2nd edition Canther

PhD Students' Day

Canther

Cancer Heterogeneity, Plasticity
and Resistance to Therapies

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Dear All, dear PhD students,

Today is the 2nd PhD Day of the UMR CANTHER laboratory. It is an important moment in the life of a research unit and during PhD student's training. Sharing data during oral communication, seminar or conference is the way scientists interact in national and international meetings and often set-up collaborations from these discussions. English is of course the international language in Life Sciences and it is thus important that PhD students get used to speaking English to present their data but also to daily interact with foreigner students/scientists in the laboratory. Also, since sharing Science with the public becomes an important part of our activity, this year we have invited 1st and 2nd year PhD students to talk about their project in 180 seconds. This conciseness exercise will help you improve the clarity of your talks.

In CANTHER this year, there are 35 PhD students (12 D1, 9 D2, 11 D3 and 3 D4) among which 21 females (M/W ratio: 40%). As we have to pay attention to the parity, we will have to try to recruit more male PhD students in the future ;).

I want to thank the PhD participants: 24 out of 35 (68.57%). Am glad to see that the percentage has increased since last year (63.63%) but am sure we can do better next time.

I wish our PhD students long life and great success in Science. It is a long winding road to getting a permanent position as a scientist and thus it is also important to start looking at other opportunities that your PhD degree may give you and participating to meetings/seminars/conferences/workshops may be one way of doing it.

I wish you all a great, enjoyable and successful PhD Day,

Isabelle Van Seuningen

CANTHER laboratory director

PhD Students' Day
March, 19th 2020

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

9 h 30 – 9 h 45 ***Welcoming and opening session by Isabelle VAN SEUNINGEN (Director of UMR Canther)***

9 h 45 – 11 h 05 **Session 1 (Genetic and epigenetic landscapes) -** Chair: **Erwan GOY & Audrey VINCENT**

Ludivine RABY (D1)
Polycomb Repression and zebrafish model in cancerology

Marie DENOULET (D2)
Plasticity mechanisms involved in radio-induced reprogramming of cancer stem cells (CSC) from non-CSC in breast cancer.

Laurène FENWARTH (D3)
Genomic landscape of acute myeloid leukemia in younger adults: results from the ALFA-0702 study

Amélie DECOURCELLE (D3)
The OGT-EZH2 axis regulates the expression of UNC5A in colon cancer cells

Quentin BAILLEUL (D3)
Impact of H3.3K27M mutation on Diffuse Intrinsic Pontine Glioma's resistance to treatment

11 h 05 – 11 h 35 ***Coffee break***

11 h 35 – 12 h 40 **Session 2 (Chemoresistance and resistance to targeted therapies) –**
Chair: **Quentin BAILLEUL & Marie-Hélène DAVID**

Meriem BEN KHOUD (D1)
Characterization and follow-up of exhausted T cells in acute myeloid leukemia-affected patients pre- and post-chemotherapy treatment

Clara LEWUILLON (D1)
Deciphering the PD-1/PD-L1 calcium signature in the immunological synapse: microfluidic single cell technology towards precision immunotherapies

Nicolas STOUP (D1)
3D structural resolution and therapeutic targeting of the MUC4-ErbB2 oncogenic complex in the treatment of pancreatic cancer

Sarah TROUVILLIEZ (D2)
Interactions between TrkA/CD44vX and their signaling partners in Triple Negative Breast Cancer (TNBC) : new target therapy ?

Marie WINTER (D2)
Vimentin contributes to the enhanced agressiveness of triple negative breast cancer cells surviving combined and sequential chemotherapeutic treatment

Thomas SWIERCZEWSKI (D3)
MUC1, an actor of chemoresistance in clear cell renal carcinoma

12 h 40 – 13 h 40 ***Lunch (Atrium)***

13 h 40 – 14 h 50 **Session 3 (Non coding RNAs) –** Chair: **X… X.... & Bernadette NEVE**

Sandy FELLAH (D1)
Role and therapeutic potential of DNM3OS a long non-coding RNA in hepatic fibrosis

Evodie PEPERSTRAETE (D1)
Role of long non coding RNA H19 in emergence of cancer stem cells and in the metastatic development of breast cancer

Julie LEMAIRE (D2)
Role and therapeutic potential of ncRNA MIR17HG in pulmonary carcinogenesis

Clément LECERF (D3)
The long non-coding RNA H19 impairs p53 and disrupts the DNA damage response to promote genetic instability in breast cancer cells.

Nihad BOUKROUT (D3)
Roles of MUC4-miR-210 in pancreatic carcinogenesis and resistance to chemotherapy

14 h 50 – 15 h 35 **Session 4 (Metabolism) -** Chair: **Marie WINTER & X… X…**

Pierre-Damien CAUX (D1)
In vivo real-time mass spectrometry for gastric cancer guided surgery with SpiderMass

Mathilde BRULE (D1)
Redox status as a mediator of ionizing radiation-induced reprogramming of non-tumorigenic cancer cells into cancer stem cells in breast cancer

Quentin FOVEZ (D2)
Metabolic regulation of mitochondrial spare capacity: role in drug resistant AML

Raeeka KHAMARI (D3)
Mitochondrial targeting of persistent myeloid leukemia cells after exposure to iFLT3.

15 h 35 – 16 h 00 ***Coffee break***

16 h 00 – 17 h 00 **Session 5 (Mechanisms of carcinogenesis) -** Chair: **Quentin FOVEZ & Alessandro FURLAN**

Lama HASAN BOU ISSA (D1)
Gene dependencies in MYC OE myeloma

Martine PALMA (D2)
The involvement of AKT1 protein in nonsense-mediated mRNA decay

Erwan GOY (D3)
DNA Single-Strand breaks and cell senescence : the two steps towards second cancer post-radiotherapy

Marie FERNANDES (D3)
Co-activation of invasion and survival contributes to transformation induced by exon 14 skipping of MET receptor in lung cancer

17 h 00 – 17 h 15 ***Selection of the best oral communications by the jury***

17 h 15 - 17 h 30 ***Awards for the best oral communications***

Closing remarks

Ma thèse en 180 secondes – le concours (D1)

Abstract n° 1
Meriem Ben Khoud. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 2
Mathilde Brulé. Team *Cell Plasticity and Cancer*
Abstract n° 3
Pierre-Damien Caux. Team *Mucins, Cancer and Drug Resistance*
Abstract n° 4
Sandy Fellah. Team *Senescence, Fibrosis and Cancer*
Abstract n° 5
Lama Hassan Bou Issa. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 6
Clara Lewuillon. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 7
Evodie Peperstraete. Team *Cell Plasticity and Cancer*
Abstract n° 8
Ludivine Raby. Team *Cell Plasticity and Cancer*
Abstract n° 9
Nicolas Stoup. Team *Mucins, Cancer and Drug Resistance*

La science en 180 secondes (D2)

Abstract n° 10
Marie Denoulet. Team *Cell Plasticity and Cancer*
Abstract n° 11
Quentin Fovez. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 12
Julie Lemaire. Team *Senescence, Fibrosis and Cancer*
Abstract n° 13
Martine Palma. Team *Efficacy & Resistance to anti-tumor Therapies*
Abstract n° 14
Sarah Trouvilliez. Team *Cell Plasticity and Cancer*
Abstract n° 15
Marie Winter. Team *Cell Plasticity and Cancer*

Oral communications (D3)

Abstract n° 16
Quentin Bailleul. Team *Cell Plasticity and Cancer*
Abstract n° 17
Nihad Boukrout. Team *Mucins, Cancer and Drug Resistance*
Abstract n° 18
Amélie Decourcelle. Team *Senescence, Fibrosis and Cancer*
Abstract n° 19
Laurène Fenwarth. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 20
Marie Fernandes. Team *Efficacy & Resistance to anti-tumor Therapies*
Abstract n° 21
Erwan Goy. Team *Senescence, Fibrosis and Cancer*
Abstract n° 22
Raeeka Khamari. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 23
Clément Lecerf. Team *Cell Plasticity and Cancer*
Abstract n° 24
Thomas Swierczewski. Team *Mucins, Cancer and Drug Resistance*

Supplementary abstracts

D1

Abstract n° 25
Nicolas Germain. Team *Factors of Persistence of Leukemic Cells*

D2

Abstract n° 26
Léa Fléchon. Team *Factors of Persistence of Leukemic Cells*
Abstract n° 27
Charles Herbaux. Team *Factors of Persistence of Leukemic Cells*

D3

Abstract n° 28
Philippe Jamme. Team *Efficacy & Resistance to anti-tumor Therapies*
Abstract n° 29
Frédérick de Miollis. Team *Mucins, Cancer and Drug Resistance*

D4

Abstract n° 30
Anthony Turpin. Team *Efficacy & Resistance to anti-tumor Therapies*

D1

Abstract n°1

Authors: **Meriem Ben Khoud[1],** Suman Mitra[1], Bruno Quesnel[1,2] and Carine Brinster[1]

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CANTHER team:☐**CA**☐**XLB**☐**DT**☐**IVS** ☒**BQ**

PhD supervisor: **Dr. Carine BRINSTER**

Characterization and follow-up of exhausted T cells in acute myeloid leukemia-affected patients pre- and post-chemotherapy treatment

Abstract

Introduction. Acute myeloid leukemia (AML) is characterized by an increased proliferation of hematopoietic progenitors or precursors (blasts) of the different myeloid lineages (granulocytic, monocytic, erythrocytic or platelet). Exhaustion of T cells (TEX), leading to a gradual descrease of their effector functions, was observed in AML-affected patients due to their continous stimulation by persistent AML-associated antigens.

