ADDENDUM

SUMMARY
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SUMMARY

In this project, mechanisms of PARP inhibitor (PARPi) resistance due to alterations in the DNA damage response have been explored in mouse BRCA1-deficient mammary tumors that are deficient in homology-directed DNA repair. The overall goal was to understand the mechanisms that impair the efficacy of PARP inhibition and thereby find new therapeutic strategies that may improve current therapies. Thus far, olaparib is the only PARP inhibitor that has been approved by both the EMA and FDA as therapy for BRCA-mutated ovarian cancer. In mouse models for BRCA1- or BRCA2-associated breast cancer, high sensitivity of the tumors to the PARPi olaparib was found. Despite the initial sensitivity, tumors were not eradicated and eventually acquire olaparib resistance. This provides a useful preclinical model to investigate mechanisms of PARPi resistance. As analytical tool I focused on using loss-of-function shRNA screens in cell lines derived from the BRCA1-deficient mouse mammary tumors. My aim was to identify genes that cause resistance when deleted. In particular, I was interested in genes that encode proteins involved in the DNA damage response.

In Chapter 1, we discussed potential mechanisms of PARPi resistance in BRCA1/2-mutated cancers. Thus far, a few mechanisms of PARP inhibitor resistance have been identified in preclinical models or the clinic. For example, secondary mutations restoring the BRCA open reading frame resulting in truncated but still HR-competent BRCA isoforms. To identify new mechanisms of resistance I used for my PhD project a model with a large intragenic Brca1 deletion, which makes it impossible to get a genetic reversion of Brca1. Our lab previously identified olaparib as a substrate of P-gp(MDR1a/b), and found that increased expression of the mouse Mdr1a/b genes contributed to olaparib resistance in BRCA1/2-deficient mouse mammary tumors that acquired olaparib resistance. Nonetheless, upregulation of P-gp or alteration of other drug transporters has not yet been confirmed to correlate with drug resistance in cancer patients. In addition, our lab previously found that loss of 53BP1 restores HR and causes PARPi resistance in BRCA1-deficient mouse mammary tumors. Since this mechanism does not explain all cases of resistance, it is still unclear
what other mechanisms can explain the lack of sensitivity that eventually emerges.

In Chapter 2, we report that loss of REV7 (also known as MAD2L2) in mouse and human cells rescues CTIP-dependent end resection of DSBs in BRCA1-deficient cells, leading to partial restoration of HR and PARP inhibitor resistance. REV7 is recruited to DSBs in a manner dependent on the H2AX–MDC1–RNF8–RNF168–53BP1 chromatin pathway, and appears to block HR and promote end joining. We uncover that REV7 counteracts end resection to promote NHEJ during immunoglobulin class switch recombination in B cells. Our findings reveal a crucial function of REV7 in the choice of DSB repair pathways in BRCA1-deficient cells. Importantly, we also present evidence that the resistance caused by loss of REV7 can be reversed by ATM inhibition.

In Chapter 3, we report that the ATP-dependent motor DNA Helicase B (also known as HDHB or HELB) is recruited to resected DNA ends by interacting with RPA to inhibit EXO1- and BLM-DNA2-dependent end resection in human and mouse cells. HELB acts independently of 53BP1/RIF1 and is exported from the nucleus in a CDK2-dependent manner near the G1/S transition, thereby enabling long-range resection. Consistent with its novel role as a resection antagonist, loss of HELB partially restores HR and results in resistance to PARP inhibition in BRCA1-deficient tumor cells, which is reversed by ATM kinase inhibition. Our findings thus indicate a cell cycle regulated inhibition mechanism on the process of end resection of DSBs mediated by HELB.

In Chapter 4, we report that Loss of 5′-3′ Exoribonuclease 2 (Xrn2) causes cisplatin resistance of Brca1;p53-deficient mouse mammary tumor cells but does not affect the cell growth. In addition, XRN2 loss also causes PARP inhibitor resistance of Brca1;p53-deficient tumor cells both in vitro and in vivo.

In Chapter 5, we discuss the potential future implications of the work presented in this thesis. In addition, we formulate remaining and newly arisen questions especially on the deep mechanisms of processing end resection at DSBs and how to translate our findings into the clinic.