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Bulletin de l'Association pour la Recherche sur les Tumeurs de la Prostate

Editorial

L'ARTP a vocation de se faire rencontrer tous les acteurs travaillant sur les pathologies prostatiques et de subventionner des travaux de recherche. Sa journée scientifique se poursuit cette année encore au sein du congrès de l'Association Francaise d'Urologie ce qui rendra les interactions vers les cliniciens encore plus faciles.

Pour la 25ème fois, nous nous réunissons pour échanger sur plusieurs thèmes traitant de l'hétérogénéité tumorale et de la médecine de précision. Nous aurons aussi l'occasion de remettre plusieurs prix/subventions grâce au soutien de l'industrie pharmaceutique Pierre Fabre Médicament, Astellas, Takeda, Janssen-Cilag, Bouchara Recordati, Ferring, GSK et l'Association Française d'Urologie. Enfin les lauréats des prix ARTP 2014 viendront présenter l'avancée de leurs travaux.

L'ouverture vers l'Europe est toujours assurée par le prix ARTP/ESUR.

Très bon congrès à tous.

Pr Alexandre de la Taille

Président de l'ARTP

Palais des Congrès de Paris
2 place de la Porte Maillot
75017 Paris

Comité d'Organisation

Alexandre de la Taille
Olivier Cuvillier
Palma Rocchi
Jocelyn Céraline
Virginie Vlaeminck-Guillem
Laurent Morel

Programme scientifique de la 25ème journée

'Hétérogénéité tumorale et Médecine de précision dans le cancer de la prostate'

'Tumor heterogeneity and Precision medicine in prostate cancer'

Ce programme 2016 n'aurait pas été possible sans le dévouement des co-modérateurs et des membres du Comité d'Organisation. Nous tenons à exprimer notre reconnaissance pour les entreprises et les organisations qui ont fourni un soutien financier généreux pour la réunion annuelle 2016 de l'ARTP.

09h00 - 09h15
Message de bienvenue
Pr Alexandre de la Taille, Président de l'ARTP

09h15 - 10h30
Session I : Hétérogénéité tumorale/Tumor Heterogeneity
Modérateurs : Dr J. Ceraline, Dr V. Vlaeminck-Guillem
Orateurs :
Pr Lluis Fajas (UNIL, Lausanne, Switzerland) *Cell cycle regulators as key factors in the metabolism of cancer cells*
Dr Pete Nelson (Fred Hutchinson Cancer Center, Seattle, USA) *The molecular taxonomy of metastatic prostate cancer*
Dr Jack Schalken (Radboud University Medical Center, Nijmegen, Netherlands) *Cancer heterogeneity : the number one challenge !*

10h30 - 10h45 Pause Café

10h45 - 12h00
Session II : Médecine de Précision/Precision Medicine
Modérateurs : Dr Palma Rocchi, Pr Laurent Morel
Orateurs :
Pr Yuzhuo Wang (University of British Colombia, Vancouver, Canada) *PDX Models : for cancer discovery, precision medicine and beyond*

Dr Max Chaffanet (CRCM Inserm U1068, Marseille, France) *Oncogenomics analysis of advanced cancers and precision medicine*
Dr Dimitris Visvikis (LaTIM Inserm U1101, Brest, France) *Imagerie de l'hétérogénéité tumorale*

12h00 - 13h30 Pause Déjeuner (réservé aux adhérents de l'ARTP)

13h30 -14h45
Session III : Session Clinique/Clinical Session
Modérateurs : Pr Alexandre de la Taille, Pr Armauld Villers
Orateurs :
Dr Gwenaëlle Gravis (Institut Paoli-Calmettes, Marseille, France) *Prise en charge du cancer de la prostate métastatique homono-sensible*
Pr Christophe Hennequin (Hôpital Saint Louis, Paris, France) *Interaction de la radiothérapie avec le système immunitaire*
Dr Yohann Loriot (Institut Gustave Roussy, Villejuif, France) *Predictive signatures and therapeutic targets*

14h45 - 15h00 Pause Café

15h00 - 16h00
Session IV : Lauréats ESUR/ARTP Laureates
Modérateurs : Dr Olivier Cuvillier, Dr Vincent Goffin
Orateurs :
Maria Luna Velez (Radboud University Medical Center, Nijmegen, Netherlands)
Mechanisms of nuclear import of the androgen receptor splice variants and their contribution to progression of castration-resistant prostate cancer
Pauline Berthelemy (Inserm U1113, Strasbourg)
Voies de signalisation des variants autonomes du récepteur des androgènes et rôle dans la progression du cancer de la prostate
Audrey Serre (Inserm U990, Clermont-Ferrand)
Développement d'analogues d'acides aminés radiohalogénés pour l'imagerie TEP des cancers de la prostate

16h00 - 17h00
Session V : Session Poster/Poster Session
Jury : Dr Edith Bonnelye, Dr Jocelyn Ceraline, Dr Vincent Goffin, Dr Daniel Metzger, Dr Stéphane Terry, Dr V. Vlaeminck-Guillem

17h00 - 17h30
Session VI : Auditions Lauréats Prix Poster ARTP 2015
Modérateurs : Dr Daniel Metzger, Dr Stéphane Terry
Orateurs :
Elhsan El Sayed (Inserm U955, Créteil)
CRIPTO is associated with tumor aggressiveness in prostate cancer
Allan Fouache (GRed Inserm U1103/CNRS UMR6293, Clermont-Ferrand)
Environmental chemical disruptors, Liver X receptors and prostate cancer

Adrien Guerard (IPBS, CNRS UMR5089, Toulouse)
Role of chemokines from adipose tissue in local and distant dissemination of prostate cancer

17h30
Remise des 3 prix Poster de l'ARTP 2015

17h30-18h00
Assemblée Générale de l'ARTP (réservé aux adhérents)

Prix Poster ARTP

Les 3 meilleurs posters recevront un prix Poster de l'ARTP d'un montant de 500 euros et présenteront leurs travaux lors de la 26ème Journée Scientifique l'ARTP en 2017

Orateurs

Session I : **Hétérogénéité tumorale/Tumor Heterogeneity**

Pr Lluis Fajas
Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Cell cycle regulators as key factors in the metabolism of cancer cells

Analysis of genetically engineered mice deficient for cell cycle regulators, including E2F1or cdk4 showed that the major phenotypes are metabolic perturbations. These key cell cycle regulators contribute to lipid synthesis, glucose production, insulin secretion, and glycolytic metabolism and we show how deregulation of those pathways can lead to metabolic perturbations and related metabolic diseases, such as obesity and type II diabetes. Cell cycle regulators are activated by insulin and glucose, even in non-proliferating cells. Most importantly these cell cycle regulators trigger the adaptive metabolic switch that normal and cancer cells require in order to proliferate. These changes include increased lipid synthesis, decreased oxidative, and increased glycolytic metabolism. In summary, these factors are essential regulators of anabolic, biosynthetic processes, blocking at the same time oxidative and catabolic pathways, which are the metabolic hallmarks of cancer.

