Association pour la Recherche sur les Tumeurs de la Prostate

ARTP BULLETIN

November 16th, 2022

31st Annual Meeting Association pour la Recherche sur les Tumeurs de la Prostate

Foreword

With this 31st annual meeting, the ARTP continues a tradition of bringing together researchers, academics and clinicians from all over the world, experts in prostate cancer. Once again, the funding of the new projects selected for 2023 as well as the organization of this meeting would have not been possible without the generous contributions of Pierre Fabre Médicament, Astellas, Janssen, AstraZeneca/MSD, Bayer and the AFU foundation. We thank all speakers and participants for their contributions.

Prof Alexandre de la Taille

ARTP President

Program

Organizing Committee

Frédéric Bost, Jocelyn Céraline, Olivier Cuvillier, Vincent Goffin, Palma Rocchi & Alexandre de la Taille

9:00 a.m.

Welcome

Alexandre de la Taille
ARTP President

9:05 a.m.

Session I : ARTP 2021 laureates

Chairs : Dimitra Gkika, Laurent Morel

Thierry Capiod (Institut Necker - Inserm U1151 - Université Paris Descartes)
'GPRC6A, macrophages et cancer de la prostate'

Khahn Le-Thi (CRCM, Inserm UMR1068, Université d'Aix-Marseille)
'DDX5 mRNA-targeting antisense oligonucleotide as a new promising therapeutic in combating castration-resistant prostate cancer'

Anne Chotteau (Institut de Biologie de Lille, UMR 8161, Université de Lille)
'Récepteur MET et fusions ETS : co-acteurs de la progression tumorale du cancer de la prostate'

9:50 a.m.

Coffee Break
& Visit of Exhibition Booths

10:05 a.m.

SESSION II : MICROBIOME

Chairs : Frédéric Bost, Stephane Terry

Karina Dalsgaard Sørensen (Dpt of Molecular Medicine, Aarhus University, Denmark)
'Molecular analyses of the prostate tumor microenvironment and microbiome: Clinical implications? '

Aurora Perez-Cornago *(Oxford University, UK)*
'Diet, nutrition, and prostate cancer risk'

Nicolo Pernigoni *(IOR, Bellinzona, Switzerland)*
'Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis'

11:35 a.m.

Lunch

Restaurant Rural
by Marc Veyrat,
Palais des Congrès
niveau 0

1:15 p.m.

Session III : Clinical session

Chairs : Alexandre de la Taille, Arnaud Villers

Pascal Pujol

(CHU de Montpellier)

'Testing BRCA et HR dans le cancer de la prostate : qui quand comment ?

Gwenaëlle Gravis

(Institut Paoli Calmette, Marseille)

'Place de l'immunothérapie dans le cancer de la prostate métastatique'

Gaëlle Fromont

(CHRU, Inserm UMR 1069, Tours)

'Cribriform and intraductal prostate cancer: pathological, clinical and molecular specific features'

2:30 p.m.

Coffee Break & Visit of Exhibition Booths

2:45 p.m.

Session IV : Cellular heterogeneity and lineage plasticity

Chairs : Clémentine Le Magnen, Vincent Goffin

Douglas Strand

(UT Southwestern Medical Center, Dallas, USA)

'Prostate lineage plasticity and stem cells'

Wouter Karthaus

(Ecole Polytechnique fédérale de Lausanne, Switzerland)

'Understanding and targeting lineage plasticity in prostate cancer'

3:45 p.m.

Session V : Poster Session

Chairs of Selection Committee:
Jocelyn Céraline, Edith Bonnelye

5:00 p.m.

Session VI : 2021 ARTP Poster Prize Laureates

Chairs : Palma Rocchi, Dimitra Gkika

Manon Baurès

(Institut Necker Enfants Malades, Paris)

'Cytokine and growth factor regulation of castration-tolerant luminal progenitors of the mouse prostate'

Kateryna Len

(Institute of Genetics and Molecular and Cellular Biology, Strasbourg)

'Impact of vitamin D signaling on prostatic precancerous lesions'

Raphaëlle Servant

(University Hospital Basel, Switzerland)

'Prostate cancer patient-derived organoids: detailed outcome from a prospective cohort of 81 clinical samples'

Marine Vialat

(Institut GreD, Clermont- Ferrand)

'Intratumoral sex steroid synthesis is involved in basal extrusion during prostate-like tumorigenesis, and its deprivation promotes the emergence of a new tumor cell population'

5:35 p.m.

Assemblée Générale ARTP

Poster Prize Distribution

Alexandre de la Taille, ARTP President
Olivier Cuvillier, ARTP Treasurer

5:45 p.m.

End of the meeting

Table of contents

Session I :
ARTP 2021 Laureates

1

Thierry CAPIOD

Institut Necker - Inserm U1151- Université Paris Descartes

GPRC6A, macrophages et cancer de la prostate

Prostate cancer is the most frequent cancer in men after 50 years of age and the second cause of death by cancer in men. While most of the prostate cancer are not lethal, around a fifth of them will cause death. Emerging evidence show that G protein-coupled receptors are important players of the interaction between tumor cells and microenvironment. Among them, one of the predominant markers is GPRC6A, whose expression in prostate tumors is correlated with aggressive cancers. Our preliminary data show for the first time that GPRC6A is expressed in macrophages within the tumor microenvironment (TME) of prostate cancers. Further analyses showed that GPRC6A+ macrophages in prostate tumor are anti-inflammatory and respond to its ligand, Osteocalcin, by promoting autophagy machinery. Therefore, we hypothesize that the presence of GPRC6A+ macrophages within the TME is of bad prognosis for patients and that Osteocalcin could promote GPRC6A-mediated signaling pathways, which participate to the progression and bone metastasis initiation of prostate cancer.

Session I :
ARTP 2021 Laureates

1

Khahn Le-Thi

CRCM, Inserm 1068, Université d'Aix-Marseille

'DDX5 mRNA-targeting antisense oligonucleotide as a new promising therapeutic in combating castration-resistant prostate cancer'

The heat shock protein 27 (Hsp27) has emerged as a principal factor of the castration-resistant prostate cancer (CRPC) progression. Also, an antisense oligonucleotide (ASO) against Hsp27 (OGX-427 or apatorsen) has been assessed in different clinical trials. Here, we illustrate that Hsp27 highly regulates the expression of the human DEAD-box protein 5 (DDX5), and we define DDX5 as a novel therapeutic target for CRPC treatment. DDX5 overexpression is strongly correlated with aggressive tumor features, notably with CRPC. DDX5 downregulation using a specific ASO-based inhibitor that acts on DDX5 mRNAs inhibits cell proliferation in preclinical models, and it particularly restores the treatment sensitivity of CRPC. Interestingly, through the identification and analysis of DDX5 protein interaction networks, we have identified some specific functions of DDX5 in CRPC that could contribute actively to tumor progression and therapeutic resistance. We first present the interactions of DDX5 and the Ku70/80 heterodimer and the transcription factor IIH, thereby uncovering DDX5 roles in different DNA repair pathways. Collectively, our study highlights critical functions of DDX5 contributing to CRPC progression and provides preclinical proof of concept that a combination of ASO-directed DDX5 inhibition with a DNA damage-inducing therapy can serve as a highly potential novel strategy to treat CRPC.

