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DNA ligases as therapeutic targets

Alan E. Tomkinson[1,2,3], **Timothy R.L. Howes**[4], and **Nathaniel E. Wiest**[2,4]

[1]Department of Internal Medicine, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

[2]Department of Molecular Genetics and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

[3]University of New Mexico Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

[4]Biomedical Sciences Graduate Program, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

Abstract

During DNA replication, DNA joining events link Okazaki fragments on the lagging strand. In addition, they are required to repair DNA single- and double-strand breaks and to complete repair events initiated by the excision of mismatched and damaged bases. In human cells, there are three genes encoding DNA ligases. These enzymes are ATP-dependent and contain a conserved catalytic region. Biophysical studies have shown that the catalytic region contains three domains that, in the absence of DNA, are in an extended conformation. When the catalytic region engages a DNA nick, it adopts a compact, ring structure around the DNA nick with each of the three domains contacting the DNA.

Protein-protein interactions involving the regions flanking the conserved catalytic regions of human DNA ligases play a major role in directing these enzymes to participate in specific DNA transactions. Among the human *LIG* genes, the *LIG3* gene is unique in that it encodes multiple DNA ligase polypeptides with different N- and C-termini. One of these polypeptides is targeted to mitochondria where it plays an essential role in the maintenance of the mitochondrial genome. In the nucleus, DNA ligases I, III and IV have distinct but overlapping functions in DNA replication and repair.

Small molecule inhibitors of human DNA ligases have been identified using structure-based approaches. As expected, these inhibitors are cytotoxic and also potentiate the cytotoxicity of DNA damaging agents. The results of preclinical studies with human cancer cell lines and mouse models of human cancer suggest that DNA ligase inhibitors may have utility as anti-cancer agents.

Keywords

Cancer; DNA replication; DNA repair; genome instability; mitochondria; DNA ligase

Corresponding to: Alan E. Tomkinson. Cancer Research Facility, 915 Camino de Salud, University of New Mexico, Albuquerque, NM 87131, USA. atomkinson@salud.unm.edu.

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Introduction

The identification of DNA repair defects in inherited human diseases that are characterized by predisposition to cancer, including inherited forms of colon and breast cancer, provides compelling evidence that the cellular mechanisms that maintain genome stability play a critical role in suppressing cancer formation (1). Since genomic instability is a hallmark feature of sporadic as well as hereditary cancers, it is likely that alterations in one or more of the mechanisms that maintain genome stability occur at some stage during the development of most cancers. Although it has been assumed that these alterations in the DNA damage response contribute, at least in part, to the therapeutic activity of cytotoxic DNA damaging agents such as cis-platinum and doxorubicin, they remain poorly characterized, particularly in sporadic cancers. The recent development of poly (ADP-ribose) polymerase inhibitors as therapeutics that selectively target the DNA repair defect in hereditary breast cancers has stimulated interest in defining abnormalities in the DNA damage response in sporadic cancers (2) and the development of inhibitors of other DNA repair proteins that may have utility as anti-cancer agents (2). Since DNA joining is required to complete almost all DNA repair events and there are three human genes encoding DNA ligases with different but overlapping functions in DNA replication and repair (3), DNA ligase inhibitors with defined specificity can potentially be combined with different DNA damaging agents to target a wide variety of DNA repair pathways. In this review, we summarize our current understanding of the cellular functions of human DNA ligases (Table 1) and recent studies that identify DNA ligases as potential biomarkers for abnormal DNA repair and demonstrate the potential clinical utility of DNA ligase inhibitors in cancer treatment.

Structure and function of human DNA ligases

DNA ligases maintain the integrity of the phosphodiester backbone of duplex DNA by catalyzing phosphodiester bond formation (3). All DNA ligases utilize the same three-step reaction mechanism (Figure 1). In humans, the DNA ligases encoded by the three *LIG* genes are ATP-dependent (3). In step 1, ATP is hydrolyzed, resulting in the covalent linkage of an AMP moiety to a specific lysine residue within the DNA ligase active site and the release of pyrophosphate. Next, the AMP moiety is transferred from the adenylated ligase to the 5' terminus of a DNA nick with 5' phosphate and 3' hydroxyl termini, generating a DNA-adenylate intermediate. Finally, the non-adenylated DNA ligase interacts with the DNA adenylate and, using the 3' hydroxyl as a nucleophile, links the termini via a phosphodiester bond, releasing AMP.