Pr Yuzhuo Wang

University of British Colombia, Vancouver, Canada

PDX Models : for cancer discovery, precision medicine and beyond

At the Living Tumor Laboratory (www.livingtumorlab.com), we have developed a routine procedure for successfully grafting and serially transplanting primary human cancer tissues into immuno-deficient SCID mice. It is based on grafting patients' biopsy or excised primary cancer tissue into the subrenal capsule graft site. The high vascularity of this site, compared to subcutaneous and orthotopic sites, allows more adequate supply of nutrients to the graft important for maintaining tumor heterogeneity. Using the effective xenografting methodology, the group has developed over 280 transplantable patient-derived "high fidelity" xenograft models (> 40 are prostate cancer models). These xenografts retain all the salient features of the donor tumor. These features include pathology, growth dynamics, global gene expression, genome structure, and response to therapy including the development of resistance. Consequently, these high fidelity models can be used for gaining detailed knowledge of human tumor development, progression and metastasis. The xenografts are powerful tools for development of novel therapeutics, cancer discovery and personalized cancer therapy. In this presentation, foci will be on the properties of such next generation models and examples of their applications.

Dr Pete Nelson

Fred Hutchinson Cancer Center, Seattle, USA

The molecular taxonomy of metastatic prostate cancer

Personalized medicine and precision oncology are dependent on two related concepts to achieve success. First, the molecular composition of cancers between individual patients must be different such that a therapy selected for one individual would be different than a treatment selected for a different individual, based on underlying biology. Second, tumor diversity within an individual must be limited, such that a selected treatment would target all or most tumor cells within an individual. This presentation will discuss current information capable of classifying subtypes of prostate cancer amenable to specific therapeutics and assess the extent of tumor heterogeneity in metastatic prostate cancer.

Session II :
Médecine de Précision/ Precision Medicine

Session III :
Session Clinique/ Clinical Session

Dr Gwenaëlle Gravis
Institut Paoli-Calmettes, Centre de Recherche en Cancérologie de Marseille (CRCM) - Inserm U1068, Marseille, France

Prise en charge du cancer de la prostate métastatique hormono sensible

Le cancer de la prostate métastatique sensible à la castration touche moins de 10% des cancers de la prostate au diagnostic. Le traitement de référence était jusqu'en 2015 la castration seule. Trois études de phase III ont évalué l'impact du docetaxel associé à la castration versus la castration seule, qui est le traitement standard. La première étude le GETUG-AFU 15 n'a démontré qu'un bénéfice en survie sans progression (22.9 mois vs 12.9 mois, HR 0.72, [IC95% : 0.57-0.91])(1). Les études CHAARTED et STAMPEDE ont démontré un avantage de survie sans progression mais surtout un avantage de l'association en terme de survie globale (CHARTEED : n=790; 57.6 mois versus 44.0 mois, HR 0.61, [IC 95% : 0.47-0.80]; STAMPEDE : n=1086; 60 mois versus 45 mois; HR 0.76, [IC95% : 0.62 - 0.92]) (2,3).

Les patients inclus dans l'étude CHARTEED avaient une masse tumorale importante (65%), définie par une atteinte viscérale et ou une atteinte osseuse avec au moins 4 métastases osseuses dont une en dehors du rachis ou du pelvis. Cette population bénéficiait particulièrement de l'ajout du docetaxel (49.2 mois vs. 32.2 mois; HR, 0.60; [IC 95% : 0.45- 0.81]). Une mise à jour récente de l'étude CHARTEED [HR 1.04 IC95% : 0.7-1.55] (ESMO 2016), concernant les patients avec une faible masse tumorale, n'a pas mis en évidence de bénéfice évident de l'apport du docetaxel sur la survie globale (4).

Un grand pas vient d'être franchi dans la prise en charge de cette population métastatique sensible à la castration. Les résultats des nouvelles approches évaluées dans le cadre d'essais cliniques sont attendus : l'ajout des anti-androgènes de 2ème génération ou le traitement de la maladie locale qui pourrait avoir un impact sur la maladie métastatique au diagnostic… Cependant au sein de cette population il existe certains patients qui ne vont pas bénéficier de ces thérapeutiques. Des éléments encore très grossiers sont à notre disposition pour individualiser ces différentes populations : le volume tumoral, les phosphatases alcalines (5)…. Il est nécessaire d'affiner le profil de ces patients par des caractéristiques cliniques, biologiques moléculaires peut être immunologique ou autres, pour nous orienter vers des traitements personnalisés.

1. Gravis G, et al. Lancet Oncol. 2013;14:149-58.
2. Sweeney CJ, et al. N Engl J Med. 2015;373:737-46
3. James ND, et al Lancet 2016;387:1163–77.
4. Sweeney CJ, et al Abstract 720PD ESMO 2016.
5. Gravis G, et Europ Urol. 2015 : 68 : 196-204.