Session I :
ARTP 2021 Laureates

1

Anne CHOTTEAU

Institut de Biologie de Lille, UMR 8161, Université de Lille

Récepteur MET et fusions ETS : co-acteurs de la progression tumorale du cancer de la prostate

Prostate cancer (PCa) is the most common male cancer in France and in Western countries. It is a slowly progressing cancer, with an average age of diagnosis of 70 years and for which the treatment is very dependent on the stage of evolution. The appearance of metastases, most of which are in the bone, is a sign of the dramatic evolution of the disease and the incidence of mortality.

There are few good markers for the diagnosis and/or prognosis of the disease and its various stages. Certain players have been identified, including MET receptor signaling and the involvement of ETS fusion genes, particularly ERG and ETV1 fusions, resulting from chromosomal changes.

The program we have developed aims to decipher the functional interface between the MET receptor and ETS fusions in CaP progression and particularly in the metastatic progression associated with androgen resistance.

For that, we used different prostate cancer cell lines, PC3 and PC3M (up to now) from which we established overexpressing ERG and ETV1 models.

Phenotypic evaluations showed that ERG as well ETV1 overexpression increases the migration and invasion properties and that the activation of MET signalisation enhances these responses.

In vivo, subcutaneous injection of these cellular models in mice knock-in with human HGF depicted more aggressive abilities of ERG and ETV1 overexpressing cells. Interestingly, treatment with the TKI (tyrosine kinase inhibitor) Capmatinib reverses these tumorigenic properties, highlighting an intricated interaction between MET signaling and ERG/ETV1 fusions in CaP aggressiveness.

To complete these approaches, large scale comparative transcriptomic analyses of these PC3 and PC3M models are in progress to identify pertinent target genes and signatures.

We will now seek to validate these data in human by studying the clinical relevance of the results obtained on cohorts of patients and TMA of different stages of CaP.

Knowledge of the mechanisms involved should lead to identify new partnerships and molecular players that could be used in therapy or serve as diagnostic and prognostic tools.

Session II : Microbiome

2

Karina Dalsgaard Sørensen

Department of Molecular Medicine, Aarhus University, Denmark

Molecular analyses of the prostate tumor microenvironment and microbiome: Clinical implications?

Prostate cancer (PC) is characterized by extensive molecular heterogeneity that is also reflected in highly variable disease courses. Currently used prognostic tools for early stage PC risk stratification are inaccurate and new biomarkers are needed to facilitate better and more personalized treatment. It is becoming increasingly clear that solid tumors are complex ecosystems and that the disease course of PC is controlled not only by the molecular profile of the cancer cells, but also affected by interactions with and between various stromal and immune cell types in the tumor microenvironment (TME). In addition, recent lines of evidence point to a role of the human microbiota in PC development and/or progression, although this area of research is still in its infancy. Based on this, we hypothesized that a deeper understanding of the TME and microbiome of PC may enable discovery of new biomarkers that better reflect the complexity of PC biology and hence also more accurately predict disease aggressiveness. As a first step towards development of such biomarkers, we performed bulk tumor RNA sequencing and spatial molecular analyses of primary tumor tissue samples from early stage PC patients. Based on this, we identified and validated new immune cell profiles prognostic for PC patient outcome after radical prostatectomy. In addition, our metatranscriptomic analyses identified several microbial species to be in dysbiosis in PC tissue and between different pathological stages of PC. Dysbiosis was associated with altered immune regulation in the TME, indicating host-pathogen interactions in the TME. Together, our findings point to the future possibility of using TME profiling and/or certain microbial species as prognostic biomarkers in PC, or even as novel treatment targets.

Session II : Microbiome

2

Aurora Perez-Cornago
Oxford University, UK

Diet, nutrition, and prostate cancer risk

Session II : Microbiome

2

Nicolo Pernigoni

IOR, Bellinzona, Switzerland

Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis

The microbiota comprises the microorganisms that live in close contact with the host, with mutual benefit for both counterparts. The contribution of the gut microbiota to the emergence of castration resistant prostate cancer (CRPC) has not yet been addressed. We found that androgen deprivation in mice and humans promotes the expansion of defined commensal microbiota that contributes to the onset of castration resistance in mice. Specifically, the intestinal microbial community in mice and patients with CRPC was enriched for species capable of converting androgen precursors into active androgens. Ablation of the gut microbiota by antibiotic therapy delayed the emergence of castration resistance even in immunodeficient mice. Fecal microbiota transplantation (FMT) from CRPC mice and patients rendered mice harboring prostate cancer resistant to castration. In contrast, tumor growth was controlled by FMT from hormone-sensitive prostate cancer patients and Prevotella stercorea administration. These results reveal that the commensal gut microbiota contributes to endocrine resistance in CRPC by providing an alternative source of androgens.

Session III :
Clinical Session

3

Pascal PUJOL

CHU Montpellier

Testing BRCA et HR dans le cancer de la prostate : qui quand comment ?

Session III : Clinical Session

3

Gwenaëlle GRAVIS

Institut Paoli-Calmette, Marseille

Place de l'immunothérapie dans le cancer de la prostate métastatique

Le cancer de la prostate est la première cause de cancer chez l'homme et 3ème cause de décès, par évolution métastatique, dont le traitement est actuellement la suppression androgénique associée aux hormonothérapies de nouvelle génération et/ou les chimiothérapies à base de taxanes (docetaxel, cabazitaxel). Plus récemment l'efficacité des radiothérapies métaboliques par PSMA lutetium et Rad 223, ont étoffé l'arsenal thérapeutique ainsi que des traitements ciblant les altérations des gènes de la réparation de l'ADN comme l'olaparib. La médiane de survie reste cependant aux alentours de 3 ans en situation de cancer de la prostate métastatique résistant à la castration (mCRPC). De nouvelles thérapeutiques efficaces sont nécessaires dans cette pathologie toujours mortelle.

Le sipuleucel, immunothérapie cellulaire autologue a été la première immunothérapie vaccinale qui a démontré un bénéfice de survie dans le cancer mCRPC peu ou pas symptomatique. Malheureusement les inhibiteurs des points de contrôles de l'immunité (ICI) n'ont pas fait la preuve de leur efficacité dans le cancer de la prostate métastatique. La faible charge mutationnelle et la faible expression de néo antigènes tumoraux sont en partie responsable de cette faible réponse. Le rôle immunosuppressif du micro-environnement tumoral prostatique contribue également à cette rare efficacité de l'immunothérapie. Ces éléments participent à qualifier le cancer de la prostate comme une tumeur froide.

De nombreuses associations d'ICI avec de la chimiothérapie, hormonothérapie de nouvelle génération ou anti-PARP ont été évalués en phase II/III avec des résultats décevants. De nouvelles approches sont en développement pour tenter de contourner les résistances tumorales comme la manipulation du micro environnement ou l'utilisation de CAR-T cell ou d'antigènes bispécifiques.