Methods. Blood and bone marrow samples of AML patients will be collected at diagnosis and at different time points of post-chemotherapy treatment. A multicolor flow cytometry (FC) analysis will be used to identify the different T cells subsets followed by a functional characterization of the exhausted T cells. Their TCR (T-cell receptor) and CD3 sequences will then be assessed using a SMART (Switching Mechanism at 5' End of RNATemplate)- RT- PCR and next generation sequencing.

Results/expected results. We will identify exhausted T cells (TEX) in AML-patients at diagnosis and after chemotherapy treatment. As TEX can present different degrees of exhaustion according to the expression of different inhibitory receptors, we will test their functionality and the capacity of partial IL-2 agonists to reverse their impairment. The analysis of their TCR and CD3 sequences will allow us to better understand whether their functionality can be restored post-chemotherapy in the presence of lower amounts of antigens.

Conclusion. The follow-up of TEX in AML-patients pre- and post-treatment will allow us to analyze the mechanisms and markers of a potential functional restoration and thus understand their role in complete remissions or relapses of patients. The use of partial IL-2 agonists and the efficacy in exhaustion phenotype could lead to new therapeutic strategies in AML-affected patients.

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PhD student funding: Université de Lille, Ligue contre le cancer
Acknowledgements: Nathalie Jouy and Emilie Floquet for cell sorting

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Abstract n°2

Authors: **Mathilde Brulé**[1], Marie Denoulet[1], Nadège Bidan[1], François Anquez[2], Xuefen Le Bourhis[1] & Chann Lagadec[1]

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CANTHER team:☐**CA**☒**XLB**☐**DT**☐**IVS** ☐**BQ**

PhD supervisor: **Dr. Chann Lagadec**

Redox status as a mediator of ionizing radiation-induced reprogramming of non-tumorigenic cancer cells into cancer stem cells in breast cancer

Abstract

Introduction. Cancer Stem Cells (CSCs) are particularly resistant to chemotherapy and radiotherapy. A relative phenotypic plasticity has been observed in some non-tumorigenic cancer cells (non-CSCs) able to re-acquire a CSC phenotype under the effect of these treatments. The laboratory recently discovered that this conversion involved inflammatory cytokines. Furthermore, cytokines and Reactive Oxygen Species (ROS) lead to an autoregulation loop between them. Interestingly, while CSCs have a higher expression of ROS scavengers that make them resistant to ROS produced by anticancer treatments, their mitochondrial metabolism results in a higher level of mitochondrial proton leakage than non-CSCs and generates ROS.
Considering the close relations between cytokines, ROS and reprogramming, this research project aims to identify molecular mechanisms that determine the redox balance in CSCs to annul the reprogramming and thus improve the efficiency of cancer treatments.

Methods. Classical methods to study CSCs is often destructive and allow analysis of cell population at specific time points. However, as a rare event, reprogramming is an unsynchronized dynamic process. To better understand it with the goal to prevent it, we developed a protocol including microscopy and algorithms to allow the dynamic of reprogramming non-CSCs into CSCs. Thus, individual cell can be track over five days in multiple fluorescence channels in parallel. These tools enable us to evaluate, with fluorescent sensors, the stem state, the redox state and the energy metabolism at a single cell level.

Results/expected results. We generated three breast cancer cell lines stably expressing redox biosensors, such as Grx1roGFP2 and CSC reporter. Interestingly, we observed that the moment of the CSC transition seemed to be preceded by an abrupt drop in the oxidation level, ten hours before this reprogramming.

Conclusion. This results have to be validated and confirmed using pharmaceutical inhibitors, in order to prevent reprogramming and increase anti-tumor efficiency.

Number of characters: ***2058***

PhD student funding: Inserm, Région Hauts-de-France
Acknowledgements: PhLAM (Physique des Lasers, Atomes et Molécules), Plateforme BiCel

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Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°3

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CANTHER team:**☐CA☐XLB☐DT☒IVS ☐BQ**

PhD supervisor: **Pr Fournier Isabelle/ Dr Florence Renaud**

In vivo real-time mass spectrometry for gastric cancer guided surgery with SpiderMass

Abstract

Introduction. In 2011, the number of esogastric cancers were estimated at 1,500,000 new cases and 2,110,100 new cases are expected in 2025. The detection of the esogastric cancer is generally at a late stage and a surgical procedure is usually carried out. To avoid any risk of relapse, surgeons take between 5 and 8 cm of excision margins controlled by intraoeprative pathology diagnostic. However, this process show several drawbacks of which, time (30-45 min), error rate (up to 30%) and the fact that it is operator dependent. This is particularly true for poorly cohesive carcinoma (PCC) which is an aggressive form of esogastric cancer occuring in younger population that poses issues for intraoepative diagnostic. There is thus a need to improve the intraoperative diagnostic.Since 2014, Prism laboratory has been developing, the SpiderMass, a new mass spectrometry system designed for in vivo and real time guided surgery. SpiderMass was shown to provide adequate sensitivity and specificity for tumor typing and grading and its inocuity was proven on a pre-clinical trial conducted from dog patients.

Methods. Here, we performed analyses of ex vivo esogastric human tissue sections provided by FREGAT (French Research Esophageal and Gastric Tumors) using SpiderMass technology. Briefly the technology is a mini-invasive system for in vivo analysis by mass spectrometry (MS).The system is composed of a laser microprobe that perform tissue micro-sampling by excitation of tissue water molecules. The generated aerosol is transferred to the MS instrument for real-time analysis.

Results/expected results. Thanks to the SpiderMass, we were able to discriminate the different subtypes of esogastric cancer and in peculiar the PCC. Molecular profiles of the analyzed tissue sections together with the classification bring an accurate and quick diagnosis.

Conclusion. These results show the ability of SpideMass to bring a new molecular diagnosis to the surgeons.

Number of characters: 1964

PhD student funding: ISite Sustain / Région Hauts de France/OCR
Acknowledgements: ISite ULNE, Satt Nord, IUF

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Abstract n°4

Authors: **Sandy FELLAH**[1], Corentin DE SOUSA[1], Grégoire SAVARY[1], Edmone DEWAELES[1], Cynthia VAN DER HAUWAERT[1,2], Nicolas POTTIER[1,3], Christelle CAUFFIEZ[1]

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CANTHER team: ☒**CA** ☐ **XLB** ☐ **DT** ☐ **IVS** ☐ **BQ**

PhD supervisor: **Christelle CAUFFIEZ**

Role and therapeutic potential of DNM3OS a long non-coding RNA in hepatic fibrosis

Abstract

Introduction. Liver myofibroblasts, which derived from activated hepatic stellate cells (HSC), play a critical role in fibrogenesis. However, targeting these cells has been challenging due to the lack of specific receptors or motifs on these cells. In addition, targeting TGF-β, one of the most potent stimuli for HSC activation, remains a major challenge for the development of antifibrotic therapy, as systemic inhibition of TGF-β can provoke inflammation and increase the risk of neoplasia. Recently, our lab demonstrated that targeting the non-coding RNA DNM3OS using LNA-based oligonucleotides represents a new powerful approach to selectively inhibit TGF-β signaling in lung myofibroblasts. In this thesis project, we now aim at demonstrating that pharmacological approaches interfering with DNM3OS may also represent new effective therapeutic strategies in liver fibrosis.

Methods. This project will be addressed using a combination of experimental approaches including cultured cell lines, two complementary mouse models of hepatic fibrosis (Bile Duct Ligation and CCl4 injections) as well as state-of-the-art technologies such as single cell genomics and next generation RNA-FISH.

Results/expected results. Our results indicate that DNM3OS is strongly induced in both mouse models of hepatic fibrosis and selectively expressed in liver myofibroblasts. Moreover, the systemic administration of anti-miR-199a-5p, directed against one of the mature miRNAs derived from DNM3OS, limits liver fibrosis lesions. Data obtained in the LX-2 human HSC line show an overexpression of both DNM3OS and its three mature miRNAs in cells exposed to TGF-β. Finally, modulation of miR-199a-5p expression showed implication of this miRNA in the fibrotic phenotype of these cells (fibroblast to myofibroblast differentiation, proliferation, migration, collagen synthesis...).

Conclusion. Interfering with DNM3OS function could constitute a real therapeutic solution for patients with liver fibrosis.

Number of characters: 1985

PhD student funding: University of Lille
Acknowledgements:

Abstract n°5

Authors: **Lama Hasan Bou Issa**[1], Katarina K. Jovanović[1], Léa Fléchon[1], Xavier Leleu4, Romanos Sklavenitis Pistofidis[2,3] , Irene M. Ghobrial[2,3], Thierry Facon[5], Bruno Quesnel[1,5], Salomon Manier[1,5]

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PhD supervisor: **Dr. Salomon MANIER, MD, PhD**

Gene dependencies in MYC OE myeloma

Abstract

Introduction. MYC alterations trigger transition from premalignant stages to multiple myeloma (MM). However, MYC remains undruggable. To overcome this, we sought to determine the main dependencies in MYC overexpressing MM by analyzing large-scale knockdown screening followed by functional validations.