Maria Luna Velez
Radboud University Medical Center, Nijmegen, Netherlands

Mechanisms of nuclear import of the androgen receptor splice variants and their contribution to progression of castration-resistant prostate cancer

Persistent androgen receptor (AR) transcriptional activity underlies resistance to AR-targeted therapy and progression to lethal castration-resistant prostate cancer (CRPC). One emerging mechanism of CRPC progression is the elevated expression of constitutively active truncated AR splice variants (AR-Vs) that lack the AR ligand-binding domain. In variants-enriched cell lines, AR-Vs drive resistance to AR-targeted therapy by functioning as constitutive and ligand-independent effectors of the androgen/AR transcriptional program. A prerequisite for transcriptional activation mediated by full-length AR (AR-FL) is its nuclear import, which depends on androgen binding, AR dimerization and recognition by importing proteins like KPNA2. However, the mechanisms of the androgen-independent nuclear translocation of AR-Vs and its contribution to AR-FL-related functions is still unclear. The objectives of this study were to unravel the mechanisms behind AR-Vs constitutive nuclear import in prostate cancer models. Mutagenesis studies in AR-V7 revealed a novel bipartite nuclear localization signal (NLS), located in the DNA binding domain and the variant-specific C-terminal end. These two clusters of basic amino acids, were shown to be responsible for AR-V7 constitutive nuclear import. Moreover, transient co-transfection experiments of AR-Vs and AR-FL have demonstrated that AR-V12 can promote androgen-independent AR-FL nuclear translocation. Yeast2Hybrid assay will be used to determine interactions between the protein transporter KPNA2 and AR proteins. A cryptic NLS in AR-V7 contributes to the understanding of the mechanism of action of AR-Vs and should be considered during development of new AR-targeted therapies. Involvement of AR-V12 in AR-FL nuclear localization suggests a partnership role of this constitutively active variant with AR-FL and is a reflection of the complexity in this late stage of disease progression.

Session IV :
Lauréats ESUR ARTP/ ESUR ARTP Laureates

Pauline Berthelemy
Inserm U1113, Strasbourg

Voies de signalisation des variants autonomes du récepteur des androgènes et rôle dans la progression du cancer de la prostate

Blocking androgen receptor (AR) axis via medical castration and by AR antagonism remains a cornerstone of advanced prostate cancer (PCa) treatment. Naturally occurring gain-of-function AR variants are known to trigger PCa to a castration resistance disease (CRPC).
We focus on constitutively active AR variants that come from either nonsense mutations in exon 4 or alternative splicing. Besides their role in CRPC, we showed that these AR variants play a role in epithelial mesenchymal transition (EMT). Indeed, a higher expression of EMT markers such as N-cadherin, vimentin and SNAIL has been associated with AR variants.
ChIP analysis, miRNA-seq and RNA-seq approaches have enabled us to decipher how AR variants could upregulate N-cadherin in PCa cells. We propose a model in which full-length AR represses expression of specific EMT genes, while on the contrary AR variants favour their expression.
PCa cells expressing AR variants further demonstrated higher migration capacity and by using a 3D culture of PCa cells, a difference in desmosomal proteins and membranes expansions have been evidenced.
When put together, these data highlight the importance to focus on AR amino-terminal domain (NTD) to further block AR variants in CRPC.

Audrey Serre
Inserm U990, Clermont-Ferrand

Développement d'analogues d'acides aminés radiohalogénés pour l'imagerie TEP des cancers de la prostate

L'incidence du cancer de la prostate est aujourd'hui la plus forte chez l'homme avec 56800 nouve cas en 2012 en France. Il est le troisième cancer en termes de mortalité (8900 décès)1. L'espérance vie étant dépendante de l'avancement de la maladie, seule une prise en charge thérapeuti précoce basée sur un diagnostic fiable et précis pourrait améliorer les chances de guérison patients. Parmi les méthodes d'imagerie permettant de réaliser un bilan d'extension corps en l'imagerie TEMP (tomographie d'émission mono-photonique) et l'imagerie TEP (tomographie émission de positons) sont particulièrement performantes. Malheureusement pour le cancer de prostate, les radiotraceurs développés à ce jour sont soit inadaptés (e.g. [18F]fluorodésoxyglucose), controversés (e.g. [18F]fluorocholine) ou bien encore limités à la détection des métastases osseu (e.g. 99mTc-HMDP, [18F]NaF). Notre projet de recherche s'inscrit dans ce contexte et concern développement de nouveaux radiotraceurs performants pour l'imagerie TEMP et/ou TEP du cancer la prostate. Il est basé sur des résultats prometteurs obtenus par l'équipe de Samnick sur un acide an non naturel cyclique, dénommé ITIC(OH)1,1,1. Les synthèses des dérivés radiofluorés (TEP) ou radioic (TEMP) ont été réalisées à partir d'intermédiaires communs de type organostannane, soit radioiodation électrophile directe soit via l'utilisation de sels de diaryle iodonium pour les dér radiofluorés. Leurs évaluations biologiques seront réalisés tant in vitro (captation cellulaire) qu'in vivo modèle animaux et ce comparativement aux radiopharmaceutiques de référence actuellement.

NEUROPILINE-1, UN NOUVEAU BIOMARQUEUR D'AGRESSIVITÉ ET DE RÉSISTANCE THÉRAPEUTIQUE DU CANCER DE LA PROSTATE

Charly Blanc[1], Anissa Moktefi[2], Fannie Semprez[1,2], Pascale Maille[1,2], Nathalie Nicolaiew[1], Pascale Soyeux[1], Virginie Firlej[1], Francis Vacherot[1], Alexandre De La Taille[1,3], Arturo Londono-Vallejo[4], Yves Allory[1,2], Jean Delbe[1], Yamina Hamma-Kourbali[1]

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Introduction

Le cancer de la prostate (CaP) représente un problème majeur de santé publique. L'hormonothérapie constitue aujourd'hui la seule arme thérapeutique efficace pour les formes avancées. Cependant, une forme de résistance s'installe inéluctablement et conduit à des cancers plus agressifs. Les mécanismes moléculaires impliqués dans la résistance à la castration associent l'activation du récepteur des Androgènes (RA), l'activation de voies de survie ou encore l'implication de gènes influençant la transdifférenciation neuroendocrine (NE).

L'objectif de la recherche actuelle repose sur l'identification de biomarqueurs de la résistance thérapeutique afin de proposer de nouvelles cibles permettant de contrecarrer la résistance.

Méthodes

Nous avons mis en œuvre le profilage moléculaire de 180 tumeurs à différents stades du CaP afin d'identifier de nouveaux biomarqueurs. Les échantillons ont été analysés par des approches à haut débit pour des études transcriptomiques et protéiques. Parallèlement, l'utilisation de lignées cancéreuses du CaP a permis de retracer les mécanismes moléculaires.

