Homologous recombination-deficient cancers: approaches to improve treatment and patient selection

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Introduction and outline of the thesis

Chapter 1

1

Introduction

DNA damage maintenance

In order for cells to proliferate, the genomic material of a cell needs to be copied without errors and subsequently distributed over two daughter cells. However, the DNA in our cells continuously encounters different types of lesions, either from exogenous sources (e.g. UV rays from sunlight) or endogenous sources (e.g. radical species as byproducts of metabolism, and errors during DNA replication). To maintain genomic integrity, cells are therefore equipped with a broad spectrum of pathways that can detect and repair DNA lesions, together called the DNA damage response (DDR)[1].

A very toxic type of DNA lesion that needs to be repaired to maintain genomic integrity and cellular viability, is the DNA double-stranded break (DSB). Cells have two main pathways to repair DSBs, namely non-homologous end-joining (NHEJ) and homologous recombination (HR) (**Figure 1**). NHEJ is able to repair DSBs throughout the cell cycle and directly ligates DNA-ends together[2]. Although NHEJ is very efficient, it is also error-prone and can induce mutations. In contrast, HR is an error-free repair mechanism that is only able to repair DSBs in S- or G2-phase of the cell cycle, as it uses the sister chromatid as a template for DSB repair[3]. HR is slower and more complex when compared to NHEJ because HR requires more extensive processing of the DNA-ends[4]. Ultimately, HR involves the loading of the recombinase enzyme RAD51 onto single-stranded DNA (ssDNA) stretches that are created around the break site. The formation of RAD51 filaments initiates invasion of the broken DNA ends into the sister chromatid to search for a homologous sequence to repair the break without introducing mutations. The loading of RAD51 onto the ssDNA is frequently used as a readout for functional HR and is explored as a diagnostic tool to identify DNA repair-defective cancers[5]. If DNA lesions are not properly repaired, for instance, due to defects in DNA repair, this may lead to genomic instability, defined as structural alterations in the genome involving the accumulation of mutations or larger genomic rearrangements, with consequent chromosomal defects.

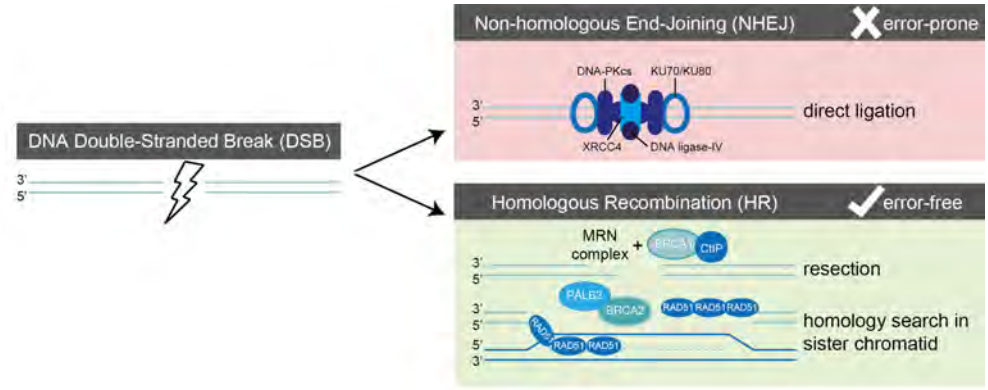

Figure 1. DNA double-strand break repair pathways. *For repair of DSBs by NHEJ, breaks are recognized and bound by Ku70-Ku80 heterodimers which activate DNA-PKcs. XRCC4, DNA ligase-IV, and polymerases (μ/λ) are recruited to complete DNA-end joining. During HR repair, DSBs are recognized by the MRN complex, which initiates DNA-end resection in conjunction with CtIP and BRCA1. EXO1 and DNA2 generate extensive ssDNA stretches, which are coated with RPA. In a PALB2-dependent fashion, BRCA2 is recruited, which loads RAD51 onto the ssDNA to invade the sister chromatid and to find sequence homology.*