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Session III : Clinical Session

3

Gaëlle FROMONT

CHRU, Inserm UMR1069, Tours

Cribriform and intraductal prostate cancer: pathological, clinical and molecular specific features

Cribriform growth pattern is a specific form of invasive prostate cancer (PCa), while intraductal carcinoma (IDC) is characterized by proliferation of cancer cells within distended pre-existing prostate acini or ducts. Cribriform pattern and IDC are often indiscernible on HE staining without immunohistochemistry and are therefore frequently analysed together. IDC must be differentiated from high grade PIN, a precancerous lesion with a radically different prognosis.

The presence of either IDC or cribriform pattern is associated with adverse pathologic parameters, including lymph node metastasis, and represents an independent predictor of reduced progression-free survival in hormone naïve and CRPC patients.

Cribriform pattern and/or IDC are also associated with specific molecular features, including increased PTEN loss, and more frequent alterations of DNA repair genes, homologous recombination related (HRR) and mismatch repair (MMR) genes. These molecular characteristics could confer to cribriform/IDC patterns increased sensitivity to targeted treatments, including PARP inhibitors and immunotherapy.

Session IV : Cellular heterogeneity and lineage plasticity

4

Douglas STRAND

LIVE VISIO

UT Southwestern Medical Center, Dallas, USA

Prostate lineage plasticity and stem cells

The ability of the prostate to regenerate after castration and androgen replenishment has sparked a historical debate about whether regeneration is mediated by a pre-existing proximal niche of progenitors or survival and growth of pre-existing distal luminal cells. Recent data from single cell RNA sequencing and spatial transcriptomics experiments provide a nuanced interpretation. Pre-existing proximal cells are actually extensions of the urethra that survive castration. While they form organoids at higher frequency than distal prostate luminal cells, lineage tracing shows they do not regenerate the prostate. Instead, clonal analysis shows that distal prostate luminal cells undergo plasticity to resemble urethral luminal cells, which is confirmed by spatial and single cell transcriptomics in human prostate.

Session IV : Cellular heterogeneity and lineage plasticity

4

Wouter KARTHAUS
École polytechnique fédérale de Lausanne, Switzerland

Understanding and targeting lineage plasticity in prostate cancer

In many cancers, treatment failure is linked to changes in tumor cell state or lineage, and often involves the reactivation of stem-like or developmental transcriptional programs. How this plasticity unfolds at a molecular level and whether it plays a causal role in drug resistance remains unclear. In this talk I present the origin and dynamics of lineage plasticity in the benign prostate epithelium and prostate cancer and its relationship to antiandrogen-based therapeutic resistance. In time course experiments utilizing genetically engineered mouse models and murine organoid cultures of prostate cancer, we find that plasticity initiates in an epithelial population defined by mixed luminal and basal lineage gene expression, and that it depends on elevated JAK and FGFR kinase activity. Organoid cultures from patients with late-stage castration-resistant disease harboring mixed-lineage cells reproduce the dependency we observe in mice, by upregulating luminal gene expression upon JAK and FGFR kinase inhibitor treatment. Single-cell analysis of human tumor samples confirms the presence of mixed lineage cells with elevated JAK/STAT and FGFR signaling in a subset of patients with metastatic disease, with implications for stratifying patients for clinical trials.

Session V : Poster session

5

MECHANIMS OF EPITHELIAL MESENCHYMAL TRANSITION BY LXRS IN ADVANCED PROSTATE CANCER

Bouchareb E, Bunay J, Lobaccaro J.M , Degoul F , De Joussineau C, Morel L, Kocer A , Baron S

iGReD, Clermont-Ferrand, France

iGReD, Clermont-Ferrand, France

Background: Most prostate cancer related deaths correlates with the metastatic potential of the tumor. One of the main process involved in the metastatic process is the "Epithelial-Mesenchymal Transition" (EMT) in which epithelial cells lose their adhesive properties and acquire a mesenchymal-like morphology. LXRs (Liver X Receptors) are nuclear receptor that have been shown to downregulate EMT processs in many cancers. In this project, we are defining how LXRs pathway controls EMT in prostate cancer using in vitro and in vivo models.

Methods: To study the underlying molecular mechanisms by which LXRs signaling controls EMT, we use several metastatic prostate cancer cell lines treated with LXRs agonist. In vivo, we use Nod-Scid Gamma immunodeficient mice, allowing us to study metastatic spread of xenograft cells and the effect of LXRs activation in this process.

Results: Activation of LXRs increases migration of PC3 cells and EMT markers, notably Vimentine.

In vivo, LXRs activation augments metastatic spread in our mice model. Finally, there is a positive correlation between LXRs target genes and Vimentine in human prostate cancer samples.

Conclusions:

LXRs have been described to inhibits EMT process in several cancers. However, many studies reporting LXRs protumoral effect have emerged, especially in Breast Cancer research. Here, we show that activation of LXRs significantly increases migration of human metastatic prostate cancer PC3 cell line and Vimentine accumulation, suggesting an activation of EMT by LXRs. It results to higher metastatic spread in vivo.. Interestingly, there is a positive correlation between LXRs target genes and Vimentine expression In human metastatic samples, suggesting a protumoral effect of LXRs in advanced prostate cancer.

MET RECEPTOR AND ETS FUSIONS: CO-ACTORS IN THE METASTATIC PROGRESSION OF PROSTATE CANCER

Elisa Carouge1, Audrey Dengremont1, David Tulasne1, Anne Chotteau-Lelièvre1

1Univ.Lille, CNRS, Inserm,CHU Lille, Target teams « efficacy & Resistance to anti-tumor targeted Therapies", UMR9020-U1277-CANTHER-Cancer Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France

Background: Prostate cancer (PCa) has the highest incidence among male cancers in European and American countries. In advanced stages, which may be metastatic, there is a high mortality rate. MET receptor and ETS transcription factors (ERG and ETV1) are important actors in PCa progression. MET is overexpressed in hormone-resistant tumors and in bone metastasis. ETS can be overexpressed throughout the disease. Interestingly, there are many functional links between MET receptor and ETS transcription factors suggesting their belonging to the same regulatory pathway. The aim of our study is to understand the collaboration between these two actors in PCa.

Methods: Cellular models expressing ERG or ETV1 fusions and MET by retroviral infection, in vitro phenotypic with Incucyte® also migration and invasion in transwell assay, comparative transcriptomic analyzes (RNAseq) and in vivo tests in humanized SCID mice (expressing human HGF).

Results: In vitro phenotypic tests showed that, ERG and ETV1 transcription factor induce more migration/invasion capacities and activation of MET signalling amplified these responses. In vivo subcutaneous injection in humanised HGF mice showed that of ERG/ETV1 overexpressing cells leads to bigger tumors. Moreover, administration of a Tyrosine Kinase Inhibitor (capmatinib) blocking MET signalisation, considerably reduced the tumoral volume induced by the expression of ERG and ETV1.

Conclusion: Our results show for the first time, a collaboration between MET receptor and ERG/ETV1 transcription factor in our PCa models. Our goal is to know whether MET receptor signaling uses ERG or ETV1 transcription factors as a relay to induce cancerization and metastasis formation. If it is really so, it could be advantageous for patient presenting ETS fusions and MET receptor to have a treatment by TKI. It will be a big step in PCa knowledge and that also should lead to new therapies for patients.