Résultats

L'analyse d'une signature moléculaire au sein de notre cohorte a permis d'identifier la Neuropiline-1 (NRP1) au cours de la résistance à la castration. NRP1 est une glycoprotéine transmembranaire impliquée dans le développement neuronal et vasculaire. Nous montrons pour la première fois que l'expression de NRP1 est dynamiquement régulée par l'axe du RA et favorise la neuro-transdifférenciation du CaP in vitro et in vivo. En outre, NRP1 favorise l'acquisition d'une hormono-résistance, l'activation de voies de survie cellulaire et par conséquent la résistance aux traitements

Session V : **Posters**

De surcroit, nous montrons pour la première fois que le ciblage de cette voie moléculaire bloque l'évolution tumorale vers la résistance à la castration et augmente l'efficacité d'une chimiothérapie à base de docétaxel sur des modèles précliniques in vivo.

Conclusion

L'ensemble de ces travaux apporte de nouvelles connaissances sur la caractérisation de la résistance thérapeutique du CaP, et fournisse un réel intérêt clinique porteur d'espoir dans la prise en charge de la maladie résistante à la castration.

LIVER X RECEPTORS LIMIT INVASIVENESS OF CASTRATION-RESISTANT PROSTATE CANCER

Laura Bousset, Anthony Alioui, Amalia Trousson, Cyrille de Joussineau, David Volle, Laurent Morel, Jean-Marc Lobaccaro et Silvère Baron

UMR Génétique, Reproduction et Développement - GReD, CNRS 6293 INSERM U1103 Clermont Université, 10 Avenue Blaise Pascal 63171 Aubière

Background

Therapeutic treatment of advanced and metastatic prostate cancer is based on tumor androgen deprivation, inducing their regression. However, most of the patients experience tumor growth recovery, corresponding to the appearance of a castration-resistant prostate cancer.

Deciphering the molecular mechanisms underlying castration-resistance appearance is mandatory.
To support tumor growth and metastatic dissemination, cancer cells adopt new metabolic strategy. Among metabolic alterations that occur during prostate cancer progression, cholesterol accumulation is now considered as a hallmark of cancer aggressiveness. Liver X Receptors (LXRs) are major sensors of intracellular cholesterol. These nuclear receptors exert anti-proliferative and pro-apoptotic effects on tumor cells. Preliminary work from our lab shows that LXRs reduce tumor invasiveness and limit metastatic dissemination of prostate tumor cells. Moreover, LXRs can modulate transcriptional activity of Androgen Receptor in normal mouse prostate.

Methods
We analyse the tumor response to castration of genetically-engineered mouse model invalidated for *Pten* and *Lxrs* (*Pten*$^{pc-/-}$;*Lxr*$\alpha\beta^{-/-}$) in terms of invasive and metastatic potential in comparison to a model invalidated for *Pten* alone (*Pten*$^{pc-/-}$).

Results
Analysis of gene expression in normal mouse prostate shows that LXRs and AR share a common regulated gene set. We further characterize *Pten*$^{pc-/-}$ mouse model phenotype in response to castration. *Lxr*$\alpha\beta$ knockout leads to an increase in cell proliferation and expression of markers of prostate cancer aggressiveness in absence of androgens. This correlates with an *in vivo* altered expression of androgen-regulated genes. Moreover, castration of *Pten*$^{pc-/-}$;*Lxr*$\alpha\beta^{-/-}$ mice increases metastatic dissemination of prostate tumor cells.

Conclusions
We showed that *Lxr*$\alpha\beta$ knockout leads to the establishment of a very aggressive castration-resistant prostate cancer in *Pten*$^{pc-/-}$ mouse model, with an enhanced metastatic dissemination. This correlates with an altered expression of androgen-regulated genes. Further experiments will be performed to determine how LXRs can modulate androgen sensitivity and metastatic dissemination properties of prostate cancer cells.

IMPLICATION DE LA PHOSPHOLIPASE D DANS LA PROGRESSION TUMORALE DU CANCER DE LA PROSTATE

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Contexte
Le cancer de la prostate est le 4ème cancer le plus fréquent tout sexe confondu et le 2ème cancer le plus fréquent chez l'homme. Il métastase dans 80% des cas en un seul site détectable : l'os.
La phospholipase D (PLD) hydrolyse préférentiellement la phosphatidylcholine, pour générer de la choline et de l'acide phosphatidique. Il existe 6 isoformes mais seulement la PLD1 et PLD2 sont directement impliquées dans le cancer. La PLD régule plusieurs processus physiologiques fondamentaux au sein de la cellule comme la prolifération, la migration ou la réorganisation du cytosquelette. De nombreuses études ont impliqué la PLD dans les aspects importants de l'oncogenèse mais de manière surprenante, peu d'études ont été conduites sur l'implication de la PLD dans la progression tumorale du cancer de la prostate.

Méthodes
- Lignées de cancer de la prostate avec différente agressivité (LNCaP, C4-2B, PC-3)
- Lignée non-tumorale prostatique (WPMY-1)
- Western blot, RT-qPCR
- Test de viabilité cellulaire (MTT), test de clonogénie
-Tests de migration « scratching », zymographie

Résultats
Nos résultats préliminaires montrent que la PLD1 et la PLD2 sont impliquées dans la viabilité et la prolifération cellulaire ainsi que dans la capacité des cellules à cloner (potentiel métastatique). Par ailleurs, le degré d'implication de la PLD1 et la PLD2 semble être différente selon l'agressivité de chaque lignée tumorale. De plus, nous avons démontré que l'expression de la PLD1 et la PLD2 (ARN et protéine) ainsi que l'activité PLD sont augmentées dans les lignées tumorales prostatiques par rapport à une lignée non-tumorale prostatique (WPMY-1).
De plus, nos expériences montrent que les inhibiteurs de la PLD ont très peu d'effet sur la migration de la lignée contrôle. *A contrario*, l'inhibiteur de PLD1 bloque fortement la migration dans la lignée PC-3. De plus, la PLD2 serait impliquée dans la sécrétion des métalloprotéinases MMP2/9 (essentielles pour l'invasion) dans les PC-3.

Conclusion
L'ensemble de ces résultats met en évidence un rôle prépondérant de la PLD1 et la PLD2 dans différents aspects de la prolifération tumorale prostatique et la dissémination métastatique.

