Molecular Pathways

Molecular Pathways: Exploiting Tumor-Specific Molecular Defects in DNA Repair Pathways for Precision Cancer Therapy

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Abstract

Disabling mutations in genome maintenance and DNA repair pathways are frequently observed in cancer. These DNA repair defects represent genetic aberrations that are specific to cancer cells and not present in healthy tissues. It is thought that these molecular defects produce a “mutator phenotype,” which allows incipient cancer cells to accumulate additional cancer-promoting mutations. In recent years, our molecular understanding of DNA double-strand break (DSB) repair mechanisms has led to the development of targeted therapeutic approaches to selectively eradicate cancer cells that display defects in homologous recombination–mediated DNA DSB repair. These regimens for the treatment of homologous recombination–defective tumors predominantly aim at pharmacologically repressing the activity of PARP1, which is crucial for base excision repair, or to inhibit the nonhomologous end joining kinase DNA-PKcs (DNA-dependent protein kinase, catalytic subunit). Normal tissue can bypass PARP1- or DNA-PKcs inhibitor–induced genotoxic lesions via homologous recombination–mediated DNA DSB repair. In contrast, homologous recombination–defective cancer cells are unable to properly repair DNA DSBs, in the presence of PARP1 or DNA-PKcs inhibitors, ultimately leading to apoptotic cancer cell death.

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Learning Objectives

Upon completion of this activity, the participant should know the different DNA repair pathways that are active in human cells. In addition, the participant should have a better understanding of the actionable molecular liabilities that are associated with cancer-specific defects in these DNA repair pathways and the advancement of targeted therapeutics derived against these pathways.

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Background

A central task of modern-day cancer research is the identification of cancer-specific (epi)genetic alterations that are amenable for targeted therapeutic interventions with the ultimate goal to selectively eradicate cancerous cells, while sparing healthy tissue. Oncogenic driver mutations, such as mutant EGFR or fusions of EML4–ALK or BCR–ABL, represent prime examples for actionable genetic alterations in human cancer (1–3). Pharmacologic interception of these signaling pathways in oncogene-addicted hematologic or solid cancers has resulted in impressive responses, even in sometimes massively pretreated disease. However, many known oncogenic drivers exist for which no targeted therapeutic intervention is available, to date. Prominent examples for such nondruggable oncogenic mutations include nonkinase oncogenes, such as RAS and MYC.
family members, as well as several inactivated tumor-suppressor genes (4). Hence, novel therapeutic concepts have been developed to indirectly target these oncogenic driver lesions.

**Synthetic lethality**

In particular, the concept of synthetic lethality has been successfully revisited to indirectly target primarily nondruggable cancer-specific genomic alterations (5). By definition, two genes are said to engage in a synthetic lethal genetic interaction, when activating or inactivating mutations of either gene alone allow viability of the affected cell, whereas combined mutation of both synthetic lethal partners is detrimental (5). In recent years, two approaches have been widely used to identify such tumor cell–specific molecular liabilities. Large-scale RNAi-mediated loss-of-function screens typically silence specific gene products (6, 7). Using this technology, the cellular survival of a genetically well-defined cell line in vitro or a well-characterized cancer model in vivo is analyzed. RNAi screens bear the major advantage that the knockdown of a single gene can be studied separately. These screens are, however, frequently hampered by acute toxicities of individual knockdowns. In addition, the typically incomplete loss-of-function achieved by RNAi does not necessarily mirror the effect of a homozygous, protein-damaging mutation that is observed in tumor cells. Finally, the currently available RNAi libraries suffer from an uneven representation of individual genes with varying degrees of knockdown efficiency. In contrast, cell line–based screens analyze the effects of defined small-molecule compounds in a large collection of genomically annotated cell lines that represent individual cancer genomes (8). The broad availability of massive parallel DNA sequencing allows an unprecedented high-resolution analysis of the complex cancer genomes represented in each cell line included in these screens. Such a fine-grained deconstruction of genomic alterations facilitates the association of certain tumor genotypes with compound sensitivity.

