Targeting Poly(ADP-Ribose) Polymerase: A Two-Armed Strategy for Cancer Therapy

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Abstract

The DNA repair pathways are protective of the host genome in normal cells; however, in cancer cells, these pathways may be disrupted and predispose to tumorigenesis or their activity may overcome the potentially cytotoxic damage caused by anticancer agents and be a mechanism of resistance. Poly(ADP-ribose) polymerase inhibitors, which block base excision repair of single-strand breaks, have entered the clinic in the last few years. This article discusses the interactions between the pathways of single- and double-strand break repair, which explain the two clinical development strategies for this class of drugs.

Background

Poly(ADP-ribose) polymerases (PARP) are a family of highly conserved enzymes found in plants and animals, first described more than 40 years ago (1). Potent inhibitors of PARP have entered early clinical trials over the last 5 years in a range of clinical indications (2, 3); in cancer therapy, these agents are emerging both as potentiators to improve existing DNA-damaging therapy and also, tantalizingly, with single-agent activity in biologically selected tumors (4). This article attempts to explain the biology behind their potential dual action and the molecular pathway underlying this.

PARP-1 (EC 2.4.2.30) was the first of this enzyme family to be described and is the most abundant, being a nuclear enzyme intimately involved in DNA repair. There are five conserved DNA repair pathways that protect the integrity of the genome: nucleotide excision repair, mismatch repair, double-strand break (DSB) repair (comprising nonhomologous end joining in GO and homologous recombination in dividing cells), base excision repair (BER), and direct repair (5, 6). PARP-1 has a critical role in signaling DNA single-strand breaks (SSB) as part in the BER pathway (7). PARP-1 also binds strongly to DNA DSBs (8) and has an emerging role in their repair (9–11).

The enzyme has three functional domains, the DNA binding, automodification, and catalytic subunits, and is inactive in the absence of DNA damage. The DNA binding domain, which contains two zinc finger motifs, recognizes and binds to DNA strand breaks activating the enzyme, to make long and branched polymers of poly(ADP-ribose) from NAD⁺ (Fig. 1). These negatively charged polymers are formed on various acceptor proteins, including histones, p53, and the central automodification domain of PARP-1 itself, which includes a BRCA1 C-terminal domain. X-ray crystallography has shown that the negative charge of the polymer helps open up the damaged DNA to allow access to other components of the repair process (12). The polymer is then rapidly removed by poly(ADP-ribose) glycohydrolase (13), allowing release and inactivation of PARP-1 to “search and signal” further DNA damage—a molecular-nick sensor (12, 14–16). It is the most highly conserved third functional domain (17), the NAD⁺ binding catalytic subunit, which has presented medicinal chemists with the tools to develop potent PARP inhibitors. The early inhibitors (e.g., 3-aminobenzamide; ref. 18) were based around the structure of nicotamide, a by-product of the poly(ADP-ribose)lation reaction and a weak PARP inhibitor in itself. Purification of chicken PARP allowed X-ray crystallography of the NAD⁺ binding site (19) leading to the identification of structure activity relationships and the development of more potent inhibitors (20–24). It is these agents that have entered early clinical trials.

The Fate of the SSB when PARP Is Inhibited

Throughout the life of a mammalian cell, there are various situations in which DNA bases may be damaged, either by exogenous toxins, exposure to ionizing radiation, and from endogenous sources such as products of cellular metabolism. It is estimated that the average rate of damage is about 10⁴ events per cell daily (25). It is therefore essential that the cell has mechanisms in place to preserve its genomic integrity. BER has a key role in the repair of damaged bases, being subdivided into short patch and long patch repair and involving the removal of relatively short stretches of DNA, between 1 and 15 nucleotides. Short patch BER involves removal of one base only, whereas long patch repair removes 2 to 15 nucleotides; both share a common pathway. It is thought that short patch repair is involved in the repair of methylated-induced DNA damage, whereas long patch repair corrects oxidative damage (26). These mechanisms having evolved to correct endogenous DNA...
damage (27), but also protect cancer cells from radiotherapy or alkylating agent induced DNA damage.