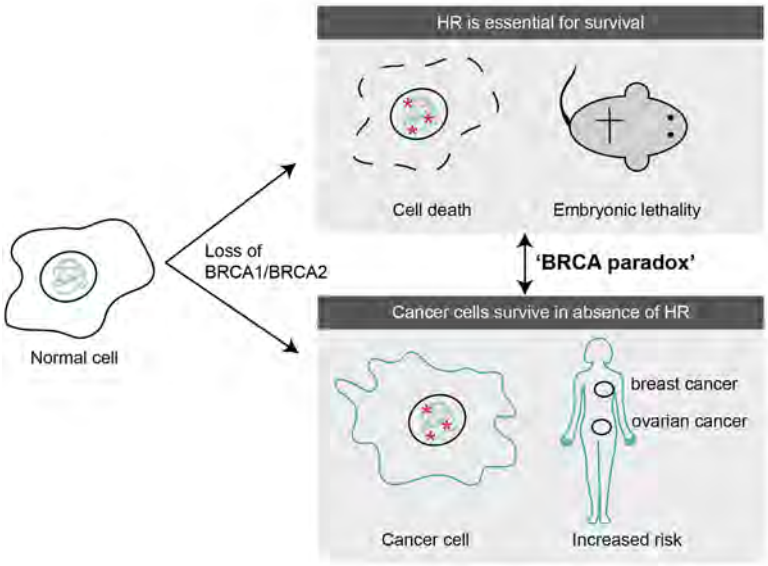

Figure 2. BRCA paradox.
Loss of BRCA1 or BRCA2 in normal cells results in cell death and embryonic lethality in mice. Surprisingly, cancer cells are viable in the absence of BRCA1 or BRCA2. Individuals harboring a BRCA1/2 mutation have an increased lifetime risk to develop breast- or ovarian cancer.

Loss of DNA damage repair in cancer

Genetic defects in DNA repair pathways leading to the accumulation of DNA lesions and have been associated with a range of diseases, including neurological disorders, accelerated aging, and play an important role in the development of cancer[1]. A significant subset of cancers has been described to have defects in DNA repair, including HR[6]. In line with this notion, DNA repair deficiencies and the resulting genomic instability has been described as a hallmark of cancer[7,8].

A link between defective DNA repair and cancer was first established when specific gene mutations were identified to underlie cancer-predisposing syndromes and hereditary breast cancer, and led to their gene names, for instance, Ataxia Telangiectasia Mutated (ATM) and Breast cancer 1, early onset (BRCA1)[9,10]. Individuals who harbor a germline mutation in *BRCA1* or the subsequently identified *BRCA2* gene have an increased lifetime risk of up to 70% to develop breast cancer[11]. Furthermore, germline *BRCA1/2* mutations are also associated with an increased risk to develop ovarian cancer and a range of other cancer types[12]. In the decades that followed the initial discovery of the *BRCA1/2* genes, numerous germline mutations in other HR genes have been associated with cancer predisposition, including *PALB2* and *RAD51C*[13,14].

To study BRCA1/2-mediated cancers, *Brca1* and *Brca2* genes emerged to be essential for development while developing mouse models, pointing towards HR as an essential process in proliferating normal cells[15,16]. Additionally, BRCA1 and BRCA2 serve important functions in the protection of stalled replication forks to maintain genomic stability[17]. These observations formed a clear contrast with the notion that *BRCA1* or *BRCA2* mutant cancer cells are viable in the absence of functional HR and replication fork protection. How cancer cells survive in the absence of BRCA1/2 is still incompletely understood and is called the 'BRCA paradox'[18] (**Figure 2**). Increasing evidence suggests that secondary (epi)genetic events, such as mutations or overexpression of other genes, might allow these cancer cells to survive in the context of HR deficiency. Also, the role of the immune system is increasingly recognized to play a role in the survival and growth of HR-deficient cancer cells.

Treatment of HR deficient cancer

If cancer is still localized, it is preferably surgically removed. If surgery is not possible, most cancer types are being treated with radiotherapy, chemotherapy, or with a

combination of both. Radiotherapy and most chemotherapeutic agents induce high levels of DNA damage, which kills rapidly dividing cancer cells but is also harmful to normal cells. Furthermore, like normal cells, many cancer cells have residual repair activity and are not properly sensitive to these treatment options or become resistant.

