with overall survival in the range of weeks to few months. Therefore, there is urgent need for targeted therapies. Our lab has recently developed a PDAC therapeutic mouse model that recapitulates the human pathology and enables the study of therapeutic strategies in fully developed tumors. In this concept, we have demonstrated that genetic ablation of Egfr and Raf1, two main mediators of the RAS signaling, results in complete regression of a significant fraction of Kras/Trp53 driven tumors of small sizes. Yet, tumors of bigger sizes remain refractory to this combined deletion (Blasco et al., 2019).

In the present study we have characterized by OMIC methods the molecular signaling of PDAC cells sensitive and resistant to this combined elimination. Of note, sensitive and resistant cells have distinct transcriptional profiles that define their response and resemble those of Classical and Basal subtypes of human PDAC. We have recently discovered that these differences derive from different methylation patterns, as shown by genome-wide methylation analysis. More specifically, among the differentially methylated genes various known STAT3 interactors were detected hypermethylated in the sensitive PDAC cells. Therefore, we have identified and validated the transcription factor STAT3 as the key gene that drives survival of the resistant tumor cells after elimination of Egfr and Raf1. Indeed, combined in vivo and in vitro genetic ablation of Egfr, Raf1 and Stat3 leads to complete and irreversible regression of tumors induced by Kras/Trp53 mutations. Importantly, we have also validated in vitro and in vivo the efficacy of this therapeutic strategy using combinations of pharmacologic compounds with minimal toxicities, such as EGFR inhibitors and a STAT3 PROTAC degrader.

Overall, we have developed a novel therapeutic strategy against mouse PDAC based on the simultaneous inhibition of EGFR, RAF1 and STAT3. We are currently validating our findings in our collection of human 3D cultures, the so-called patient-derived organoids (PDO), as well as in PDO-derived xenografts.

Keywords : PDAC, EGFR, RAF1, STAT3, therapy

Identification and Targeting of New Tumor Vulnerabilities

Mode of action of H-1PV oncolytic virus on pancreatic tumor microenvironment

Margaux Vienne [1*], *Adèle Nevot* [1], *Guillaume Labrousse* [1], *Charlène Lopez* [1], *Corinne Bousquet* [1], *Christine Jean* [1], *Pierre Cordelier* 1

Pancreatic ductal adenocarcinoma (PDAC) is a disease with no cure due to the lack of early detection and effective treatments. The PDAC tumor mass represents a persistent, scar-like proliferation of cellular tissue within a dense fibrous stromal microenvironment. This expansion hinders the effective penetration of therapeutic agents into the tumor, while also generating substantial immunosuppression and fostering resistance to both drugs and radiation. Oncolytic viruses are a relatively recent but extremely promising approach in the fight against cancer, as they induce selective lysis of tumor cells and may promote potent anti-tumor immune response. Among them, the rodent protoparvovirus H-1 (H-1PV) is oncolytic in several experimental models of cancer and is safe in early clinical trials for patients with PDAC or glioblastoma. Still, the role of H-1PV on the PDAC tumor microenvironment is poorly understood, as its potential as a novel immunotherapy for this disease.

For this study, we generated murine PDAC tumors derived from KPC cells implanted in immune competent mice. We characterized the immune landscape of these tumors, revealing a significant proportion of neutrophils and macrophages and to a lesser extent, of helper and cytotoxic T cells.

We then injected purified H-1PV intratumorally and demonstrated a transient, yet significant inhibition of PDAC tumor growth, with fewer viable cells in treated tumors. We next investigated the antitumoral mechanism of action of H-1PV in PDAC experimental tumors. We found that murine PDAC cells can be infected by H-1PV. They can support viral genome replication but they do not die from infection, ruling out a possible direct antitumor effect of the virus.

In addition, we found that the growth of PDAC subcutaneous tumors implanted in immune-deficient mice was not inhibited by H-1PV intratumoral delivery, strongly suggesting that a functional immune system is essential to virotherapy efficacy. We then analyzed the consequences of H-1PV infection on the immune landscape of murine PDAC tumors established in immune competent mice. While the total number of intratumoral immune cells was unchanged following infection, we found that H-1PV treatment resulted in a significant decrease in immune cells with immunosuppressive phenotype.

We then explored if the decrease in live cells in tumors following H-1PV therapy could be due to interactions with other tumor components, such as cancer-associated fibroblasts (CAF). We demonstrated that normal murine pancreatic stellate cells were not impacted by H-1PV infection. On the contrary, H-1PV induced the robust oncolysis of IL-1- or TGF-β-induced inflammatory and myofibroblastic CAFs, respectively.

Altogether, our results highlight a new and unexpected role of H-1PV on the experimental PDAC tumor microenvironment, suggesting a possible mechanism to overcome tumor resistance to treatment and to improve the efficacy of virotherapy.

Keywords : H-1PV, oncolytic virus, antitumor immunity, PDAC microenvironment

Identification and Targeting of New Tumor Vulnerabilities

TNFR2 blockade promotes anti-tumoral response in PDAC by decreasing Treg and T cell exhaustion

Ilaria Cascone [1]

1. University of paris est, Imrb, inserm u955, Creteil, FRANCE

Background

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers, highly resistant to standard chemotherapy treatment and immunotherapy. Regulatory T cells (Tregs) highly express TNFα receptor 2 and display strong T cell suppressive capacities in different types of human tumors and mice models of cancer. In PDAC stroma, Treg infiltration correlates with poor survival and tumor progression in PDAC patients. We hypothesized that TNFR2 inhibition using a blocking mAb could reverse the balance of effector and regulatory T cells in PDAC tumors thus triggering a more efficient anti-tumoral response.

Results

In human tumors, the main cell population expressing Tnfrs1b are regulatory T cells, macrophages and endothelial cells. Importantly, Tnfrs1b was highly upregulated in tumor infiltrated CD4+ T cells and Tregs compared to healthy tissue. Mice carrying orthotopic PDAC were treated by a blocking anti-TNFR2 mAb. By both flow cytometry and single cell RNAseq analysis, we showed that blocking the TNFα/TNFR2 pathways induced a significant decrease of activated Treg infiltrated into the tumor, and of the expression of T cell exhaustion markers in CD8+ T cells. However, anti-TNFR2 treatment was not sufficient to activate CD8+ T cells and only slightly reduced the tumor growth compared to control mice. We improved CD8+ T cell activation by adding an agonist anti-CD40 mAb to the anti-TNFR2 treatment. This combined treatment promotes stronger tumor growth inhibition and survival of PDAC carrying mice. Survived mice acquired immunological memory and rejected PDAC cells.

Conclusion

These results suggest that decreasing Treg activation and T cell exhaustion by the anti-TNFR2 remodel the TME and allow PDAC carrying mice to become better responder to the anti-CD40.

Keywords : TNFR2, immune tumor microenvironment, Treg

Identification and Targeting of New Tumor Vulnerabilities

Mechanics & Genetics of pancreatic cancer Mechanical compressive stress favors selective mutational contexts and intracellular signaling during pancreatic cancer development

Mickael Di-Luoffo [1*], Silvia Arcucci [1], Tristan Marty [2], Nicole Therville [1], Sanzhar Aitbay [1], Pauline Lefebvre [3], Benoit Thibault [1], Pascal Swider [4], Pauline Assemat [4], Morgan Delarue [5], Julie Guillermet-Guibert [1]

1. Crct, Inserm, Toulouse, FRANCE
2. Crct/imft, Inserm/cnrs, Toulouse, FRANCE
3. De lorraine, Université, Nancy, FRANCE
4. Imft, Cnrs, Toulouse, FRANCE
5. Laas, Cnrs, Toulouse, FRANCE
* Auteur correspondant : mickael.di-luoffo@inserm.fr

Context

Mechanical compressive stress arises during pancreatic cancer progression (PDAC)1. In vitro, compression forces decrease PDAC cell proliferation, increase invasiveness, and induce resistance to chemotherapies. In vivo, the importance of compression is unknown. In PDAC, increased compressive stress happens simultaneously with the second wave of genetic alterations (p53 mutations/truncations) after KRAS oncogenic mutations; it is also linked with overexpression/activation of the PI3-Kinases (PI3K) pathway2. We think that compression favors selective genetic backgrounds that modify the signaling environment in cells and thus cell fate.

Experimental design

We generated compressive stresses to spheroids derived from PDAC cells with KRASG12D mutation, in which p53R172H mutation or p53R172H;R210* truncation are induced sequentially. Further, we applied a compressive stress to KRASG12D±p53R172H/p53R172H;R210* mutated mouse allografts using a compressive device. We also used the punch method in order to evaluate the relaxation of tumors depending on their genetic background.

Results

Compression decreased the spheroid growth (<30%)3. However, p53R172H mutated PDAC cells developed a resistance to compression and continued to proliferate. This mutation associated with a truncation of p53 accentuated this resistance, even bringing a proliferative advantage. A transcriptomic analysis of KRASG12D, p53 mutated and p53 truncated spheroids under compression was performed. This analysis showed a modification in adhesion properties via plasma membrane and RTK signaling activity, mechanisms regulated by PI3K pathway. In parallel, we observed, in vivo, that the growth of KRASG12D mutated tumors decreased by 40% under compression, whereas the size of tumors with p53R172H;R210* truncated form was similar with or without compression. Finally, KRASG12D tumors relaxed more easily compared to the p53R172H and p53R172H;R210* tumors; this was due to a greater cellular and matrix homogeneity in these tumors compared to p53R172H and p53R172H;R210* tumors.

Conclusion

Growth under pressure can influence the progression of PDAC promoting selective genetic background and activation of oncogenic signaling pathways. These observations open the way to integrate the mechanical context in the management of patients with PDAC.

Keywords : Pancreatic cancer, Mechanical stress, Mechanotransduction, Oncogenes, PI3K

Identification and Targeting of New Tumor Vulnerabilities

Single-cell immune multi-omics and repertoire analyses in pancreatic ductal adenocarcinoma reveal differential immunosuppressive mechanisms within different tumour microenvironments

Ashwin Kumar Jainarayanan [1*], Shivan Sivakumar [1], Edward Arbe-Barne [2], Michael Dustin [1], Enas Abu-Shah [1], Rachael Bashford-Rogers [3]

1. Ndorms, Kennedy institute of rheumatology, Oxford, UNITED KINGDOM
2. Department of paediatrics, John radcliffe hospital, Oxford, UNITED KINGDOM
3. Department of biochemistry, Department of biochemistry, Oxford, UNITED KINGDOM
* Auteur correspondant : ashwin.jainarayanan@gmail.com

Pancreatic ductal adenocarcinoma (PDAC) has an extremely poor prognosis. Understanding the multiple mechanisms by which the tumour evades immune control, and how these mechanisms may be disrupted is critical to developing better targeted immunotherapies. Previous studies have shown that higher lymphocyte infiltration is associated with better survival, and here we investigated what mediates these differences. We performed a comprehensive analysis of PDAC-associated immune cells using single cell multi-omics coupled with re-analysis of public PDAC scRNA-seq datasets. We introduce novel single-cell and repertoire analyses that have uncoupled diverse roles and contributions of various immune cell populations within different tumour microenvironments (TMEs). They revealed clear distinctions in the clonal characteristics among different patient groups, provided valuable insights into the mechanisms of immune cell migration and tissue adaptation underlying these disparities. Overall, we identified two major distinct themes for future immune intervention within PDAC patients highlighting patients with higher myeloid infiltration, and those with higher adaptive immune cell infiltration.

