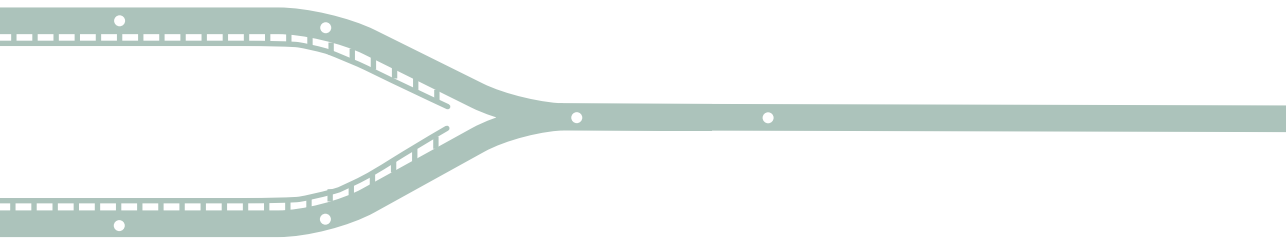
Using functional genetic screens to understand and overcome PARP inhibitor resistance

Paes Lobo Lopes Dias, M.



Chapter 1

Scope of the thesis

Developing targeted therapies that kill cancer cells while sparing non-malignant tissues is one of the main goals of current cancer research. Many of the currently available targeted therapeutics are inhibitors of oncogenes such as kinases, which have been one of the most successful classes of cancer drugs developed to date. However, strategies to specifically target cancers cells carrying loss-of-function mutations in tumor suppressor genes are less straightforward. Afterall, how can a mutated protein that is no longer working, or even produced at all, be targeted? The answer to this question relies on the identification of synthetic lethal interactions. Synthetic lethality in a cell or organism describes the situation where a defect in either one of two genes has little or no effect, whereas the combination of defects in both genes results in cell death. This phenomenon was discovered exactly a century ago, in 1922, by the Drosophila melanogaster geneticist Calvin Bridges, and the term synthetic lethality was coined a couple of decades later by Theodor Dobzhansky, in 1946[1,2]. Synthetic lethal relationships create drug discovery opportunities to selectively kill cancer cells carrying loss-of-function mutations while leaving nonmalignant cells largely unaffected. To date, the use of poly(ADP- ribose) polymerase (PARP) inhibitors in patients with homologous recombination (HR)-deficient tumors, such as *BRCA1/2*-mutated cancers, provides one of the best examples of synthetic lethality that has been successfully translated into the clinic[3,4].

BRCA1 and *BRCA2* are tumor suppressor genes that, when heterozygously mutated in the germ line, confer a considerably higher risk of breast and ovarian cancer. It is estimated that the cumulative breast cancer risk to age 80 years is 72% for *BRCA1* mutation carriers and 69% for *BRCA2* carriers, whereas the cumulative ovarian cancer risk to age 80 years is 44% for *BRCA1* carriers and 17% for *BRCA2* carriers[5]. While initially associated with hereditary breast and ovarian cancer (HBOC) syndrome, inherited mutations in *BRCA1/2* also predispose to other types of cancer, including prostate and pancreatic cancer[6]. Functional BRCA1 and BRCA2 proteins are crucial for the error-free repair of DNA double-strand breaks (DSBs) by HR and, consequently, for the maintenance of genomic stability[7]. Tumors arising in *BRCA1/2* mutation carriers frequently show loss of the wild-type allele and therefore cannot employ HR to repair DSBs, a defect that renders cells vulnerable to PARPi. Inhibition of PARP leads to collapsed replication forks and consequently to an increase in DSBs during the S phase of the cell cycle. BRCA1/2-deficient tumor cells can only resolve PARPi-induced DSBs via error-prone mechanisms, resulting in an accumulation of chromosomal rearrangements that ultimately leads to mitotic catastrophe.

The success of PARPi-induced synthetic lethality in BRCA1/2-deficient tumors has so far led to the approval of four different PARPi for the treatment of patients with advanced breast, ovarian, pancreatic and prostate cancers. Unfortunately, despite initial and often dramatic responses, patients receiving PARPi often develop resistance to the treatment. The major focus of my thesis is to understand the molecular mechanisms behind PARPi resistance in order to improve patient stratification and to find combination treatments to overcome resistance to PARPi by preventing, delaying or targeting resistant clones.