**The DNA-damage response**

In response to genotoxic stress, cells activate a complex signaling cascade, which is commonly referred to as the DNA-damage response (DDR; ref. 9). DDR signaling prevents further cell-cycle progression and thus allows time for DNA repair, or, if the lesions are beyond repair capacity, leads to the activation of cell death pathways (9). A common feature of numerous distinct cancer entities is an impaired DDR and a reduced DNA repair capacity (4, 10). In fact, genes encoding for different components of the DDR and specifically of DNA repair pathways are among the most frequently mutated genes in cancer (4, 10). Concordantly, various cancer entities display an impaired DDR and a reduced DNA repair capacity (4, 10). It is thought that inactivating mutations in these genome maintenance pathways lead to progressive genomic instability by fueling a so-called "mutator phenotype," which ultimately leads to the accumulation of additional cancer-driving genomic aberrations (11–15).

Exploiting these lesions for targeted cancer therapy holds great promise but requires a fundamental understanding of the DNA repair collaterals.

Mammalian cells evolved at least seven distinct DNA repair pathways to remove a plethora of structurally distinct DNA lesions in different cell-cycle phases. Mismatch repair corrects mispaired bases and insertion or deletion loops, which arise as the result of slippage during replication of repetitive DNA sequences (16). Nucleotide excision repair is essential for the correction of helix-distorting lesions, and base excision repair (BER) removes chemically modified bases (16). Translesion synthesis uses low-fidelity DNA polymerases, such as Polk and Polh, to allow (error-prone) replication over damaged DNA templates (17). The Fanconi anemia pathway is used to remove interstrand crosslinks (18).

Specifically in response to DNA double-strand breaks (DSB), human cells use two distinct repair mechanisms—error-prone nonhomologous end joining (NHEJ) and error-free homology-mediated DSB repair pathways, namely homologous recombination (16). As the NHEJ pathway does not depend on an intact DNA replication product as a template for repair, this pathway is available in all cell-cycle phases, but unable to repair DSBs in an error-free manner. The NHEJ pathway critically hinges on the catalytic activity of the protein kinase DNA-PK, which is recruited to the sites of the DSB through direct interactions with its noncatalytic subunits Ku70 and Ku80 (19). In contrast, homologous recombination, the second major DSB repair pathway in mammalian cells, requires the presence of an intact template for repair, and is, thus, largely restricted to the S- and G2-phases of the cell cycle (20). The homologous recombination mechanism requires an initial DSB resection step. Next, the homologous recombination core protein Rad51 is loaded onto the resulting single-stranded DNA 3’-overhangs to mediate homology searching, strand exchange, and Holliday junction formation (20). As the intact sister chromatid is used as template for DNA repair, the homologous recombination pathway is able to repair DSBs in an error-free fashion.

Single-strand annealing represents a distinct and Rad51–independent form of homology-mediated DSB repair, which typically mediates repair of DSBs located between repetitive DNA elements, in which two homologous DNA sequences are located on either side of the break on the same chromatid (21). Thus, single-strand annealing–mediated DNA repair does not involve sister chromatid exchange. Single-strand annealing–mediated repair is initiated by recruitment of RPA and Rad52 to the 3’-DNA overhangs (21). Complementary sequences up- and downstream of the DSB are then annealed by the RPA/Rad52/ssDNA ternary complex (21). The annealing products are typically flanked by displaced nonhomologous 3’-flap DNA tails, which are removed by ERCC1/XPF and MSH2/MSH3 complexes, which are generally considered to be components of the nucleotide excision repair and mismatch repair machinery, respectively (21). Single-strand annealing–mediated DSB repair ultimately leads to the deletion of the DNA stretch between the homologous DNA repeat sequences.
used for annealing (21). Thus, single-strand annealing represents an error-prone homology-mediated mechanism for DSB repair between repetitive sequences.

