CANCER THERAPY

Beyond PARP: POL θ as an anticancer target

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Since the discovery that DNA is unstable and prone to decay, targeting DNA repair deficiencies has become a proven and effective strategy in the fight against cancer. Over the past decades it has become increasingly apparent that selective loss of DNA repair pathways is an early and frequent event in tumorigenesis, occurring in 40–50% of many cancer types. Loss of DNA repair likely provides a selective growth advantage to tumor cells as this results in genetic instability and/or enhanced mutation rates, which can drive tumour evolution. However, DNA repair deficiencies represent an “Achilles heel” that can be targeted to overwhelmingly weaken cancer cells, as exemplified by the success of poly(ADP-ribose) polymerase (PARP) inhibitors in treating *BRCA*-deficient breast and ovarian cancers. However, novel therapeutic strategies are urgently needed to overcome acquired and innate PARP inhibitor (PARPi) resistance and to exploit other DNA repair deficiencies. Optimism is increasing that targeting DNA polymerase θ (POL θ), which is involved in DNA repair, will not only synergise with PARPi's but may have broader utility in cancer treatment.

The realization that tumor-specific alterations in DNA repair pathways could be exploited therapeutically was first established in 2005 (1, 2). Inactivating *BRCA* mutations, which account for ~10% of hereditary breast and ovarian cancers, are defective for homologous recombination (HR), a key pathway of DNA double strand break (DSB) repair. *BRCA*-deficient cancer cells, but not the wild type controls, were found to be exquisitely sensitive to inhibitors of the single strand break (SSB) repair enzyme PARP (1, 2). Recent studies have established that PARPi's act by trapping PARP on DNA. This stalls replication forks, which require HR for stabilization and replication restart (3). Hence, cells deficient in HR (for example, with *BRCA* mutations) are unable to dislodge PARP from DNA, leading to cell death.

Currently, there are five PARP inhibitors

in clinical trials, which have clear benefit in some patients with HR-deficient (HRD) cancers. *BRCA* mutations represent the most widely studied HRD subtype and have previously been shown to be associated with the greatest progression-free survival benefits from PARPi therapy (4). Unfortunately, of the HRD cancers that do respond to PARPi's, ongoing studies suggest that these tumors ultimately progress due to acquired PARPi resistance. It is also clear that PARPi's do not work in all DNA repair deficient tumors, such as gastric cancers defective for DNA damage signaling by ataxia-telangiectasia mutated (ATM) (5), nor do they target heightened spontaneous DNA damage caused by DNA replication stress, which is a hallmark of many tumors. POL θ has emerged as a promising target for the treatment of various DNA repair deficient cancers, including HRD cancers. Evidence suggests that POL θ inhibitors could synergize with PARPi's (6, 7), as they act by a different mechanism, and may also provide a means to selectively sensitize tumors to radiotherapy (8).

DSBs are predominantly repaired by the non-homologous end joining (NHEJ) DNA repair pathway in G1 of the cell cycle and by HR in S-G2 of the cell cycle. Micro-homology mediated end joining (MMEJ), is a 'backup DSB repair pathway in the event that HR is compromised (see the figure). HR and MMEJ share the same substrate, namely a resected DSB that contains a 3' single stranded DNA (ssDNA) overhang bound by replication protein A (RPA). Hence, if HR fails or is deficient then MMEJ is the favored option to repair resected DSBs. POL θ possesses both DNA polymerase and helicase domains, which are important for MMEJ in vivo (9). Biochemical evidence suggests that the helicase domain displaces RPA at resected DSBs to facilitate annealing of ssDNA containing micro-homologies (short segments of complementary DNA sequence). The polymerase activity then completes DNA synthesis to fill in the resected gap before ligation completes the MMEJ repair reaction (9). MMEJ is error-prone as it often results in loss of genetic information at the break site and can promote repair between DSBs from different parts of the genome, leading to translocations and other complex rearrangements. Although other proteins such as RPA and CtBP-interacting protein (CTIP) are important for

MMEJ, their roles in DNA damage recognition and in other DNA repair processes arguably make them less attractive targets for drug development because of a lack of specificity.

