2023-12-20

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Exploring functional interactome of Androgen Receptor and RNA in the early onset of the castration-resistant prostate cancer

General information

Call	2024
Reference	2024-08-MORILLON_PINSKAYA
Keyword(s)	Castration-resistant prostate cancer; RNA; Androgen Receptor; spatial interactomics

Director(s) and team

Thesis director(s)	Antonin Morillon & Marina Pinskaya
Research team	Non-coding RNA, Epigenetics, and Genomes Fluidity
Research department	UMR3244 - Dynamics of Genetic Information: fundamental bases and cancer (DIG-Cancer)

Description of the PhD thesis project

Abstract

Androgen Receptor (AR) is a key androgen-activated transcription factor driving prostate cancer progression. In clinics, the androgen deprivation therapy (ADT) is used to block AR activity but often fails leading to the onset of aggressive and incurable castration-resistant prostate cancer forms (CRPC). CRPC tumors display significant heterogeneity and cell plasticity with cells maintaining or restoring AR signaling. Emerging evidence suggests that RNA regulates the activity and stability of AR, even if it doesn't contain a canonical RNA-interacting domain. In this project, we aim to explore AR interactions with RNAs and their functional relevance in response to androgen deprivation in cell prostate cancer models and patient-derived tissues. For this purpose, specific RNA-protein molecular biology and imaging technics will be used to define structural determinants of AR binding to RNAs and their spatial cellular localization, in bulk and single-cell resolution. The RNA-AR interactome will be further studied for function in acquisition of the CRPC phenotype using conventional genetics, transcriptomics, molecular and cellular biology approaches. Collectively, performed experiments will help to elucidate a novel non-canonical mechanism of AR signaling regulation by RNAs that may contribute to cell survival and tumor growth in the early onset of CRPC.

Background

Inhibition of the Androgen Receptor activity by the conventional Androgen Deprivation Therapy (ADT) of advanced prostate cancer (PCa) induces the incidence of the lethal Castration-Resistant Prostate Cancer (CRPC) in more than 30% of cases. CRPC tumors display high heterogeneity and cell plasticity evolving into various subtypes including tumors with still functional AR signaling. Recently, AR was reported to interact

with RNAs in various cancer cell models. In particular, long noncoding (lnc)RNAs as SLNCR or HOTAIR regulate AR signaling by tethering it to specific gene promoters in melanoma and renal carcinoma or protecting it from proteolysis in prostate cancer. All these transcripts share a consensus AR-binding motif. We are using a dynamic *in vitro* cell model, recapitulating an early response to ADT, to investigate how AR regulates cell plasticity upon androgen deprivation. AR immunoprecipitation and RNA-sequencing (RIP-seq) revealed that AR is associated with distinct sets of RNAs in the presence and absence of androgen. More than 60% of these transcripts also possess the AR-binding motif (unpublished). This finding supports a hypothesis that RNAs may regulate the AR signaling in CRPC. However, it is still not clear which of AR domains interacts with RNAs, and whether this interaction is important for the AR activity and PCa progression in acquisition of androgen independence.

Objectives

The aim of this project is to explore whether and how RNAs modulate the AR activity contributing to cell plasticity upon androgen withdrawal. This will base further investigation of AR-RNA interactomes at bulk, single-cell and space resolutions to define:

(I) AR-RNA interactomes in bulk, single-cell and spatial resolutions.

Primarily, a direct binding of AR to RNAs will be assessed in prostate cancer cells in the presence and absence of androgen in bulk. Then, structural determinants of these interactions will be assessed, in particular, AR domain(s) and RNA motif(s) involved in binding. Finally, spatial localization of AR- RNA interactome will be explored for several examples by imaging analysis in cells and tissues revealing their subcellular residence, heterogeneity.

(II) Functional relationship between RNAs and AR in response to androgen deprivation.

RNA expression or RNA-AR interactions will be disrupted genetically in cells and studied for consequences on CRPC-related phenotypes (proliferation, drug resistance, apoptosis, gene expression pattern).

The study will be conducted at three levels of complexity:

- In cells: a dynamic system with cells growing in the presence of androgen and following androgen deprivation.
- In patient-derived xenografts: 4 models provided by our industrial partner, including 2 primary samples from treatment-naïve patients, 1 hormone-sensitive and 1 CRPC samples.
- In FFPE tissues: hormone-sensitive and CRPC tumor tissues will be provided by our collaborators.