Methods. We performed in silico analyses from the Dependency Map (Achilles) with CCLE, CLUE and MM patient datasets to look for gene dependencies. We generated an isogenic model of MYC OE in U266 cell line by using EF1A-C-MYC lentiviruses, and performed RNA-seq, quantitative proteomic analysis and a drug screening with ~2000 compounds. The GLS1 dependency can be validated, using both CB-839 for pharmacological inhibition and siRNA targeting GLS1.

Results/expected results. By analyzing correlations between MYC expression and ATARiS scores from a genome-wide shRNA screening, we identified main dependencies of MYC OE cells for genes involved in glutamine (Gln) metabolism and cell cycle. Top dependencies were observed with MYC binding protein MAX, GLS1 and SLC1A1 involved in Gln metabolism and E2F6 involved in cell cycle. GSEA analysis of RNA seq data using isogenic model showed strong enrichments of translation and cell cycle pathways, with similar results observed in CCLE and MM patient data and Tandem Mass Tag mass spectrometry analysis. To explore the therapeutic potential of these dependencies, we performed a drug screening and identified 47 compounds with potent activity on U266/MYC. Validation screen identified 3 leading compounds (Torin-2, LY2835219 and AT7519) to which U266/MYC showed highest sensitivity at 10 µM. To validate GLS1 dependency, siRNA-mediated GLS knockdown and CB-839 will be tested on several cell lines with various MYC expressions and the sensitivity will be assessed.

Conclusion. Our results demonstrate that MYC OE MM cells are dependent on Gln metabolism and cell cycle, and these findings can represent a keystone in identifying novel therapeutic str

Number of characters: 2000

PhD student funding: IRCL
Acknowledgements:

Abstract n°6

Authors: **Clara Lewuillon**[1], Aurélie Guillemette[1], Shaik Faruk Azam[2], Nathalie Jouy[3], Dominique Collard[2], Mehmet Cagatay Tarhan[2], Bruno Quesnel[1], Loïc Lemonnier[4], Carine Brinster[1] & Yasmine Touil[1]

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PhD supervisor: **Yasmine Touil**

Deciphering the PD-1/PD-L1 calcium signature in the immunological synapse: microfluidic single cell technology towards precision immunotherapies

Abstract

Introduction. Tumor dormancy and the escape of cancer cells from the immune response represent the major causes of relapse in patients with cancer including acute myeloid leukemia (AML), however the mechanisms are still poorly understood. Cytotoxic lysis of tumor cells by CD8+ T cells is dependent on calcium signaling. However, this cytotoxic activity can be modulated by the expression of PD-L1 by the tumor cells. This project will consist in understanding how PD-1/PD-L1 signaling established during the immunological synapse (IS) between T lymphocytes and AML cells modulates calcium signaling.

Methods. For this project, we propose to investigate, at the IS scale, the calcium signature of the IS involving PD-1/PD-L1 signaling in a clinical context of AML. Using blood and bone marrow samples from patients, we will assess the impact of the PD-1/PD-L1 axis on the calcium signaling of CD8+ T and leukemia cells according to their phenotype. PD-L1+ leukemic cells will be sorted according to their proliferative status and brought into contact with CD8+ T cells expressing PD-1 in microfluidic platforms, enabling (i) optimization of IS formation and (ii) molecular studies of cellular events at the single cell level. The calcium signaling and the cellular events will be evaluated via calcium imaging and confocal microscopy.

Results/expected results. Restoring the functionality of T lymphocytes could lead to lysis of PD-L1+ tumor cells. This project could provide additional knowledge on the impact of the anti-PD-1 antibody at the IS scale. A specific calcium signature could also help to predict the efficacy of anti-PD-1. Recently, we carried out the first experiments on the IS calcium signaling starting from T lymphocytes PD-1 and PD-L1 cancer cells, within microfluidic devices.

Conclusion. Better knowledge of these regulatory mechanisms may provide new perspectives for immunotherapies or optimization of chemotherapy protocols currently used in the clinic.

Number of characters: 1976

PhD student funding: Région Hauts de France/CHR de Lille
Acknowledgements: Ligue contre le cancer, ARC, Université de Lille, Ecole Doctorale Biologie Santé, IRCL, INSERM

Abstract n°7

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CANTHER team: ☐ **CA** ☒**XLB** ☐ **DT** ☐ **IVS** ☐ **BQ**

PhD supervisor: **Eric ADRIAENSSENS**

Role of long non coding RNA H19 in emergence of cancer stem cells and in the metastatic development of breast cancer

Abstract

Introduction. Breast cancer is a public health problem. Despite the progress made in terms of screening and treatment, the mortality rate is stable since 15 years. Mortality results from the occurrence of metastasis. The appearance of metastasis requires mechanisms allowing tumor proliferation, migration, invasion, resistance to anoikis, the epithelio-mesenchymal transition but also appearance of mammary cancer stem cells.
The long non-coding RNA H19 (lncRNA) is involved in breast tumorigenesis. It is a precursor of microRNA, the miR-675. The aim of this study is to understand the relative part of H19 and miR-675 in the phenotype observed during tumor progression and metastatic development of breast cancer.

Methods.

Results/expected results. We demonstrate that breast cancer cell lines (MCF-7, MDA-MB-231, SUM159PT) overexpressing H19, form more spheres and migrate more than cell lines which do not overexpress H19 by transwell. The results of the clonogenicity assay show an increased formation of clones in the cell lines which overexpress H19. However, the cells that overexpressed miR-675 did not form more clones than their control cell lines. In addition, H19 participates in the epithelio-mesenchymal transition. We obtain a breast cancer cell line (SUM159PT) expressing the pH19-mCherry vector (fluorophore mCherry under the control of H19 promoter). The pH19-mCherryhigh cells form more spheres, more clones and migrate more than the pH19-mCherrylow cells. In vivo tests on zebrafish confirme the results obtained in vitro.

Conclusion. The long non-coding RNA H19 and the miR-675 participate in the appearance of mammary cancer stem cells and in the metastatic development of breast cancer.

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PhD student funding:
Acknowledgements:

2nd Ph.D. Day
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Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°8

Authors: **Ludivine Raby**[1], Pamela Völkel[1], Xuefen Le Bourhis[1] & Pierre-Olivier Angrand[1]

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CANTHER team: ☐ **CA** ☒**XLB** ☐ **DT** ☐ **IVS** ☐ **BQ**

PhD supervisor: **Pierre-Olivier Angrand**

Polycomb Repression and zebrafish model in cancerology

Abstract

Introduction. The epigenetic mechanisms that change chromatin environment regulate in part gene expression. Among the epigenetic regulators, proteins of the Polycomb Repressive Complex 2 (PRC2) trimethyls the lysine 27 of the histone H3, chromatin compaction and repression of many genes involved in cell proliferation and cell differentiation. Polycomb repression is also involved in human pathologies such as cancer. In 80% of cases of high grade gliomas (HGG) and diffuse intrinsic pontine gliomas (DIPG) in children, a K-to-M mutation at lysine 27 of histone variant H3.3 is found. This mutation inhibits the activity of the PRC2 and lead to oncogenesis. The aim of my thesis project is to model this human H3.3-K27M oncogenic mutation in zebrafish.

Methods. We will use a transgenic approach to generate new lines of zebrafish carrying the H3.3-K27M mutation and model this type of cancer. In order to express the mutation in specific neuronal cells, we will use the GAL4/UAS system and will take advantage of available zebrafish lines expressing GAL4 under the control of specific neuronal promoter. We will generate a transgenic line expressing H3.3-K27M under the control of UAS. Crosses between these different zebrafish lines will drive the expression of the oncogenic mutation in defined brain cells.

Results/expected results. Expression of the human mutation in transgenic fish lines will give new insights into the molecular mechanisms involved in glioma biology. With our system, we will investigate whether the differentiation state of cells have an impact on tumorigenesis. Our transgenic construction will allow us to isolate cancer cells and to do transcriptomic analyses.

Conclusion. Finally, the transgenic lines could be used to test new molecules to fight against this disease. This strategy will be the first attempt to model pediatric gliomas in zebrafish.

Number of characters: 1880

PhD student funding: University of Lille
Acknowledgements: Cancéropôle Nord-Ouest ; La Ligue contre le Cancer – Pas-de-Calais

Abstract n°9

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CANTHER team: ☐ **CA** ☐ **XLB** ☐ **DT** ☒ **IVS** ☐ **BQ**

PhD supervisor: **Isabelle VAN SEUNINGEN, Nicolas LEBEGUE**

3D structural resolution and therapeutic targeting of the MUC4-ErbB2 oncogenic complex in the treatment of pancreatic cancer

Abstract

Introduction. Pancreatic cancer remains one of the cancers for which no diagnosis or no efficient therapy exists. The incidence for this cancer is increasing since 1980 (+247.7% globally between 1980 and 2012) without clearly identified risk factors, which makes pancreatic cancer a real problem of public health. Moreover, conventional therapies and/or targeted therapies often remain inefficient and fail. In the laboratory, we have shown the pro-tumorigenic role of the MUC4-ErbB2 complex in a murine pre-clinical model of pancreatic cancer. This makes the MUC4 mucin an alternative therapeutic target to slow down tumorigenesis. Moreover, we have also identified the EGF domains of MUC4 as involved in the direct interaction with ErbB2 and in the tumor progression.