Conflict of interest

Rachael Bashford-Rogers is a co-founder of Alchemab Therapeutics Ltd and consultant for Alchemab Therapeutics Ltd, Roche, Enara Bio, UCB and GSK. Shivan Sivakumar held a personal fellowship from BMS during this study with a grant provided to conduct experiments. BMS did not have any intellectual input into the study design or analysis. Enas Abu- Shah reports no conflict of interests. Michael Dustin is on the SAB for Adaptimmune and Singula Bio, consults for Molecular Partners, Enara Bio, Labgenius and Astra Zeneca, and undertakes research supported by BMS, Cue Biopharma, Boehringer Ingelheim, Regeneron and Evolveimmune outside the submitted work.

Keywords : Single-cell immune multi-omics, Immunosuppressive mechanisms, Tumour microenvironment, Repertoire analyses

Identification and Targeting of New Tumor Vulnerabilities

Targeting Stress Granule Formation as a Synthetic Lethality Strategy for Kras-Dependent Pancreatic Cancer Initiation

Santofimia Patricia [1*], *Fraunhoffer Nicolas* [1], *Liu Xi* [1], *Fernandez-Bessone Ivan* [1], *Pasca Di Magliano Marina* [2], *Audebert Stephane* [1], *Camoin Luc* [1], *Modesti Mauro* [1], *Lomberk Gwen* [3], *Urrutia Raul* [3], *Soubeyran Philippe* [1], *Neira Jose Luis* [4], *Iovanna Juan* [1]

1. Pancreatic cancer, Cancer research center of marseille, Marseille, FRANCE
2. Department of surgery research, University of michigan, Michigan, UNITED STATES
3. Medical college of wisconsin, Medical college of wisconsin, Milwaukee, UNITED STATES
4. Instituto de biología molecular y celular, Universidad miguel hernández, Elche, SPAIN
** Auteur correspondant : patricia.santofimia@inserm.fr*

Purpose

Stress granules (SGs) are membrane-less organelles formed by liquid-liquid phase separation (LLPS) that play a critical role in regulating RNA metabolism, and protein synthesis, in a wide range of stress responses. These granules are required to support pancreatic cancer (PDAC) transformation, cell survival, growth, and chemotherapy resistance, making them an attractive target for new cancer therapies. Here we report a novel mechanism for SG formation, which is induced by NUPR1 (an intrinsically disordered stress-associated protein), in response to KrasG12D oncogenic stress.

We provide a preclinical proof of concept for using SG inhibition as an efficient strategy for targeting PDAC initiation and development, by using NUPR1 inhibitors.

Methods

We have used recombinant proteins for in vitro tests (LLPS tests), human and mouse PDAC-derived cell lines, and doxycycline-inducible Kras transgenic cell lines for in cellulo assays (cell viability tests, evaluation of gene expression, and SGs quantification), a transgenic Pdx1-Cre;LSL-KrasG12D mouse model for the in vivo experiments (evaluation of precancerous lesions).

Results

In this study, we show that NUPR1, has the capacity to produce droplets through LLPS, but not its mutants or under the treatment of its inhibitor (ZZW-115). We observed that LLPS induced by rNUPR1 is essential for SGs formation since genetic or pharmacological inhibition of NUPR1 activity hampers SGs formation in pancreatic cancer cells. In addition, we found that KrasG12D mutation causes significant oncogenic stress which in turn induces a strong overexpression of NUPR1, promoting the formation of SGs as a stress-dependent mechanism of cell survival.

Consistently, forced KrasG12D expression in pancreatic cells elicits a strong sensitivity to NUPR1 inactivation by genetic or pharmacological means. Finally, inhibition of SGs formation with the NUPR1 inhibitor ZZW-115 in Pdx1-Cre;LSL- KrasG12D mice blocks the transformation process indicating that SGs formation is necessary for PDAC development.

Significance

In this work, we provide a preclinical proof of concept showing that SG formation is a targetable step in the KrasG12D signaling pathway, thus suggesting that inhibiting NUPR1 or SG formation can be utilized as a synthetic lethality therapeutic strategy for KrasG12D-dependent tumors.

Keywords : Stress Granules, Kras, NUPR1

Exploration of the desmoplastic reaction and the tumor microenvironment

Elevated levels of Apobec3B results in acceleration of pancreatic ductal adenocarcinoma but no increase in adaptive immunity

Karen Pickering [1*]

1. Cruk beatson institute, Glasgow, UNITED KINGDOM

Pancreatic cancer has one of the worse prognoses of all cancers, with a 10-year survival rate of 1%. The survival rate has improved only slightly over the past 50 years and lags far behind that of other cancers.

Despite showing promise in other tumour types, immunotherapy trials in pancreatic cancer have been disappointing. This is partly due to pancreatic tumours exhibiting a paucity of infiltrating T cells. Recent data from other tumour types have suggested that mutation burden may correlate with immunogenicity and predict the efficacy of immunotherapy. The APOBEC family of cytidine deaminase enzymes, whose normal function is to protect from viral infections, have been revealed as drivers of mutation in a variety of human tumours, including pancreatic cancer. In order to model this, we have engineered mice to express cre-inducible APOBEC3B and crossed these with the KPC genetically engineered mouse model of pancreatic cancer. We have found that overexpressing APOBEC3B results in faster initiation of pancreatic cancer, decreased survival, and changed tumour microenvironment characterized by an increase in cancer- associated fibroblasts and tumour-associated macrophages. Interestingly, genomic analyses failed to detect any increase in overall mutation burden in APOBEC3B expressing tumours. However, transcriptomic and phenotypic data suggest that overexpression of APOBEC3B results in an increased proliferative capacity, which could be responsible for driving accelerated tumorigenesis. Analysis of these tumours at an early stage suggests that whilst we do not observe increased T-cell infiltration we see an early expansion of the macrophages which may stimulate an immunosuppressive environment. We are currently evaluating how APOBEC3B can drive increased proliferation and how this class of tumour might be rendered susceptible to therapy.

Keywords : APOBEC, TME, Stromal reaction

Exploration of the desmoplastic reaction and the tumor microenvironment

Inflammation-induced epithelial plasticity can be by-passed through Vps34 inactivation to limit pancreatic cancer initiation

Hala Shalhoub [1*], *Julie Guillermet-Guibert* [1], *Benoit Thibault* [1], *Fernanda Ramos-Delgado* [1], *Camille Guyon* [1], *Nicole Therville* [1], *Carine Joffre* [1], *Marlène Dufresne* [1], *Elisa Boutet-Robinet* [2]

1. Toulouse cancer research center, Toulouse, FRANCE
2. Université ttoulouse cancer research centeroulouse iii - paul sabatier, Toulouse, FRANCE

Context

Pancreatic ductal adenocarcinoma (PDAC) will become the second cause of cancer related mortalities by 2030. PI3K/Akt/mTORC1 pathway is among the major oncogenic signaling pathways that are activated in PDAC. Whether class III PI3K also tightly control mTORC1 in physiological settings remains controversial; besides, the role of class III PI3-kinase remained underexplored both in PDAC and in pancreatitis, a potential risk factor for PDAC development. Class III PI3K comprises only one isoform named Vps34. Through its kinase activity, Vps34 plays a role in regulating autophagy. Autophagy is an evolutionarily conserved process, which plays an important function in both pancreatic physiology and pathophysiology.

Methods

In our study, we developed Vps34+/+, Vps34KI/KI(=V34), KC and KCVps34KI/KI (=KCV34) mice modelsto understand the role of class III of PI3-kinase in pancreatic diseases. Single-cell RNA sequence analysis, western blot analysis, immunofluorescence analysis and immunohistochemistry analysis were performed for collected tissues or acini cultured ex-vivo. Data were confirmed on Human samples.

Results

Vps34 inactivation in exocrine pancreas resulted in fibrogenesis and lipid accumulation. In vivo and ex vivo, acinar cells had heterogeneous levels of autophagy ; Vps34 inactivation showed blocked flux of autophagy and differential expression levels of autophagy regulating proteins compared to WT acini. Autolysosome surface decreased in acinar cells with Vps34 inactivation. Surprisingly, despite a blockage of autophagy, those cells appeared resistant to mutant Kras oncogenicity as full inactivation of Vps34 in KC mice led to absence of precancer lesions in aged mice. ScRNA-seq showed that Vps34 inactivation prompted a selective loss of a subset of acinar cells with high mitochondrial and autophagy-related genes.

Moreover, acinar cells with Vps34 inactivation showed enrichment in the expression of regenerating islet-derived 3 beta (Reg3b) gene. Acinar cells with Vps34 inactivation showed difference in Regs protein levels both at the basal levels and in response to autophagy modulators compared to WT acini. Finally, acinar cells with Vps34 inactivation showed also decreased levels of p-Akt levels, known to be necessary to pancreatic plasticity.

Conclusion

Vps34 full inactivation may protect from initiation of precancer lesions in the context of inflammation by bypassing epithelial plasticity. These finding may be a key to understand pancreatic cancer initiation.

Keywords : PDAC initiation, autophagy, inflammation, PI3K class III (Vps34), KRAS, Reg proteins family.

Exploration of the desmoplastic reaction and the tumor microenvironment

Do mechanical forces induce a protumoral dialogue between the tumor and the adjacent healthy tissue?

Claire Lac [1], Julie Foncy [2], Corinne Bousquet [1], Laurent Malaquin [2], Morgan Delarue [2], Christine Jean [1]

1. 31, Crct, Toulouse, FRANCE
2. 31, Laas, Toulouse, FRANCE

Pancreatic ductal adenocarcinoma (PDAC) is characterized by an abundant stroma composed predominantly of activated fibroblasts (CAFs) and dense extracellular matrix (ECM). Tumor cell growth and ECM modifications generate forces named "solid stress" documented to promote tumor progression. While it is recognized that these forces also result in compression of surrounding healthy tissue, their impact on the biology of adjacent normal cells, especially normal fibroblasts (Pancreatic Stellate Cells, PSCs) remain poorly described.

In order to mimic the compressive force transmitted by the tumor to the surrounding healthy tissue, we added an agarose cushion on a monolayer of PSCs isolated from human healthy pancreas. We show that a low pressure (85 Pa) induces a strong expression of at least three of the main markers of activated fibroblasts. This phenomenon is associated with a reorganization of smooth muscle actin into stress fibers associated with drastic changes in cell morphology, mitochondrial network rearrangement, and the activation of the mechanosensor FAK. We identified that Akt and ERK are two main pathways involved. The consequence of this mechano-induced fibroblast activation is an important increase of their ECM production and secretion. Finally, we show that the ECM secreted by the "mechanically activated fibroblasts" induce epithelial-mesenchymal transition of tumor cells.

We are currently developing an original 3D device, allowing the application of a quantifiable and homogeneous pressure on cells embedded in a gelatin methacrylate (GelMA) hydrogel, to verify our results in a context more relevant to the pathology. This device, compatible with a wide range of techniques (IF, WB, RNAseq, live imaging...) will enable us to deeply characterize the impact of tumor-generated pressure on normal adjacent pancreatic fibroblasts cultivated alone or in co-culture with tumor cells.

In conclusion, we obtained the proof of concept in 2D, and will verify it in 3D, that solid stress activates PSCs in a durable manner and confer them protumoral properties that most likely will support and favor tumoral progression.