To increase the effectiveness of cancer treatment, strategies are needed that specifically target characteristics that are unique to cancer cells. This treatment strategy is called 'targeted therapy'. A specific type of targeted therapy is based on the principle of 'synthetic lethality' (**Figure 3**). A combination of genes is termed synthetic lethal, when defects in these genes are combined (e.g. simultaneous loss of function of gene A and gene B) and result in cell death, whereas loss of only one of these genes is not enough. The principle of synthetic lethality can be applied to cancer therapy when in cancer cells with a loss-of-function mutation in gene A, gene B is therapeutically targeted[19]. Notably, a synthetic lethal interaction between *BRCA1/2* and Poly-(ADP-Ribose)-polymerase 1 (*PARP1*) was discovered and led to the finding that cancers that are deficient for HR can be targeted with PARP inhibitors[20,21]. Healthy cells still have functional HR and will therefore be less sensitive to PARP inhibitors. Additionally, PARP is trapped onto the DNA during PARP inhibition resulting in replication fork stalling[22]. In 2014, the first PARP inhibitor olaparib (Lynparza) was approved by the FDA to treat *BRCA1/2*-associated advanced ovarian cancer patients[23]. In 2016, rucaparib and niraparib were also approved for the treatment of patients with recurrent *BRCA1/2*-mutated ovarian cancer[24]. Most recently, olaparib has also been approved for *BRCA1*/2 mutant HER2-negative metastatic breast cancers[25].

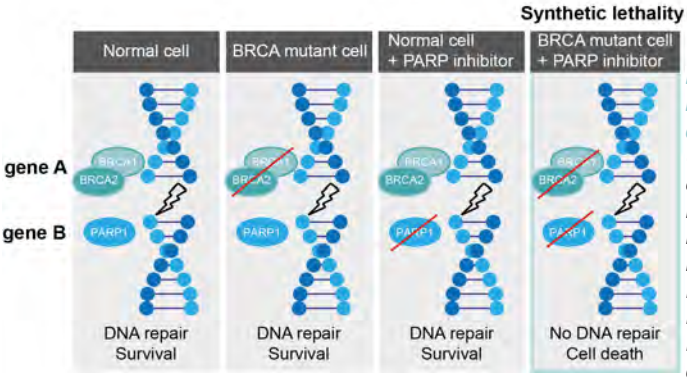

Figure 3. Principle of synthetic lethality between BRCA1/2 and PARP. *Middle panels: Upon either loss of BRCA1/2 or PARP1, maintenance of replication forks and DNA repair is still sufficient, resulting in cell survival. Right panel: In the absence of BRCA1/2, PARP inhibition results in failed DNA repair, replication stress, and ultimately cell death. Simultaneous loss of function of gene A (BRCA) and gene B (PARP1) ultimately results in cell death, defined as synthetic lethality.*

Unfortunately, many cancers eventually develop resistance to PARP inhibitor treatment. Resistance might occur when cells restore HR function by deregulation of HR suppressor genes such as *53BP1*, *REV7*, *RIF1, JMJD1C,* and *SHLD* components in a *BRCA1*-mutant background[26–30]. Furthermore, loss of *BRCA1* promoter methylation or secondary mutations can restore BRCA1/2 function[31,32]. Finally, the protection of stalled replication forks can be restored in a *BRCA2*-mutant background[33]. To prevent resistance, it is important to increase our knowledge of the exact mechanisms of action of PARP inhibitors and to improve their efficacy by developing combination strategies with other drugs. Combination trials have thus far focused on combining PARP inhibitors with chemotherapy, anti-angiogenic agents, and most recently immunotherapy. Unfortunately, dose-limiting toxicity is frequently observed in trials that combine chemotherapy with PARP inhibitors[34]. To develop tolerable and effective combination therapies, it is imperative to understand the cellular and molecular

consequences of BRCA defects in cancer cells. In this context, the combination of PARP inhibitors with immunotherapy is increasingly studied, as the role of the immune system is recently suggested to play an important role in the survival of HR-deficient cancer cells.

Whereas PARP inhibitors are currently approved to treat *BRCA1/2*-mutated ovarian and breast cancer, HR deficiency can also be caused by mutations in other DNA repair genes, beyond *BRCA1* or *BRCA2*[35]. These patients are currently not eligible for PARP inhibitor treatment but may benefit from this treatment as has already been shown in clinical trials[24]. Therefore, the selection of patients that could benefit from PARP inhibitors beyond *BRCA1/2* mutations is needed and tools to do this are currently suboptimal. Such patient selection tools will likely also be relevant for identifying tumors that might have become PARP inhibitor-resistant, to avoid unnecessary treatment.

The overall **aim of this thesis** is to dissect the molecular mechanisms and cellular consequences of HR-deficient cancer cells to improve the effectiveness of treatment modalities and patient selection for PARP inhibitors.