The key role of PARP-1 in signaling and initiating this process means that this enzyme is ideally placed as a target for drug inhibition to alter function of the repair pathway. The illustration below explains the potential fates of a SSB in DNA (Fig. 2).

Damaged bases are removed by DNA glycosylases, generating an apurinic/apyrimidinic site, which is cleaved by an apurinic/apyrimidinic endonuclease, 3'-phosphodiesterase, leaving a SSB (27). PARP-1 binds to the exposed ends of the DNA, leading to activation and production of negatively charged polymer, which introduces flexibility into the damaged region of DNA and allows access of the other proteins of the repair complex (12). Replacement of the damaged base and religation of the DNA involves recruitment of a complex including DNA polymerase β, DNA ligase I or III, and X-ray repair cross-complementing 1 (28).

Inhibition of PARP-1 will mean persistence of the SSB. In replicating cells, this will be converted to a DSB at the replication fork. The persistence of multiple DSBs within a cell is a known potent stimulus for apoptosis. The involvement of poly(ADP-ribosylation) in DNA repair and the fact that its inhibition in proliferating cells potentiated DNA-damaging agents was first shown more that 20 years ago (29), and the first inhibitors have entered clinical trials to explore this hypothesis in cancer patients (see below).

DNA DSBs are usually efficiently repaired in cells. In replicating cells, the predominant pathway used is the error-free homologous recombination pathway. The damage is signaled by the recruitment of ATM or ATR to the strand break; these kinases are activated and phosphorylate a cascade of proteins including CHK1 and CHK2, the histone H2AX, the Fanconi anemia protein FANCD2, and BRCA1 and BRCA2 inducing cell cycle arrest and subsequent DNA repair (reviewed in refs. 5, 30). This pathway will repair some of the additional damage introduced by PARP inhibition in the dividing cell treated with a DNA-damaging agent, but preclinical data confirm that despite this, potentiation of cytotoxicity does occur (31–34).

It is well recognized that homologous recombination is defective in several familial cancer syndromes. This germ-line mutation and subsequent loss of heterozygosity by mutation or methylation of the remaining functional allele is the cause of the inherited genetic instability and high life-time risk of cancer in these individuals. Cells that are homozygous for a

**Fig. 1.** Schematic diagram of the functional domains of PARP-1 and its binding to DNA. 1014 aa protein (inset) with three functional domains (DNA binding, automodification, and NAD⁺ binding) binds either side of DNA strand break, activating the polymerase and forming negatively charged polymers of poly(ADP-ribose) and opening strand break to allow access of other components of BER.
defect in homologous recombination are exquisitely sensitive to PARP inhibition even in the absence of a DNA-damaging agent (4, 35). In this situation, the background endogenous base damage discussed above, which is normally repaired by BER, will lead to collapsed replication forks as there is no effective DSB repair to compensate (36).

PARP inhibitors have entered clinical trials at a time when the pathways of DNA repair are becoming much better understood. The elucidation of these molecular pathways has allowed them to emerge into early clinical drug development both as chemopotentiators and as single-agent therapy.

**Clinical-Translational Advances**

**PARP inhibitors to potentiate cytotoxic treatment.** There is a wealth of preclinical data, both in vitro and in vivo, to support the premise that inhibition of PARP-1 will potentiate the cytotoxicity of DNA-damaging agents. When acting as potential adjuncts to anticancer treatment, it is hypothesized that PARP inhibitors are preventing DNA repair via BER, blocking the pathway shown on the left-hand side of the diagram. Therefore, the DNA damage caused by the cytotoxicity is protected from repair by PARP-1 inhibition, potentially overcoming one of the causes of resistance to anticancer treatment (37, 38).