Human DNA ligases contain a common catalytic core consisting of an oligonucleotide/oligosaccharide binding-fold (OB-fold) domain and an adenylation domain (AdD) that are found in all DNA ligases and other nucleotidyl transferases including RNA ligases and mRNA capping enzymes (3). While these two domains comprise the minimum unit that can perform the DNA ligation reaction, the activity of the catalytic core of human DNA ligases is greatly enhanced by an additional conserved N-terminal DNA-binding domain (DBD) (3,4). In the absence of DNA, the catalytic region of human DNA ligases encompassing the OB-fold, AdD and DBD adopts an extended, asymmetric conformation (3,5). As shown in Figure 2A, the structures of the catalytic regions of DNA ligase I and DNA ligase III bound to non-ligatable nicked DNA, which were determined by X-ray crystallography (4,6), revealed that these enzymes form similar ring-shaped structures around nicked DNA. Thus, the catalytic regions of the DNA ligases are flexible and undergo large conformational changes during the ligation reaction (Figure 1). It is assumed that the catalytic region of DNA ligase IV will behave in a similar manner. Recently, the structure of the DBD of DNA ligase IV was determined by X-ray crystallography in the absence of DNA (7). As expected, the DBD of DNA ligase IV has a similar overall structure to the DBDs of DNA ligases I and

III (Figure 2B). Notably, the availability of atomic resolution structural information has permitted the use of rational structure-based approaches to identify small molecule inhibitors of human DNA ligases. The properties of these inhibitors and their potential as cancer therapeutics are discussed later in this article.

In contrast to the DBD and catalytic core of human DNA ligases I, III and IV, the regions in these enzymes adjacent to the catalytic region are much more diverse. Furthermore, unlike the *LIG1* and *LIG4* genes, the *LIG3* gene encodes multiple DNA ligase polypeptides that have different N-and C-terminal regions (Figure 3). An alternative translation initiation mechanism generates polypeptides that either have or lack an N-terminal mitochondrial localization signal from *LIG3* mRNA (8). This signal sequence is removed during entry into mitochondria and so the nuclear and mitochondrial versions of DNA ligase III encoded by this mRNA transcript are very similar in size. In addition, a germ cell-specific alternative splicing mechanism generates polypeptides with different C-terminal sequences (9). At the C-terminus of the DNA ligase IIIα polypeptide there is a breast and ovarian cancer susceptibility protein 1 C-terminal (BRCT) domain that mediates an interaction with the C-terminal BRCT domain of nuclear DNA repair protein X-ray cross complementing protein 1 (XRCC1) (9–12). Structural analysis of the BRCT-BRCT heterodimer revealed that residues adjacent to the XRCC1 domain contribute to heterodimer interface, favoring formation of the DNA ligase IIIα/XRCC1 heterodimers rather than homodimers of DNA ligase IIIα and XRCC1 (13). In the DNA ligase IIIβ polypeptide, the C-terminal BRCT domain is replaced by a short sequence that acts as a nuclear localization signal (9). All the DNA ligase III polypeptides have an N-terminal zinc finger domain (ZnF) that, in concert with the DBD, plays a key role both in the initial recognition of DNA strand breaks and intermolecular ligation (14–16). BRCT domains also occur in DNA ligase IV. In this case, there are two tandemly arrayed BRCT domains within the C-terminal region of DNA ligase IV. As with DNA ligase IIIα, the DNA ligase IV BRCT domains are critical for an interaction with a partner protein, the DNA repair protein X-ray cross complementing protein 4 (XRCC4) (17–19). Structural studies have shown that within the DNA ligase IV/XRCC4 complex, the two BRCT domains encircle the coiled-coil region of the XRCC4 homodimer and that the linker region between the BRCT domains of DNA ligase IV makes the majority of the contacts with XRCC4 (20–22). The only recognizable motifs within the N-terminal region of DNA ligase I are a nuclear localization signal and a proliferating cell nuclear antigen (PCNA) interacting protein (PIP) box which, as the name suggests, interacts with PCNA (23–25).