Methods. The aims of this PhD project are:
1- Resolve the 3D structure of the MUC4-ErbB2 complex using carbene footprinting or X-ray diffraction,
2- Identify the ligands of the EGF domains of MUC4 using virtual or fragment-based library screening,
3- Measure the affinity (MicroScale Thermophoresis) and the biological activity of the ligands in vitro (proliferation, migration, invasion), and in vivo (transgenic mouse model of pancreatic cancer).

Results/expected results. This project should lead to the structural resolution of the MUC4-ErbB2 complex, still unknown and propose efficient ligands targeting the EGF domains of MUC4 to reduce the tumor progression in pancreatic cancer.

Conclusion. This strategy could provide new therapeutic options for this deadly cancer, aiming at increasing patient survival, quality of life and decrease treatment costs.

Number of characters: 1625

PhD student funding: Région Hauts de france, Inserm
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D2

Abstract n°10

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PhD supervisor: **Chann Lagadec**

Plasticity mechanisms involved in radio-induced reprogramming of cancer stem cells (CSC) from non-CSC in breast cancer.

Abstract

Introduction. Cancer stem cells (CSC) are a tumor subpopulation characterized by their ability of self-renewal and their pluripotency, allowing them to generate new tumors, and are the major cause of cancer recurrence. Moreover, anticancerous treatments, such as radiotherapy, have been described to induce the reprogramming of non-CSC into CSC in breast cancer. Pluripotent factors such as KLF4, NANOG, OCT4 and SOX2, are reexpressed during this process.This study aims to decipher the plasticity mechanisms such as cell-to-cell communication through extracellular vesicles as well as epigenetic changes as effectors of reprogramming and enabling the stemness phenotype reacquisition.

Methods.On the one hand, extracellular vesicles from irradiated cells are purified in order to characterize their abundance, their content and their impact on non-irradiated cells. On the other hand, CSC and non-CSC populations are sorted by FACS before and after irradiation, and global epigenetic changes are evaluated by reduced representation bisulfite sequencing (RRBS) and ChIP-seq in order to characterize the involvement of both DNA methylation and histones modifications (H3K27me3 and H3K4me3).

Results/expected results. After irradiation, we observed an enhanced production of extracellular vesicles and an enrichment of their RNA content in EMT related genes, leading to an increased stemness related genes expression in non-irradiated treated cells. Global analysis of epigenetic modifications reveals differentially methylated regions (DMR) and differential peaks of H3K4me3 and H3K27me3. The validation of these key differences should lead to new insights of pathways implicated in reprogramming.

Conclusion. The study of cell-to-cell communication and epigenetic regulation occurring throughout reprogramming will provide therapeutic possibilities to radiosensitize tumors and new features in understanding the plasticity mechanisms of cancer cells.

Number of characters: 1948

PhD student funding: University of Lille
Acknowledgements: BiCeL Plateform, Bilille Plateform, IRCL

Abstract n°11

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PhD supervisor: **Dr Jérôme Kluza**

Metabolic regulation of mitochondrial spare capacity: role in drug resistant AML

Abstract

Introduction. Spare respiratory capacity (SC) is a mitochondrial metabolic characteristic used to describe the level of maximal oxygen consumption that can be reached by oxidative phosphorylation in case of a sudden increase in energy demand. Depletion of SC has been described in acute myeloid leukemia (AML), but the link between this metabolic feature and its impact on leukemia phenotype remains unclear. This project aims to unravel the mechanisms underlying SC regulation in AML and to evaluate SC assessment by oximetry as a potential biomarker.

Methods. SC from healthy hematopoietic cells has been compared to AML blasts from patients (n=35) or to AML cell lines (n=8) by oximetric methods. SC of AML was also assessed in normoxia or hypoxia. Metabolites modifications have been determined during SC by mass spectrometry (using Glucose-13C6 and Glutamine-13C5). Finally, we have determined the cell death induced by anticancer or pro-oxydant drugs in AML treated or not with SC inhibitors.

Results / expected results. We have shown that a subpopulation of patients (60%) exhibit blasts with a lower SC than healthy hematopoietic cells. DNA sequencing revealed that low SC group has more mutations than normal SC group which correlated with poor survival. Oximetry experiments showed that SC can still be recruited under hypoxia. Mass spectrometry and oximetry approaches revealed that SC was dependent on pyruvate oxidation. Finally, we have shown that the mitochondrial pyruvate carrier inhibitor UK5099 reduced SC in AML and increased cell death induced by pro-oxidative drug.

Conclusion. Using oximetric analysis of blast, we have shown that AML patients can be divided in two subgroups: low SC or normal SC. Survival of patients is actually under investigation to determine if SC could be considered as a new predictive biomarker. We will now focus on molecular targets involved in SC regulation of AML.

Number of characters: 1913

PhD student funding: Université de Lille et Région Haut-de-France

Acknowledgements: Salim Dekiouk, Dr. Thierry Idriozek, Nathalie Jouy, Emilie Floquet, Dr. Samuel Meignan, Dr. Bart Ghesquière, Jean-Claude Vienne, Catherine Cimetta, BioImaging Center Lille, IRCL, Ligue contre le cancer

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°12

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PhD supervisor: **Nicolas POTTIER**

Role and therapeutic potential of ncRNA MIR17HG in pulmonary carcinogenesis

Abstract

Introduction. Lung cancer is a leading cause of cancer death in the world. Non Small Cell Lung Cancer is a common subtype (85%), associated with a low 5-year survival rate. Adenocarcinoma is the predominant histological phenotype. Therapeutic strategies rely on cisplatin therapy as first line agent. As resistance to treatment occurs frequently, identifying new therapeutic targets, such as microRNAs, is necessary.

Methods. We have identified miR-92a-3p, a member of 17-92 miARN cluster, produced by the polycistronic non coding RNA MIR17HG, that is upregulated in lung cancer. We developed a strategy based on LNA GapmeR Antisense Oligonucleotides, able to inhibit expression of this primary transcrit in nucleus by RNAse H action. Two lung adenocarcinoma cell lines, mutated KRAS A549 and PC9, without KRAS mutation, were used. Overexpression of MIR17HG should confirm its oncogenic effect and that targeting this non coding RNA with GapmeR is an efficient strategy.
To evaluate the feasibility to target MIR17HG in vivo, a model of mutant-KRAS driven pulmonary adenocarcinoma is developed.

Results/expected results. MIR17HG nuclear expression A549 was confirmed by RNA FISH. Furthermore, MIR17HG inhibition was achieved using GapmeR both in A549 and PC9 cells.
Finally, we are assessing the loss of function of mature miARNs belonging to MIR17HG, such as miR-92a-3p, in various oncogenic processes.

Conclusion. GapmeR efficacy was demonstrated in two distinct cell lines and additional experiments are currently performed to characterize the anticancer effects resulting from MIR17HG targeting.

Number of characters: 1595

PhD student funding: EDBSL
Acknowledgements:

2nd Ph.D. Day
March 19th, 2020

Abstract n°13

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PhD supervisor: **Fabrice Lejeune**

The involvement of AKT1 protein in nonsense-mediated mRNA decay

Abstract

Introduction. Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism ensuring the fast degradation of mRNAs harboring a premature termination codon (PTC) to prevent the synthesis of truncated proteins. Different proteins are implicated in the NMD and to date, only one kinase has been identified to be involved in NMD. This kinase is SMG1, a member of the phosphatidylinositol 3-kinase-related protein kinase family, with the phosphoprotein UPF1 as target. Other proteins involved in NMD are phosphorylated but their kinase has not be identified yet.
By using a particular screening system, we selected three putative NMD inhibitors from a kinase inhibitor library, with the same target: the AKT1 protein. We are now proving that AKT1 is a kinase involved in the NMD but also we are searching the role of AKT1 in the NMD regulation.

Methods. By using the CRISPR/Cas9 technology, we realized the silencing of AKT1 gene with the creation of a HEK293FT cell line, named HEK 293 FT Delta AKT1. After the characterization of this novel cell line, we analyzed the expression of different mRNAs by RT-PCR compared to wild-type cells, to analyze the NMD efficiency. We also studied the interactions between AKT1 and NMD factors by immunoprecipitations and microscopy.

Results/expected results. The absence of AKT1 protein leads to a slower proliferation of cells compared to wild-type cells. In addition, the NMD is inhibited in cells when AKT1 is silenced, demonstrating that AKT1 is involved in the NMD mechanism. My results also indicated that AKT1 interacts with UPF1 and UPF3X proteins, two central NMD factors.

Conclusion. I clearly demonstrated that AKT1 is involved in NMD. My preliminary results suggest that AKT1 could phosphorylate UPF1 and/or UPF3X. We have now to understand the close connection between the PI3K/AKT/mTOR signaling pathway and the NMD, and explore the molecular mechanism of this regulation.