Keywords : PDAC - Solid stress - Fibroblast activation - 3D model

Exploration of the desmoplastic reaction and the tumor microenvironment

Immuno-modulation of the tumor microenvironment of pancreatic adenocarcinoma following isotoxic high-dose stereotactic body radiotherapy (iHD-SBRT)

Christelle Bouchart [1*], *Oier Azurmendi Senar* [2], *Julie Navez* [3], *Laurine Verset* [4], *Anaïs Boisson* [5], *Matthieu Hein* [6], *Nicky D'haene* [7], *Dirk Van Gestel* [1], *Luigi Moretti* [1], *Pieter Demetter* [8], *Jean-Baptiste Bachet* [9], *Karen Willard-Gallo* [5], *Rémy Nicolle* [10], *Tatjana Arsenijevic* [2], *Jean-Luc Van Laethem* [11]

1. Radiation-oncology, Institut jules bordet hub, Brussels, BELGIUM
2. Laboratory of experimental gastroenterology, Ulb, Brussels, BELGIUM
3. Digestive surgery, Hopital erasme hub, Brussels, BELGIUM
4. Pathology, Institut jules bordet hub, Brussels, BELGIUM
5. Laboratory of molecular immunology, Institut jules bordet hub, Brussels, BELGIUM
6. Sleep laboratory, Hopital erasme hub, Brussels, BELGIUM
7. Pathology, Hopital erasme hub, Brussels, BELGIUM
8. Pathology, Ulb, Brussels, BELGIUM
9. Gastroenterology, Hopital la pitié salpétrière, Paris, FRANCE
10. Inserm, Crcn, Paris, FRANCE
11. Digestive oncology, Hopital erasme hub, Brussels, BELGIUM

Introduction/Objectives

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest tumors. Unlike other tumors, the progress of systemic therapies has been very slow, mainly due to the peculiar and resistant tumor microenvironment (TME) of PDAC. The addition of ablative stereotactic body radiation therapy (SBRT) in a total neoadjuvant strategy is promising for the treatment of localized PDAC and is currently being explored in several clinical trials. However, if radiation therapy possesses the ability to modulate the TME, the impact of high-dose SBRT is still poorly known in PDAC. Here, we aim to characterize by immunohistochemistry (IHC) and RNA sequencing (RNAseq) analysis the immuno-modulations of the TME following isotoxic high-dose stereotactic body radiotherapy (iHD-SBRT).

Material/Methods

Paraffin-embedded residual tumoral tissues of 50 localized PDAC resected between 2011 and 2020 were used: seventeen patients had surgery first, seventeen received an induction chemotherapy with FOLFIRINOX (FFX) only and sixteen with FFX followed by an iHD-SBRT designed to individually maximize the dose prescribed to the tumor and vessels interfaces up to Dmax(0.5cc)<53Gy in 5 fractions. After verification by an experienced pathologist, a quantitative analysis of the different IHC labelings was carried out using the Visiopharm™ software on the tumor area.

RNA from the tumoral area was extracted using ALLPrep FFPE tissue kit (Qiagen®) and NGS libraries prepared using the QuantSeq Library Prep Kit for Illumina (Lexogen®). Differential gene expression (DGE) analyses were performed using Limma and edgeR packages from Bioconductor. RESULTS: DGE analyses of RNAseq data revealed that induction treatments modulate the molecular landscape of PDAC (Figure 1). Gene set enrichment analysis (GSEA) demonstrate that, while FFX is associated with changes in TME (significant enrichment in myCAF/ iCAF cells) (Fig 2A), iHD-SBRT is associated with a significant enrichment in basaloid cells (a subtype of basal cells associated with immunomodulatory stroma and better clinical outcomes), and negatively associated with activated and inflammatory stroma (Fig. 2B). Additionally, cell type enrichment analysis using xCell algorithm reveal that M1/M2 macrophages and CD8+ naïve T cells are increased while CD4+Th2 and γδ T cells are decreased after iHD-SBRT (Fig. 2C). The gene ontology (GO) analysis shows 10 terms significantly enriched after iHD-SBRT involving the mitochondrial system suggesting the potential of mitochondrial inhibitors as candidates for combination therapy (Fig. 2D). Furthermore, our IHC data demonstrate that although collagen deposition increases significantly after iHD-SBRT (COL1: 83.27 vs 78.60 vs 68.04%, $p<0.001$ for iHD-SBRT, FFX and non-treated cohort respectively), the intra-tumoral lymphocyte infiltration is globally not altered, including for cytotoxic CD8+ lymphocytes, with the exception of CD4+ T helper population that was significantly decreased. After iHD-SBRT, the IHC expression levels of FOXP3+ cells and PD-L1 are significantly increased while PD-1 expression is significantly decreased and no difference is observed for CD68+ expression.

Conclusion/Significance

iHD-SBRT is able to durably immuno-modulate the TME of PDAC. If the overall T-cell infiltration is preserved, we have also identified some increased pro-tumoral cells and pathways after iHD-SBRT that could improve the development of better-oriented combination trials involving high-dose SBRT.

Keywords : Pancreatic cancer, Stereotactic radiotherapy, Tumor Microenvironment

Exploration of the desmoplastic reaction and the tumor microenvironment

A Comprehensive Study of a Pancreatic Cancer Subtype: The Interplay between Translation and Transcription in the Tumor Microenvironment

Jacobo Solorzano [1*], *Mehdi Liauzun* [1], *Sauyeun Shin* [1], *Alexia Brunel* [1], *Christine Jean* [1], *Ola Larsson* [2], *Remy Nicolle* [3], *Corinne Bousquet* [1], *Vera Pancaldi* [1], *Yvan Martineau* [1]

1. Centre de recherche en cancérologie de toulouse (crct), inserm u1037, Toulouse, FRANCE
2. Department of oncology?pathology, karolinska institutet, Stockholm, SWEDEN
3. Centre de recherche sur l'inflammation (cri), université de paris cité, inserm, u1149, Paris, FRANCE
jacobo.solorzano@inserm.fr

Over a decade after the initial transcriptomic classification of Pancreatic Ductal Adenocarcinoma (PDAC), tumors of Basal and Classical subtypes still don't have a differential treatment. A plethora of alterations in mRNA translation machinery affecting the proteome without change in the transcriptome have been described in PDAC (Shin et al. 2022). Yet, tumor profiling based protein synthesis rate, has never been explored for PDAC classification.

Using a collection of 27 Patient Derived Xenografts (PDX), a hybrid model, where human tumor and mice stroma RNA can be computationally separated, our lab has analyzed polysome-associated RNA. Application of Independent Component Analysis (ICA) to tumor translated RNA revealed a subset of tumors displaying a specific Integrated Stress Response (ISR) translational program, and resistance to chemotherapy (Shin et al. 2020). My PhD aims at computationally analyzing (1) the stromal compartment of this new ISR activated (ISRact) subtype and (2) to project ISRact signature in other datasets, including human cohorts.

(1) Through ICA and a comparative analysis of the stroma transcriptomics with cell type deconvolution and transcription factor activity analysis, I uncovered a potential role of immunity in ISRact stroma, confirmed by protein-protein interaction network analysis. In particular, we found that an independent component related to ISRact is highly associated with the Interferon response and antigen presentation (MHC-II). In the same way, the stroma translatomics ICA in comparison with a list of curated RNA binding proteins reveals a possible relation of ISRact with m6A methylation events and mRNA processing in the stroma.

On the other hand, (2) using a Matrix factorization approach directed to the tumor transcriptomics, I developed a classifier (ISRactPCA) able to detect the tumor ISRact subtype in the transcriptome of other PDX and importantly, in publicly available datasets of PDAC patients. Notably, ISRactPCA shows correlation with a signature described as related to chemoresistance (GSEA glucuronidation, Fraunhoffer et al 2022). Extended projection of ISRactPCA to a multi omics dataset of PDAC cohorts will be discussed (Cao et al, 2021).

In vitro results also have proved that some Cancer Associated Fibroblasts (CAFs), the most abundant stromal cells in PDAC, can support the growth of ISRact cancer cells. Thus, we are collecting and curating CAF subtype markers, aiming to identify some of these populations on the PDX stroma as well as in the human datasets of (2) using deconvolution and enrichment approaches.

Overall, this project allowed to deepen into a promising PDAC signature, and explore the associated microenvironment, which will potentially favor the transition towards the clinics by generation of in silico models as well as in vitro and in vivo validation.

Keywords : mRNA-translation, micorenvironment, PDAC, bioinformatics, classification

Exploration of the desmoplastic reaction and the tumor microenvironment

PD-1 blockade induces reactivation of non-productive T cell responses characterized by NF-kB signaling in patients with pancreatic cancer

Stephanie Dougan [1*]

1. Cancer immunology and virology, Dana-farber cancer institute, Boston, UNITED STATES

Pancreatic ductal adenocarcinoma (PDAC) trials have evaluated CTLA-4 and/or PD-(L)1 blockade in patients with advanced disease where bulky tumor burden and limited time to develop anti-tumor T cells may have contributed to poor clinical efficacy. Here we evaluated peripheral blood and tumor T cells from patients with PDAC receiving neoadjuvant chemoradiation plus anti-PD-1 (pembrolizumab) versus chemoradiation alone. We analyzed whether PD-1 blockade successfully reactivated T cells in the blood and/or tumor to determine whether lack of clinical benefit could be explained by lack of reactivated T cells versus other factors. We used single cell transcriptional profiling and TCR clonotype tracking to identify TCR clonotypes from blood that match clonotypes in the tumor. PD-1 blockade increases the flux of TCR clonotypes entering cell cycle and induces an IFNγ signature like that seen in patients with other GI malignancies who respond to PD-1 blockade. However, these reactivated T cells have a robust signature of NF-κB signaling not seen in cases of PD-1 antibody response. Among paired samples between blood and tumor, several of the newly cycling clonotypes matched activated T cell clonotypes observed in the tumor. Cytotoxic T cells in the blood of PDAC patients remain sensitive to reinvigoration by PD-1 blockade and some have tumor-recognizing potential. Although these T cells proliferate and have a signature of IFN exposure, they also upregulate NF-κB signaling, which potentially counteracts the beneficial effects of anti-PD-1 reinvigoration and marks these T cells as non-productive contributors to anti-tumor immunity.

Keywords : neoadjuvant PD-1 blockade, clinical trial

Exploration of the desmoplastic reaction and the tumor microenvironment

CD169+ macrophages orchestrate immune reaction and matrix deposition in pancreatic cancer

Kevin Thierry [1*], Zhichong Wu [1], Sophie Bachy [1], Pia Gamradt [1], Hector Hernandez-Vargas [1], Ana Hennino [1]

1. 69008, Cancer research center of lyon, umr inserm 1052, cnrs 5286, lyon, f-69373, france, Lyon, FRANCE

PDAC is both a stromal and immune disease. Macrophages and T lymphocytes are the most abundant immune cells in the tumor microenvironement alongside mesenchymal-derived cells for non-immune cells. We identified that CD169+ macrophages were very abundant in pancreatic cancer in human and mice settings. We previously described the identification of a pericyte stem cells (PeSC) population with immunosuppressive properties (Wu et al., 2023) in PDAC microenvironment. We show here that PeSC were able to polarize CD11b+F4/80+ macrophages towards an immunosuppressive phenotype expressing CD169. CD169+ macrophages were able to produce large amounts of stromal βig-h3/TGFBI that directly stimulated CXCL12 production by PeSC that attracted CD8+ T cells. Invalidation of CD169 by a DTR-approach in orthotopic pancreatic cancer model led to diminished stromal reaction area and impaired CD8 T cell recruitment at the tumor site. The structure and the amount of the collagen was drastically reduced in absence of the crosstalk between PeSC and CD169+ macrophages leading to a shrinkage of the stromal area within the tumoral bed. The data reported here pinpoint for the first time that CD169+ macrophages play an important role in sustaining the stromal reaction in pancreatic cancer.