Cellular functions of human DNA ligases

Nuclear DNA replication

There is compelling evidence that DNA ligase I is the replicative DNA ligase (3,26). Human DNA ligase I is recruited to nuclear DNA replication foci via its PIP box-dependent interaction with PCNA (24). In addition, it also interacts with replication factor C (RFC), a clamp loader that loads the trimeric PCNA ring onto DNA during Okazaki fragment synthesis (27,28). Finally, cell lines (46BR and 46BR.1G1) established from an immunodeficient human patient with mutated *LIG1* alleles have reduced DNA ligase I activity and severely impaired Okazaki fragment joining (28–30). As expected, DNA ligase I activity is essential for embryogenesis in mice but, surprisingly, it was possible to establish cell lines from *LIG1* null embryos that had a defect in joining Okazaki fragments similar to the human 46BR cell lines (31,32). The viability of *LIG1* null cells indicates that another DNA ligase participates in nuclear DNA replication in the absence of DNA ligase I. Recent studies in chicken DT40 and human cells have shown that DNA ligase IIIα but not DNA ligase IV, is essential for nuclear DNA replication in the absence of DNA ligase I (33,34). XRCC1, which interacts with and stabilizes nuclear DNA ligase IIIα (10,12,35), and poly (ADP-ribose) polymerase 1 (PARP-1), which initiates the repair of DNA single strand

breaks (SSB)s (36), are also required for nuclear DNA replication in DNA ligase I-deficient cells (33). Thus, it appears that, in the absence of DNA ligase I, Okazaki fragment joining is accomplished, at least in part, through a SSB repair mechanism in which ADP-ribosylated PARP-1 recruits the DNA ligase IIIα/XRCC1 complex via its interaction with XRCC1 (33).

Mitochondrial DNA replication and repair

Genetic inactivation of the mouse *LIG3* gene result in embryonic lethality at an even earlier stage than *LIG1* and *XRCC1* null embryos (31,32,37,38). Furthermore, although *LIG1* and *XRCC1* mouse embryonic cell lines were established from the embryos (31,32,37), it was not possible to establish a *LIG3* null cell line, suggesting that the *LIG3* gene is required for cell viability (38). This has been confirmed in recent studies showing that the *LIG3* gene is indeed essential because it encodes the only mitochondrial DNA ligase (39,40). These observations were predicted by earlier studies showing that alternative translation initiation generates nuclear and mitochondrial versions of DNA ligase IIIα and that mitochondrial DNA ligase IIIα functions independently of XRCC1 (8,41). Furthermore, depletion of DNA ligase IIIα levels by siRNA resulted in reduced numbers of copies of the mitochondrial genome and increased accumulation of SSBs in mitochondrial DNA (42). Thus, mitochondrial DNA ligase IIIα plays an essential and unique role in the replication and repair of the mitochondrial genome (Table 1).

Nuclear DNA excision repair

There are three pathways that excise mismatched and/or damaged bases from DNA: mismatch repair (MMR), base excision repair (BER) and nucleotide excision repair (NER). The major function of the MMR pathway is to correct mistakes in newly synthesized DNA made by the DNA replication machinery (1). Although significant progress has been made in elucidating the molecular mechanism of MMR, the identity of the DNA ligase (or DNA ligases) that completes this repair pathway has not been established definitively.

There is compelling evidence linking both DNA ligase I and DNA ligase IIIα with BER. This repair pathway is initiated when a DNA glycosylase recognizes and excises a damaged base (1). After the resultant abasic site is cleaved, there are two possible subpathways to complete the repair (3,43–45). In short-patch BER, DNA polymerase β removes the remaining 5' sugar-phosphate residue and inserts a single nucleotide, generating a ligatable nick that is sealed by the DNA ligase IIIα/XRCC1 complex (3,44). This pathway is thought to be active across the entire genome in non-dividing cells and throughout the cell cycle. In contrast, the long-path BER pathway, in which a longer repair patch is inserted by either DNA polymerase δ or ε and repair is completed by the action of DNA ligase I in conjunction with FEN-1, appears to occur only during S phase and to be linked to the DNA replication machinery (3,43,45). Analysis of DNA ligase-deficient human and mouse cells have given paradoxical results regarding the contribution of DNA ligase I- and DNA ligase IIIα-dependent BER to cell survival after DNA alkylation damage. For example, human DNA ligase I-deficient 46BR cells are sensitive to DNA alkylating agents (28,46,47) whereas *LIG1* null mouse embryonic fibroblasts and mouse embryonic fibroblasts that express a mutant version of DNA ligase I that is equivalent to the enzyme expressed in 46BR cells are not (31,32,48). This could be explained if DNA ligase I-dependent BER predominates in human cells whereas DNA ligase IIIα-dependent BER predominates in mouse cells. However, the characterization of mouse cells lacking nuclear DNA ligase IIIα led to the conclusion that DNA ligase I-dependent BER is the major pathway in the mouse cell-types examined (39,40). These discrepancies may reflect differences in the relative contributions of DNA ligase I- and DNA ligase IIIα-dependent BER and the extent of the functional redundancy between these BER subpathways in different cell types.