One of the principle reasons why POL θ has become a major focus for drug discovery is the fact that its expression is largely absent in normal cells but is significantly up-regulated in many cancers, both with and without HRD (6). Indeed, POL θ overexpression in multiple HR-proficient tumor types such as lung, gastric and colorectal cancer, is associated with adverse clinical outcomes (10). Critically, tumor cells harboring DSB repair defects develop a pronounced dependency on MMEJ to the extent that synthetic lethality has been shown to occur when POL θ is disrupted in cells deficient in either HR or NHEJ (6, 7, 11). The reasons why POL θ is up-regulated and associated with poor outcomes in many tumors is not well understood, but one possibility is that POL θ repairs spontaneous DNA damage present in cancer cells, and therefore affords them a growth advantage (12). Alternatively, error-prone DNA repair associated with POL θ activity could also contribute to greater genomic instability, and lead tumors to develop greater genetic diversity and evolutionary plasticity, which can drive resistance to cytotoxic therapies.

Despite the substantial clinical benefits observed with PARPi monotherapy in *BRCA*-mutated cancers, treatment resistance is a common occurrence and POL θ inhibition might be beneficial in treating those tumors that progress on PARPi's. PARPi resistance can arise from a variety of genetic alterations, including those that reactivate HR. Although POL θ inhibition would not be expected to cause tumor response in cases where secondary reversion *BRCA* mutations cause restoration of HR activity, over half of recurrences arise from other changes such as decreased expression of DNA repair proteins such as PARP1 or p53-binding protein 1 (53BP1) (13). It is possible that POL θ inhibitors would induce tumor response in these instances of PARPi resistance. The mechanisms by which tumors might eventually develop resistance to POL θ inhibitors are not yet clear, although it is anticipated that the primary mechanism would be secondary *BRCA* mutations causing reinstatement of HR function.

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Due to their distinct mechanisms of action, combined PARPi and POL θ inhibitor therapy might be expected to have a synergistic effect; simultaneously driving DSB formation whilst inhibiting DSB repair. Dual POL θ and PARP inhibition might therefore lead to more prolonged, clinically meaningful responses, and possibly delay the emergence of resistant disease compared with either therapy alone. In vitro data supports this optimism, since PARPi treatment, combined with POL θ knockdown, has been shown to synergistically reduce colony formation in BRCA1-deficient cells (6). The dramatic tumor growth delay seen in xenograft studies, whereby human HRD tumor cells are subcutaneously engrafted in mice, also points to a synergistic effect of POL θ and PARP deficiency in tumors with impaired HR (6).

In addition to the synthetic lethal effects, disruption of POL θ has also been shown to induce sensitization of tumors to radiotherapy in HR-proficient tumor cells at a level that would be expected to have considerable clinical benefit (8). Radiotherapy is widely used in the curative management of cancer patients, either as an adjunct to surgery, or as definitive treatment, either alone or in combination with cytotoxic chemotherapy. POL θ is overexpressed in several common cancers that are routinely treated with radiotherapy, such as lung and rectal cancer. Despite developments in the precision and accuracy of radiotherapy, the unavoidable irradiation of healthy tissues surrounding tumors can induce side-effects that limit the dose of radiation that can be delivered and thus the tumor cell-killing that can be induced. The marked disparity in POL θ expression between tumor cells and most normal tissues has raised the exciting prospect that specific POL θ inhibitors, delivered concurrently with radiotherapy, could improve tumor control without exacerbating the associated side effects of radiotherapy.

The clinical potential for POL θ inhibition to prolong the survival of patients with BRCA-mutated tumors, HRD tumors in general, and to improve the cure rates of patients receiving radiotherapy are substantial. It is hoped that the ability to define those patients likely to benefit from POL θ inhibitor treatment should enable this emerging class of anticancer therapies to be rapidly translated into the clinic.

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Figure Legend

Figure 1. Shown is a schematic of the double strand break (DSB) repair processes of homologous recombination (HR, left) and micro-homology mediated end-joining (MMEJ, right). HR is initiated by CtIP-dependent resection of the DSB to produce a 3' single stranded DNA overhang, which is bound by RPA (Green). BRCA1/2, PALB2 and the Rad51 paralogs facilitate the replacement of RPA with the recombinase RAD51 (yellow), which forms a filament on the 3' ssDNA. The RAD51 filament catalyzes strand exchange between the broken end and an intact template duplex, after which DNA synthesis copies the missing genetic information leading to error-free repair of the DSB. In the absence of HR, such as in cancers defective for the BRCA genes, MMEJ is up-regulated and serves as a key backup repair pathway. MMEJ is initiated by the displacement of RPA by POL η on the processed DSB to reveal micro-homologies (grey box) that can be annealed to other DSBs. Following flap processing, POL δ promotes DNA synthesis to fill the gaps, and following ligation, repair by MMEJ is completed. MMEJ is error-prone as it results in loss of genetic information at the DSB site and also promotes repair of DSBs originating from different parts of the genome (leading to translocations and chromosome rearrangements).