Keywords : Pancreatic cancer, Macrophages, Stroma

New Modalities and Technologies for Pancreatic Cancer Research

Sensitizing the PDAC tumor microenvironment to immune checkpoint therapies: characterization of a PDAC 3D model to decipher immune infiltration

Thomas Bessede [1*], *Freixinos Clara* [1], *Garombois Véronique* [2], *Viea Nadia* [3], *Michaud Henri-Alexandre* [1], *Faget Julien* [4], *Gongora Céline* [5], *Bonnefoy Nathalie* [1], *Gros Laurent* [1], *Larbouret** *Christel* [3]

1. Ircm, Inserm, Montpellier, FRANCE
2. Ircm, University of montpellier, Montpellier, FRANCE
3. Ircm, Icm, Montpellier, FRANCE
4. Ircm, Inserrm, Montpellier, FRANCE
5. Ircm, Cnrs, Montpellier, FRANCE

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal solid tumors, with an extremely unfavorable prognosis. The dense stroma rich in Cancer-Associated Fibroblasts (CAFs) and the immunosuppressive microenvironment confer resistance to current treatments including combination chemotherapies, targeted therapies or immunotherapies. Moreover, PDAC tumors are poorly infiltrated by T cells, and the majority of immune cells present at the tumor site are immunosuppressive. This cellular context leads to the failure of clinical trials using immune checkpoint inhibitors in PDAC [1]. The immune infiltrate within the tumor is dynamic and depends in particular on soluble factors secreted in the tumor microenvironment (cytokines, chemokines etc.).

The objective of this project is to develop a combinatorial approach using a monoclonal antibody that targets the tumor microenvironment (anti-AXL) combined with interleukin-15 (IL15) associated with conventional chemotherapies (Gemcitabine or Folfirinox) in order to increase immune infiltration. In clinical applications IL15 activates T lymphocytes (LT) and Natural Killer (NK) cells and promotes their infiltration into tumors [2]. However, IL15 treatment causes significant side effects with high toxicity reported in patients. Thus, we have generated an anti-AXL antibody fused to IL15 to associate the immunomodulatory properties of IL15, combined with the target specificity of the anti-AXL antibody. We thus developed a PDAC three-dimensional in vitro spheroid model composed of xenograft-derived tumor cells from PDAC patients, CAFs (primary and immortalized), and peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, with the aim of closely reproduce the complex pathophysiological features of the cancer-stroma found in pancreatic TME. Human 3D models were first set up and characterized by cytometry, imaging mass spectrometry and immunohistochemistry.

We showed that in our heterospheroid models, CAFs promote tumor cell growth, improve resistance to chemotherapy and are able to down-regulate immune cell infiltration and modulate the nature of infiltrated immune cells. These CAF- dependent resistance mechanisms, immune suppression and cancer progression also described in patients, are one of the trademarks of pancreatic cancer. In parallel we showed that, upon anti-AXL-IL15 treatment, PBMCs infiltrate cell line-derived heterospheroids, whatever the tumor cell line, kill tumor cells and disrupt the three-dimensional structure. Moreover, immunophenotyping experiments showed a modification of the nature of immune infiltration, with a strong increase of CD4+ and CD8+ T lymphocytes and NK cell populations infiltration. We also obtained combinatorial effects that positively modulated immune infiltration and allowed a control of spheroid growth by combining chemotherapy (gemcitabine) with our anti-AXL-IL15. Thus, the heterotypic spheroids described in our study are a suitable model to both characterize the influence of CAF on therapeutic effects and the mechanisms that drives immune suppressive microenvironment. Next step will be to perform in vivo tests using an orthotopic model to verify whether this approach enhances effector immune cells infiltration in vivo, thereby sensitizing the PDAC microenvironment to anti-PD1 therapies.

Keywords : Pancreatic duct adenocarcinoma (PDAC), immune infiltration, 3D heterotypic models, Tumor microenvironment (TME), IL15, therapeutic combination, cancer associated fibroblast (CAFs), antibody.

New Modalities and Technologies for Pancreatic Cancer Research

Development of an amphicrine pancreas-on-a-chip to study tumor initiation in a diabetic microenvironment

Flora Clement [1*], *Monika Hospodiuk-Karwowski* [1], *Vincent Haguet* [1], *Amandine Pitaval* [1], *Anastasia Papoz* [1], *Emily Tubbs* [1], *Stéphanie Combe* [1], *Xavier Gidrol* [1]

1. Irig-bge-biomics, Cea, Grenoble, FRANCE
Univ. Grenoble Alpes, CEA, Inserm, IRIG-BGE, Biomics, F-38000, Grenoble, France
** Auteur correspondant : flora.clement@cea.fr*

Purpose

In 2021, 537 million of the global adult population worldwide were living with diabetes [1]. Type 2 diabetes, which accounts for more than 95% of diabetes mellitus, is associated with an increased risk of pancreatic ductal adenocarcinoma (PDAC) [2]. PDAC is a destructive disease with an unoptimistic prognosis. It ranks 9th in the incidence of solid cancers but 4th for cancer-related deaths [3]. While several studies described potential molecular links between diabetes mellitus and PDAC, there is a lack of pertinent biological models to better elucidate the molecular mechanisms involved [2]. Three-dimensional cell cultures emerge as more relevant models towards organ-emulating constructs superseding the traditional two-dimensional format. Here, we created a perfusable microtissue-laden device using 3D printing technology to investigate the molecular links between diabetic microenvironment and PDAC initiation.

Methods

We use a 3D-printed device in which fugitive ink has been extruded to form a pancreatic duct. The duct is lined with a hydrogel from healthy or diabetic origin that also contain islets of Langerhans. The duct-mimicking channel is then filled with human pancreatic epithelial cells either healthy or harboring preneoplastic abnormalities.

Results

We developed an amphicrine pancreas-on-a-chip model that recapitulate diabetic condition and allow immunostaining labelling in 3D, as confirmed by ELISA quantification of insulin secretion in response to glucose stimulation, and light- sheet microscopy. We have also developed a decellularized extracellular matrix of healthy tissue that allows perfusion and good cell survival, a necessary first step towards the preparation of matrix from diabetic tissue. We maintain the perfused devices over several weeks, enabling the study of tumor initiation over long periods. We will further analyze the link between the diabetic environment and the cell activity (proliferation, migration) in cells harboring preneoplastic abnormalities (such as genetic mutations, inflammatory/hyperglycemia exposition) to study the link between cells abnormalities in diabetic patients and the risk for development of pancreatic cancer.

Conclusion and significance

Our approach allows the fabrication of semi-automatized and standardized devices that can be used for multiple organ models. The advantages of hydrogel-based devices are the possibility of cell self-arrangement in the relatively large bulk of hydrogel. The self-organized cells will then synthesize their own extracellular matrix, and will mature over time. The results are promising to generate glucose-responsive, functional Langerhans islet-based device for a breakthrough research in the early stages of pancreatic adenocarcinoma in diabetic patients, to identify new early diagnostic biomarkers.

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Keywords : diabetes, PDAC, early disease, organ-on-chip, in vitro model

New Modalities and Technologies for Pancreatic Cancer Research

Role of internal brachytherapy for pancreatic cancer

Cirolla Virginia [1*]

1. Medicina clinica e molecolare, Universita' la sapienza, Roma, ITALY

Pancreatic cancer is one of the most aggressive and difficult tumors to treat and cure, also because it is often diagnosed when it is already in an advanced stage. The best treatment option is surgery, which is currently only possible when the disease is localized. In the future the scenario could change: in recent days, in fact, for the first time in Italy, OncoSil, a microparticle device labeled with Phosphorus-32, was used to reduce the volume of an inoperable locally advanced pancreatic tumor.
The procedure performed at the San Camillo Forlanini Hospital in Rome made use of OncoSil, a particular brachytherapy device, a radiotherapy technique in which the radiation source is placed inside the patient's body, inside or proximity to the tumor.

This device is made up of microparticles of Phosphorus-32, a radioisotope capable of emitting highly curative beta particles in the short range, and of a specially formulated diluent, which serves to transport and facilitate the implantation of the radioactive microparticles into the tumor mass.

OncoSil is approved in various states, including those that form the European Union, and is indicated for the treatment of patients with locally advanced unresectable pancreatic cancer, in combination with chemotherapy, with the aim of rendering initially inoperable masses attributable to possibility of radical surgery.

The team from the Roman hospital - which involved the Nuclear Medicine, Gastroenterology, Oncology, Transplant Surgery and General Surgery and Health Physics units of San Camillo-Forlanini - implanted the brachytherapy device endoscopically last May 11th , therefore minimally invasive, on a 65-year-old patient with locally advanced pancreatic cancer. The intervention, the result of a year of work, is part of the European Osprey study, which in Italy also involves the Gemelli Polyclinic, the Pancreas Institute of Verona and the Tumor Institute of Milan.

The procedure was a perfect success, the patient was discharged the following day and is doing well. Now the oncology team will take him back for subsequent diagnostic re-evaluations and decide whether or not to proceed with the surgical operation.
Pancreatic cancer is the fourth most common in incidence; in 2022, 14,500 new cases were estimated in Italy. The mortality rate has not changed significantly in recent years: it is the tumor with the lowest survival both one year after diagnosis and five year

New Modalities and Technologies for Pancreatic Cancer Research

Development and validation of transcriptomic signatures for predicting the response to individual drug of the mFOLFIRINOX regimen in patients with pancreatic ductal adenocarcinoma

Nicolas Nicolas Fraunhoffer [1*], *Carlos Teyssedou* [2], *Patrick Pessaux* [3], *Martin Bigonnet* [4], *Nelson Dusetti* [1], *Juan Iovanna* [5]

1. Crcm-u1068, Inserm, Marseille, FRANCE
2. Endocrine and visceral surgery department, University hospital angers, Angers, FRANCE
3. Department of general, digestive, and endocrine surgery, Nouvel hôpital civil, Strasbourg, FRANCE
4. Predictingmed, Marseille, FRANCE
5. Crcm-u1068, Inserm, Marseille, FRANCE

Purpose

Current therapeutic options for patients with pancreatic ductal adenocarcinoma (PDAC) implicate monotherapy (gemcitabine) or combined therapies (modified FOLFIRINOX). mFFX treatment is accompanied by high toxicity; therefore, the administration of mFFX is conditioned by the performance of the patients instead of by rational criteria. In this work, we developed transcriptomic signatures for each mFFX regimen drug and validated their clinical interest.

Methods

To extract biologically relevant signatures for 5FU, oxaliplatin, and irinotecan, we integrated transcriptomic data from patient-derived primary cell cultures (PDC), patients-derived organoids (PDO) and patient-derived xenografts (PDX) with their corresponding chemo-response profiles to capture the biological components responsible for the response to each drug. The genes displaying the highest contribution levels defined 5FUCore, OxaCore, and IriCore signatures. We further validated the signatures in a pooled cohort of 167 patients with advanced and metastatic PDAC (94 patients from the COMPASS cohort and 73 from the Angers-Strasbourg cohort).