TARGETING MENIN AS A NEW THERAPEUTIC STRATEGY IN CASTRATION-RESISTANT PROSTATE CANCER

Chaima Cherif[1-5], *Abdesmad El kawtheri*[1-4], *Tan Nguyen dang*[1-4], *Ladan Fazli*[6], *Martin Gleave*[6], *Philippe Barthélémy*[7], *Palma Rocchi* [1-4]
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Background

Prostate cancer (PC) is one of the most common malignancies in industrialized countries, and the second leading cause of cancer-related deaths in the United States. Previously we have shown that Hsp27 is highly overexpressed in castration-resistant prostate cancer, and developed a Hsp27 inhibitor (OGX-427) currently tested in phase I/II clinical trials as a chemosensitizing agent in different cancers.
Using large scale proteomic approach, we found that Menin is a new Hsp27 client protein that might drive cancer progression. Interestingly, we found that CRPC progression correlates with Menin overexpression. To define Menin's function, we have developed and worldwide patented a Menin antisense oligonucleotide (ASO). The treatment with our Menin ASO inhibits cell proliferation and induces cell cycle arrest in castration-resistant (CR) PC3 cultured cells.

Methods
After submission of proteomic data, we have established the Hsp27-protein interaction network using the basis of KEGG. Expression of Menin has been confirmed by western blot. Using co-immunoprecipitation we have demonstrated an interaction between Hsp27 and Menin. Immunohistochemical analysis of Menin expression has been performed using tissue micro-array (TMA). We also analyzed in vitro the effect of Menin ASO treatment on CR PC3 tumor cells.

Results
Menin is overexpressed in LNCaP-Hsp27. Menin interacts with Hsp27. Moreover, the results of TMA showed a higher expression of Menin in CRPC: Expression of this protein is absent or weak in hyperplastic prostate cells, and becomes uniformly and strongly expressed in CRPC. This expression correlates with increased risk of recurrence and disease malignancy.
Finally, treatment of CR PC-3 cells with Menin ASO downregulates Menin protein level, inhibits proliferation, induces cell cycle arrest and enhances docetaxel cytotoxicity.

Conclusion
These results suggest that Menin could be considered as a relevant specific target for molecular therapy of CRPC.

FATTY ACID COMPOSITION OF PERI-PROSTATIC ADIPOSE TISSUE (PPAT) : ASSOCIATION WITH TUMOR AGGRESSIVENESS, ETHNO-GEOGRAPHICAL ORIGIN AND MIGRATION OF PROSTATE CANCER CELLS.

S. Figiel[1], I. Domingo[1], R. Guibon[1,2], P. Besson[1], M. Pinault[1], F Bruyère[2], R. Mathieu[3], AR. Azzouzi[4], MA. Perrouin-Verbe[5], J. Rigaud[6], P. Blanchet[7], L. Multigner[8], K. Maheo[1] and G. Fromont[1,2].
[1]*Inserm U1069, Tours ;* [2]*CHRU Bretonneau, Departments of Pathology and Urology, Tours ;* [3]*CHU Rennes ;* [4]*CHU Angers ;* [5]*CHU Brest ;* [6]*CHU Nantes ;* [7]*CHU Pointe à Pitre ;* [8]*IRSET Rennes*

Background
Both genetic (ethnic origin) and environmental factors (nutrition) are linked to the risk of aggressive prostate cancer (PCa). The fatty acid composition of PPAT, whose reflects the past fatty acid intake, is potentially involved in PCa progression. The objectives of the study were to analyze the fatty acid composition of PPAT and to correlate these results with the ethno-geographical origin of the patients and clinical markers of tumor aggressiveness. The bioressources of "Prostate Grand-Ouest" network have been used.

Methods
The fatty acid composition of PPAT was analyzed in a cohort of 156 patients with PCa (106 Caucasians and 50 African-Caribbeans), 78 with an indolent tumor (Gleason score 6 + pT2 + PSA <10 ng/mL), and 78 with an aggressive tumor (Gleason 8 + pT3). Each population included as many indolent tumors as aggressive tumors that were matched for age. The effect of fatty acids extracted from PPAT on migration of prostate cancer cells DU145 was studied (72 patients : 36 Caucasians, 36 African-Caribbeans).

Results
In African-Caribbean patients, the LA rate is associated with tumor indolence, and the rates of saturated and monounsaturated acid are associated with aggressiveness.

In the Caucasian population, indolence is associated with eicosapentaenoic acid levels (EPA, a n-3 PUFA). *In vitro*, cancer cell migration of prostate cancer cells is negatively correlated to the LA rate in the PPAT of African-Caribbean patients.

Conclusion
These results highlight an important ethno-geographic variation of PPAT, in their fatty acid composition and an association with tumor aggressiveness.

THE BXL-72 VITAMIN D ANALOGUE IMPACTS PROSTATE CANCER PROGRESSION IN MICE.

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Prostate cancer is the second leading cause of male cancer related deaths in western societies and is characterized by tumors which progress slowly over several decades. Treatments of aggressive forms, based on anti-androgenic therapies, are poorly effective. Thus, a better understanding of the mechanisms underlying carcinogenesis and new treatments are required.
PTEN (Phosphatase and TENsin homolog) is the most often tumor suppressor mutated or deleted in prostate cancer. PTENpe-/- mice, with a selective invalidation of PTEN in prostatic epithelial cells at adulthood, develop prostatic intraepithelial neoplasia (PINs) within 1 month after PTEN invalidation. PTEN-null epithelial cells become progressively senescent during the following 4-5 months, and adenocarcinomas are formed only after one year. Thus, these mice reproduce the early steps of human prostate cancer.
The bioactive vitamin D (1,25 dihydroxy-vitamine D3 ou calcitriol) has anti-inflammatory and anti-proliferative effects in many cancers. However, its clinical use is limited since required therapeutic doses induce hypercalcemia causing soft tissue calcification and multiple organ failure. Interestingly, some vitamin D analogues, including BXL-72, exert at normocalcemic concentration a higher anti-proliferative effect than calcitriol. Our results show that a three-week treatment of PTENpe-/- mice with BXL-72, at a stage where epithelial cells of PINs are mostly senescent, induces apoptosis of these cells, and improves the architecture of PINs. Thus, vitamin D analogs with low calcemic effects might be a promising therapy to eliminate PTEN-null prostatic epithelial cells and prevent the development of adenocarcinomas.