The potent quinazoline and benzimidazole PARP inhibitors potentiate monofunctional alkylating agents, topoisomerase I poisons, and radiotherapy in a range of cell lines (31, 39, 40). Chemopotentiation of temozolomide, cisplatin, and irinotecan has been shown by the PARP-1 and PARP-2 (a less abundant nuclear PARP enzyme with close homology to PARP-1) inhibitor CEP-6800 (a 3-aminomethyl carbazole imide) both in tumor xenografts and cell lines (33). Potentiation of temozolomide has also been reported in preclinical xenograft models with other novel inhibitors GPI 15427 (41) and INO-1001 (42).

It was as an attempt to show the potentiation of a monofunctional alkylating agent that PARP-1 inhibitors first entered clinical trials in cancer patients. The potent tricyclic indole PARP inhibitor, AG014699, developed in a collaboration between Newcastle University, Cancer Research UK, and Agouron Pharmaceuticals (part of Pfizer GRD), entered clinical trials in combination with temozolomide in 2003. This study...
was driven by a pharmacodynamic end point, establishing a PARP-inhibitory dose of the novel agent, before attempting to evaluate the maximum tolerated dose of the combination. Inhibition of the target enzyme was shown in peripheral blood cells and tumor biopsies and the combination was taken into a phase II study in metastatic melanoma (43). This second trial showed enhanced temozolomide-induced myelosuppression when full-dose temozolomide was combined with a PARP-inhibitory dose of AG014699; however, a 25% dose reduction of the temozolomide dose meant that the regimen was well tolerated and this small phase II study reported a doubling of the response rate and median time to progression compared with temozolomide alone (44). These encouraging data need to be confirmed in a phase III setting.

Following these initial studies, there are several PARP inhibitors also scheduled to begin clinical trials as chemopotentiating or radiopotentiating agents. Much of the current interest in the field is centered around the treatment of glioblastoma multiforme. The elegant work of the European Organization for Research and Treatment of Cancer and National Cancer Institute of Canada brain tumor research teams establishing combined treatment with temozolomide and radiotherapy as the new standard of care in this poor prognosis disease (45–47) coupled with the fact that PARP inhibitors are known to potentiate both temozolomide (48, 49) and radiotherapy (50) has stimulated interest in this research area, and the novel agents INO-1001 (Inotek, now part of Genetec), ABT888 (Abbott), and GPI 21016 (MGI Pharma) are in late preclinical development in this indication.

PARP inhibitors to exploit tumor biology. The publication of paired Nature articles in 2005 where two independent groups showed the exquisite sensitivity of BRCA1- and BRCA2-defective cells to PARP inhibition (4, 35) has led to clinical investigation of their use in this indication. The orally available PARP inhibitor KU-0059436 (KuDOS, AstraZeneca; ref. 51) is completing phase I studies and has already shown indications of activity in BRCA-defective patients with metastatic disease using a continuous oral dosing schedule. The potent intravenous inhibitor, AG014699, will enter phase II studies in this indication on an intermittent dosing schedule in the near future. Should these studies confirm significant activity in the metastatic setting in these familial cancers, it could be postulated that intermittent use of a PARP inhibitor at an early “preventative” stage could eliminate cells with a biallelic loss of BRCA function before they develop into tumors. It is also becoming clear that a wider group of patients may benefit because a significant number of tumors have a BRCA-like phenotype due to promoter methylation or loss of other elements of the DSBR repair pathway (36, 52, 53).

Conclusions

The close interaction between BER and DSBR repair, which is so essential to protect the integrity of the human genome, has therefore allowed PARP inhibitors to emerge over the last 3 years as an exciting potential addition to the cancer armamentarium (54, 55), with one primary mechanism of drug action preventing the repair of damaged DNA but two different scenarios where this can be lethal to the cancer cell. In the presence of a DNA-damaging agent (chemotherapy or radiotherapy), PARP inhibitors protect additional damage and may overcome resistance and potentiate cell kill. However, in those cancer cells with defective DNA, repair inhibition of another repair pathway seems sufficient alone to trigger apoptosis—the concept of synthetic lethality.

References


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