Number of characters: 1917

PhD student funding: University funding (Ecole Doctorale)
Acknowledgements: Ligue contre le cancer

2nd Ph.D. Day
March 19th, 2020

Abstract n°14

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PhD supervisor: **Pr. Robert-Alain Toillon**

Interactions between TrkA/CD44vX and their signaling partners in Triple Negative Breast Cancer (TNBC) : new target therapy ?

Abstract

Introduction. Breast cancer is the most frequent malignancy among woman worldwide (OMS). But, breast cancer is a heterogeneous disease and according to the molecular characteristics, prognosis is different. Especially, Triple Negative Breast Cancer (TNBC) remains a clinical challenge due to the absence of specific and efficient therapy. In this context, CANTHER unit CNRS 9020 - INSERM U1277 highlights the role of Nerve Growth Factor (NGF) in TNBC. In fact, NGF binding to its receptor, TrkA, induces its phosphorylation and promotes CD44vX interaction. TrkA/CD44vX complex activates a phospho-TrkA independent signaling pathway which is involved in resistance of Trk kinase inhibitors (Larotrectinib). To target such resistance mechanism, we depicted TrkA/CD44vX interactions and deciphered TrkA signaling pathways.

Methods. Immunoprecipitation were performed to characterize kinetic of complex patners binding. By biolayer interferometry, we set up direct interactions. At least, molecular determinant of protein interaction were identified by mutagenesis.

Results/expected results. Immunoprecipitation of TrkA showed that CD44 inhibition does not affect signaling partners recruitment. Then, Biolayer interferometry reveals, for the first time, that TrkA directly interacted with a peculiar signaling molecule (so called W). Moreover, we showed that the interaction TrkA/W implies a four amino-acid motif of TrkA and a functional domain of W. Inhibition of W and TrkA directed mutagenesis both inhibited migration/invasion of MDA-MB-231 TBNC line.

Conclusion. All Together my phD results reveal that TrkA binding to W promotes breast cancer aggressiveness and resistance of Trk kinase inhibitors. Moreover, in order to block TrkA oncogenic functions, we determinated that if current Tki are not efficient, our results suggest the potential of new Trk inhibitors targeting this resistance mechanism.

Number of characters: 1902

PhD student funding: Région Haut de France, Université de Lille
Acknowledgements: Ligue contre le Cancer, i-Site, e-Zyvec

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°15

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PhD supervisor: **Xuefen Le Bourhis**

Vimentin contributes to the enhanced agressiveness of triple negative breast cancer cells surviving combined and sequential chemotherapeutic treatment

Abstract

Introduction. Tremendous in vitro and preclinical data have been accumulated in the effort to understand chemoresistance of triple negative breast cancer (TNBC). However, modifications in cancer cells surviving combined and sequential treatment still remain poorly described.

Methods. In order to mimic clinical neoadjuvant treatment, we first treated MDA-MB-231 and SUM159-PT TNBC cell lines with epirubicin and cyclophosphamide for 2 days, and then with paclitaxel for another 2 days. After 4 days of recovery, persistent cells surviving the combined and sequential treatment were characterized in terms of growth under 2D and 3D conditions, invasion in Matrigel culture and in zebrafish embryos. Molecular expression profile of persistent cells was also evaluated by qRT-PCR, immunoblotting and flow cytometry analyses.

Results/expected results. Persistent TNBC cells exhibited decreased colony formation in 2D culture and increased growth in Matrigel. These cells formed also more tumourspheres and were more invasive in Matrigel culture and in zebrafish. At the molecular level, persistent TNBC cells were enriched for vimentinhigh sub-population. Vimentin silencing using siRNA approach decreased the invasive and sphere forming capacities as well as Akt phosphorylation in persistent MDA-MB-231 cells, suggesting that vimentin could be considered as a new targetable player in the ever-elusive status of drug resistance and recurrence of TNBC.

Conclusion. For the next step, we will treat MDA-MB-231 and SUM159-PT TNBC cell lines in a long-term manner by treating cells with drugs during several cycles according to the clinical protocol of neoadjuvant treatment. The resulted persistent cells will be then evaluated at both the cellular and molecular levels. Particularly, we will perform functional "omics" analysis to identify potential targetable modifications implied in tumour resistance and recurrence.

Number of characters: 1913

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Acknowledgements: We thank the flow cytometry core facility of BioImaging Center of Lille (BICeL), which is a shared ressource of INSERM/CHRU of Lille, and particularity Nathalie Jouy for her help with the FACS experiments. We are grateful to William Laine for his help in the quantification of cells using a Seahorse XFe24 analyzer and to Mélanie Arcicasa for her help in confocal analyse.

D3

Abstract n°16

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PhD supervisor: **Dr Samuel Meignan**

Impact of H3.3K27M mutation on Diffuse Intrinsic Pontine Glioma's resistance to treatment

Abstract

Introduction. DIPG is one of the worst pediatric brain tumors regarding prognosis due notably to intrinsic cell resistance to radio and chemotherapy. One of the main characteristics of DIPG cells is the presence of a mono-allelic mutation on the lysine 27 of histone H3 (H3K27M). This mutation inhibits the trimethylation of this lysine that leads to strong modifications of gene expression. Until now, its role in cell resistance to treatment has not been deciphered, due to a lack of relevant cellular models.

Methods. In order to evaluate the role of the mutation on resistance to treatment, we first induced the mutation in three H3K27 WT pediatric glioma cell lines. In parallel, using gene editing, we are establishing DIPG cellular models in which the mutation is reverse.These model constitute original tools to study the impact of H3K27M mutation in DIPG cells resistance to treatment.

Results/expected results. For the model of induction, the transfected cell lines exhibit the mutation accompanied by a loss of H3K27me3 mark. For now, we showed an increased cell growth due to the mutation in two cell lines. On contrary there was no impact on resistance to selected chemotherapies or ionizing radiation. In the third cell line, we didn't observe any impact on cell growth, but an increase of cell radioresistance. These two subtypes are also different according to their epigenetic profil. We currently evaluate the mutation's impact on cell migration/invasion, sensitivity to a large panel of chemotherapies and targeted therapies, in vivo cell growth in parallel to gene expression analysis by RNASeq. Concerning the mutation reversion, we confirmed the establisment of one reversed DIPG cell lines which will be caracterized as well.

Conclusion. To sum up, these different models would allow us to decipher cellular and molecular mechanism induced by the H3.3K27M mutation in DIPG cells, especially concerning resistance to treatment, in order to identify putative therapeutic targets.

Number of characters: 1997

PhD student funding: Centre Oscar Lambret / Région Hauts de France
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Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°17

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PhD supervisor: **Nicolas Jonckheere**

Roles of MUC4-miR-210 in pancreatic carcinogenesis and resistance to chemotherapy

Abstract

Introduction. Pancreatic adenocarcinoma is one of the most deadly cancers in western countries with an extremely poor prognosis because of a lack of efficient therapy. The MUC4 membrane-bound mucin is normally not expressed in pancreas but is neoexpressed in early pancreatic intraepithelial neoplastic lesions and its expression correlates with the disease progression and chemoresitance to gemcitabine. Our aim is to characterize the cellular mecanism including the microRNAs (miRNAs) by which MUC4 regulates these processes. Our preliminary data show that MUC4 inhibition impaired expression of miR-210.

Methods. We evaluated miR-210 expression level by RT-qPCR in: (1) LstopLKrasG12D, Pdx1-Cre preclinical pancreatic cancer mouse tissues (2) patients tissues (n=20). We firt demonstrated a regulation loop between miR-210 and MUC4. For that, we focused on miR-210 trasncriptional regulation by NFkB factor, known to be regulated by MUC4. We performed NFkB chromatin imminuprecipitation experiments (ChIP) on miR-210 promoter in pancreatic cancer cells. In order to evaluate miR-210 biological roles, we generated stable cell lines overexpressing miR-210 or anti-miR-210 and then measured cell proliferation and migration in vitro and tumor growth in vivo using subcutaneous xenograft models.

Results/expected results. Our results showed a miR-210 overexpression in mice and patient tissues. Our ChIP experiments confirmed the interaction between NFkB and miR-210 promoter which is lost in MUC4–KD cells. We also demonstrated that miR-210 suppresses MUC4 expression via TGFb pathway. miR-210 Overexpression inhibits pancreatic cancer cells proliferation of. Similarly we observed tumor growth inhibition of xenografts overexpressing miR-210. Finally we highlighted that cell proliferation regulated by miR-210 involves the AKT pathway.

Conclusion. Our work show the existence of miR-210-MUC4 regulation loop and that miR-210 regulates pancreatic cancer cells proliferation.

Number of characters: 1973

PhD student funding: Lille university
Acknowledgements: I would like to thank the Bio Imaging Center of Lille

2nd Ph.D. Day
March 19th, 2020

Abstract n°18

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PhD supervisor: **Vanessa DEHENNAUT**

The OGT-EZH2 axis regulates the expression of UNC5A in colon cancer cells.