Chapter 2 provides a general introduction to this topic and a comprehensive summary of the currently known processes underlying PARPi resistance. Chapter 2 also discusses the potential strategies that might overcome PARPi resistance, such as combinations with chemotherapies, targeting the acquired vulnerabilities of PARPi-resistant tumors and suppressing the mutator phenotype of *BRCA1/2*-mutated tumors.

Preclinical studies have identified several PARPi resistance mechanisms, such as upregulation of drug efflux transporters, drug target-related mechanisms, restoration of HR via BRCA1/2 reactivation or via loss of DNA end-protection, and restoration of replication fork stability[7]. Yet, for most of these mechanisms, their relevance in the clinic remains elusive. In **Chapter 3**, we combined molecular profiling and functional analysis of the HR status of a unique collection of matched PARPi-naïve and PARPi-resistant tumors derived from genetically engineered mouse models (GEMMs) of *BRCA1/2*-associated breast cancer to investigate which BRCA1/2-independent mechanisms drive spontaneous resistance *in vivo*. While HR restoration was observed in the majority of PARPi-resistant *BRCA1*-mutated tumors, our data strongly suggests that HR cannot be reactivated in the absence of BRCA2. Moreover, our data suggests that 53BP1 loss is the prevalent resistance mechanism in HR-restored BRCA1-deficient tumors, whereas resistance in BRCA2-deficient tumors is mainly induced by PARG loss. These results may help the investigation of BRCA1/2-independent PARPi resistance in the clinic.

Preclinical studies have shown that HR can be restored in BRCA1-deficient cells through the loss of components of the 53BP1-RIF1-Shieldin DNA end-protection complex, resulting in PARPi resistance[8–16]. In **Chapter 4** and **Chapter 5**, we used functional genetic dropout screens to identify vulnerabilities of BRCA1/53BP1 double-deficient cells that could potentially be exploited therapeutically to overcome PARPi resistance. In **Chapter 4**, we used a DNA damage response (DDR) library and identified nuclear DNA ligase III (LIG3) as a critical mediator of PARPi resistance in BRCA1/53BP1 double-deficient cells *in vitro* and *in vivo*, rendering LIG3 a potential therapeutic target. Importantly, loss of nuclear LIG3 does not revert HR restoration but exposes cells to MRE11-mediated post-replicative single-stranded DNA (ssDNA) gaps upon treatment with PARPi, leading to accumulation of chromosomal abnormalities and cell death. Moreover, this work suggests that ssDNA gaps are a novel determinant of PARPi response which creates new opportunities for designing combination therapies **(Chapter 4 addendum)**. In **Chapter 5**, we looked beyond the DDR domain by using a genome-wide library to screen for acquired vulnerabilities of BRCA1/53BP1 double-deficient cells. We found that loss-of-function of multiple candidate genes improved response to PARPi, with the majority of the hits associated with DDR, including recombination and meiosis genes such as *GGNBP2* and *SWSAP1*. These results suggest that rewiring of DDR is the most promising approach of reverting PARPi resistance in BRCA1/53BP1 double-deficient cells. Nevertheless, we also identified candidate genes not involved in DDR, including many subunits from mitochondrial Complex I. Unfortunately, individual validation of these subunit genes was technically hard to achieve and further

studies are required to understand how inactivation of complex I of the mitochondrial respiratory chain can modulate PARPi response.

Finally, in **Chapter 6** we describe a detailed protocol for the use of three-dimensional (3D) mouse mammary tumoroids (tumor-derived organoids) to carry out functional genetic dropout screens and orthotopic transplantations in mice. These 3D tumoroids may be used to screen for new therapeutic targets to improve response to PARPi. In addition, they allow rapid and straightforward *in vivo* validation of candidate genes.

Altogether, the work described in this thesis extends our knowledge of the mechanisms behind PARPi response and resistance, and identifies potential therapeutic candidates to improve response to this tailored therapy. **Chapter 7** provides a general discussion in which we highlight some of the remaining questions.

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