Results: All the signatures captured high-responder patients for overall survival (OS) and progression-free survival (PFS) in the mFFX arm but not in the gemcitabine arm. Then, we studied patients' responses to 0, 1, 2, and 3 drugs of mFFX. We identified a positive correlation between the number of drugs predicted as sensitive, the OS and PFS, and the objective response rate (ORR). Higher OS and PFS were observed in the patients sensitive to 2 and 3 drugs. The patients sensitive to 2 drugs showed a median OS of 13.6 months (95% CI, 9.6- not reached [NR] months) with HR of 0.23 (95% CI, 0.11-0.49; P<0.001) and a PFS of 6.0 months (4.4-NR months) with HR of 0.32 (95% CI, 0.15-0.68; P=0.003). The patients sensitive to 3 drugs displayed an OS of 23.0 months (11.6-NR months) with HR of 0.09 (95% CI, 0.03-0.27; P<0.001) and a PFS of 15.8 months (8.7-NR months) with a HR of 0.09 (95% CI, 0.03-0.32; P<0.001). The ORR was significant in the patients sensitive to 2 (ORR=0.35; 95% CI, 0.20-0.52; P=0.006) and 3 (ORR=0.61; 95% CI, 0.32-0.86; P<0.001) drugs. Next, we evaluated the association level between the PurIST classifier and our signatures. We found an association between OxaCore, IriCore, and the classical subtype but none with 5FUCore. However, we observed in a multivariate Cox regression that our model based on independent signatures displayed higher predictive values for the OS and PFS.

Conclusion

We developed and validate in a retrospective cohort of PDAC patients three novel transcriptome-based signatures that define sensitivity for each mFFX drug that can be used to rationalize the administration of the mFFX regimen and could help avoid unnecessary toxic effects.

Significance

These results support the use of transcriptomic signatures to optimize the treatment allocation and could determine the combination of mFFX drugs according to the sensitive profile of the patients in the future.

Keywords : PDAC, Transcriptomic Signatures, mFOLFIRINOX

New Modalities and Technologies for Pancreatic Cancer Research

Telemonitored circadian rhythm metrics towards personalized care and (chrono)therapy: intermediate assessment in patients with pancreatic ductal adenocarcinoma (PDAC) (MultiDom, NCT04263948)

Francis Levi [1*], *Rodrigo Balp* [2], *Ayhan Ulusakarya* [3], *Rachel Bossevot* [3], *Imène Hadj-Naceur* [3], *Xiao-Mei Xiao-Mei.li@inserm.fr* [1], *Sandrine Dulong* [1], *Sonia Boudriche* [3], *René Adam* [4], *Bärbel Finkenstädt-Rand* [5], *Mohamed Bouchahda* [6], *Pascal Hammel* [3]

1. Research team chronotherpay, cancers & transplantation, Faculty of medicine, paris-saclay university, Villejuif, FRANCE
2. Health research lab, Cap gemini, Meudon, FRANCE
3. Gastro-intestinal and general oncology service, Ap-hp hop paul brousse, Villejuif, FRANCE
4. Faculty of medicine, paris-saclay university, Faculty of medicine, paris-saclay university, Villejuif, FRANCE
5. Department of statistics, University of warwick, Warwick, UNITED KINGDOM
6. Mousseaf.u clinics, Ramsay-santé, Evry, FRANCE

Purpose

To determine between-patients differences in telemonitored circadian rhythms in PDAC patients and their relations with patient characteristics before chemotherapy.

Methods

The MultiDom study evaluates whether mFOLFIRINOX safety can be improved using a novel circadian-based telemonitoring-telecare platform in 67 PDAC patients at home (1). Study participation is 7 weeks for each patient, including a reference week before chemotherapy onset. Accelerometry and body temperature are measured q1-min using a continuously-worn telecommunicating chest surface sensor, and daily body weight is self-measured with a telecommunicating balance. Hidden Markov model (HMM), spectral analyses and other algorithms (2) automatically compute physical activity, body weight, and 12 circadian sleep/activity parameters, including the dichotomy index I<O (% activity 'In-bed' below median activity 'Out-of-bed') in near-real time (3). I<O values <97.5% independently predicted for both worse survival and increased risk of emergency admission in cancer patients (3,4). Here we report median and IQR values of 12 circadian parameters from telemetered measures in the 25 initial Multidom patients from Paul-Brousse hospital during the reference week. We determine their associations with patient characteristics using Spearman correlations.

Results

Patients were mostly females (N=14, 56%), had a median age of 67 years (range, 38-82), a WHO performance status of 0 (N=11, 44 %) or 1 (N=14, 56%). Primary PDAC was in the head (N=9, 36%), body (N=8, 32%), tail (N=7, 28%), or not specified (N=1, 4%). Metastases were found for 22 patients (88%), mostly in liver (N=14, 52%), lung (N=8, 30%) and/or lymph nodes (N= 5, 18.5%). Circadian disruption was found for 7/25 patients (28%), whose I<O value was <97.5%. Median values of 10/12 parameters differed significantly between-patients, with variations ranging from 5.6-fold for the circadian temperature amplitude (range, 0.3 to 1.8°C); to 2.4-fold for the rest-activity Rhythm Index, a measure of the regularity and quality of sleep spans inferred from observed low activity patterns in the daily rest/activity cycle (0.38 to 0.89). The median circadian acrophases (daily times of rhythm maximum) differed significantly between-patients for both rest-activity (by 4h19min) and body temperature (by 7h31min), thus revealing major between-patients differences in circadian timing system function.
Both low median Out-of-Bed activity and weak rest-activity rhythm amplitude were significantly associated with female sex ($p<0.034$), PS of 1 ($p<0.007$), worse liver function tests, (ASAT, ALAT, gamma-GT and Alk. Phosph., $0.005<p<0.03$). Performance status was correlated with most circadian rest-activity parameters ($0.008<p<0.04$), but not with any circadian temperature parameter.

Conclusion and significance

A remarkable patient engagement and >90% compliance supports the feasibility of circadian rhythms telemonitoring in PDAC patients. The apparent independence of most circadian metrics from several patient and all tumor characteristics suggest they could serve for near real-time personalization of care and treatment timing, i.e. chronotherapy.

Keywords : telemonitoring, circadian rhythms, telecare, mFOLFIRINOX safety

Early disease and metastasis

Pancreatic Cancer Cells Expand Tumor Burden via Direct Instruction on Tumor Adjacent Normal Acinar Cells to Triger The Acinar-Ductal-Metaplasia Dependent of KRAS Mutation

Wu Zhichong [1*], *Li Fanlu* [1], *Wang Xue* [2], *Tang Xiaomei* [3], *Shi Mingming* [3], *Xu Haimin* [2], *Luo Yakun* [4], *Zhao Yajie* [5], *Peng Chenghong* [1], *Deng Xiaxing* [1], *Hennino Ana* [6], *Shen Baiyong* [1]

1. General surgery department - pancreatic disease diagnosis and treatment center, institute of pancreatic diseases, Ruijin hospital affiliated to shanghai jiao tong university school of medicine, Shanghai, CHINA
2. Department of pathology, Ruijin hospital affiliated to shanghai jiao tong university school of medicine, Shanghai, CHINA
3. Institute of pancreatic diseases, Ruijin hospital affiliated to shanghai jiao tong university school of medicine, Shanghai, CHINA
4. Nhc key laboratory of molecular probes and targeted diagnosis and therapy, Harbin medical university, Harbin, CHINA
5. Department of gerontology, Ruijin hospital affiliated to shanghai jiao tong university school of medicine, Shanghai, CHINA
6. Stromal immune interactions in cancer, Cancer research center of lyon, inserm u1052, centre léon berard, université claude bernard lyon 1, Lyon, FRANCE

* Auteur correspondant : zhichong.wu@shsmu.edu.cn

Previously, we reported the discovery and definition of the pericyte stem cell (PeSC) subpopulation, characterized by the expression of CD106 dominantly localized in pancreatic ductal intraepithelial neoplasia (PanIN). Intriguingly, we found that pancreatic cancer stem cells (CD24+CD44+CD133+ CSCs) express CD106, which is the mesenchymal marker, to varying degrees. Transcriptome analysis and qPCR confirmed that the CSC subpopulation are characterized with epithelial, stem cell and EMT property. Tissue microarray and single cell-seq analysis showed that these CSCs, dominantly distributed in the highly differentiated precancerous lesions and tumor adjacent regions, are virtually absent in the poorly differentiated areas of the tumor core. in vitro 2D culture and 3D organoids comparative experiments verified their EMT signature including the stromal cell characteristic and invasive property. in vivo experiments in mouse models showed that the kinetic expression of surface markers CK19 (epithelial) and CD106 (mesenchymal) is correlated to biological behavior of the CSCs, i.e., morphology, differentiation and invasiveness. Orthotopic transplantation implied that the CSCs promoted tumor growth not only by self-proliferation, but also by triggering the adjacent normal acinar cells to initiate acinar-to-duct metaplasia in a KRASG12D mutation dependent manner. This finding may challenge the habitual concept of the current pathological staging system in pancreatic cancer and instruct surgeons to determine a definitive resection margin. Our study suggests a novel feature as a complement to comprehensively understand the hallmark of cancer cells exemplified in pancreatic cancer.

Keywords : Pancreatic Cancer, Cancer Stem Cells, Kras Mutation, Acinar-Ductal-Metaplasia

Early disease and metastasis

Pancreatic infiltration in pancreatic oncogenesis

Sonia Frendi [1]

1. Cri-u1149, Inserm, Paris, FRANCE

Pancreatic precancerous lesions are associated with obesity and fatty pancreatic infiltration but the mechanisms remain unclear. We aimed to assess the role of fatty infiltration in the pancreatic oncogenesis process in obesity context.

We developed a combined transcriptomic, lipidomic and pathological approach to explore neoplastic transformations.

We analyzed 1/ the intralobular (ILF) and extralobular (ELF) lipidomic profiles to search for lipids associated with pancreatic intraepithelial neoplasia (PanINs) and obesity; 2/ the effect of ILF and ELF fat infiltration on acinar tissue from obese (OB) and non-obese patients; and 3/ histopathological aspects of pancreatic parenchyma changes in OB patients. This study showed that the lipidic composition of ILF was different from ELF. ILF was related to obesity and ELF-specific lipids were correlated with PanINs. We showed that acinar cells exhibit different phenotypes depending on the presence and proximity to ILF in the context of obesity. Several lipid metabolic pathways, oxidative stress and inflammatory pathways were upregulated in acinar tissue upon ILF infiltration in OB patients. Early acinar transformation called acinar nodules were linked to obesity but not with ELF and ILF suggesting that they are the first reversible pancreatic precancerous lesion occurring in obese patients. In contrast, the number of PanINs was higher in OB patients, and was positively correlated to ILF, ELF score and to fibrosis.

Our study suggests that two types of fat infiltration must be distinguished, ELF and ILF. ILF plays a major role in the acinar modifications and in the development of precancerous lesions linked to obesity and ELF may be implicated in PDAC progression.

Early disease and metastasis

Single-nuclei multiome reveals permanent reprogramming of epithelial cells after pancreatiti

Carrer Alessandro [1*]

1. Biology, University of padova, Padova, ITALY

Acute pancreatitis (AP) is a local inflammation of the pancreatic parenchyma that resolves spontaneously, without clinical complications. However, epidemiological evidence shows that individuals who suffered an AP event are at elevated risk for developing pancreatic cancer, even decades after hospitalization.

We hypothesized that AP induces permanent changes in the epigenome or subtype distribution of acinar cells, which can initiate pancreatic carcinogenesis, setting up a pro-oncogenic memory of inflammation.