Abstract

Introduction. Numerous studies support the existence of a close relationship between nutritional disorders, epigenetic changes and the etiology of colorectal cancers (CRC). However, the underlying mechanisms remain poorly understood. The UNC5 gene family includes 4 members: UNC5A-B-C-D that act as Netrin-1 receptors to regulate the survival/apoptosis balance and are often down-regulated in CRC partly through epigenetic mechanisms not fully deciphered. Lately, the post-translational modification O-GlcNAcylation, has been defined as a new CRC hallmark and is considered as a nutritional sensor. Moreover, OGT, the unique enzyme that catalyzes protein O-GlcNAcylation, has also emerged as an important epigenetic regulator of gene expression notably by modifying EZH2, the catalytic subunit of the Polycomb Repressive Complex 2. In this context we investigated the involvement of the OGT-EZH2 axis in the regulation of the expression of the UNC5 family members.

Methods. We first tested in vivo the impact of a short-term nutritional disorder on the colonic expression of the UNC5 family members. For that, mice were subjected to a high carbohydrates or a normal diet for 8 weeks. Then, we used a combination of pharmalogical inhibitions and siRNA approaches coupled to RT-qPCR analyses and promoter activities studies, to test in vitro whether the OGT-EZH2 axis could play a role in the epigenetic regulation of UNC5 genes.

Results/expected results. We showed that the high sugar diet impacts the colonic expression of all members of the UNC5 family. Moreover, our in vitro results strongly suggest that OGT/O-GlcNAcylation and EZH2 act in synergy to repress UNC5A transcription in colonic cancer lines.

Conclusion. Our data suggest that the O-GlcNAcylated form of EZH2 represses the colonic transcription of UNC5A and support the hypothesis that hyper-O-GlcNAcylation may contribute to aberrant EZH2 activity leading to the repression of key tumor suppressor genes including UNC5A resulting in CRC.

Number of characters: 2000

PhD student funding: University of Lille
Acknowledgements: Financial partners: CNRS, la ligue contre le cancer, Cancéropôle Nord-Ouest and GEFLUC Lille Flandres-Artois

Abstract n°19

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PhD supervisor: **Claude Preudhomme**

Genomic landscape of acute myeloid leukemia in younger adults: results from the ALFA-0702 study

Abstract

Introduction. Over the past decade, molecular insights from high-throughput sequencing (HTS) have helped to characterize molecular landscape of acute myeloid leukemia (AML) and identify new prognostic factors. Other high resolution technologies such as the single-nucleotide polymorphism array (SNP-array) have emerged but their impact on daily practice remains unclear. Here we aimed at 1) deciphering the molecular landscape of a large academic AML cohort from the ALFA-0702 trial (n=713 patients) by HTS and SNP-array technologies, 2) validating the personalized approach recently developed by Gerstung et al. 3) refining the molecular landscape of adolescents and young adults (AYA, aged 15-25 years).
Methods. Patients aged 18-59 years, enrolled in the ALFA-0702 trial with available DNA at diagnostic were screened by HTS and SNP-array. The molecular landscape of AML in AYA was retrospectively addressed gathering data from both adult and pediatric protocols.
Results/expected results. Among the 656-analyzed patients, NPM1 (35.7%), DNMT3A (26.8%), FLT3-ITD (22.4%), NRAS (22.0%) stood for the most commonly mutated genes. The ELN 2017 classification discriminated 3 distinct prognostic subgroups. Five-year OS was 77.0%, 57.0% and 40.0% in patients with favorable, intermediate and adverse risk, respectively (P<10-5). However, the knowledge bank approach outperformed OS predictions of the ELN 2017 stratification (concordance index 69.0 versus 63.1). Interestingly, AML in AYA presented a higher mutational burden compared to children (P=0.014).
In the randomized cohort of the ALFA-0702 trial (n=187 patients), defining « micro-complex » karyotype from at least 4 SNP–array lesions defined a new subset of adverse AML-patients that may benefit from new alternative consolidation regimen such as clofarabine.
Conclusion. HTS along with SNP-array have refined AML prognostication. These results raise new hope to personalize treatment decision-making.

Number of characters: 1956

PhD student funding: I-SITE ULNE
Acknowledgements: To all authors' contribution

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°20

Authors: **Marie Fernandes**[1], Sonia Paget[1], Zoulika Kherrouche[1], Martin Figeac[4], Anne Chotteau-Lelièvre[1], Luca Grumolato[3], Alexis Cortot[1,2] and David Tulasne[1]

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PhD supervisor: **David Tulasne**

Co-activation of invasion and survival contributes to transformation induced by exon 14 skipping of MET receptor in lung cancer.

Abstract

Introduction. Recently, mutations leading to MET exon 14 skipping (METex14) have been discovered in 3% of lung cancers. This exon skipping causes the loss of several negative regulatory sites such as the Y1003 implicated in MET degradation and the ESV1001D motif, a site of caspases cleavage associated with MET pro-apoptotic activity. Our objective is to characterize the transforming potential of METex14 through evaluation of the relative contribution of these regulatory mechanisms.

Methods. We have developed by stable transfection and by CRISPR-Cas9, two models of epithelial cell lines expressing versions of MET WT, METex14, mutated separately or simultaneously on the residues Y1003 and V1001.

Results/expected results. Our results show that the mutation of Y1003 site like METex14 induces a greater activation of the signaling pathways and invasion with a similar transcriptional program. Furthermore, the mutation of the caspases cleavage like METex14 induces the loss of the MET pro-apoptotic capacities.
Since these data suggest a combined consequence of the loss of these different regulatory mechanisms, I will first assess whether the increase in the invasive capacities associated with resistance to apoptosis of METex14 can cooperate in the development of tumors in vivo. Second, we hypothesize that resistance to apoptosis may represent a mechanism of resistance to chemotherapy still widely used for these patients. Thus, mice injected with cells expressing the different versions MET will be treated with Cisplatin in order to evaluate tumor regression and the rate of apoptosis.

Conclusion. A better understanding of the METex14 mechanisms of activation and its potential implication in resistance to chemotherapy can provide a strong argument to accelerate the transition from the use of chemotherapy to targeted therapies for patients carrying METex14 mutations.

Number of characters: 1882

PhD student funding: University of Lille - EDBSL
Acknowledgements: ANR, BiCel Platform.

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°21

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PhD supervisor: **Corinne ABBADIE**

DNA Single-Strand breaks and cell senescence: the two steps towards second cancer post-radiotherapy

Abstract

Introduction. Secondary sarcomas are a rare complication of radiation therapy. Clinical data reveal that they occur mostly in the margin of the Planning Target Volume (PTV), an area receiving less than 80% of the dose, deposited by low energy photons.

Methods. To characterize the DNA damages affecting normal cells surrounding the PTV, we positioned culture plates of Normal Human Dermal Fibroblasts (NHDFs) straddling the limit of a field which was irradiated at 2Gy daily for 2 weeks with a linear accelerator (Clinac, VARIAN), hence mimicking a classical treatment.

Results/expected results. Cells inside the PTV underwent as expected lethal DNA double-strand breaks (DSBs) and DNA single-strand breaks (SSBs). In contrast, cells in the margin underwent only SSBs. These SSBs accumulated with successive irradiations because of a decrease in the activity of PARP1, the enzyme that initiates SSB repair. This SSB accumulation did not induce cell death, but senescence.
Senescence is a specific cell cycle arrest state mainly induced by oxidative and genotoxic stresses during therapy or physiological aging. To examine the role of senescent cells as sarcoma's cell source, we sorted the senescent cells of the margin and kept them in culture. After 10 days, proliferating cells re-appeared in the culture. These post-senescent cells were more invasive than proliferating NHDFs and were mutated. Tumorigenic assays are ongoing to study their potential to form sarcoma.
To strengthen these results in vivo, we positioned mice at the margin of a phantom that was irradiated once at 2Gy. The results confirm the specific induction of SSBs without DSBs. We will soon evaluate the possible induction of senescent phenotype in the margin using a p16-LUC model and its impact on sarcomas development by using a senolytic drug, the Navitoclax.

Conclusion. This work evidence the specific induction of SSBs and senescence at the margin of an irradiated field and its possible role in secondary cancer induction.

Number of characters: 1999

PhD student funding: EDBSL
Acknowledgements: Project funded by "la Ligue contre le cancer" and the "Canceropole Nord Ouest"

Abstract n°22

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PhD supervisor: **Jérôme Kluza**

Mitochondrial targeting of persistent myeloid leukemia cells after exposure to iFLT3.

Abstract

Introduction. FLT3-ITD acute myeloid leukemia (AML) is a poor survival disease driven by a constitutively activated receptor tyrosine kinase. Although FLT3 inhibitors (iFLT3) show significant antitumor activities, they don't always succeed in eliminating all tumor cells, limiting the effectiveness of long term treatments. The tumor relapses in patients are related to the persistence of leukemic cells that survive during drug treatment. To address this issue, this project proposes to identify the metabolism of persistent leukemia cells to find therapeutic targets to overcome iFLT3 resistance.

Methods. Using cytometry (to assess survival), Seahorse (to measure O2 consumption and glycolysis activity) and mass spectrophometry (with 13C glc or gln), early metabolic alterations were characterized in AML exposed to iFLT3.We have also evaluated the specific metabolism of persistent AML, surviving after short or long term exposure of iFLT3.