AN ANTI-OXIDANT DRUG IMPROVES URINARY SYMPTOMS OF A MOUSE MODEL OF BENIGN PROSTATIC HYPERPLASIA: A PROGENITOR CELL-DEPENDENT EFFECT?

PACREAU[1], Emilie NAVARRO[1], Manon BAURES[1], Stefano PALEA[2], Sékou Siramakan DIARRA[2], Natascha PIGAT[1], Marine LUKA[3,4], Mickaël MENAGER[3,4], Nicolas BARRY DELONGCHAMPS[1], Ahmed HAMAÏ[1], Jacques-Emmanuel GUIDOTTI[1], & Vincent GOFFIN[1]

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4 Labtech Single-Cell@Imagine, Imagine Institute, INSERM UMR 1163, Paris, France.

Background. Almost half of the patients with benign prostatic hyperplasia (BPH) remain unsatisfactorily addressed by current medications. Treatment with 5-ARI has been shown to promote the plasticity of androgen-dependent luminal cells towards a progenitor-like, androgen-independent molecular profile, which may support therapy resistance (Joseph et al 2021). Based on the increased hallmarks of oxidative stress in BPH, the aim of this study was to evaluate the potential beneficial effects of an anti-oxidative drug (named Z1017) in preclinical models of BPH.

Methods. Probasin-Prolactin (Pb-PRL) mice exhibit hypertrophied prostates sharing several histopathological features with human BPH (Pigat & al, 2019). Z1017 (vs vehicle) was administered per os daily for 28 consecutive days. Urodynamics (metabolic cages), histopathological/molecular characterizations (IHC, WB, qPCR), and single-cell RNA-seq profiling (10x genomics technology) were performed at the end of treatment.

The organoid assay was used to assess the effect of Z1017 on the progenitor capacities of sorted Pb-PRL prostate epithelial cells. The BPH-1 cell line was used to assess its effects on cell proliferation (Trypan blue assay)/viability (DAPI) and gene expression (qPCR and WB), enzymatic activity (Aldefluor Kit), and oxidative stress (DCFDA probe by FACS).

Results: Z1017 treatment reduced protein carbonylation (oxidative stress hallmark), epithelial cell proliferation, and significantly improved urinary symptoms of Pb-PRL mice (decreased urination frequency & increased urine volume per voiding). Single-cell transcriptomics revealed strong enrichment of epithelial intermediate/progenitor clusters in Pb-PRL vs WT mice; this cell hierarchy was altered by the treatment. Functionally, Z1017 reduced the number and/or size of organoids generated from various Pb-PRL mouse epithelial cell populations. PPARg expression (Strand & al. 2012) and ALHD activity (Burger & al 2009) are two hallmarks of prostate epithelial progenitor proprieties. In BPH-1 cells, both were drastically reduced following Z1017 treatment.

Conclusion: These results suggest that Z1017 hampers prostatic epithelial cell progenitor capacities. Z1017 appears as a promising medicine for the treatment of LUTS (lower urinary tract symptoms) in patients with BPH.

FUNCTIONAL, STRUCTURAL AND BINDING STUDIES OF THE ATYPICAL ER-RESIDENT PROTEIN FKBP7, A POTENTIAL TARGET IN CHEMORESISTANT PROSTATE CANCER

Luce DRENO[1,2,3,4], Sofian LACOSTE[1,2], Carolina TIRABOSCHI[5], Marine GARRIDO[1,2,3], Mariana TANNOURY[1,2], Eric JACQUET[3,4], Naima NHIRI[3,4], Yohann LORIOT1,[2,3], Daniel COMPAGNO[5], Nadine ASSRIR[3,4], Ewen LESCOP[3,4],*, Anne CHAUCHEREAU[1,2,3],*

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* Principal Investigator

Background: Metastatic Castration Resistant Prostate Cancer is the latest stage of Prostate Cancer (PCa) and remains lethal. We identified FKBP7 as a potential therapeutic target of interest in PCa. FKBP7 is overexpressed in taxane-resistant PCa cells, impacts both cell proliferation and Docetaxel efficacy in chemoresistant models, and interacts with eIF4F, a component of translation initiation. FKBP7 is an ER-resident FKBP still largely unknown.

Methods: FKBP7 expression was assessed by Western Blot. Impact of FKBP7 expression on clinical prognosis was examined on public cancer databases. Subcellular localization of FKBP7 was determined by digitonin-based cell fractionation. Structural modeling and sequence alignments were used to obtain FKBP7-predicted structure. Finally, we produced and purified 15N enriched recombinant catalytic domain of FKBP7 and collected high quality 15N HSQC NMR spectra in presence or absence of ligands.

Results: FKBP7 expression increased upon treatment with several cytotoxic chemotherapies in PCa-cell lines, and TCGA data underlined FKBP7 impacts survival in other solid cancers than PCa. Subcellular fractionation showed FKBP7 localizes in the Endoplasmic Reticulum (ER) but also in the cytosol, independently of proteasomal degradation. We determined FKBP7 is N45-glycosylated and observed that cytosolic FKBP7 is mainly glycosylated. At last, structural modeling predicted a potential substrate specificity for FKBP7 catalytic pocket with a distinct composition for charge and bulkiness compared to other FKBPs. Accordingly, NMR revealed FKBP7 strongly binds Rapamycin and Everolimus, but surprisingly not FK506.

Conclusions: Our results suggest FKBP7 could be a druggable target in adaptive resistance of other solid cancers. They also point atypical properties for an ER-resident protein. We propose a model in which FKBP7 could acquire new functions in the cytosol, such as the reprogramming of the proteome mediated by its interaction with its cytosolic partner eIF4F. Besides, we gathered new structural data showing FKBP7 presents a selective binding profile, encouraging the development of future drug-targeting strategies.

ANDROGENRECEPTOR-MEDIATED TRANSCRIPTIONAL REPRESSION TARGETS CELL PLASTICITY IN PROSTATE CANCER

Éva Erdmann[1], Pauline Ould Madi Berthélémy[1], Félicie Cottard[2], Charlotte Zoe Angel[3], Edwige Schreyer[1], Tao Ye[1], Bastien Morlet[1], Luc Negroni[1], Bruno Kieffer[1] and Jocelyn Céraline[1,4,5]

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Background:

Androgen receptor (AR) signaling remains the key therapeutic target in the management of hormone-naïve-advanced prostate cancer (PCa) and castration-resistant PCa (CRPC). Recently, landmark molecular features have been reported for CRPC, including the expression of constitutively active AR variants that lack the ligand-binding domain. Besides their role in CRPC, AR variants lead to the expression of genes involved in tumor progression. However, little is known about the specificity of their mode of action compared with that of wild-type AR (AR-WT).