**DSB repair defects as a therapeutic target**

The tremendous relevance of an intact homologous recombination–mediated DSB repair machinery is underscored by the observation that patients carrying germline mutations in different homologous recombination genes, such as ATM, *BRCA1*, *BRCA2*, and *RAD51C* display a dramatically elevated risk for the development of neoplastic diseases (22–28). Furthermore, recurrent somatic protein-damaging mutations or focal deletions of *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, *RAD50*, *RAD51C*, and others have been found in multiple cancer entities (29–33). However, although these homologous recombination–abrogating mutations clearly promote carcinogenesis, they are also associated with certain molecular liabilities that are specific to homologous recombination–deficient tumors. Two specific actionable liabilities in homologous recombination–defective neoplastic diseases have undergone extensive investigation in recent years (Fig. 1). First, poly-ADP ribose polymerase-1 (PARP1) inhibitors have been shown to display selective toxicity in homologous recombination–defective tumors, specifically *BRCA1*– or *BRCA2*-deficient breast and ovarian carcinomas (22, 34–37). PARP1 inhibitors are per se genotoxic, likely through interfering with the BER pathway and thus creating unrepaired single-strand lesions that are converted to DSBs when replication forks collapse at these single-strand lesions (36–38). PARP1 inhibition in *BRCA1*/2-proficient and -deficient cells results in the occurrence of nuclear γH2AX foci, an established hallmark of DSBs (37). Intriguingly, wild-type cells are capable of repairing DSBs through the homologous recombination pathway (indicated by the transient occurrence of nuclear Rad51 foci), whereas *BRCA1*/2-deficient cells fail

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**Figure 1.** Therapeutic targeting of PARP1 and DNA-PK for the treatment of homologous recombination–defective cancer cells. Depicted is a simplified model for targeted therapy of DNA repair–defective tumors by inhibitors of PARP1 and DNA-PKcs. Genomic inactivation of DNA repair pathways (e.g., HR and MMR) results in a “mutator phenotype,” which leads to the accumulation of somatic mutations. Therapeutic blockade of pathway collaterals (e.g., NHEJ and BER) abrogates the ability of repair-defective cancer cells to clear DNA lesions (catastrophic DNA repair). Hence, these inhibitors induce selective apoptosis of repair-deficient tumors. ATM, ataxia telangiectasia mutated; BLM, Bloom syndrome protein; *BRCA1*/2, breast cancer 1/2 early onset; *CHEK2*, checkpoint homolog 2; *CIP*, CIBP–interacting protein; DNA–PKcs, DNA-dependent protein kinase, catalytic subunit; *Exo1*, exonuclease 1; *LigI*, DNA ligase 1; *LigIγ*, DNA ligase 4; *MLH1*, MutL homolog 1; *MSH2/3/6*, MutS protein homolog 2/3/6; *PMS1*, protein homolog 1; *Polδ/ε*, DNA polymerase δ/ε; *RAD51*, RecA recombinate homolog 51; *SSB*, single-strand break.
to repair PARP1 inhibitor-induced DNA lesions (37). Mechanistically, it has been proposed that PARP1 inhibitor-induced repression of the BER pathway leads to the accumulation of DSBs, which are subsequently cleared through homologous recombination in BRCA1/2-proficient cells in the subsequent G2-phase of the cell cycle (37–39). However, the mechanistic details of the synthetic lethality between PARP inhibition and molecular defects in homologous recombination remain a matter of active debate and alternative models for the selective toxicity of PARP inhibitors in homologous recombination–defective cells have been proposed (40). Follow-up studies have now revealed that—apart from BRCA1 and BRCA2 deficiency—mutations in other DNA repair genes, such as ATM, ATR, CHK1, CHEK2, DSS1, FANCA, FANCC, FANCD2, NBS1, RAD51, RAD54, or RPA1 result in a similar PARP inhibitor sensitivity (38).

