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Using functional genetic screens to understand and overcome PARP inhibitor resistance

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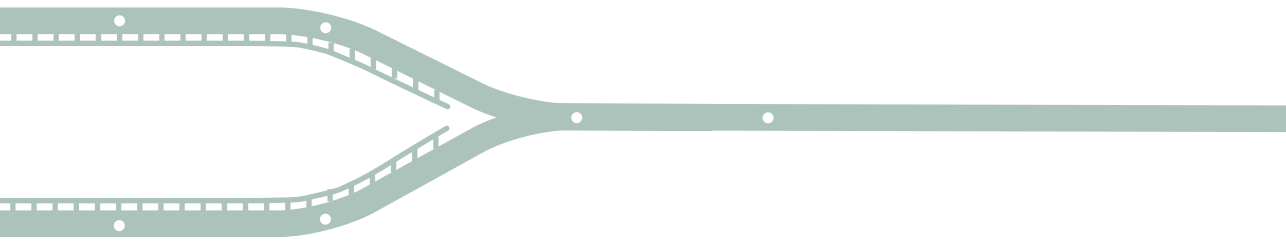

Appendices

ENGLISH SUMMARY

Heterozygous germ-line mutations in *BRCA1* and *BRCA2* predispose to several types of cancer in which the remaining wild-type allele is lost. Owing to their roles in the error-free repair of DNA double-strand breaks (DSBs) via homologous recombination (HR), lack of BRCA1/2 in these tumors results in DNA damage defects that can be specifically targeted by the inhibition of Poly-(ADP-ribose) polymerase 1 (PARP1). PARP1 is a key sensor of DNA damage and its inhibition has been shown to be synthetically lethal with deficiencies in HR, resulting in the selective killing of BRCA1/2-deficient tumor cells, while sparing BRCA1/2-proficient non-tumor cells. The success of this approach has resulted in the approval of four PARP1 inhibitors (PARPi) for the treatment of ovarian, breast, prostate and pancreatic cancers. However, drug resistance poses a major obstacle as, despite initial responses, patients receiving PARPi often develop resistance to the treatment. Understanding the molecular mechanisms behind PARPi resistance is therefore crucial to identify key determinants of PARPi response and to find combination treatment strategies to overcome resistance to PARPi by preventing, delaying or targeting resistant clones.

In this thesis, we expanded our insights into the molecular mechanisms underlying PARPi resistance by carrying out molecular profiling of mouse mammary tumors that have developed PARPi resistance *in vivo* and by conducting functional genetic screens in PARPi-resistance cell lines. **Chapter 2** provides a general introduction summarizing our current knowledge of the mechanisms behind PARPi response and resistance as well as potential strategies that might overcome PARPi resistance. In **Chapter 3**, we show that, while the restoration of HR is a frequent PARPi resistance mechanism in BRCA1-deficient tumors, HR cannot be reactivated in the absence of BRCA2. Moreover, our data suggest 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. In **Chapter 4** and **5**, we use functional genetic dropout screens to identify vulnerabilities of PARPi-resistant BRCA1/53BP1 double-deficient cells that could potentially be exploited therapeutically to overcome resistance. In **Chapter 4,** we identified DNA ligase III (LIG3) as a critical mediator of PARPi resistance in BRCA1/53BP1 double-deficient cells. We show that loss of LIG3 enhances PARPi-mediated toxicity in BRCA1/53BP1 double-deficient cells as well as in BRCA1-deficient cells, rendering LIG3 a potential therapeutic target. Moreover, we show that loss of LIG3 exposes cells to MRE11-mediated post-replicative single-stranded DNA (ssDNA) gaps upon treatment with PARPi, and suggest that ssDNA gaps are a novel determinant of PARPi response. In **Chapter 5**, we generated a comprehensive list of potential therapeutic targets, including several subunits from the mitochondrial complex I (MCI) as well as multiple genes associated with DNA damage response pathways, such as *SWSAP1*. While individual validation of the MCI subunits proved technically difficult and requires further validation, we show that loss of SWSAP1 enhances toxicity of PARPi, *in vitro* and *in vivo*. Finally, in **Chapter 6** we describe a detailed protocol for the use three-dimensional mouse tumoroids (tumor-

derived organoids) to carry out functional genetic dropout screens for the identification of new therapeutic targets and for the rapid and straightforward *in vivo* validation of these candidates.

In summary, 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 PARPi. In **Chapter 7**, we discuss the results of our research in the context of the current literature while highlighting some of the remaining questions.