TRPM8 INHIBITS ENDOTHELIAL CELL ADHESION AND MIGRATION VIA THE RAP1-β1-INTEGRIN SIGNALING PATHWAY.

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Background
Endothelial cell adhesion and migration are critical steps of the angiogenic process, whose dysfunction is associated with tumor growth and metastasis. The TRPM8 channel has recently been proposed to play a protective role in prostate cancer by impairing cell motility. However, the mechanisms by which it could influence vascular behavior are unknown.

Methods
Migration, adhesion and hapotaxis assay, biotinylation assay, immuno-precipitation and GST pull down assay, western blot analysis, calcium and confocal imaging were used for this purpose.

Results
Here we report that TRPM8 is expressed in endothelial cells and downregulated in tumor-derived endothelial cells. We reveal a novel pore independent function for TRPM8 which unexpectedly acts as a Rap1 GTPase inhibitor. TRPM8 retains Rap1 intracellularly through direct protein- protein interaction impairing the activation of a major inside-out signaling pathway known to trigger the conformational activation of integrins and, consequently, cell adhesion, migration and in vitro vascular morphogenesis.

Conclusions
Our results bring to light a novel molecular mechanism, pore independent, by which endogenous TRPM8 expression inhibits Rap1 GTPase and thus plays a critical role in the behavior of vascular endothelial cells by maintaining them in a quiescent state.

THE DOWNREGULATION OF PGC-1α PROMOTES PROSTATE CANCER CELL MIGRATION: POTENTIAL ROLE IN METASTASIS?

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Prostate cancer (PCa) is the third cause of cancer death in men and deaths are due to advanced, metastatic PCa. The metabolic pathways implicated in the formation of metastasis are poorly understood. Previous observations in other cancers have shown that primary tumor metabolism differs from metastatic tumor cells metabolism. In this context, we chose to study peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) which plays a major role in cell metabolism and more specifically the regulation of Oxidative Phosphorylation. Indeed, PGC-1α is a master regulator of mitochondrial biogenesis and energy metabolism. Furthermore, a recent paper suggests that low levels of PGC-1α may be associated with a poor prognosis for patients with PCa.

We knockdown (KD) PGC-1α in prostate cancer cell lines using different siRNA. Then, we deciphered the role of PGC-1α on cell migration and metabolism.

PGC-1α KD enhances prostate cancer cell proliferation. Moreover, it promotes cancer cell migration in LNCaP (3 fold) and DU145 (2 fold). To determine which mechanism is implicated in this phenotype, we analyzed the expression of several genes implicated in metabolism and oncogenesis. We find that c-myc and some other genes implicated in glutaminolysis are up regulated in cells KD for PGC-1α. We then performed transwell migration assay using c-myc inhibitors. These inhibitors reverse completely the pro-migratory effects induced by the downregulation of PGC-1α. In KD PGC-1α cells, several genes of the glutamine pathway are up regulated such as GLUL, GLS2, GOT2, IDH1, and IDH2. Thus, we measured glutamine, and intracellular glutamate. We notice that the KD of PGC-1α lead to an increase in glutamine consumption and intracellular glutamate compared to control cells. Finally, in accordance with the results presented here, we demonstrated that the expression level of PGC-1α is significantly downregulated in biopsies of patients with PCa.

These results show that the downregulation of PGC-1α, promotes cancer cell migration in a c-myc dependent way. Moreover, the increase of the glutamine metabolism may, at least, in part, be involved in the pro-migratory effects observed in cells with low PGC-1α expression level.

Prix Poster ARTP

Les 3 meilleurs posters recevront un prix Poster de l'ARTP d'un montant de 500 euros et présenteront leurs travaux lors de la 26ème Journée Scientifique l'ARTP en 2017

AUTONANOVECTORIZED TCTP-LIPID MODIFIED ANTISENSE OLIGONUCLEOTIDE IMPROVE STABILITY CELL UPTAKE, AND EFFICIENCY IN CASTRATION-RESISTANT PROSTATE CANCER

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Background
Castration-resistant prostate cancer (CRCP) correlates with Translationally controlled Tumor protein (TCTP) overexpression and loss of p53. Analysis showed that TCTP's expression was found to be significantly down regulated after androgen ablation to become uniformly highly expressed in 75% of CRCP.
We chose the oligonucleotide antisense (ASO) strategy to inhibit TCTP because ASOs can be used for human therapy and inhibit specifically target genes especially whose are difficult to target with small molecules inhibitors or neutralizing antibodies.
We developed and patented a second generation ASO targeting TCTP that significantly inhibits CRCP pre-clinical models that restores p53 expression and function (Baylot et al, 2012; Patent PCT 10306447.3, 2010).
We are now developing a third generation ASO by using lipid-conjugated oligonucleotide modification (LASO) via "Click chemistry" in order to improve stability, delivery and biodisponibility of TCTP ASO. Interestingly, LASOs self-assembly give spherical nano-objects of different sizes ranging from few nanometers to 500 nm in diameter. Hence, we will take advantage of the nanosystems obtained from LASOs self-assemblies to encapsulate chemotherapy to promote its delivery.

Methods
In castration resistant PC-3, we evaluated TCTP expression with different chemically modified oligonucleotides by Western Blot analysis.
Alamar blue was used to evaluate cell proliferation and flow cytometry to examine the oligonucleotide internalization mode. We also used confocal microscopy to evaluate TCTP-ASO cellular localization. Finally, in vivo experiments were performed on swiss nude mice.

Results
The addition of lipid to TCTP ASO allowed faster penetration of the oligonucleotide in the cell via micropinocytosis without any transfecting agent, inducing TCTP's inhibition and significant cell viability decrease. Moreover, LASO inhibited tumor growth in vivo, without toxicity on animal models.

Conclusion
TCTP's inhibition with specific antisense oligonucleotide lipid-moiety-modified seems to be a promising therapeutic strategy to restore chemo sensitivity for the treatment of CRCP.

