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International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Characterization of DNA repair in mitosis, a path to identify new tumor vulnerability

General information

Call	2024
Reference	2024-02-CECCALDI
Keyword(s)	DNA repair, synthetic lethality and targeting therapy, BRCA1/2-mutated breast and ovarian cancer

Director(s) and team

Thesis director(s)	Raphael Ceccaldi
Research team	Alternative DNA repair mechanisms in human cancers
Research department	U830 - Cancer, Heterogeneity, Instability and Plasticity - CHIP

Description of the PhD thesis project

Abstract

Deficiency in homologous recombination (HR)-mediated DNA repair occurs mainly through genetic inactivation of the *BRCA1* and *BRCA2* (*BRCA1/2*) genes. HR-deficiency (HRD) plays a role in the initiation and progression of many tumor types, including breast, ovarian and pancreatic tumors. HRD tumors exhibit increased genomic instability and dependence on alternative DNA repair mechanisms for survival, setting the stage for synthetic lethality-based targeted therapy. A prime example is the extreme sensitivity of HRD tumors to poly (ADP-ribose) polymerase inhibitors (PARPi).

More recently, the polymerase theta (Polθ) has recently emerged as a new, promising anti-cancer drug target. The first inhibitors including the one published by our group, are entering phase 2 clinical trial for the treatment of chemoresistant BRCA1/2-mutated ovarian tumors. Despite these advances, an exhaustive understanding of the biological function of Polθ was lacking until our lab recently solved its mechanism of action. We found that Polθ repairs DNA double strand breaks (DSBs) in mitosis to ensure genome integrity. Specific mitotic inhibition of Polθ leads to a defect in the repair of mitotic DSBs, increases genome instability and hinders BRCA1/2-mutated cell survival. The goal of this phD proposal is to study in-depth the mechanism that maintain genome stability during in mitosis to potentially uncover new tumor vulnerability.

Background

DNA double strand breaks (DSBs) are deleterious lesions that challenge genome stability. In interphase, DSBs are mainly repaired by non-homologous end joining (NHEJ) and homologous recombination (HR). However, cells can enter mitosis with DSBs that are formed during interphase or DSBs can also form in mitosis, as a

consequence of replication stress (RS). Given the fact the NHEJ and HR are inhibited in mitosis, cells must have evolved alternative ways of dealing with these lesions.
Our lab has recently found that the polymerase theta (Polθ) repairs mitotic DSBs and thereby maintains genome stability. In contrast to other DSB repair factors, Polθ function is activated in mitosis upon phosphorylation by the Polo-like kinase 1 (PLK1). Phosphorylated Polθ is recruited to mitotic DSBs, where it carries out end joining repair of broken DNA ends. Specific mitotic inhibition of Polθ leads to a defect in the repair of mitotic DSBs, increases genome instability and hinders BRCA1/2-mutated cell survival.
Our findings highlight the importance of mitotic DSB repair in maintaining genome stability and tumor survival (Gelot et al., Nature 2023). They also present the first evidence of active DSB repair in mitosis, suggesting that other DNA repair mechanisms (independent of Polθ) could exhibit their function in mitosis, opening new avenues of future investigations.

Objectives
Cells can enter mitosis with DSBs that are formed during interphase, and that DSBs can also form in mitosis, as a consequence of replication stress (RS). However, the mechanisms governing DSB transmission to mitosis remain unknown. In addition, while we showed that Polθ repairs DSBs in mitosis, little is still known about the composition, regulation and mode of action of mitotic repair. This project aims to shed light on the pathways limiting and/or resolving mitotic DSBs. We will employ two independent yet complementary approaches:

- **Aim 1: To identify factors limiting DSB formation in mitosis.** Here, we will perform an immunofluorescent-based siRNA screening for factors limiting DSB accumulation in mitosis.
- **Aim 2: To identify and study new players in mitotic repair.** Here, we will combine a Polθ immunoprecipitations (IPs) followed by mass spectrometry (MS) analyses to super resolution microscopy to gain insights into players and mechanism of action of mitotic repair.
  We posit the players preventing and/or resolving mitotic DSBs are required for survival of tumors with high levels of replication stress, such as breast and ovarian BRCA1/2-mutated tumor cells. This will be assessed in aim 3:
- **Aim 3: Clinical relevance of our findings.** A combination of in vitro and in vivo survival assays will be used to evaluate the newly identified players as potential drug target for cancer therapy.