Resistance against PARP inhibitors is, as with most other targeted anticancer therapies, a potential pitfall. To date, numerous mechanistically distinct resistance mechanisms have been identified: Loss of 53BP1 or JMJD1C, molecules that normally repress HR, was found to reverse PARP inhibitor sensitivity in BRCA1-deficient settings (41–43). Intriguingly, loss of 53BP1 and JMJD1C is frequently observed in breast cancer (41, 44). These clinical observations strongly suggest that 53BP1 and JMJD1C status should be routinely evaluated before and while treating patients with HR-defective tumors with PARP inhibitors. Secondary intragenic deletions in BRCA2 that occur after PARP inhibitor treatment and result in the expression of truncated HR-competent BRCA2 mutants constitute another mechanism of acquired PARP inhibitor resistance (45, 46).

A second actionable vulnerability of homologous recombination–defective cells is their dependence on the NHEJ pathway (47–50). This dependence has been pharmacologically exploited by inhibition of the critical NHEJ kinase DNA-PK (47, 49). A large-scale functional cancer genomics screen recently correlated DNA-PK inhibitor sensitivity with cancer-associated mutations (49). This screen revealed that cancer-associated inactivating mutations in numerous homologous recombination genes, such as ATM, BRCA1, BRCA2, CHEK2, FANCD2, MSH3, PAXIP, and RAD50 are significant genomic predictors of DNA-PKcs sensitivity (49). Mechanistic dissection of this nononcogene addiction to DNA-PK revealed that the combined functional loss of homology-mediated DSBR repair and NHEJ prevents successful rejoining of broken DNA fragments, leading to accumulation of DSBs, even in the absence of exogenous genotoxic stress (47–50). Specifically, in the case of ATM and MSH3 mutations, it has been shown that DNA-PK inhibition results in the generation of numerous unrepaired DSBs (47, 49). Furthermore, futile repair efforts in these cells lead to the generation of large single-stranded DNA repair intermediates, which ultimately trigger activation of the ATR–Chk1–p53–Puma axis, resulting in apoptotic cell death (47). Thus, both PARP1 and DNA-PK inhibition emerge as novel therapeutic approaches for the targeted treatment of human neoplastic disease with defects in homology-mediated DSB repair.

