Cancer chess: molecular insights into PARP inhibitor resistance

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"The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge"

(Stephen Hawking)

Cancer Chess:

Addendum

Molecular Insights

Into PARPi Resistance

Summary

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Curriculum Vitae

List of Publications

Acknowledgements

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Summary

Personalized medicine has great potential to improve cancer treatment. The premise of defining the genetic background of a tumor and tailoring the treatment to exploit specific tumor characteristics is especially powerful when applied to synthetic lethal interactions. Synthetic lethality occurs when the perturbation of two genes individually exerts no effect on cell survival, while simultaneous perturbation of the same two genes is highly lethal. A prime example is the BRCA/Poly (ADP-Ribose) Polymerase (PARP) paradigm in which a defect in the *BRCA1/2* genes (i.e. *BRCA1/2* mutation carriers and subsequent loss-of-heterozygosity of the remaining wild type allele) results in a remarkable sensitivity to PARP inhibition (PARPi). The clinical exploitation of this synthetic lethal interaction has achieved durable responses in a subset of patients, illustrating the potency of this interaction for cancer treatment. However, sustained responses are not universal, as tumors frequently develop resistance towards PARPi. The mechanisms that allow BRCA1 deficient cells to tolerate PARPi induced DNA damage are poorly understood. The current understanding is that PARPi induces double-strand breaks (DSBs) indirectly through replication fork collapse. The preferred route to repair these DSBs is via the error-free DSB repair pathway homologous recombination (HR). A critical step in the HR pathway is DSB end resection, which is promoted by BRCA1. In the absence of BRCA1, cells rely on error-prone repair of DSBs via non-homologous end joining (NHEJ) at the expense of an increased risk to generate toxic genomic DNA rearrangements which can subsequently lead to cell death. It is this synthetic lethal interaction that is exploited by PARPi.

Recently, the 53BP1 pathway has emerged as a key component in the response to PARPi induced DSBs by antagonizing end resection. Its loss in BRCA1 deficient cells results in the partial restoration of HR activity and drives PARPi resistance. However, since neither 53BP1 nor its downstream factors RIF1 or REV7/MAD2L2 have DNA-binding capacity, it remains elusive how this pathway blocks end resection. This framework is explained in more detail in the introductory **Chapter 1** and forms the basis for the rest of this thesis, which aims to provide insight into mechanisms that allow BRCA1 deficient cells to tolerate PARPi induced DNA damage and to find new vulnerabilities of these PARPi resistant cells. Ultimately, this knowledge might offer a rationale to act on resistance as it emerges, or ideally to anticipate on resistance prior to its appearance.

Genetically engineered mouse models (GEMMs) are invaluable in cancer research. In **Chapter 2** the major technological advancements that have been made in GEMMs of breast cancer in the last years are reviewed. This review is focused on the biological insights in cancer development that have been obtained from such model systems, and describes how these models have been used to study therapy response and resistance. The evolutionary process contained in GEMM tumors faithfully recapitulates the patient tumor setting and allows tumors to acquire resistance in an unbiased manner. However, the practical use of GEMMs has been hampered by the low success rates to

generate *in vitro* cell line systems from GEMM tumors and vice versa, the inefficiency to establish *in vivo* tumors from these cell lines. These limitations were eliminated by applying established 3D culture conditions on mammary tumor cells derived from the *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ (KB1P) mouse model. Hereby, it was possible to generate tumor organoids derived from KB1P mouse tumors at a high success rate. This work is described in **Chapter 3** and, since tumor organoids could be genetically modified *ex vivo* and re-transplanted orthotopically without compromising the tumor forming potential, allowed time- and labor-efficient examination of genetic interactions with therapy response. This is exemplified by the systematic interrogation of known factors of the 53BP1 pathway presented in this chapter. This technique was used in the forthcoming chapters, including for the *in vivo* validation of ASCIZ (**Chapter 4**). The ASCIZ-DYNLL axis promotes 53BP1 oligomerisation and recruitment to DSB ends, and thereby 53BP1 mediated NHEJ. Its depletion induces PARPi resistance in BRCA1 deficient cells.

Novel PARPi resistance mechanisms were identified by employing (genome-wide) CRISPR/SpCas9 loss-of-function screens in BRCA1 deficient cells using survival in the presence of PARPi as a readout. These screens yielded a number of interesting new factors that provided new insight into end resection regulation and metabolism. **Chapter 5** describes the identification of a novel trimeric single-stranded DNA (ssDNA) binding complex, composed of C20ORF196 (SHLD1), FAM35A (SHLD2) and FLJ26957/CTC534A2.2 (SHLD3) together forming the Shieldin complex. Shieldin was shown to be recruited to sites of DNA damage by REV7/MAD2L2 and its loss phenocopied the loss of 53BP1 pathway members. The tethering of the Shieldin complex to the upstream factor RNF8 eliminated the requirement for 53BP1-RIF1-REV7 and prohibited restoration of HR in the absence of 53BP1; unequivocally demonstrating that Shieldin is the downstream effector complex of the 53BP1 pathway. The genetic screens also implicated another trimeric ssDNA binding complex, the CST complex, as a modulator of the PARP1/BRCA1 synthetic lethal interaction. The CST complex is composed of CTC1, STN1 and TEN1 and has previously been described to regulate telomere length by counteracting resection of telomeres via the recruitment of POLA and subsequent fill-in DNA synthesis. The validation of this hit is the focus of **Chapter 6.** The loss of CST in BRCA1 deficient tumors partially restored HR activity and was a driver of PARPi resistance *in vitro* and *in vivo*. Moreover, CST promoted NHEJ activity on dysfunctional telomeres and facilitated NHEJ driven class-switch recombination. These data demonstrated that CST, besides its role on telomeres, also functions on non-telomeric DSBs, although the requirement for POLA fill-in DNA synthesis requires further study. Together, **Chapters 5** and **6** delineate the hitherto missing links between the 53BP1 pathway and DSB end metabolism.

In **Chapter 7** the knowledge on PARPi resistance mechanisms was utilized to identify and exploit new vulnerabilities of PARPi resistant BRCA1 deficient cells. The existence of such vulnerabilities was expected since the 53BP1 pathway is implicated in DSB repair via NHEJ. BRCA1 deficient cells that have lost the 53BP1 pathway are cross-resistant to topoisomerase inhibition, which, analogous to PARPi, generates DSBs

through replication fork collapse. However, these cells displayed increased sensitivity to radiotherapy (RT). This highlighted that the partial restoration of HR did not outweigh the loss of the 53BP1 pathway. Indeed, radiotherapy resistant BRCA1 deficient tumors did not restore HR activity underscoring that, despite the hypersensitivity to RT that was imposed by BRCA1 deficiency; BRCA1 independent (partial) restoration of HR was not beneficial in the context of RT. This vulnerability was exploited *in vitro* and *in vivo* to deplete PARPi resistant cells demonstrating a potential therapeutic option to treat recurrent tumors in which the 53BP1 pathway has been inactivated.

Given the encouraging response rates to PARP inhibition in the clinic and the urge to ultimately tackle clinical resistance, it is no surprise that the BRCA1/PARP1 paradigm receives great attention in the field. The high number of independent publications on PARPi resistance mechanisms testifies to the rapid pace at which the field is advancing. This thesis concludes with a general discussion of the remaining questions related to the repair of PARPi induced DNA damage and DSB end regulation in **Chapter 8**. Finally, strategies to improve the clinical utilization of PARPi are discussed. Together, this thesis provides insights into the game of cancer chess in the context of PARPi treatment of BRCA1 deficient tumors.