The aim of this project was to decipher a full landscape on distinctive transcriptional activities of AR-WT, AR-V7, and AR-Q641X in PCa cells and to specify the molecular mechanisms involved in cell plasticity during tumor progression.

independence (AI) by tumor cells. Neuroendocrine markers can be detected in many tumors subjected to ADT, but hardly detected in ADT naïve tumors, supporting the idea that ADT sets off the NED of PCa. Long non coding (lnc)RNAs play an important role in tumorigenesis, contributing to all hallmarks of cancer. Aberrant IncRNAs expression has been associated with different malignancies, PCa included. Recently, thousands of lncRNAs have been reported to be expressed specifically in neuroendocrine prostate cancer, but their role in NED is still unknown.

Methods:

We performed AR transcriptome analyses in an androgen-dependent PCa cell line as well as cross-analyses with publicly available RNA-seq datasets. The Gene Set Enrichment Analysis, Gene Ontology and Enrichr tools allowed the identification of significantly enriched pathways in different experimental conditions. Furthermore, a proximity-dependent biotin identification (BioID2) approach was used to analyze a potential difference in partner recruitment between AR-WT and constitutively active AR variants.

Results:

We established that transcriptional repression capacity that was marked for AR-WT was pathologically lost by AR variants. Functional enrichment analyses allowed us to associate AR-WT repressive function to a panel of genes involved in cell adhesion and epithelial-to-mesenchymal transition. The BioID2 approach by biotinylating proteins that interacted directly or indirectly, or were within proximity (~ 10 nm) to DHT-activated AR-WT, AR-Q641X or to AR-V7 led us to highlight a lower recruitment of corepressors by constitutively active AR variants.

Conclusion:

So, we postulate that a less documented AR-WT normal function in prostate epithelial cells could be the repression of a panel of genes linked to cell plasticity and that this repressive function could be pathologically abrogated by AR variants in PCa.

PROSTATE CANCER AND COVID-19: ROLE OF ANDROGENS IN SARS-COV-2 VIRAL INFECTION AND COVID-19 PROGRESSION

Aline Hantute-Ghesquier[1], Anthony Turpin[1], Valentin Sencio2, Anne Flourens[1], Nathalie Vanpouille[1], Cyril Robil[2], Yvan De Launoit[1], David Tulasne[1], Sandrine Belouzard[2], François Trottein[2], et Martine Duterque-Coquillaud[1]

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Background:

Men with COVID-19 are more likely than women to be hospitalized, admitted to intensive care or die from the disease. Physiological effects of androgens are among the factors that may contribute to this difference. It is also already known that the expression of the TMPRSS2 gene, which encodes a crucial protease for virus entry, is regulated by androgens in prostate. Our aim is to investigate the effect of gene expression modulation by androgens on SARS-CoV-2 infection both in vitro in a prostate cell model and in vivo in an animal model and in men.

Methods and results:

We selected a hormone-dependent cell line stably overexpressing ACE2, the protein required for SARS-CoV-2 infection to measure the effect of hormonal modulation of the TMPRSS2 gene on SARS-CoV-2 infection. The results show that the absence of androgen decreases viral infection, and particularly viral entry, via the loss of TMPRSS2 expression. Conversely, the presence of testosterone increases the rate of viral infection.

In vivo, to study the effect of androgens on SARS-CoV-2 infection and disease severity over time we used a hamster model, for which we performed androgen suppression by castration. Our results show an earlier pulmonary inflammatory response in castrated hamsters compared to non-castrated ones, assessed both by a pathological diagnosis and by monitoring inflammatory cytokines. This kinetic shift in response to SARS-CoV-2 infection reveals an effect of androgens in the lung via an altered transcriptional program.

Finally, in men, we exploited the national multicenter cohort, CACOVID-19, established by the cooperating oncology groups during the first wave of COVID-19. We evaluated the degree of severity of SARS-CoV-2 infection in patients with cancers, particularly androgen-dependent cancers such as prostate cancer, according to whether or not they were treated with androgen deprivation therapy (ADT). Data analyses suggest that ADT is associated to a less severe form of COVID-19.

Conclusion:

Our results indicate a role for androgens in the SARS-CoV-2 infection process and in the kinetics of disease progression. Our study points towards adapting the management of prostate cancer patients under hormone-suppressive therapy.

INHIBITION OF HYPUSINATION REPROGRAMS PROSTATE CANCER CELL METABOLISM AND DECREASES AGGRESSIVENESS

Michel KAHI[1], Abigaïl MAZZU[1], Mathieu CARLIER[1], Marie IRONDELLE[2], Sandra LACAS-GERVAIS[3], Amine BELAID[1], Luc CAMOIN[4], Stéphane AUDEBERT[4], Issam BEN-SAHRA[5], Nathalie MAZURE[1], Frédéric BOST[1]

Background:

Prostate cancer (PCa) is a major public health problem, and the treatment of advanced stages remains not curable. Our team is interested in innovating therapeutic approaches targeting cancer cell metabolism. Cancer cells adapt their metabolism to resist various stresses and treatments and to provide the metabolites, energy and co-factors required for their proliferation and progression. Here, we focus on the polyamine/hypusination pathway which is associated with poor prognosis in PCa. Hypusination is a unique post-translational modification of the eukaryotic translation initiation factor 5A (EIF5A). This reaction is dependent on spermidine (a polyamine) and it is regulated by two enzymes, the deoxyhypusine synthetase (DHPS) and the deoxyhypusine hydroxylase (DOHH). Hypusination is involved in several cellular processes such as autophagy, metabolism, senescence, and differentiation, however, the mechanism by which it is implicated in tumor growth and metastasis is still unclear. To elucidate its role in PCa, we inhibited the enzymes that catalyzes this reaction and investigated the effects on PCa cells aggressiveness and metabolism.

Methods:

To study the effect of hypusination on the aggressiveness of PCa we inhibited this reaction with a pharmacological compound GC7 and Ciclopirox and performed cell counting, Brdu proliferation test, cell cycle analysis and migration assay. To explore the role of hypusination on the metabolism of PCa cells, we performed steady state metabolomics, 13C-glucose tracing and measured mitochondrial respiration, glucose consumption and lactate production. We also studied the phenotype of mitochondria by electron microscopy and Mitotracker.

Results:

We have shown that inhibition of hypusination decreases cell growth, cell migration and mitochondrial respiration. In addition, our metabolomic and proteomic analysis revealed a reprogramming in cancer cell metabolism and an alteration of the mitochondria and its respiratory chain

Conclusions:

Inhibition of hypusination decreases cell growth, cell migration and mitochondrial respiration three biological processes implicated in PCa aggressiveness and metastasis. Our results highlight a potential therapeutic opportunity for PCa that target hypusination and could be used for clinical applications.

SODIUM- CALCIUM SIGNALING UNDERGOING METASTATIC POTENTIAL OF PROSTATE CANCER CELLS

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Background:

Despite considerable progress in terms of diagnosis and treatment, prostate cancer represents the third most cause of death with more than 375,000 deaths per year. The treatment options are limited after the tumor has metastasized. A new model has been generated from circulating tumor cells (CTC) which will help us to have deeper insights in understanding the mechanisms involved. Previous study from our laboratory have described that ion channels control some of the "hallmarks of cancer" such as tumor angiogenesis, migration, and metastasis, thereby paving the way to a new chapter in oncology. Recently we have performed bioinformatic analysis of prostate tumors, revealing several mutations of NALCN, a sodium channel which we previously described as actor of invasion (a crucial step before metastasis) during prostate cancer (PCa). During my thesis, I am investigating; 1) the role of ion channels in metastasis using CTC derived cell line as a pre-clinical model and 2) the role(s) of newly reported NALCN mutations during prostate cancer progression.