Clinical–Translational Advances

The preclinical data showing the efficacy of PARP inhibitors in BRCA1- and BRCA2-defective cell lines and xenograft mouse models in 2005 (36, 37) ignited tremendous excitement in the community. Consequently, the safety and tolerability of the PARP inhibitor olaparib (AZD2281 and KU-0059436) were rapidly evaluated in early clinical trials. As early as 2009, only 4 years after the initial reports, the results of a phase I trial (ClinicalTrials.gov: NCT00516373) were published (34). Patient selection within this trial was purposefully biased toward a study population enriched in BRCA1 or BRCA2 mutation carriers (22/60; ref. 34). Side effects were generally mild and included fatigue, thrombocytopenia, and mild gastrointestinal symptoms (34). Intriguingly, objective antitumor activity of olaparib was documented only in individuals with BRCA1 or BRCA2 mutation who suffered from pretreated ovarian, breast, or prostate cancer. These data strongly support the argument that the concept of synthetic lethality is applicable in the clinical setting and that PARP1 inhibition inflicts selective cytotoxicity in homologous recombination–defective, BRCA1- and BRCA2-mutant tumors. In 2012, an interim analysis of a phase II trial testing olaparib versus placebo as a maintenance regimen in platinum-sensitive relapsed ovarian cancer was published (51). The olaparib arm displayed a significantly improved progression-free survival in patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer. However, no benefit regarding the overall survival could be detected at the time of analysis, which led AstraZeneca to withhold the further clinical development of olaparib. Not before 2013 an additional subgroup analysis revealed that patients with BRCA1- or BRCA2-mutant ovarian cancer displayed the greatest benefit from an olaparib maintenance regimen. These observations prompted AstraZeneca to resuscitate the olaparib program and to launch two novel phase III studies: The SOLO 1 trial evaluates single-agent olaparib (300 mg twice daily for up to 2 years or until objective radiologic disease progression) as a maintenance regimen following first-line platinum-based chemotherapy. This trial recruits patients with advanced BRCA1- or BRCA2-mutant ovarian carcinomas (ClinicalTrials.gov: NCT01844986). The SOLO 2 trial evaluates the efficacy of a single-agent olaparib maintenance regimen in patients with relapsed high-grade serous ovarian cancer or high-grade endometrial cancer with BRCA1 or BRCA2 mutations, who initially responded to platinum-based chemotherapy (ClinicalTrials.gov: NCT01874353). Furthermore, the PARP inhibitor BMN-673 is currently being evaluated in solid tumor patients with disabling mutations in BRCA1 or BRCA2, who experienced progressive disease following at least one standard therapy (ClinicalTrials.gov: NCT01989546). In addition to these
single-agent trials, PARP inhibitors are also evaluated as part of combination therapies for advanced solid cancers. Numerous trials are validating the PARP inhibitor ABT-888 (veliparib). For instance, a combination treatment regimen consisting of the PARP inhibitor ABT-888 with the alkylating agent temozolomide is currently being evaluated in patients with solid tumors (ClinicalTrials.gov: NCT00526617). In addition, a phase III randomized, placebo-controlled trial will evaluate carboplatin and paclitaxel with or without ABT-888 in HER2-negative metastatic or locally advanced unresectable BRCA-associated breast cancer (ClinicalTrials.gov: NCT02163694). Further examples include olaparib, which is undergoing evaluation as part of a combination regimen consisting of cisplatin and gemcitabine in adults with solid tumors (ClinicalTrials.gov: NCT00678132). Finally, the PARP inhibitor BMN-673 will undergo evaluation as part of a combination regimen with either temozolomide or irinotecan in patients with locally advanced or metastatic tumors (ClinicalTrials.gov: NCT02049593).

These clinical activities highlight the rapid clinical development of PARP inhibitors for the treatment of neoplastic human disease. However, a better molecular understanding of PARP inhibitor–induced cytotoxicity and resistance mechanisms against PARP inhibitor treatment is of crucial importance for better stratification of patients into PARP inhibitor–containing treatment regimens. Specifically, the multitude of distinct mechanisms of acquired resistance against PARP inhibitors (see details above) necessitates a careful molecular evaluation of each patient undergoing PARP inhibitor treatment.

Compared with this rapid clinical evaluation of PARP1 inhibitors, the clinical development of DNA-PKcs inhibitors is lagging behind considerably. Numerous compounds with DNA-PKcs–inhibiting properties have been developed and preclinically characterized (52). However, thus far only CC-115, a combined DNA-PKcs/mTOR inhibitor, is being evaluated in a phase I trial (ClinicalTrials.gov: NCT01353625). Although patients with HR-defective neoplastic disease are actively enrolled in this study, there is no strict patient stratification to specifically enhance the recruitment of such individuals.

**Perspectives**

Results from current preclinical and clinical studies suggest that both PARP1 and DNA-PK inhibitors are well tolerated by patients and display selective toxicity in homologous recombination–defective tumors. Although PARP1 inhibitors have already undergone extensive clinical investigation, the clinical development and validation of DNA-PK inhibitors has not progressed with the same kinetics, despite the strong support of their selective antineoplastic effects by preclinical studies both in vitro and in vivo. In an extension of the previous discussion, it might be interesting to test whether combination treatments consisting of PARP1 and DNA-PKcs inhibitors might enhance the effects of either compound alone. This combination might help to reduce the dose of both individual compounds and therefore diminish the side effects associated with both inhibitors alone.

**Authors’ Contributions**

Conception and design: F. Dietlein, H.C. Reinhardt

Development of methodology: H.C. Reinhardt

Writing, review, and/or revision of the manuscript: F. Dietlein, H.C. Reinhardt

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.C. Reinhardt

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