Experimental approaches

We will use spatial microscopy-based technologies combined with genetic, molecular and cellular approaches in the already established in *vitro* system of androgen deprivation and in patient-derived samples (xenografts and tumor tissues).

(I) AR-RNA interactomes:

First, using the RNA interactome capture technique alone (2C) and coupled to AR immunoprecipitation and RNA-sequencing (CLIP-2C), we will assess if AR binds RNAs directly, by which domain and what are these AR-bound RNAs. It will be done in PCa cells expressing AR variants (full-length or truncated for the N-terminal, DNA- or Ligand-Binding domains) in the absence and presence of androgen.

In parallel, a specific microscopy-based tool of RNA *in situ* hybridization and proximity ligation assay (rISH-PLA) will be adapted to reveal AR-RNA interactions in cells. RNA candidates will be selected from our recent RIP-seq and 2C. Finally, rISH-PLA will be done in tissues.

(II) Functional relationship between RNAs and AR:

Using a loss-of-function approach to deplete RNAs or disrupt AR-RNA binding, we will explore changes in CRPC-related phenotypes as proliferation, drug resistance, and apoptosis. The most relevant conditions will be further investigated at the molecular level to assess perturbations in the AR activity by CUT&RUN and RNA-seq.

International, interdisciplinary & intersectoral aspects of the project

The project will be curated by Maïwen Caudron-Helger (DKFZ, Heidelberg, DE), the expert in RNA-protein interactomes currently in partnership through a French-German exchange network (UFA).

The project benefits from a long-date collaboration with clinical researchers: Virginie Firlej and Alexandre de la Taille (Hôpital Mondor, Créteil, FR), and Yves Allory (IC, Paris) who provided us with the expertise and patients tissues throughout recent publications, currently in the frame of a collaborative clinical trial on Prostate cancer patients (HOPE) and cofounding (Emergence-Sorbonne Université). Spacial interactomics experiments will be conducted in collaboration with the industrial partner Claire Béraud (Urosphere, Toulouse, FR).

The project appeals to skills in wet biology (cell culture and tissues manipulation, genetics, imaging and molecular biology technics) and computational analysis (NGS and imaging analyses). Technical support will be provided internally for the confocal microscopy, FACS, NGS (Curie Core Tech). The imaging analysis will be done in collaboration with Florian Mueller (Institut Pasteur, Paris, FR).

Recent publications

1. Cipolla R, Gabriel M, Ianese Regin G, Piemontese M, Szachnowski U, Firlej V, **Pinskaya M*** & **Morillon A*** A novel lncRNA PROCA11 regulates cell plasticity in response to androgen deprivation of prostate cancer cells. BioRxiv 2023, ** - Co-corresponding authors*

2. Le Hars M, Castro-Vega LJ, Rajabi F, Tabatadze D, Romero M, **Pinskaya M*** and Groisman I*. Pro-tumorigenic role of lnc-ZNF30-3 as a sponge counteracting miR-145-5p in prostate cancer. Biology Direct 2023 Jul 11;18(1):38, ** - Co-corresponding authors*

3. Jarroux J, Foretek D, Bertrand C, Gabriel M, Szachnowski U, Saci Z, Guo S, Londoño-Vallejo A, **Pinskaya M***, **Morillon A***. HOTAIR lncRNA promotes epithelial-mesenchymal transition by redistributing LSD1 at regulatory chromatin regions. EMBO Rep. 2021 Jul 5;22(7):e50193, ** Co-corresponding authors*

4. **Pinskaya M**, Saci Z, Gallopin M, Gabriel M, Nguyen HT, Firlej V, Descrimes M, Rapinat A, Gentien D, Taille A, Londoño-Vallejo A, Allory Y, Gautheret D, **Morillon A**. Reference-free transcriptome exploration reveals novel RNAs for prostate cancer diagnosis. Life Sci Alliance. 2019 Nov 15;2(6):e201900449

Expected profile of the candidate

We are looking for a PhD candidate with a strong motivation for basic research and acquisition of dual (wet biology and computational) skills, potential for independent and creative thinking. Applicants should show proof of their ability to work in different lab environments and adapt to different research topics and/or techniques (geographical/thematic/technical mobility). Knowledge and skills in RNA and cellular biology, and molecular basis of cancer are strongly recommended. Background in computational analysis of high throughput data (imaging or sequencing) is a plus, but not compulsory.