Methods:

Sodium and Calcium Imaging, Patch Clamp Technique, Drug Assays, Cell Biology techniques including qPCR and Western Blot, Proliferation Assay, Migration Assay, Invasion Assay, RNA Seq etc.

Results:

Analysing the RNA sequencing (available from Faugeroux et al. 2020) on CTC derived cells, differential expression of a number of ion channels in comparison to LNCaP is noticed. Understanding the ion homeostasis of CTC derived cells, from calcium imaging using Fura-2, it is observed that the store mediated calcium entry (SOCE) of these cells is lower than the SOCE release in androgen dependent LNCAP and neuroendrocrine derived LNCaP, LNCaP C4-2 cells. This characteristic property is in accordance with the lower expression of ORAI 1 and STIM1 seen in RNA sequencing which is confirmed by qPCR. We have preliminary data supporting this as a mechanism of these highly metastatic cells to resist cell death (Flourakis, Matthieu, et al.).

Conclusion:

It is crucial to discover new and effective therapies for advanced PCa and in this study, preliminary evidences of role of ion channels in the regulation of cell-survival pathways of highly metastatic cells are observed.

TRPM4 REGULATES CYTOSOLIC Ca2+ OSCILLATIONS AND SECRETOME IN CHEMOTHERAPY-INDUCED SENESCENCE

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Cellular senescence is characterized by a stable cell cycle arrest, various macromolecular changes, and a hypersecretory, pro-inflammatory phenotype- the senescence-associated secretory phenotype (SASP). During chemotherapeutic treatment, DNA damaging agents may induce senescence in benign cells of the tumor microenvironment resulting in SASP production that act in a paracrine manner promoting tumor resistance phenotypes. Here, by q-RT-PCR, we show that TRPM4 is upregulated in response to DNA-damaging chemotherapeutic drugs in prostate stromal cells. By western blot analysis, we identify that the isoform that is upregulated represents the channel's dominant negative, short isoform, rather than the wild type, full-length isoform. TRPM4 appears to reshape Ca2+ homeostasis and control the oscillatory behavior of persistent DNA damage-induced- senescent cells. Moreover, we show that conditioned medium from senescent stromal cells enhances the invasive capacity of epithelial prostate cancer cells, which can be limited by silencing TRPM4 in stromal cells.

Our results suggest TRPM4 as a novel tumor microenvironment regulator in prostate cancer progression in response to chemotherapy.

COLLAGEN REMODELING LEADS TO INFLAMMATION-FREE EXPANSION OF PERIPROSTATIC ADIPOSE TISSUE AND PROMOTES PROSTATE CANCER PROGRESSION

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Prostate cancer (PCa) is the second most common form of solid tumor in men worldwide and the fifth leading cause of death from cancer. It is a highly heterogeneous disease, ranging from slow-growing indolent tumors to fatal metastatic carcinomas. The adipose tissue surrounding the prostate, called periprostatic adipose tissue (PPAT), has emerged recently as an important factor in the progression of the disease. PPAT accumulates independently of body mass index and its abundance correlates with PCa progression, but the mechanism remains unexplained. Here, we used a statistical approach to define abundant PPAT by normalizing PPAT volume to prostate volume in a cohort of 351 patients with a linear regression model.

We find that tumors from patients with abundant PPAT exhibit several hallmarks of aggressiveness such as undifferentiated tumors, increased fibrosis, angiogenesis and macrophage infiltration. Multivariate analysis using a logistic regression model indicate that PPAT abundance is an independent risk factor for occurrence of aggressive PCa. We show that abundant PPAT expands by adipocyte hypertrophy but this does not result in inflammation. A proteomic study using isolated adipocytes reveals that abundant PPAT display under-representation of proteins involved in mechano-sensing and cytoskeletal contractile force generation as compared to non-abundant PPAT. Using 3D confocal microscopy, we find that abundant PPAT has a looser collagen network than less abundant PPAT and exhibits increased collagen VI degradation associated with production of endotrophin, a matrikine already known to promote breast cancer progression. We find high levels of endotrophin in the urine of patients with abundant PPAT, indicating the clinical relevance of our findings. Our new and robust definition of PPAT abundance could be applied to clinical practice to improve risk stratification. Endotrophin is a good candidate biomarker and a potential pharmacological target to dampen PCa progression. Knowledge of the mechanism(s) that initiate PPAT expansion might allow clinicians to identify preventive approaches to decrease PPAT abundance in order to improve the outcome of PCa patients.

Novel Phenazinium Compound Downregulates Stress-induced Signaling Pathways and Restores Treatment Sensitivity of Therapy-Resistant Prostate Cancer

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Background:

Prostate cancer (PC) represents one of the most common male cancers and PC progression due to Castration-Resistant Prostate Cancer (CRPC) after ablation has been seen as a menace to be controlled. Docetaxel-based regimens and, Cabazytaxel and Abiraterone are chemotherapies that have shown improved survival in CRPC but re-occur after months of treatments. A novel class of chemotherapy is needed to restore prostate cancer progression due to the over-expression of stress-induced signaling pathways and some target survival proteins like Hsp27, eIF4E, and TCTP that block apoptosis and therapy sensitivity. In this study, we carried out in vitro experiments with a novel Phenazinium 8b compound which is able to downregulate the signaling pathways and restore treatment sensitivity on PC-3 and LNCaP prostate cancer cell lines, without having adverse effects on normal epithelial prostate cell line PNT1A to serve as an excellent treatment strategy against CRPC and as theranostic compound.

Methods:

1.) MTT test for measuring proliferation, viability and cytotoxicity of cells.

2.) Western blotting to evaluate protein interactions and expressions.

3.) FACS Flow- Cytometry analysis to determine inducement of apoptosis and cell cycle arrest.

4.) RT-PCR for messenger RNA quantification.

5.) Confocal Microscopy used for Cellular Localization and Internalization of the compound

Results:

The results showed the compound has high cytoplasmic localization and the ability to inhibit protein interactions and expressions of Hsp27, eIF4E & TCTP, decrease cell viability, proliferation, cytotoxicity and messenger RNA expression, induce apoptosis and cell cycle arrest, leading to prostate cancer cell death with less effect on normal epithelial prostate cells.

Conclusion:

Phenazinium 8b compound has high concentration in cytoplasmic distribution and permeability on prostate cancer cells, which enhanced targeting and inhibiting of anti-apoptotic signaling pathways. The compound has shown in vitro anticancer abilities and in vivo analysis will be done to further buttress its clinical potential as a therapeutic alternative to remedy CRPC sensitivity.