Results/expected results. We have shown that inhibition of glucose metabolism by iFLT3 contribute to induce cell death in AML. Since the mechanism of action of iFLT3 involves a reduction of glycolysis, the hypothesis was based on the fact that resistant leukemic cells survived through mitochondrial functions. We demonstrated that glutamine metabolism, through its ability to support mitochondrial function, became a metabolic dependency of persistent AML. Therefore, glutamine depletion by L-asparaginase synergistically sensitizes leukemic cells to iFLT3. Finally, we evaluated the microenvironmental influence in this context and we have shown that association of iFLT3 with L-asparaginase increases leukemic cell death in the presence of mesenchymal cells.

Conclusion. To conclude, these data highlights the role of metabolic adaptations as a resistance mechanism to iFLT3 and suggests glutaminolysis as a therapeutically targetable vulnerabilit.

Number of characters: 1894

PhD student funding: University of Lille / Nord-Pas-de-Calais subvention.
Acknowledgements: My thanks go to Nathalie Jouy, Nicolas Germain, Emilie Floquet, Boyer, Guillaume Garçon, Antonino Bongiovanni, Anne Loyens.

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°23

Authors: **Clément Lecerf**[1], Jordan Collette[1], Evodie Peperstraete[1], Erwan Goy[1], Corinne Abbadie[1], Bruno Lefebvre[2], Fabrice Lejeune[1], Xuefen Le Bourhis[1], Eric Adriaenssens[1]

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PhD supervisor: **Eric Adriaenssens**

The long non-coding RNA *H19* impairs p53 and disrupts the DNA damage response to promote genetic instability in breast cancer cells.

Abstract

Introduction. *H19* is a long non-coding RNA described to play key roles in cancers from different tissue origins. We have previously shown that the *H19* gene is activated by E2F, repressed by p53 and RB tumor suppressors and implicated in cell cycle progression. Few studies suggest that *H19* can regulate the expression of p53, but nothing has been done in breast cancer and the functional mechanism remains undeciphered.

Methods.

Results/expected results. We demonstrated that *H19* interacts with p53 in breast cancer cells. This interaction induces p53 degradation but also impairs p53 function under DNA damage by preventing its translocation into the nucleus. We showed that *H19* interacts not only with p53 but also with MDM2 to form a ternary complex. Moreover, *H19* reduces p53 transcriptional activities and impairs cell cycle blockage, apoptosis induction and senescence of cells after DNA damage. Furthermore, we found that *H19* expression favors genetic instability, allowing for the accumulation of gene mutations as revealed by HPRT assay.
Thereafter, we hypothesized that the genetic instability observed was due to a defective DNA repair system. We next investigated the implication of *H19* during the DNA damage response, using genotoxic agents (bleomycin, irradiation) and molecular tools that allow the analysis of DNA repair. We showed that *H19* expression represses the activation of histone variant H2AX, and thus represses the signalization of DNA breaks. Interestingly, this is accompanied by enhanced repair mechanisms such as non-homologous end-joining (NHEJ) and homologous recombination (HR). Comet assays revealed that *H19* expression reduces the DNA breaks proportion, suggesting that *H19* expression accelerates DNA repair, leading to unreliable repair and thus mutations. Further studies are ongoing about the putative link of genetic instability induced by *H19* and chemoresistance.

Conclusion. Thus, our data highlight a novel mechanism of protumoral action of *H19* through the repression of p53 and the induction of genetic instability.

Number of characters: ***2055***

PhD student funding: Université de Lille
Acknowledgements:

Abstract n°24

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PhD supervisor: **Michaël PERRAIS**

MUC1, an actor of chemoresistance in clear cell renal carcinoma

Abstract

Introduction. MUC1 is a large O-glycosylated membrane-bound mucin, overexpressed in clear cell renal carcinoma (CCRC). Although representing 3% of all adult cancers, it is known to be one of the most chemo- and radio-resistant carcinoma. Our team already demonstrated that MUC1 overexpression in CCRC leads to a poor prognosis and metastatic status. It is also known that MUC1 overexpression is related to higher levels of MDR (Multi-Drug Resistance) gene expression, coding for efflux membrane transporters. The aim of this study is to reveal the underlying mechanism of MUC1-induced chemoresistance in CCRC.

Methods. Two renal carcinoma cell lines have been used: ACHN cells (no expression of MUC1) and RCC4 cells (overexpression of MUC1). Our team already has a stably transfected ACHN cell line which overexpresses MUC1. For RCC4 cells, we have used the CRISPR/Cas9 technology to generate a stable cell line deficient for MUC1. Proliferation has been assessed by real-time imagery with Incucyte® technology, and migration by wound assay. Cell viability and chemoresistance have been assessed by MTS assay and calculation of IC50 after a 72h-treatment with classical and targeted chemotherapies. MDR genes expression was evaluated by RT-qPCR and activity by flow cytometry.

Results/expected results. MUC1-overexpressing cells show higher proliferation and migration rates than MUC1-deficient cells. Moreover, MUC1-overexpressing cells are more resistant to classical or targeted chemotherapies treatments, suggesting a higher chemoresistance. Finally, overexpression of MUC1 is also correlated with an increased expression and activity of drug transporters of the MDR family.

Conclusion. In CCRC, we show that MUC1 is an actor in tumor progression (migration and proliferation) and in chemoresistance by increasing expression and activity of efflux transmembrane transporters.

Number of characters: 1876

PhD student funding: Année Recherche (CHU/ARS HdF)
Acknowledgements: Cancéropôle Nord Ouest

Supplementary abstracts

D1

2nd Ph.D. Day
March 19th, 2020

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°25

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PhD supervisor: **MARCHETTI Philippe, LANCEL Steve**

Adipocyte microenvironment and leukemia resistance to targeted therapies

Abstract

Introduction. Microenvironment plays a central role in the development, progression and resistance of cancers. Tumour microenvironment and in particular adipocyte lineage (from mesenchymal stem cells (MSC) to adipocytes), induces modifications of cancer cell metabolism mediated in particular by a transfer of mitochondria or fatty acid. This project aims to unravel the mechanisms underlying Chronic Myeloid Leukemia (CML) resistance to targeted therapies induced by different adipocyte subpopulations.

Methods. Murine MSCs (D1-ORL UVA) and preadipocytes (3T3-L1 MBX) have been differentiated into adipocytes. 3D culture microenvironment has been generated through bioprinting to study metabolism of CML cells (DA1-3B) in the presence of the adipocyte lineage. Viability and proliferation of cells has been assessed. We will then study the reversibility of the resistance but also metabolic changes by determining oxidative phosphorylation on Seahorse, fatty acid transfer (FABP4 GFP), transcriptomic and calcium metabolism.

Results / expected results. Differentiation media have been tested and optimized in order to obtain differentiation in 14 days. We have shown that the adipocyte lineage induce resistance of imatinib-treated DA1-3B in contact co-culture. This resistance increased with differentiation of adipocyte cells: DA1-3B cells co-cultured with mature adipocytes showed 80% of resistance to imatinib vs only 20% with MSCs. Furthermore those mature adipocyte population showed an increase in oxydative phosphorylation (2 times fold increase of spare capacity).

Conclusion. Our results show that DA1-3B resistance to imatinib is dependent of mature adipocyte subpopulations who have a specific oxydative phosphorylation profile. Nature of resistance induction has to be confirmed and mitochondrial metabolic changes of leukemic resistant population has to be assessed in order to be able to target this resistance.

Number of characters: 1925

PhD student funding: INSERM, La ligue contre le cancer
Acknowledgements:

D2

2nd Ph.D. Day
March 19th, 2020

Abstract n°26

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PhD supervisor: **Thierry Idziorek and Salomon Manier**

Study of the Clonal Evolution of Mycosis Fungoides

Abstract

Introduction. Cutaneous T-cell lymphomas (CTCLs) are lymphoid malignant hemopathies affecting primarily the skin. The most common CTCL is Mycosis Fungoides (MF), which can cause skin lesions due to the focal accumulation of T-cells in the epidermis. This disease can progress to involve lymph nodes, spleen, and bone marrow and targets more frequently males over 50. Generally, MF has an indolent course with slow progression from cutaneous patch/plaque-stage disease to cutaneous tumors/necrotic ulcers. Transformed MF (tMF) occurs in approximatively 20% of patients with advanced-stage MF, when tumor cells undergo molecular and/or genetic changes that cause these cells to become larger. Presence of tMF is associated with a poor prognosis with a decreased overall survival.
Nowadays, the molecular mechanisms involved in the progression and transformation of MF are still mainly unknown.

Methods. We performed Whole-Exome Sequencing (WES) of 70 MF tumor and 20 normal samples provided by the CHRU of Lille and the Dana Farber Cancer Institute (DFCI, Boston, USA), respectively. In order to better understand the accumulation of genetic mutations (clonal evolution) in MF cells, some of these samples were taken over the time from patients who progressed from MF to tMF. WES has been done by using Nova-Seq 6000 Illumina (100X of coverage) at the world's largest genomics platform (by number of samples sequenced) in the Broad Institute (BI, Cambridge, USA).

Results/expected results. Currently, I was invited by the Ghobrial team at DFCI to use the BI recommended bioinformatics pipelines to analyze sequenced data. These methods will allow to identify the somatic genomic alterations (structural variants, single nucleotide variants, copy number alterations…) involved in MF development.