We found that inflammation-primed acinar cells exhibit enhanced plasticity ex vivo despite lack of obvious histological abnormalities. To dissect molecular and cellular dynamics that outlast AP events, we performed single-nuclei multiomic sequencing of transcriptomic and chromatin accessibility profiles of mouse pancreata after induction of-, and recovery from-, experimental pancreatitis. We found marked alterations of pancreatic tissue homeostasis that endure even one month after AP induction. Mechanistically, AP induces tissue dyshomeostasis and skewing of major acinar cell subtypes, with accumulation of an idleing cell population and signs of chronic stress mediated by the unfolded protein response (UPR) pathway. We also observed substantial chromatin remodeling in all acinar cells after AP; long-lived changes occurs preferentially at regions of poised chromatin and are associated with a regeneration-primed phenotype.

Our data show that AP episodes promote both remodeling of tissue composition and epigenetic reprogramming to predispose the pancreatic epithelium for transformation even much later in life.

Keywords : Pancreatitis; acing cell; plasticity; epigenetic reprogramming; clonal evolution

PANCREATIC CANCER SYMPOSIUM

DOMAINE DU HAUT-CARRE, UNIVERSITY OF BORDEAUX

POSTER

Identification and Targeting of New Tumor Vulnerabilities

Metabolic rewiring in response to chemotherapy in pancreatic ductal adenocarcinoma

Elodie Metay [1*], *Eugénie Lohmann* [2], *Marion Dussot* [1], *Sandrine Roulland* [3], *Sara Scaramuzzino* [3], *Richard Tomasini* [4], *Ghislain Bidaut* [2], *Audrey Vincent* [5], *Fabienne Guillaumond* [1], *Sophie Vasseur* [1,]

1. Cell communication and microenvironment (comet), Centre de recherche en cancérologie de marseille (crcm), Marseille, FRANCE
2. Crcm integrative bio informatics (cibi), Centre de recherche en cancérologie de marseille (crcm), Marseille, FRANCE
3. Instabilité génomique et hémopathies humaines, Centre d'immunologie de marseille-luminy (ciml), Marseille, FRANCE
4. Centre de recherche en cancérologie de marseille (crcm), Marseille, FRANCE
5. Mucines, cancer et résistance aux drogues, Cancer heterogeneity, plasticity and resistance to therapies (canther), Lille, FRANCE

As of today, surgery is the only potential cure for PDAC. However, because most patients present with either a locally advanced or metastatic disease, tumor resection can only be performed in 20% of cases. As another option, and in a palliative setting only, nearly all patients are offered chemotherapy. Treatment with FOLFIRINOX (FOX: 5-fluorouracil, oxaliplatin, irinotecan, leucovorin), leads to a higher response rate, disease-free survival, and overall survival than other treatments. However, FOX is often associated with severe toxicity and is, consequently, only considered for patients presenting with good performance status. Moreover, even among patients who are initially responders to FOX, most of them develop therapy resistance, resulting in disease progression after treatment.

PDAC tumor cells (TCs) exist in an impenetrable tumor microenvironment (TME) composed of non-TCs (mainly Cancer-Associated Fibroblasts (CAFs)), extracellular matrix (ECM), that account for up to 90% of the tumor mass. CAFs secrete various components of the ECM that distorts the architecture of PDAC tissues, leading to collapsed blood vessels which impedes the efficient delivery of drugs. CAFs also exhibit diverse functions that support pancreatic tumor growth, including providing metabolic support to enable TCs proliferation in a hypoxic, and nutrient poor TME. This stromal metabolic support includes provision of amino acids to support biomass production by PDAC TCs. Metabolic reprogramming is considered a major hallmark of PDAC and is investigated for cancer diagnosis, prognosis, and treatment.

However, little is known about how FOX influences PDAC TCs metabolic pathways. A gap remains on the understanding of 1/the metabolic alterations activated in FOX-treated PDAC, 2/ how FOX- altered metabolic pathways influence chemoresistance, and 3/how the TME contributes to TCs response to FOX. Thus, we aim to highlight and target the metabolic pathways and associated-key metabolic actors that are deregulated in response to FOX in PDAC and leading to either chemosensitivity or chemoresistance.

Using transcriptomic datasets of PDX mice derived PDAC samples submitted or not to FOX, we identified specific metabolic pathways of pyruvate, as its transport into the mitochondria, as involved in PDACs' FOX sensitivity. We now aim to explore on 3D cell culture systems, the metabolic pathways of interest, in connection with pyruvate, that are deregulated in PDAC in response to FOX and that are promoters of FOX sensitivity. To that end, we developed a spheroid cell culture experimental approach that integrates CAFs conditioned medium (CM) to consider the impact of the TME on pyruvate deregulated metabolic pathways upon FOX. We currently target these FOX-induced metabolic pathways using pharmacological and inducible/traceable genetic silencing tools to examine TCs' response to FOX in association with their metabolic phenotype using targeted metabolomics and metabolic tracing approaches. We intend to ultimately identify pyruvate-dependent metabolic adaptations leading to FOX sensitivity and once inhibited, the ones that are rewired and lead to FOX resistance. These metabolic candidates will be considered as prognostic markers of FOX response and will constitute therapeutic targets to improve the response of PDAC patients to FOX.

Keywords : Metabolic Rewiring, Chemotherapy, Microenvironment, Mitochondrial Metabolism

Identification and Targeting of New Tumor Vulnerabilities

Metabolic Crosstalk between Serine-Dependent PDAC Tumor Cells and CAFs Revealed By Translatome Analysis

Yvan Martineau 1 *, *Sauyeun Shin [1], Mehdi Liauzun [1], Jacobo Solorzano [1], Christine Jean [1], Benjamin Fourneaux [2], Ismahane Belhabib [1], Alexia Brunel [1], Cindy Neuzillet [3], Carine Joffre [4], Stephane Rocchi [5], Vera Pancaldi [6], Laetitia Linares [2], Nelson Dusetti [7], Juan Iovanna [7], Ola Larsson [8], Remy Nicolle [9], Stephane Pyronnet [10], Corinne Bousquet [1]*

1. Micropanc, Crct / inserm u1037, Toulouse, FRANCE
2. Ircm / inserm u1194, Montpellier, FRANCE
3. Medical oncology, Curie institute, Paris, FRANCE
4. Metaml, Crct / inserm u1037, Toulouse, FRANCE
5. C3m/ inserm u1065, Nice, FRANCE
6. Netb(io)², Crct / inserm u1037, Toulouse, FRANCE
7. Crcm / inserm, Marseille, FRANCE
8. Oncology-pathology, Karolinska institutet, Stockholm, SWEDEN
9. Cri / inserm u1149, Paris, FRANCE
10. R'n blood, Crct / inserm u1037, Toulouse, FRANCE

Transcriptomic profiling unveils prognostic subtypes in Pancreatic Ductal Adenocarcinoma (PDA), yet personalized first- line treatments remain elusive. Here, PDA was profiled based on protein synthesis rate, a step of gene expression dysregulated in tumor cells. Analyzing translated mRNA from a collection of PDA Patient-Derived Xenografts (PDX) uncovered a tumor subtype characterized by a low protein synthesis rate, accompanied by robust translation of mRNAs involved in the integrated stress response (ISR), including the transcription factor ATF4. Such ISR-activated cancer cells demonstrated high drug resistance but a serine auxotrophy, which coincided with the expression loss of PHGDH and CBS enzymes. Importantly, specific cancer-associated fibroblasts (CAF) supported the growth of ISR-activated cells in a serine-depleted environment. Our study underlines the power of translatomic profiling in PDA, exposing a drug-resistant cancer cell phenotype and highlighting the potential of targeting serine-producing CAF to overcome this therapeutically challenging disease.

Identification and Targeting of New Tumor Vulnerabilities

Characterization of the mode of entry of oncolytic virus in pancreatic cancer cells

Nikolaos Kontopoulos [1*], Agathe Redoute [1], Laetitia Ligat [2], Naima Hanoun-Ziane [1], Guillaume Labrousse [1], Nelson Dusetti [3], Louis Buscail [4], Pierre Cordelier [5]

1. Haute-garonne, Equipe impact, université fédérale toulouse midi-pyrénées, université toulouse iii paul sabatier, inserm, crct, Toulouse, FRANCE
2. Haute-garonne, Cluster technologique, université fédérale toulouse midi-pyrénées, université toulouse iii paul sabatier, inserm, crct, Toulouse, FRANCE
3. Bouches-du-rhône, Centre de recherche en cancérologie de marseille, Marseille, FRANCE
4. Haute-garonne, Service de gastroentérologie du chu de toulouse, Toulouse, FRANCE
5. Haute-garonne, Equipe impact, université fédérale toulouse midi-pyrénées, université toulouse iii paul sabatier, inserm, crct, toulouse, Toulouse, FRANCE

Oncolytic viruses, which specifically infect and kill cancer cells, are part of the emerging and promising field of cancer biotherapies. We have previously demonstrated that SG33, derived from the Myxoma virus, exhibits oncolytic properties in cellular models of pancreatic cancer (PC). However, the molecular mechanisms involved in the viral tropism for PC cells remain unclear. Macropinocytosis (MPC) is an endocytic process that meets the metabolic needs of cancer cells, particularly pancreatic ones, and is also involved in viral infection. Thus, we investigated the role of MPC in the tropism of SG33. We classified different PC primary cell lines, deriving from patients, based on their MPC level (low, high) or its inducibility (treatment with EGF or glutamine starvation) using imaging techniques. In cells with "high" MPC, inhibiting MPC significantly reduces SG33 replication and oncolytic activity. Conversely, SG33 infection stimulates MPC by nearly 3-fold in "inducible MPC" cells. We combined SG33 with NabPaclitaxel, which depends on MPC for its efficacy, and demonstrated a synergistic effect on inhibiting cancer cell growth. In conclusion, in cellular models of PC, we show that MPC is involved in SG33 infectivity and can be considered a new metabolic vulnerability. Furthermore, SG33 stimulates MPC and enhances the response to a clinically relevant therapy. Conclusively, this work could lead to improved specificity and efficacy of virotherapy in pancreatic cancer.

Keywords : Pancreatic cancer, oncolytic virus, macropinocytosis, precision medicine, imaging

Exploration of the desmoplastic reaction and the tumor microenvironment

Acquired chemoresistance in pancreatic adenocarcinoma : mechanism implicating the stromal transcription factor Zbtb16 ?