Experimental approaches

- **Aim 1**: We will perform an unbiased immunofluorescent-based siRNA screening for factors limiting DSB accumulation in mitosis. BRCA2-/- and isogenic control cells will be transfected with a genomic siRNA library. Cells will be treated by replication stress (DNA polymerases inhibitor aphidicolin (APH)), which will result in DSBs formation in mitosis. 48 hours after siRNA transfection, we will use automated microscopy to score gH2AX and TOPBP1 foci formation (readout of mitotic DSBs) in mitotic cells.
- **Aim 2**: Understanding the function of a protein is often aided by the identification of its binding partners. We generated cells expressing GFP-tagged Polθ. GFP (Polθ) immunoprecipitations will be performed in mitotic cells. To induce mitotic DNA breaks, cells will be treated by APH. Quantitative MS analyses will provide high-throughput and unbiased identification of Polθ binding partners. Next, we will tag with the versatile HaloTag both alleles of newly identified mitotic repair factors. Using super-resolution microscopy, we will analyze endogenous Halo/Halo mitotic structures at high resolution to gain insights into mitotic repair mechanism of action.
- **Aim 3**: In vitro clonogenic survival assays and in vivo/xenotransplantation experiments will determine whether the newly identified substrates can represent new anticancer drug target. We expect inhibition of mitotic repair factors to death of tumor cell with high level of replication stress such as HRD tumors.

International, interdisciplinary & intersectoral aspects of the project

Based on the findings gathered by the student, the PI (Raphael Ceccaldi) will setup (if necessary) international collaboration(s), to seek the know-how required for the advancement of the research.

Our team aims at bridging the scientific bases of genome stability with patient needs in order to improve cancer treatment by fostering the development of new chemotherapeutics. We have demonstrated our potential to bridge basic to translation science (development of the first-in-class Polθ inhibitor (in clinical trials), filing of several patents, creation of a startup). We will apply the same strategy and effort towards innovation during this research program. Each novel vulnerability will be patented and its potential for innovation will be evaluated carefully with the help of the department for innovation at Institut Curie. This will facilitate the industrial exploitation of our discovery (patenting, licensing, collaboration with industrial partners) and expose the student to the private sector.

Our project is *per se* interdisciplinary since it stands at the crossroads between basic and translational research. Indeed, we will both dissect the molecular mechanisms of mitotic DNA repair (aims 1 and 2) and evaluate the clinical significance of our findings (aim 3). After identifying in aims 1 and 2 new players and reveal their function in mitotic DNA repair, a substantial part of this project will be dedicated to evaluate the clinical potential of our findings. Since mitotic repair is required for HRD tumor survival, we will perform in vitro and in vivo survival assays as well as curate existing cancer datasets to determine if and how the newly identified factors can represent novel drug target for the treatment of HRD tumors. Our project is also interdisciplinary because it seats at the interconnections between two biological disciplines: proteomics and cellular biology.

Recent publications

1. Kovacs M.T, Vallette M, Wiertsema P, Dingli F, Loew D, Nader G.P, Piel M, **Ceccaldi R**. DNA damage induces nuclear envelope rupture through ATR-mediated phosphorylation of Lamin A/C. Molecular cell. 2023.

2. Gelot C, Kovacs M.T, Miron S, Mylne E, Ghouil R, Popova T, Dingli F, Loew D, Guirouilh-Barbat J, Del Nery E, Zinn S and **Ceccaldi R**. Polθ is phosphorylated by Polo-like kinase 1 (PLK1) to enable repair of DNA double strand breaks in mitosis. Nature. 2023.

3. Zhou J, Gelot C, Pantelidou C, Li A, Yücel H, Davis RE, Farkkila A, Kochupurakkal B, Syed A, Shapiro GI, Tainer JA, Blagg BSJ, **Ceccaldi R***, D'Andrea AD*. A first-in-class Polymerase Theta Inhibitor selectively targets Homologous Recombination-Deficient Tumors. Nature Cancer. 2021 Jun;2(6):598-610.doi: 10.1038/s43018-021-00203-xs. * co-last and co-corresponding authors.

4. Kais Z, Rondinelli B, Holmes A, O'Leary C, Kozono D, D'Andrea AD, **Ceccaldi R**. FANCD2 Maintains Fork Stability in BRCA1/2-Deficient Tumors and Promotes Alternative End-Joining DNA Repair. Cell Reports. 2016 Jun 14;15(11):2488-99.

Expected profile of the candidate

Prospective applicants should have a strong desire to study molecular/cellular biology and cancer. The ideal applicant should further have either experience or interest in microscopy, genetics and/or proteomics. She/he is eager to learn, has solid capacity for independent and creative thinking. Great communication skills are needed, together with appropriate organizational capacity.