A NOVEL CYTOSTASTIC INHIBITOR OF HYPOXIC PROSTATE CANCER CELLS

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Background:

Hypoxia is a condition in which cells do not have enough supply of oxygen and is common in solid tumors. Prostate cancer (PCa), the second most common cancer in men, is also subjected to hypoxia. The hypoxic cells are resistant to radiotherapy and chemotherapy, metastasize, and thus have a very poor prognosis for patients. Hypoxia inducible factors (HIFs) are transcription factors that play a central role in detecting and adapting O2 level. They are heterodimers consisting of HIF-α and HIF-β. In mammals, HIF-α has different isoforms: HIF-1α, HIF-2α and less studied HIF-3α. HIFs help cancer cells adapt to hypoxic conditions and are involved in the establishment of treatment resistance. To date, only HIF-2α inhibitors have been tested and are currently in clinical trials. No specific HIF-1α inhibitor has yet been brought into the clinic. Thus, this project aims to characterize some novel HIF-1α inhibitors.

Methods: We tested compound A (comp. A), modified compound A (mod. comp. A) and compound B (comp. B) from marine sponge on human prostate epithelia P69 cell line, and on human PCa DU145 and PC3 cell lines. We tested effects of these compounds in hypoxia (1% O2) on HIF-1α stabilization (immunoblotting), nuclear localization (immunofluorescence), cell proliferation and viability, cell metabolism (glucose and lactate concentrations), and finally on gene expression (RNAseq).

Results: Our results show that the comp. A destabilized HIF-1a protein, blocked its nuclear translocation but was too toxic for normal cells. After modification, mod. comp. A was less toxic for normal cells, maintaining its ability to destabilize HIF-1a. Comp. B was able to destabilize HIF-1a, blocking its translocation to the nucleus and modifying the expression of HIF-1 genes. We observed a decrease in lactate production, leading to a reduction in cell proliferation in aggressive PC3 prostate cancer cells. RNASeq results showed that microtubule-related processes were affected. Interestingly, these compounds only worked in PC3 cells and not in DU145.

Conclusion: The compound B is a cytostatic-like inhibitor affecting microtubules, specific for hypoxic cancer cells, and may offer a novel therapeutic opportunity for prostate cancer.

the opportunities and challenges associated with the establishment of stable tumor organoid lines derived from PCa patients. Building upon this study, we will now explore the contribution of distinct growth factors to organoid generation with the ultimate goal of developing optimal organoid models.

NEUTROPHILS: THE IMMUNE CELL POPULATION DOMINATING MURINE PROSTATE CANCER

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Prostate cancer is the most common diagnosed cancer and the second leading cause of cancer-related death among men in western societies. Most immunotherapy trials were unsuccessful and the involvement of immune cells in tumour development has been only recently addressed. The novelty of our study lies on the deep phenotypical characterization of the heterogeneity of myeloid and lymphoid infiltrates in healthy murine prostate and during cancer progression in PTEN(i)pe-/- mice, in which the PTEN gene is selectively ablated in prostatic luminal epithelial cells at adulthood. The strict temporal control of PTEN inactivation along with the slow tumour progression allows to characterize the immune cells in the tumour microenvironment and to test therapeutic strategies.

By combining multiparametric flow cytometry, scRNA sequencing and confocal microscopy, we observed an increase in the CD45+ immune infiltration in prostate tumours compared to tumour-free prostates. We highlighted a massive influx of neutrophils, which were the most dominant immune cell population and harbored an immunosuppressive signature. Additionally, we noticed a heterogeneity in prostate macrophages, with one of the subsets being expanded during tumour progression. Moreover, we noticed an increased number of CD8+ T cells vastly expressing PD1 and CD44, indicating an activated/exhausted phenotype. Confocal microscopy revealed that neutrophils infiltrated the cancerous epithelium and accumulated abundantly in the lumen of the prostate, while macrophages and T lymphocytes were dominating the stromal area. Thus, our study established a detailed map of the myeloid and lymphoid infiltrates during prostate cancer progression in PTEN(i)pe-/- mice and unravelled potential immunotherapeutic targets which will benefit the tumour immunology field.

THERAPEUTIC TARGETING OF MENIN IN CASTRATION RESISTANT PROSTATE CANCER: A STRATEGY BASED ON LIPID-CONJUGATED ANTISENSE OLIGONUCLEOTIDES

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Background : Despite advances in prostate cancer (PCa) diagnosis and management, its morbidity remains high. The androgenic male hormone suppression (castration) remains the only effective therapy for PCa but the majority of patients will progress to castration resistant disease within 2-3 years which make castration resistant prostate cancer (CRPC) represent a serious challenge both to clinicians and drug developers. One of the strategies to improve current therapy for advanced CRPC involves targeting overexpressed survival genes. To achieve this, we first showed that the tumor suppressor Menin (MEN1) is overexpressed in high grade PCa and CRPC and its targeting by antisense oligonucleotide (ASO) technology could be a promising strategy in PCa therapy. For the past years, the intracellular delivery and silencing activity of oligonucleotides have been essentially completely dependent on the use of a delivery technology. Here we developed a lipid-modified ASO (LASO), evaluated its properties and examinated its efficiency in Menin knockdown without transfection reagent.

Methods : Western Blotting : To evaluate proteins expression after cell transfection with ASOs. MTT test : For Cell viability analysis. Dynamic Light Scattering (DLS) and Transmission Electronic Microscopy (TEM) : For physicochemical characterization of ASOs.

Results : First we showed that Menin-ASO down regulates Menin and causes a significant decrease in cell proliferation in both PC-3 and LNCaP cell lines in presence of transfection reagent which can be toxic to living organisms. Then we developed a lipid-modified ASO (LASO) and we showed that lipid-modification improves its penetration, but also its stability and efficiency in inhibiting Menin expression in vitro without transfection reagent. Finally, we showed that the coformulation with PSMA-Ligand conjugated allows forming hetero-nanomicelles that could increase Menin knockdown and provides specificity toward PCa cells.

Conclusions : Taken together, our results suggest that Menin plays an oncogenic role in CRPC which can make it a potential therapeutic target and its inhibition with ASO technology is a promising strategy in PCa therapy.

Session VI : 2021 ARTP Poster Prize Laureates

6

Growth Factor Regulation of Castration-Tolerant Luminal Progenitors of the Mouse Prostate

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Castration-resistant prostate cancer (CRPC) is a lethal disease. Once diagnosed, current therapeutic strategies do not improve overall survival more than a few months. The molecular and cellular mechanisms that drive cancer relapse remain poorly understood, which has prevented the development of efficient therapies for CRPC.

Our laboratory recently identified a population of castration-tolerant luminal progenitor cells that we called LSCmed according to their Lin-/Sca-1+/CD49fmed FACS profile. LSCmed cells are rare in WT mouse prostates, but are massively amplified in prostate cancer driven by loss of Pten tumor suppressor gene (Pten-null mice, a model of CRPC). This suggests that LSCmed cells contribute to pathological overgrowth of prostatic tissue. The goal of this study was to identify potential regulators of LSCmed progenitor ability.