THE SIGMA 1 RECEPTOR: A NEW PARTNER OF SOC CHANNELS IN PROSTATE CANCER

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Background

Prostate cancer (PCa) is one of the most frequently diagnosed cancer in industrialized countries, representing the third cancer-related cause of death. Its development is characterized by a dependency to androgens. While androgen deprivation therapy is a commonly used treatment, it can lead to the acquisition of an androgen-resistant phenotype for which there is no curative treatment available. The Store Operated calcium Channels (SOC), composed of Orai1 and STIM1, have been implicated in different cellular mechanisms. In PCa, our previous data show an implication of SOC channels in cell proliferation and in apoptosis resistance. Although SOC expression is steady throughout PCa, its activity decreases in advanced stages.
Initially identified as an opioid receptor, the Sigma 1 receptor (S1R) can actually bind many different ligands. This protein has been linked with several cancers, with an increased expression in many of them. S1R is mainly known as an ER chaperone, but recent publications also show a plasma membrane localization and a new role as ion channel modulator. We therefore asked ourselves if S1R could modulate the activity of SOC channels thus impact PCa cell physiology.

Methods
We used quantitative PCR, western-blotting, immunohistochemistry, fluorescence Ca^{2+} and confocal imaging, patch-clamping, gene silencing and transfection, cell proliferation assays.

Result
We show an increase of S1R expression in PCa as compared to healthy prostates, and the regulation of S1R expression by steroids. We also show that upon ER calcium store depletion, S1R translocates to the plasma membrane where it forms a complex with SOC channels leading to their increased activity. Moreover, we have associated this increase with the promotion of PCa cell proliferation.

Conclusion
Taken together our results show that S1R is a potential new partner of SOC channels in PCa. Indeed, S1R appears to positively modulate SOC channels through their interaction in PCa cells, leading to an increased cell proliferation. S1R could therefore represent a unique therapeutic marker allowing for the targeting of key players in cancer, thus potentially increasing the efficiency of future innovative treatments targeting these channels.

SEQUENTIAL ACTIVATION OF RAS/MAPK AND PI3K/AKT/TOR PATHWAYS VIA AN EGFR-AUTOCRINE LOOP RESUMES PROSTATE CANCER HALLMARKS IN THE DROSOPHILA ACCESSORY GLAND.

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Background
Prostate cancer is the most common cancer in men in Europe. Many factors play a role in prostate cancer etiology and progression, such as genetics or environmental factors. Particularly, analyses of human prostate cancer specimens have revealed the importance of Ras/MAPK and PI3K/AKT/mTOR signalling pathways for the late stages of this disease. However, human and biological data lack to understand their implication in earlier stages of tumorigenesis.

Methods
To study the underlying molecular mechanisms by which these signalling pathways operate during initiation and progression of the disease, we propose an alternative *in vivo* model of prostate tumorigenesis in the accessory gland of *Drosophila*, which is a functional homolog of the human prostate.

Results
Hyperactivation of AKT/TOR pathway induces cell overgrowth but not tumorigenesis. Conversely, hyperactivation of Ras/MAPK pathway by expression of Ras^{V12} induces formation of cells masses that recapitulate many cancer cells hallmarks such as uncontrolled cell growth and proliferation, loss of cell identity, tissue disruption, enhanced matrix metalloproteinase expression, neovascularisation-like tracheogenesis.
In this model, we furthermore demonstrate that Ras/MAPK pathway may induce tumor development by establishment of an autocrine feedback loop (EGFR dependent signaling). More, this tumorigenesis required AKT/TOR pathway recruitment to allow tumor growth and extravasation.

Conclusions
We have developed a *Drosophila* model which seems to be efficient to study phenomena that are describe in human prostate tumorigenesis.
As our results suggest that Ras/MAPK pathway deregulation may be a promoter of prostate tumorigenesis, the next step is to observe if this really happens in human samples with a Tissu MicroArray analysis.
We also plan to use our model to search for the molecular mechanisms that could be implicated in this tumorigenesis and, in particular, implication of metalloproteinases, which seem to be expressed on a short time window during the tumorigenic process.

STROMA CORRUPTION BY ANDROGEN RECEPTOR VARIANTS IN PROSTATE CANCER

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Background

Androgen ablation therapy remains the most common treatment for patients with advanced prostate cancer (PCa). However, most patients will relapse and develop a castration-resistant PCa. The emergence of androgen receptor (AR) variants, such as constitutively active ARs, has been involved in this failure to androgen deprivation.

Nevertheless, the tumour microenvironment is another necessary feature driving PCa progression. Actually, there's a strong cooperation between cancer cells and the surrounding stromal cells.

Cancer associated fibroblasts (CAFs) are one of the specialized stromal cells that favour tumour progression. Interestingly, CAFs represent a very heterogeneous population, according to their origins. They can be derived from different cell types, and 25% of CAFs originate from bone marrow-derived mesenchymal stem cells (MSCs). Hence, MSCs are attracted to the tumour site and differentiate into CAFs under tumour cells influence.

In this study, we investigated the effects of AR variants on the surrounding prostate tumour microenvironment by focusing on MSCs differentiation into CAFs.

Methods

We used an *in vitro* co-culture system of human MSCs together with LNCaP cells, expressing or not AR variants, to analyse CAFs differentiation markers expression in MSCs by RT-qPCR. These differentiation markers were also analysed with a FISH approach in MSCs exposed to conditioned medium of LNCaP cells expressing or not AR variants.

Results

RT-qPCR data revealed an upregulation of several CAFs differentiation markers in MSCs such as FSP-1. These results were confirmed with a FISH approach showing an increase (2-fold) in FSP-1 fluorescent spots number for MSCs exposed to conditioned medium from LNCaP cells expressing AR variants.

Conclusions

Together, our data would highlight an unknown property of AR variants in prostate tumour cells that is their ability to induce MSCs differentiation into CAFs. Studies are going on to validate these data using an *in vivo* PCa model.

CONDITIONNAL *MEN1*-DISRUPTION IN MOUSE *NKX3.1*-DEFICIENT PROSTATIC LUMINAL CELLS RESULTS IN ACCELERATED TUMORIGENESIS

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Background

MEN1 mutations are known to be responsible for Multiple Endocrine Neoplasia type 1 (MEN1), a hereditary disease affecting mainly endocrine organs. Recent advances highlighted the involvement of the *MEN1* gene in the development of prostate cancer. Nevertheless, the role played by the *MEN1* gene in prostate tumorigenesis remains elusive, described as an oncogenic factor by some studies, but as a tumor suppressor by others.

