Resistance to PARP inhibition by DNA damage response alterations in BRCA1/2-deficient tumors

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English summary

Inactivating mutations in *BRCA1* or *BRCA2* genes predispose to several types of cancer. Owing to their roles in maintaining genomic stability, lack of BRCA1/2 results in DNA damage repair defects, a vulnerability that can be exploited therapeutically by the inhibition of poly(ADP-ribose) polymerase 1 (PARP1). Indeed, inhibition of PARP1, a key sensor of DNA damage, has been shown to be synthetically lethal with deficiencies in homologous recombination (HR), a DNA double-strand break (DSB) repair pathway mediated by the BRCA1/2 proteins. This concept has been clinically validated over the last decade and nowadays several PARP inhibitors (PARPi) are approved for the treatment of BRCA-associated malignancies. Unfortunately, clinical benefit of PARPi therapy is often limited by emerging drug resistance. Identification of PARPi resistance mechanisms is therefore crucial to improve the clinical outcome and design strategies that would ultimately prevent or target resistant tumors.

Here, we address the problem of PARPi resistance using genetically engineered mouse models (GEMMs) of BRCA1/2-associated breast cancer. We combine the analyses of mouse mammary tumors that have developed PARPi resistance *in vivo* with genetic screens and functional assays in tumor-derived cell lines. Additionally, we describe a generation of tumor-derived organoids, a novel system that displays the advantages of traditional *in vitro* cultures, such as ease of genetic modification, yet retains the heterogeneity and drug sensitivities of the original tumors (**Chapter 3**).

As we show in **Chapter 5**, PARPi resistance profiles differ markedly between BRCA1- and BRCA2-deficient tumors. While the restoration of HR is a frequent mechanism that triggers drug resistance in BRCA1-depleted cells, our data strongly suggest that HR cannot be reactivated in the absence of BRCA2. The context-specificity of PARPi-induced HR rescue underlines that the functions of BRCA1 and BRCA2 in HR are distinct. As we show in **Chapters 4** and **5**, HR rewiring in BRCA1-deficient cells is primarily driven by the loss of the 53BP1-RIF1-shieldin pathway, which inhibits HR and is typically antagonized by BRCA1. In contrast, the role of BRCA2 is HR seems indispensable, thus tumor cells must employ alternative, HR-independent mechanisms to evade PARPi-toxicity. As described in **Chapter 6**, PARPi resistance can be induced in BRCA1/2-deficient cells by restoring their ability to protect stalled replication forks (RFs) from nucleolytic degradation. The burden of stalled RFs is increased upon PARPi treatment, and failure to prevent RF collapse render cells reliant on HR for DNA repair and survival. Moreover, PARPi resistance might be triggered by a partial restoration of PARP1 function. As we demonstrate in **Chapter 7**, clinical PARPi do not completely inhibit PARP1 at maximal tolerable dose and allow residual PARP1 activity. Our data suggest that PARP1 signaling can build up in PARPi-treated cells that lack poly(ADP-ribose) glycohydrolase (PARG), the main enzyme that counteracts catalytic function of PARP1. Because this process does not involve BRCA1/2, loss of PARG represents a generic

PARPi resistance mechanism which might be relevant in a broader spectrum of cancers. In summary, work described in this thesis extended our understanding of the molecular basis of PARPi resistance. General introduction and discussion, included in **Chapters 2** and **8**, respectively, put our research in the context of the current literature and highlight some of the remaining questions.