We first used in silico approaches. Comparative transcriptome analysis of LSCmed, luminal, basal and stomal cells FACS-enriched from WT was performed in combination with an interactome study (CellPhone DB). These studies highlighted the expression of several genes associated with EGFR, IGF-1R and c-Met pathways and their potential interactions, respectively. These growth factor signaling pathways have been shown to regulate stemness and proliferation in various contexts including cancer. We challenged the effects of these ligands on LSCmed cell progenitor and proliferative abilities using the organoid assay. As expected, EGFR signaling was mandatory for organoid formation by Pten-null LSCmed cells. Remarkably, our results show that IGF-1/IGF-1R and HGF/c-Met signaling pathways are able to rescue organoid formation and growth in the absence of EGF in culture medium, suggesting redundancy.

While EGFR-targeting therapies have been proposed to affect cancer stem cells, our experiments using natural ligands and pharmacological inhibitors suggest that alternative pathways may overcome EGFR signaling blockade.

Session VI : 2021 ARTP Poster Prize Laureates

6

Impact of vitamin D signaling on prostatic precancerous lesions

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Prostate cancer is the most frequent visceral cancer and the second leading cause of cancer-related deaths in male of western societies. Due to late diagnosis and poor treatment efficiency of aggressive forms, a better understanding of the mechanisms underlying tumor initiation and progression is required to improve prostate cancer prevention and clinical care. Epidemiological studies indicate that low levels of circulating vitamin D and reduced expression of vitamin D receptor (VDR) correlate with prostate cancer risk and severity. Moreover, our team previously demonstrated that a treatment with a vitamin D analog reduces the severity of prostate cancer in PTEN(i)pe-/- mice, a mouse model that recapitulates the human disease, and in which the most frequently mutated tumor suppressor gene (PTEN) is selectively inactivated in prostatic epithelial cells (PECs) at adulthood.

To investigate the role of VDR signaling in prostatic tumorigenesis, we generated and characterized PTEN/VDR(i)pe-/- mice, in which PTEN and VDR are selectively inactivated. Our results showed that the proliferation and the associated DNA damage response are enhanced in VDR-deficient PECs, 1-month after gene inactivation (AGI). In addition, immunophenotyping revealed that the frequency of myeloid-derived suppressive cells (MDSC) and of exhausted CD8+ T cells are increased in PTEN/VDR(i)pe-/- prostates compared to PTEN(i)pe-/- ones, 3 months AGI. Moreover, at 9 months AGI, histological analysis revealed a more severe phenotype in PTEN/VDR(i)pe-/- prostates compared to PTEN(i)pe-/- ones. Importantly, metastasis are selectively detected in lymph nodes and livers of PTEN/VDR(i)pe-/- mice. Thus, vitamin D signaling in PTEN-deficient PECs limits their proliferation during PINs initiation and slows down prostate cancer progression.

Session VI : 2021 ARTP Poster Prize Laureates

6

Intratumoral sex steroid synthesis is involved in basal extrusion during prostate-like tumorigenesis, and its deprivation promotes the emergence of a new tumor cell population.

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Background:

Sex steroids and particularly androgens trigger prostate development, differentiation, but also sustains prostate cancer progression. Hormonal therapy based on androgen deprivation is then used to treat prostate cancer in its localized, locally advanced and metastatic stages. However, the role of deprivation in the early phases of tumorigenesis as well as in the appearance of castration resistant prostate cancer remains unassessed.

Material and methods:

Drosophila accessory gland is a functional equivalent of human prostate. In this organ, we have defined the conditions to reproduce early epithelial tumorigenesis by mimicking initiation, where rare clones of cells expressing oncogenic hits are surrounded by normal cells. These clonal cells then undergo a basal extrusion, the first step of invasion, to form primary tumors outside the epithelial compartment represented by the gland.

Ecdysone is the steroid hormone that sustains accessory gland reproductive function. In the adult fly, this steroid is produced by the accessory gland itself, and interacts with its specific Ecdysone Receptor (EcR). By RNAi interference, we have downregulated either ecdysone receptor or ecdysone synthesis only in the clonal cells.

Results:

In both cases, we observe an impairment of basal extrusion and extraepithelial tumor formation, a phenomenon to compare to prostate tumor regression in human undergoing androgen deprivation therapy. However, a new kind of tumorigenesis appears independently of the previously observed tumors, which bares characters of higher aggressiveness.

Conclusions:

We conclude that accessory gland early tumorigenesis relies on steroids. We furthermore demonstrate that, in vivo, this is mostly an intratumoral ecdysone synthesis that sustains basal extrusion. Finally, we show that clone-specific ecdysone deprivation induces the emergence of a new type of resistant tumorigenesis, two phenomena that are reminiscent of the suspected mechanisms of resistance to androgen deprivation therapy.

Our aim is now to understand which molecular mechanisms are promoted by ecdysone to induce early tumorigenesis and which ones induce the emergence of a new tumoral cell population in case of ecdysone deprivation, and then to evaluate their relevance for human pathology.

Developing new patient-derived models to study advanced prostate cancer

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Introduction & objectives

A better understanding of the mechanisms underlying PCa progression and treatment resistance is a prerequisite to improve survival of men with advanced metastatic prostate cancer (PCa). It is currently limited by the scarcity of experimental models. Here, we aimed at establishing and characterizing new patient-derived organoids xenograft (PDOX) models of advanced PCa.

Materials & methods

We used two patient-derived organoid lines (PDOs), previously generated in our laboratory. P20-11 PDOs were derived from a lung metastasis obtained from a patient with hormone-naïve PCa. P20-23 PDOs were derived from a transurethral prostate resection obtained from a patient with metastatic castration-resistant PCa, priorly treated with goserelin, docetaxel, and enzalutamide. PDOX were generated by subcutaneous injection of PDOs in NSG male mice. Organoids were derived from the PDOX tumors (PDOX-O). Matched patients' tumor, PDOs, PDOX and PDOX-O samples were characterized using immunohistochemistry (IHC), immunofluorescence (IF), and whole exome sequencing (WES). PDOs and PDOX-O were treated for 5 days with specific drugs and cell viability was measured using CellTiter-Glo 3D.

Results

P20-11 PDOs developed tumors in 2 out of 6 mice. IHC analysis highlighted a loss of PTEN expression, overexpression of ERG and P53, as well as strong AR and NKX3.1 expression in the tumor, PDOs, PDOX and PDOX-O samples. WES analysis uncovered mutations in CTNNB1, PTEN and TP53 in all samples. Similar to the original PDOs, P20-11 PDOX-O displayed androgen sensitivity in vitro.

P20-23 PDOs formed tumors in 3 out of 3 mice. IHC and IF analyses highlighted a strong expression of CK8, PSMA, AR and NKX3.1, as well as a loss of PTEN expression all matched samples. WES identified a pathogenic mutation in the PCa-associated gene ZMYM3 in all samples. The activating AR point mutation L702H, previously linked to AR signaling inhibitors resistance, was detected in the tumor and in 2 out of 3 PDOX and their PDOX-O. Finally, P20-23 PDOX-O did not respond to

docetaxel or enzalutamide but exhibited sensitivity to the PI3K/AKT inhibitor ipatasertib.

Conclusion

We have successfully generated two novel PDOX models, which highly resemble the original patients' tumor and can be further cultured as organoids. These models are representative of relevant clinical and molecular subtypes of advanced PCa, providing further opportunities for translational studies.

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