Conclusion. This study of MF sequential WES sample will allow us to interpret the emergence of resistant clones and to define molecular markers of transformation and potential therapeutic targets.

Number of characters: 1985

PhD student funding: This project was funded by IRCL. Additional financial supports were provided by the FFRMG (Fondation Française pour la Recherche sur le Myélome et les Gammapathies) and a fellowship from DFCI.

Acknowledgements: The IRCL members, the Ghobrial team members (DFCI), Chip Stewart and Gad Getz (Getz team, BI), Stéphanie Poulain (Service biologie-hématologie, CBP, CHRU de Lille), Martin Figeac (Plateau de Génomique Fonctionnelle et Structural, CBP) and the engineers of the bioinformatics plateform bilille.

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°27

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PhD supervisor: **Stéphanie Poulain**

A functional precision medicine based approach to identifying optimal combination treatments for T-PLL

Abstract

Introduction. Response to conventional therapies for patients with T-cell prolymphocytic leukemia (T-PLL) is usually poor and is associated with short survival. The BCL-2 antagonist venetoclax (VEN) was recently found to have some clinical activity in this disease; however, these early data suggest that this drug will not provide prolonged response when given as monotherapy.

Methods. Clinically annotated primary T-PLL patient samples were obtained. Baseline BH3 profiling to measure cytochrome C (cyto-C) release was performed. We performed NGS on a panel of 29 genes, including ATM and TP53, as well as Sanger sequencing to assess for IL2R, JAK1, JAK3, STAT5B mutations.

Results/expected results. Baseline BH3 profiling revealed that, compared to CLL cells, T-PLL cells are less primed for apoptosis but have comparable dependency on MCL-1. BCL-2 dependency was found to be significantly lower in T-PLL than CLL (cyto-C release 48.8%; 62.7% p=0.0005). Consistent with our BH3 profiling results, the degree of BCL-2 dependency in T-PLL cells was strongly associated with the amount of apoptotic cell death induced by VEN (R2 -0.58, p=0.004), whereas MCL1 dependency was strongly associated with the cell death induced by the MCL1 inhibitor AZD5991 (R2 -0.68, p=0.0005 respectively). We next performed DBP to assess the changes in apoptotic priming in T-PLL cells induced by HDACi, JAK/STATi and TCRi. HDACi and JAK/STATi increased overall T-PLL cell priming and BCL2 dependency (delta cyto-C release of 26.8%, p=0.004 and 14.8%, p=0.01 respectively), with no effect on MCL1 dependency. TCRi had no significant effect on priming. Consistent with the DBP data, our viability assays showed that HDACi and JAK/STATi induced significantly more cell death when combined with VEN.

Conclusion. We report the first data for BH3 profiling in T-PLL. We found that this disease is heterogeneously dependent on both BCL-2 and MCL-1. HDACi and JAK/STATi both enhance BCL-2 dependence, thereby sensitizing T-PLL cells to VEN.

Number of characters: ***2011***

PhD student funding: Faculté Médecine de Lille, Dana Farber Cancer Institute, SFH
Acknowledgements:

D3

Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°28

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PhD supervisor: **Alexis Cortot and Zoulika Kherrouche**

Alterations in the PI3K pathway drive resistance to MET inhibitors in NSCLC harboring MET exon 14 skipping mutations

Abstract

Introduction. MET TKIs (tyrosine kinase inhibitors) have demonstrated efficacy against advanced NSCLC with mutations causing MET exon 14 skipping (METex14 mutations), but primary resistance seems frequent, as response rates are lower than for targeted TKIs of other oncogene-addicted NSCLC. Given the known interplay between MET and PI3K, we hypothesized that in METex14 NSCLC, PI3K-pathway alterations might contribute to primary resistance to MET TKIs.

Methods. We reviewed clinical data from 65 patients with METex14 NSCLC, assessing PI3K-pathway alterations by targeted NGS (mutations) and immunohistochemistry (loss of PTEN). Using a cell line derived from a patient with primary resistance to a MET TKI and cell lines harboring both a METex14 mutation and a PI3K-pathway alteration, we assessed sensitivity to MET TKIs used alone or with a PI3K inhibitor and investigated relevant signaling pathways.

Results/expected results. We found a PIK3CA mutation in 2/65 samples (3.0%) and loss of PTEN in 6/26 (23%). All three of the MET-TKI-treated patients with a PI3K pathway alteration had shown progressive disease at first assessment. Likewise, MET TKIs had no effect on the proliferation of METex14-mutated cell lines with a PI3K-pathway alteration, including the PTEN-lacking patient-derived cell line. Treatment combining a MET TKI with a PI3K inhibitor caused inhibition of both PI3K and MAPK signaling and restored sensitivity to MET TKIs.

Conclusion. PI3K-pathway alterations are common in METex14 NSCLC and may confer primary resistance to MET TKIs. In preclinical models, PI3K inhibition restores sensitivity to MET TKI.

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Canther
Cancer Heterogeneity, Plasticity and Resistance to Therapies

Abstract n°29

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Development of a 3D in vitro microfluidic culture system to study tumor-stroma interactions and drug resistance of pancreatic adenocarcinoma

Abstract

Introduction. Pancreatic cancer (PC) is a deadly cancer for which no diagnostic or prevention plan currently exists. In order to contribute to the development of more predictive preclinical models, we are developing a 3D microfluidic model of PC to study the role of the ECM as well as the interaction between tumour cells (TC) and stromal cells (SC) in chemoresistance. PC is characterized by a high intra-tumoral pressure. Organ-on-a-chip microfluidic systems aim at faithfully recapitulating the physiology and microenvironment of tissues through spatial control of the tissue architecture and the addition of fluid control.

Methods. Our chip is made of three adjacent parallel microchannels connected by micropillars. The 3D tissue is 100µm thick. The central channel is filled with PC cells (Capan-2) and SC cells (PS-1) mixed in Biomimesys supplemented with Collagen 1 (2mg/ml) and the lateral channels allow controlled alimentation with cell culture medium containing either drugs or oncogenic molecules. They also allow us to generate interstitial flow and chemical gradient in the central part.

Results/expected results. We have shown that Matrigel unactivate SC and is fastly degradated is the micropores area. We have shown both theoretically and experimentally that we can create chemical gradient of oxygen and glucose within Biomimesys and generate relevant interstitial flow (5µm/s). We have aslo shown that proliferation is decreased under micro stream. We have quantified various proteins expressed both in SC and TC to validate the activation of the fibroblast (a-SMA), the production of matrix by the activated fibroblasts (Fibronectin) and the mesenchymal or epithelial feature of TC (Vimentin, E-cadherin). We also performed IC50 experiments to quantify the reduction of the TC clusters as a function of the concentration of FLOFIRINOX. We see that protein expression and IC50 are affected by flow and co-culture.

Conclusion. We have validated our system both at physical and biological level.

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D4

Abstract n°30

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Axon guidance Neuropilin1 and PlexinA2 genes are involved in ERG-associated prostate cancer

Abstract

Introduction. Prostate cancer (PCa) is the second leading cause of death from cancer in men. We previously identified PLXNA2 as a directly-regulated gene by ERG belonging to axon-guidance process.Our aim is to identify axon-guidance genes involved in ERG-associated PCa.

Methods. We first performed an in silico study to identify axon guidance genes associated with ERG-fusion expression. We selected the most relevant genes and then performed a Chip-Seq data set analysis that allowed us to identify androgen-receptor (AR) and ERG-related direct binding-sites, which paved the way for Chromatin Immunoprecipitation Assay (ChIP) experiments. Secondly, we did functional tests to show the impact of the most relevant genes on migration and invasion. Thirdly, using RNAs of human PCa sample cohort, we performed qRT-PCR to detect and correlate candidate genes to fusion expression. Finally, we used immunohistochemistry experiments to study the expression of these genes in tissue samples.

Results/expected results. Neuropilin1 (NRP1) was found to be a directly-regulated gene by both ERG and AR. NRP1 expression was strongly associated to primary and metastastic PCa. We established a significant correlation between both PLXNA2 and NRP1 gene expression and the presence of ERG fusion. NRP1 was highly detected in human lymph nodes and bone metastasis samples. Since neuropilin proteins were known to interact with plexins to act as coreceptors for semaphorin ligands, protein colocalization in stable clones suggested a functional association of PLXNA2 and NRPs in tumor cells via its ligands such as Semaphorin 3A (SEMA3A) and VEGFA.We demonstrated that SEMA3A acts as a repellant ligand. We also found a modification of localization of PLXNA2 and NRP1 receptors in function of the presence or absence of the ligands in PCa clone cells.

Conclusion. ERG fusion gene expression is able to deregulate axon guidance genes such as NRP1 and PLXNA2. These results reinforce the interest in targeting neuropilins in PCa.

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Missing abstracts

Jean-Baptiste Gibier, Team *Mucins, Cancer and Drug Resistance*
Xiangu Meng. Team *Cell Plasticity and Cancer*
Marion Gradwohl. Team *Factors of Persistence of Leukemic Cells*
Manon Cruette. Team *Factors of Persistence of Leukemic Cells*
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