Ludmila Recoules 1 *, *Jérome Raffenne [1], Hippolyte Audureau [1], Rémi Samain [1], Amandine Alard [1], Alexia Brunel [1], Marjorie Fanjul [1], Ismahane Belhabib [1], Charles Theillet [2], Stéphane Pyronnet [1], Yvan Martineau [1], Remy Nicolle [3], Audrey Vincent [4], Corinne Bousquet [1]*

1. Centre de recherche en cancérologie de toulouse, Toulouse, FRANCE
2. Centre de recherche en cancérologie de montpellier, Montpellier, FRANCE
3. Carte d'identité des tumeurs, ligue contre le cancer, Paris, FRANCE
4. Centre de recherche jean-pierre aubert, Lille, FRANCE

Pancreatic adenocarcinoma (PDA) remains lethal, mainly due to patient relapse post-chemotherapeutic care. Rich in stroma, PDA comprises a diverse population of cancer-associated fibroblasts (CAFs), which play a crucial role in PDA chemoresistance. Our project is centred around the identification and characterization of stromal targets that may contribute to the acquisition of tumor resistance during chemotherapy, and then to relapse. To achieve this, we have established a model using patient-derived xenografts (PDXs, n=8 patients), where tumours have been rendered resistant to Gemcitabine (Gem) through long-term treatment. Upon unsupervised bioinformatical analysing of RNA-seq data generated from these PDX tumors, we identified a stromal gene signature correlated with PDX tumor growth response to Gem, and comprising a transcription factor whose expression is enriched in the stroma of tumors that remain "sensitive" to Gem. Given the described significant role of this transcription factor in regulating cell identity, yet outside the PDA field, we hypothesized that it controls the activation status of CAFs. Through in vitro experiments where we overexpressed this transcription factor in CAFs, we demonstrated that this reduced CAF basal activation state, and hindered their ability to be activated into pro-tumoral and chemoprotective CAFs upon cytokine treatments. Additionally, when inferred to public PDA RNAseq databases, our identified stromal gene signature comprising this transcription factor was significantly enriched in tumors of patients with best survival prognosis. Our research sheds light on the crucial role played by this transcription factor in the development of stroma-mediated chemoresistance. We are optimistic that our findings will lead to the development of additional stroma-targeting drugs specifically designed to prevent therapeutic relapse.

Keywords : PDAC, Cancer associated fibroblast, chemoresistance, cell identity

Exploration of the desmoplastic reaction and the tumor microenvironment

The extracellular matrix influences pancreatic cancer cell sensitivity to chemotherapy by modulation of purine metabolism.

Georgios Efthymiou [1] *, *Coralie Compare* [1], *Eugénie Lohmann* [1], *Issam Ben-Sahra* [2], *Richard Tomasini* [1], *Fabienne Guillaumond* [1], *Sophie Vasseur* [1], *Juan Iovanna* [1],

1. Centre de recherche en cancérologie de marseille, Marseille, FRANCE
2. Northwestern university, Chicago, UNITED STATES

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with an increasing incidence and a mortality rate projected to almost double by 2040. This dismal prospective arises from the absence of early symptoms, resulting in late diagnosis, and the failure of current therapies due in part to the presence of extreme desmoplasia around the tumor. This thick and dense extracellular matrix (ECM) functions as a physical barrier causing oxygen and nutrient deprivation, but also limiting the delivery of chemotherapeutic drugs. As a result, cancer cells adapt their metabolism to maintain their proliferative capacity and such adaptation may also contribute to chemoresistance.
Recent reports suggest that apart from the biophysical protective properties of the desmoplasia in PDAC, ECM-relayed signaling may play a role in chemoresistance. In this study, we set out to understand how the biochemical properties of the ECM shape the metabolic landscape of PDAC cells and how this ECM-mediated metabolic adaptation may modulate chemosensitivity.

By using a multi-omics approach in a tissue-mimicking 2D in vitro setting, we show that a CAF-derived desmoplasia- mimicking bio-scaffold tunes purine metabolism in PDAC cells and influences sensitivity to chemotherapy by means of DNA repair. Our results couple the ECM with PDAC cell metabolic plasticity and suggest a link between purine metabolism and drug resistance. Identification of the key players in this cross-talk will pave the way for the discovery of novel targets for combinatorial therapeutic strategies.

Keywords : pancreatic cancer, metabolism, extracellular matrix, purine metabolism, chemosensitivity

Exploration of the desmoplastic reaction and the tumor microenvironment

Helicobacter pylori induces pancreatic lesions in a mouse model of gastric carcinogenesis

Élodie Sifré [1] *

1. 33, Université de bordeaux bric équipe4, Bordeaux, FRANCE

Gastric cancer, the 4th cause of cancer mortality worldwide, is mainly caused by a chronic infection with the bacterium Helicobacter pylori, which colonizes the stomach lifelong. It induces chronic gastritis, evolving in some cases to intestinal metaplasia, dysplasia and adenocarcinoma. Many studies have tried to correlate Helicobacter infection with disease in extra-gastric digestive organs like the pancreas. It has been reported in H. pylori infected patients that this infection could affect the physiology of the pancreas without colonising it directly.
In this study, we evaluated the consequences of mice infection with different strains of gastric Helicobacters on the histopathology of their pancreas. We performed histopathological analysis of HES-stained paraffin-embedded pancreas tissue sections to evaluate fibrosis, inflammation and other lesions.
Preliminary results suggest that mice infected with H. pylori for 12 months developed chronic pancreatitis and fibrosis, known precursor lesions of pancreas cancer.
Understanding the impact of H. pylori infection on lesions of extra-gastric organs could help in fine prevent the emergence of other digestive-track related diseases.

Keywords : Pancreas, mouse model, Helicobacter pylori

Exploration of the desmoplastic reaction and the tumor microenvironment

Elimination of Hyaluronic acid in Pancreatic Ductal Adenocarcinoma (PDAC) as a potential Strategy for immunotherapy

Sun Pian [1] *

1. Group of experimental oncology, Centro nacional de investigaciones oncologicas, Madrid, SPAIN

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths, with a five-year survival rate around 5–7%. In PDAC, stromal cancer-associated fibroblasts (CAFs) play a vital role in promoting the desmoplastic and immunosuppressive tumor microenvironment (TME), as well as tumor growth and malignancy, and have emerged as cancer targets (1). Has proteins are responsible for the production of hyaluronic acid (HA). Has overexpression results in accumulation of HA which leads to high pressure on neighboring structures as well as elevated interstitial fluid-pressure (IFP) within PDAC, which can interfere with drug delivery (2) and has also been linked with tumor escape from immune surveillance. Previous results from our lab showed that Has1 and Has2 was differentially expressed between PDGFRa+ CAFs and PDGFRα+ normal pancreatic fibroblasts (NPFs) (3).
We have developed developed genetically modified mouse models (GEMMs) of Has triple knocked-out (Has1/2/3 TKO) based on Has2 conditional KO mice (4) to study the role of HA in PDAC development and progression. We aim to explore the influence of HA in tumor development and in the desmoplastic and immunosuppressive TME. In allograft studies, the TKO mice environment significantly inhibited proliferation of tumor cells competent for these genes. These studies may help to design future therapeutic strategies.

Keywords : Pancreatic ductal adenocarcinoma (PDAC), Cancer-associated fibroblasts (CAFs) Hyaluronic acid (HA).

New Modalities and Technologies for Pancreatic Cancer Research

Bioprinting early-stage pancreatic cancer models: a new tool to decipher tumor initiation mechanisms

Aurélien Mazet [1]*, *Charles Handschin* [1], *Nathalie Dusserre* [1], *Léo Compérat* [1], *Marie-Laure Stachowicz* [1], *Jean- Christophe Fricain* [2], *Julie Guillermet-Guibert* [3], *Hugo De Oliveira* [1],

1. *U1026, bioningénierie tissulaire, université de bordeaux, Inserm, Bordeaux, FRANCE*
2. *U1026, bioningénierie tissulaire, université de bordeaux, chu bordeaux, Inserm, Bordeaux, FRANCE*
3. *Centre de recherches en cancérologie de toulouse (crct), université toulouse iii paul sabatier, laboratoire d'excellence labex toucan, Inserm, Toulouse, FRANCE*

Introduction and Objectives:
Pancreatic cancer remains one of the most aggressive malignancies with late diagnosis, limited therapeutic options and low survival rates, emphasizing the critical need for early detection and improved understanding of its initiation mechanisms. In this regard, the application of 3D bioprinting technology presents a compelling approach for developing physiologically relevant cancer models. This innovative technique allows for the replication of various microenvironments and niches during the early stages of pancreatic cancer development, offering valuable insights into its progression.
Methods:
In this study, we present the development and characterization of a novel bioprinting methodology capable of replicating distinct matrix stiffness gradients that correspond to various stages of pancreatic cancer. Our approach combines inkjet bioprinting, an extracellular matrix-derived bioink, and primary pancreatic cells extracted from wild-type and genetically modified mice to create highly realistic 3D bioprinted pancreatic models.
Results:
Rheological assessment showed our ability to finely modulate the properties of the bioinks, enabling us to accurately replicate the matrix stiffness observed in vivo. Image analysis showcase the successful replication of the bioprinted model while maintaining cell viability. Additionally, we show that the model facilitates large-scale image analysis, highlighting its utility in capturing phenotypic changes with high statistical power.
Discussion/Conclusion:
Moving forward, our research aims to delve deeper into the dynamic crosstalk between cancer cells and their microenvironment within the 3D model, utilizing advanced techniques such as secretome analysis and multi-omics approaches.
By closely mimicking the in vivo tumor microenvironment, this model offers a valuable platform for investigating the underlying mechanisms involved in cancer initiation and therefore, pancreatic cell tumor phenotype acquisition.

Keywords : Bioprinting, Biofabrication, Pancreatic cancer, cancer initiation, matrix stiffness

New Modalities and Technologies for Pancreatic Cancer Research

Histology-based Deep Molecular Profiling using Artificial Intelligence in Pancreatic Adenocarcinoma

Taib Bourega [1] *, *Jérome Cros* [2], *Rémy Nicolle* [1],

1. *Inserm, Paris, FRANCE*
2. *Inserm - aphp, Paris, FRANCE*

Purpose
Our team has described the molecular intratumoral heterogeneity of pancreatic adenocarcinoma (PDAC). We developed PACpAInt [1], a deep learning model capable of predicting transcriptomic phenotypes from histology demonstrating on a massive scale that different tumor and stromal subtypes can coexist. This work combines morphological features extracted from targeted tumor subregions (average 2mm2) with matched microdissection-based transcriptomic data through Deep Learning. The aim is to decipher the complexity of pancreatic adenocarcinomas and better understand the spatial distribution of its components.

Methods
Our cohort comprises 100 patients with resected PDAC. All slides were reviewed and 2 to 8 morphologically distinct regions of interest per tumor were selected. These areas were microdissected for RNA extraction and RNA sequencing. This preliminary dataset encompasses 252 RNAseq profiles from 67 patients linked with their corresponding histological images. To extract the morphological features of each tile (112x112µm) within the selected areas we developed a deep learning tool, based on a fine-tuned model that was pre-trained on histological datasets [2]. Multiple Instance Learning was then used. Extracted features were fed into a trainable shallow neural network (3 layers, 2.6M parameter) to predict molecular transcriptomic signatures in each tile. An aggregation mechanism centered on a trainable latent space gave the prediction per region.

Results
Preliminary results showed correlations (Spearman R) surpassing 0.6 in the validation sets for 24 out of 124 transcriptomic signatures tested. The best results were obtained with the major phenotypes (e.g. Classic and Basal tumor cells, Active and Inactive Stroma), but also with CAF subtype signatures (e.g. TGFβ, ECM, and Wound) together with immune infiltrates. The models can be applied massively on large datasets of histological slides.
Previously, many phenotypes such as immune and inflammatory CAF subtypes were not predictable using whole slide approaches, yet our matched-subregion approach achieved a significant improvement in predicting many of the signatures. This indicates the importance of targeting sub-tumoral regions for specific morpho-molecular linkage.

Conclusion
We had shown that we could predict the main PDAC transcriptomic phenotypes by AI from histology slides conserving the spatial information that is classically lost in bulk analyses. This will offer morpho-molecular insights into the spatial heterogeneity within pancreatic cancer, enabling the integration of histological and cellular morphologies with molecular phenotypes.
Significance
This type of approach will allow the spatial molecular dissection of PDAC at a scale never achieved before, allowing us to understand the extent and role of tumor plasticity both in the stromal and tumoral compartments.