Methods

To further address this issue, we generated a novel and inducible mouse model, $Men1^{F/F}$-$Nkx3.1Cre^{ERT2-/+}$, in which the *Men1* gene could be specifically disrupted in *Nkx3.1*-deficient luminal prostatic cells upon tamoxifen injection.

Results

Anatomo-pathologic examination of our model showed that all double mutant mice displayed early development of precancerous lesions, Prostatic Intraepithelial Neoplasia (PIN), at 3 months of age (n=7), whereas only one mouse displayed PIN in the control (n=5). The analyses performed on the mice at 6 and 10 months of ages, showed that several double mutant mice developed more advanced lesions, microinvasive adenocarcinoma (7/14), whereas no control mice developed such lesions. Moreover, epithelial cells in the lesions were less differentiated and hyperproliferative relative to control mice.

Conclusion

Taken together, these data suggest a tumor suppressor role played by the *Men1* gene in prostatic luminal cells.

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Role of CRIPTO in epithelial-mesenchymal transition in prostate cancer and its impact on the modulation of the intercellular communication

Prostate cancer (PCa) remains the most dominant cause of mortality and morbidity in men worldwide. Detection of tumor biomarkers may aid to differentiate indolent from severe PCa cases and well-choose patients at high risk for intensive treatment. The founding member of EGF-CFC protein superfamily, CRIPTO, is widely implicated in embryonic development and is found to be expressed in a wide spectrum of human tumors. As its role in PCa was still unclear, we aimed to investigate expression profile of CRIPTO in PCa and relate its potential impact on prostate malignancy.
CRIPTO showed to be upregulated in 37.9% of PCas. Our results displayed that CRIPTO overexpression promoted epithelial-mesenchymal transition (EMT) associated with enhanced migration capacity and survival under stress conditions due to propensity to stimulate PI3K/AKT and FGFR1/ERK signaling pathways.
More interestingly, tumor mesenchymal like cells overexpressing CRIPTO secreted vesicles excessively. Uncovering the role of these vesicles in PCa progression, they showed to be highly capable to render the recipient prostatic cells more aggressive by acquisition of mesenchymal features. Our results highlight a new substantial function of CRIPTO in PCa and emphasize on an original role of mesenchymal extracellular vesicles in the interclonal communication to carry and transfer tumorigenic contents and enhance PCa progression.

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Caractérisation de perturbateurs chimiques environnementaux des liver x receptors impliqués dans le cancer de la prostate

Introduction: Ces dernières années ont été marquées par l'augmentation de l'incidence du cancer de la prostate. L'exposition continue, à faible dose, à des perturbateurs chimiques environnementaux (PCEs) a été associée à ce phénomène. Notre équipe a montré le rôle protecteur des récepteurs nucléaires LXRs *(Liver X Receptors*) dans la carcinogenèse prostatique épithéliale par le contrôle de la balance prolifération/apoptose. Sur ces bases, nous avons recherché, *in silico* et en culture de cellules, l'existence de PCEs pouvant, en partie, altérer les voies de signalisation régulées par les LXRs et expliquer, *in vivo*, le développement tumoral prostatique.

Matériels et méthodes: 1. A partir d'une banque industrielle de molécules, nous avons réalisé un criblage *in silico* par modélisation moléculaire prenant en compte la fixation potentielle d'une molécule dans le domaine de liaison du ligand des LXRs, associé à une recherche bibliographique. 2. Les molécules sélectionnées ont été testées en culture cellulaire avec le système UAS-GAL4 pour évaluer leurs effets sur le domaine de liaison du ligand des LXRs et les comparer avec des agonistes canoniques et des antagonistes connus. 3. Leurs capacités à moduler l'activité transcriptionnelle de l'hétérodimère LXR-RXR a été testée dans des cellules HeLa. 4. Des souris sauvages (wt) ou déficientes en LXRs (*lxr*$^{-/-}$) ont été exposées aux molécules testées.
Résultats: Le BPA et le Chlordécone sont des antagonistes des LXRs. Les IC50 sont de 3,34 µM pour le Chlordécone et 0,75 µM pour le BPA sur LXRa, et de 1,06 µM pour le Chlordécone et 2,12 µM pour le BPA sur LXRβ, en accord avec les concentrations retrouvées chez l'homme exposé. *In vivo* les deux molécules altèrent la voie de signalisation régulée par LXR-RXR.
Conclusions: Nous montrons pour la première fois que le BPA et le Chlordécone, décrites comme associées au risque de développement d'un cancer prostatique, sont des PCEs des LXRs.
Ce travail bénéficie du soutien du PNRPE 2013 « Projet X-SLiMs ».

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The medullary adipocytes contribute to the bone metastasis of prostate cancer and this effect is regulated by obesity

Background: We have recently demonstrated that mature adipocytes of the periprostatic adipose tissue act as a driving force for the local dissemination of prostate cancer (PCa) through the secretion of the CCL7 chemokine, and that this effect was amplified by obesity. Then, PCa cells metastasize to distant site such as bone. During this dissemination, PCa cells interact with bone marrow where the main components are medullary adipocytes (MedAd)
Objective: We investigated the role of the MedAd secretions in the bone metastasis process of PCa. We also explored the amplification of this effect in obesity and aging, two known risk factor for bone metastasis in PCa.
Methods and results: Using a series of 35 samples from patients, we first showed *in vitro* (Boyden chamber assay) that conditioned mediums from human MedAd (MedAd-CM) were able to chemoattract PCa cells (by contrast to paired conditioned medium obtained from subcutaneaous adipocytes) with a strong amplification by obesity or aging. The chemoattractive potential of medAd-CM was mediated by the chemokine CCL7 which interact with one of its receptor CCR3 on tumor cells, as shown using pharmacological inhibitors, blocking antibodies and gene repression strategies. To validate this effect *in vivo*, we used the murine cell line RM1-BM able to localize to the bone after intra-cardiac injection. We observed that the loss of CCR3 in tumor cells abrogates their bone metastatic homing.
Conclusions: This study show for the first time a mechanism that could explain the increased bone metastatic dissemination of prostate cancer linked to obesity and aging. These data highlight the fact that medullary adipocytes, using the CCR3/CCL7 axis, are able to control the distant dissemination of PCa cells to the bone. In a context of obesity or aging, medullary adipocytes show a different phenotype leading to an increased secretion of CCL7 and enhanced dissemination.

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