Outline of the thesis

Alterations in the ability of cells to repair their DNA can lead to genomic instability, which occurs frequently in cancer. As a result of genomic instability, DNA can end up in the cytoplasm of cells where it triggers a cell-intrinsic immune response via cGAS/STING signaling. In **chapter 2** of this thesis, several mechanisms are described by which genomic instability leads to cGAS/STING-mediated inflammatory signaling, and how this influences tumor development and interferes with the tumor microenvironment. Tumor cells that are characterized by genomic instability, for example, due to loss of HR, have evolved to escape these innate immune responses to overcome clearance by the immune system. Possible mechanisms by which tumors can adapt to inflammatory signaling are described. Finally, we outline how cGAS/STING-mediated inflammatory signaling can be therapeutically targeted to improve therapy responses.

PARP inhibition is an established treatment strategy for HR-deficient cancers. However, not all tumors respond to PARP inhibitors and many tumors ultimately develop resistance which results in tumor regrowth after an initial response. More insights into how PARP inhibitors kill HR-defective cancer cells are needed to improve therapy responses and to design new combination strategies. In **chapter 3**, we studied the mechanisms of PARP inhibitor cytotoxicity in multiple HR-deficient *in vitro* and *in vivo* cancer models. Using these models, we studied how cells deal with PARP inhibitor-induced replication stress throughout the cell cycle and if HR-defective cells maintain replication fork stability upon PARP inhibitor treatment. Furthermore, we assessed whether progression through mitosis influences PARP inhibitor-induced cytotoxicity.

Loss of HR -for example due to a *BRCA1/2* mutation- is tolerated in cancer cells, while this is lethal to normal cells. In **chapter 4**, we performed a loss-of-function haploid genetic screen to identify gene mutations that can rescue cellular viability upon inactivation of BRCA2 in *TP53*-mutant tumor cells. We validated whether the loss of the identified genes can indeed rescue the cellular viability upon BRCA2 depletion in various murine and human cancer *in vitro* models. Furthermore, we studied the molecular mechanisms by which the identified gene mutations influence cell viability in a BRCA2-defective context.

Overexpression of oncogenes is described to promote cell proliferation and to activate pathways that are beneficial for the survival and metastasis of cancer cells. Specifically, the *MYC* oncogene is often amplified in genomic unstable tumors, such as triple-negative breast cancer (TNBC), and often co-occurs with a *BRCA1/2* mutation. Based on our results in chapter 4 and other recent findings, *BRCA1/2*-mutant tumor cells were

hypothesized to circumvent cell-intrinsic inflammatory responses to evade clearance by the immune system. In **chapter 5**, we investigated the role of *MYC* in *BRCA1/2*-defective cells using *in vitro* and *in vivo* models for TNBC. Specifically, we assessed how amplification of *MYC* alters cGAS/STING-mediated inflammatory signaling in *BRCA1/2*-depleted cells, and how this subsequently affects the tumor microenvironment and activity of immune cells.

In **chapter 6**, the recent literature is reviewed on how the HR pathway is mechanistically wired, and current treatment options for HR-deficient cancers are represented with a focus on PARP inhibitors. As resistance to PARP inhibitors often occurs, the currently known PARP inhibitor resistance mechanisms are described. To optimally implement PARP inhibitor treatment in the clinic, patients with HR-deficient tumors must be adequately selected. Currently, only patients with germline or somatic *BRCA1/2* mutations are eligible for PARP inhibitor treatment and only a proportion of patients respond. Therefore, we discussed possible new combination therapies with PARP inhibitors and patient selection methods.

It is thought that a large proportion of patients with high-grade serous ovarian cancer (HGSOC) have HR-deficient cancer but do not harbor a *BRCA1/2* mutation. These patients are therefore not eligible for PARP inhibitor treatment, whereas they may benefit from this treatment. To further improve patient selection, in **chapter 7** we determined genomic features, including *BRCA1/2* mutation status and copy nymber variations (CNVs) profile, in a cohort of 30 patient-derived xenograft (PDX) models of ovarian cancer. In a subset of PDX models, we assessed *ex vivo* HR functionality and replication fork stability and correlated all genomic and functional outcomes with *in vivo* olaparib responses.

In **chapter 8**, the obtained results and conclusions of all previous chapters are summarized and discussed.

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