Keywords : Histology, Artificial Intelligence, Molecular Phenotyping

New Modalities and Technologies for Pancreatic Cancer Research

Interest of circulating cell-free DNA fragmentation as biomarker in pancreatic cancer

Sophie Gilbert [1]*, *Louis Buscail* [2], *Audrey Boutonnet* [3], *Barbara Bournet* [2], *Frederic Ginot* [3], *Pierre Cordelier* [1],

1. *Crct, Toulouse, FRANCE*
2. *Chu de rangueil, Toulouse, FRANCE*
3. *Adelis, Toulouse, FRANCE*

Pancreatic cancer remains a disease with no cure due to late diagnosis and the lack of effective therapies. Thus, the identification of non-invasive diagnostic and/or prognostic biomarkers shows potential to improve patient care.
Circulating cell-free DNA (ccfDNA) in the blood was recently demonstrated as a source of biomarkers for patients with cancer. However, the detection of tumoral ccfDNA using KRAS mutation analysis shows limited clinical potential for the diagnosis of patients with pancreatic cancer at early stage. Recent studies demonstrated that patients with cancer had altered DNA fragmentation profiles, that could facilitate non-invasive cancer detection. In this context, we evaluated in this pilot study the interest of ccfDNA fragmentation analysis as a biomarker in pancreatic cancer. We selected 29 plasma samples from patients with locally advanced or metastatic pancreatic cancer from the BACAP cohort. These samples were analyzed by capillary electrophoresis using the BIA-Booster system. This device allows to concentrate, purify and separate circulating DNA directly from a few microliters of plasma in a few hours. A size profile was obtained to determine the size and concentration of the fractions corresponding to mononucleosomes (160 base pairs) and dinucleosomes (320 base pairs). Our results demonstrate that ccfDNA concentration is significantly increased in the metastatic group as compared to the locally advanced group of patients with pancreatic cancer. Levels of ccfDNA fragmentation were inversely correlated with overall and progression-free survival of patients with pancreatic regardless of the tumor staging. Finally, our results show that cfDNA fragmentation combined with CA19.9 makes distinguish metastatic patients from locally advanced patients. In conclusion, this pilot study highlights the potential of ccfDNA fragmentation analysis as a promising biomarker for pancreatic cancer diagnosis and prognosis. The findings suggest that this approach may offer insights into disease stage, prognosis, and potentially aid in differentiating between metastatic and locally advanced cases when combined with other markers like CA19.9. Further research and validation on a larger scale will be necessary to confirm these findings and establish the clinical utility of ccfDNA fragmentation analysis in pancreatic cancer management.

New Modalities and Technologies for Pancreatic Cancer Research

Proteomic prognostic signature of resectable ductal adenocarcinoma

Mehdi Boubaddi [1]*

1. Hepatobiliary and pancreatic surgery unit, Chu de bordeaux, Bordeaux, FRANCE

Introduction:
Early identification of patients with resectable PDAC at high risk of early mortality has strong clinical and prognostic value. The aim of this work is to compare proteomic expression in tumor tissues from good-prognosis (GP) and poor-prognosis (PP) patients in order to determine a proteomic signature that can predict prognosis after CPP for readily resectable PDAC.

Methods:
During the study period, 9 good-prognosis and 7 poor-prognosis patients were analyzed. The clinical and anatomopathological characteristics of both groups were indistinguishable. A comparison of protein abundance between tumor tissues and healthy tissues was performed for each patient.

Results: Among over 1800 proteins analyzed, 47 were differentially expressed between the two groups, including the translation elongation factor eIF5A. This factor plays a major role in the gene expression of proteins belonging to the interactome drawn from the differentially expressed proteins. Expression inhibition of this protein and exploring the eIF5A-dependent proteomic expression link were studied.

Downregulation of eIF5A transcripts induced a drop in cell numbers after 20 days of culture compared to cells proficient in eIF5A expression (79.6±6.7% versus 41.4±1.8%, p=0.004). Several ribosomal proteins, identified as functionally linked to eIF5A, had significantly higher expression in the cell population transduced in eIF5A downregulated cells (RNA abundance respectively 0.18 versus 0.03; 1.43 versus 0.21; 3.62 versus 0.50; 0.19 versus 0.05, p<0.05).

Conclusion: The eIF5A factor has a demonstrated role in the aggressiveness of PDAC and controls a network of proteins sharing the same functional pathway. The present signature deserves to be explored in patients' tumor biopsies or blood to evaluate its value for pancreatic cancer therapeutic decisions. More studies are ongoing on eIF5A factor involvement in regulating the expression of the proteins identified in the signature.

Keywords : PDAC, prognosis, proteomic, pancreatic cancer, surgery

New Modalities and Technologies for Pancreatic Cancer Research

The CRISPR/CAS Technology for the detection of rare KRAS mutant alleles

Samuel Amintas [1]*

1. Inserm bric 1312, Bordeaux, FRANCE

Introduction:
The diagnosis of pancreatic ductal adenocarcinoma necessitates tumor biopsy by endoscopic ultrasound fine needle aspiration (EUS-FNA), which shows insufficient negative predictive value. As mutations in the KRAS oncogene are very common in PDAC, their detection from circulating tumor material may provide a powerful diagnostic tool for PDAC formal diagnosis but this requires a level of sensitivity challenging the available molecular tools. We assessed the emerging CRISPR/Cas13a SHERLOCK technology promising the high discrimination of genetic alterations, from large intragenic rearrangement to single nucleotide polymorphism. Methods: Guides hybridizing various positions on the KRAS target were designed to detect G12V, G12C and G12C mutant alleles. Mismatches around the single mutation of interest, as well as hairpin spacer sequences were tested to prevent hybridizing the wild-type allele. Mutant allele detection was tested on matrix containing known concentrations and compared to Q-PCR, allele-specific PCR and ddPCR. The sensitivity of a large intragenic rearrangement and a 15nt deletion of the EGFR were compared to that of single KRAS mutation. The combination of an allele specific PCR and a CRISPR/Cas detection (AS-SHERLOCK) of the related products was finally set-up and tested with pancreatic cancer and non-small cell lung cancer liquid biopsy patients' samples. Results: The position of RNA guide affected the ability of Cas13a to detect KRAS alleles and the possibility to discriminate between different alleles. We observed efficiency variations between mutations, possibly related secondary structures of the matrices and the nature of the mismatches between the guide and the matrix. Hairpin spacer strategy only slightly improved specificity for KRASG12D mutant detection. The detection of a large intragenic rearrangement and a 15 nt deletion mutation in the EGFR gene reached a total specificity and a sensitivity similar to the one of ddPCR. Moreover, AS-SHERLOCK reached performance similar to ddPCR for KRASG12D mutant detection.
Finally, AS-SHERLOCK technology allowed the efficient detection of KRAS and EGFR mutant in patients' samples.

Conclusion:
As other sensitive tools, CRISPR/Cas13a technology is challenged to detect mutant variants outnumbered by WT alleles. However, the use of highly discriminant guides reached or outperformed the gold standard ddPCR for the detection of rare alleles. It is implemented with a simple workflow, without expensive equipment, and applicable to patients' samples analysis. Efforts are still needed to increase the specific detection of single mutations to avoid initial allele-specific PCR.

Keywords : KRAS, SHERLOCK, CRISPR Cas13a, liquid biopsy, pancreatic cancer

New Modalities and Technologies for Pancreatic Cancer Research

A pancreatic carcinogenesis model for translational research and drug testing

Jinan Al Tarrass [1] ******, *Frederique Mittler* [1], *Maya Mokrani* [1], *Sebastien Dergan Dylon* [2], *Robin Terrisse* [3], *Vincent Haguet* [1], *Xavier Gidrol* [1], *Gaelle Saint-Auret* [3], *Maxim Balakirev* [1],

1. *Irig, bge, biomics, Univ. grenoble alpes, cea, inserm, Grenoble, FRANCE*
2. *Institut de biologie structurale (ibs), Univ. grenoble alpes, cea, cnrs, Grenoble, FRANCE*
3. *Irig, Cea, genel (the rnai switch company), Grenoble, FRANCE*

Introduction & Objectives:
Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a 5 year overall survival of only 11% [1]. The lethality of PDAC lies behind late prognosis and rapid progression to metastasis [2]. Several large-scale genomic efforts have identified multiple potential driver mutations in PDAC with KRAS (K), TP53 (T), CDKN2A (C) and SMAD4 (S) genes displaying the highest mutation frequencies. Notably, KRAS mutations are found in >90% of PDAC tumors [3].
Our work aims to design a precancerous model that retraces PDAC development by introducing the four driver mutations into pancreatic epithelial cells. We characterize the model in 2D and in 3D cultures and evaluate its efficacy for drug testing. Specifically, we are developing a video holography device "VideoCell" that allows for label-free monitoring of drug effect in 3D cultures.

Methods:
We performed CRISPR/Cas9 gene editing technology on human immortalized pancreatic ductal epithelial cells (H6c7) to knock out the three tumor suppressor genes (C-T-S) and to edit native KRAS locus. We also designed plasmid-encoded KRAS vectors for continuous and conditional (Cre-loxP and TetR systems) expressions of mutant KRAS. Where the enhanced efficiency of mutant KRAS integration is required, PiggyBac transposase is used. In parallel, we knocked out KRAS gene to investigate cellular pathways affected by KRAS loss and to identify potential KRAS-complementary targets. Following each genetic modification, clonal selection is performed and genetic mutations are validated by biological assays.

Results:
For C-T-S tumor suppressor genes, we successfully obtained cell lines with single and triple gene mutations. The experiments to modify the native KRAS locus are ongoing. Continuous expression of KRAS mutants using plasmid vectors initiated senescence both in wild type and triple mutant cells precluding the establishment of stable clones. To address this problem, Cre-loxP vectors for conditional KRAS expression were used and validated in transfection assays. We found that the ectopic expression of KRAS mutants significantly affected cell morphology and dynamics suggesting alteration of cytoskeleton. Regarding the KRAS KO experiments, we have obtained the clones both on wild-type and triple C-T-S mutation backgrounds. We found that the loss of KRAS increases cellular size in wild-type cells, whereas it results in shrinking of cells bearing the triple C-T-S mutation. Further morphological and functional studies are enduring. Discussion & Conclusion
Although p53 and p16 (CDKN2A gene product) have been identified as the main factors responsible for KRAS oncogene-induced senescence [4], our preliminary results with triple C-T-S mutants suggest that the deletion of these genes is not sufficient neither to initiate carcinogenesis nor to overcome senescence induced by KRAS. Therefore, other yet-to-be-identified factors are essential for KRAS-induced carcinogenesis. Our preliminary data with KRAS- overexpressing and KRAS KO cells suggest that KRAS affects multiple cellular pathways involving cell size/morphology/dynamics and cell cycle regulations. Interestingly, triple C-T-S mutation significantly modifies the outcome of KRAS effects, providing new insight on the role of this lesion in KRAS-driven carcinogenesis.

Keywords : pancreatic cancer, KRAS, cellular model

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GENERAL INFORMATIONS

INFORMATION & ORGANIZATION

COM&CO, Johanna GAUDE
POST 15, Bd Grawitz
13016 Marseille, France
TEL +33 (0)4 91 09 70 53
FAX +33 (0)4 96 15 33 08
EMAIL j.gaude@comnco.com

CONGRESS VENUE

Domaine du Haut-Carré
43 Rue Pierre Noailles
33400 Talence