Nucleotide excision repair (NER) removes helix-distorting lesions such as ultraviolet light (UV)-induced photoproducts (1). In contrast to BER, the DNA lesion is removed by the excision of an oligonucleotide 24–32 nucleotides in length, followed by repair synthesis and ligation (1). For many years it was assumed that human DNA ligase I completed NER ligation because of the UV light sensitivity of 46BR cells (46) and the activity of DNA ligase I in conjunction with the replicative DNA polymerases δ and ε in reconstituted NER reactions (49,50). However, more recent studies have shown that DNA ligase I only accumulates at NER sites in proliferating cells, whereas DNA ligase IIIα and XRCC1 are recruited to NER sites regardless of cell cycle stage (51). Thus, DNA ligase I appears to function in a S phase-specific subpathway of NER, whereas the DNA ligase IIIα/XRCC1 complex functions in an NER subpathway that is active in all phases of the cell cycle and presumably in non-dividing cells (51). As with BER, the relative contributions of DNA ligase I-and DNA ligase IIIα-dependent NER and the extent of the functional redundancy between these NER subpathways may vary between different cell types.

Nuclear SSB repair

SSBs are generated in numerous different ways, including as repair intermediates during excision repair, by erroneous topoisomerase I activity, and by DNA damaging agents such as reactive oxygen species (ROS) and alkylating agents (52). As mentioned above, DNA SSBs are predominantly detected by PARP-1 although PARP-2 may also contribute (53,54). The binding of PARP-1 to SSBs activates its polymerase activity resulting in the synthesis of poly (ADP-ribose) chains on PARP-1 itself and other nearby proteins (1). The DNA ligase IIIα/XRCC1 complex is then recruited to SSBs primarily by an interaction between XRCC1 and poly (ADP-ribosylated) PARP-1 (36,55). Given the recruitment of XRCC1 and DNA ligase IIIα to SSBs, it was surprising that cells lacking nuclear DNA ligase IIIα did not exhibit a defect in SSB repair similar to cells with reduced levels of XRCC1 (39,40,56). Thus, it appears that there is an as yet poorly defined DNA ligase I-dependent SSB repair pathway (56).

Nuclear DSB repair

As with SSBs, DSBs are produced by many different mechanisms. For example, they arise during programmed cell events including meiotic recombination and immunoglobulin gene arrangements (1). They are also generated during normal DNA replication and, to an even greater extent, during replicative stress. Finally, they can be generated either directly by the action of a DNA damaging agent or indirectly as a consequence of the replication fork encountering an unrepaired SSB (1). There are multiple repair pathways for these highly cytotoxic lesions that can be divided into two groups depending upon whether or not the repair reaction involves extensive DNA sequence homology (57,58). While the DNA ligases that participate in the homology-dependent DSB repair pathways have not been definitively identified, it is well-established that DNA ligase IV is a key component of the major non-homologous end-joining (NHEJ) pathway, which is functional throughout the cell cycle, repairs most DSB lesions and completes V(D)J recombination (18). This pathway is initiated by the Ku70/Ku80 complex, which binds to DNA DSB ends and recruits the other components of the repair pathway, including DNA-dependent protein kinase catalytic subunit (DNA PKcs), Artemis and DNA ligase IV/ XRCC4 (57). A key step in NHEJ is the juxtaposition of DNA ends that is mediated by interactions between DNA PKcs molecules (59). In addition, there are multiple end processing activities that act on the juxtaposed DNA ends to generate ligatable termini (57). As a consequence of this end porcessing, NHEJ is characterized by small insertions and deletions at the break site but usually the previously linked DNA ends are joined back together correctly (57).

Several DNA ligase IV-deficient individuals have been identified with symptoms that include radiation sensitivity, immunodeficiency and developmental delay (60–62). As with the *LIG1* and *LIG3* genes, genetic inactivation of *LIG4* resulted in embryonic lethality in the mouse (63,64). Cells that lack *LIG4* are viable (18,57), demonstrating that this repair pathway is not essential. Analysis of NHEJ-deficient cells revealed the presence of an alternative (alt) NHEJ pathway that also appears to be active in wild type cells albeit at a low level (65–68). Repair of DSBs by alt NHEJ is characterized by large deletions, resulting from extensive resection, and frequent chromosomal translocations (69,70). DNA ligase IIIα is the major enzyme acting in alt NHEJ but, surprisingly, this pathway does not appear to involve XRCC1 (71–73). There is also evidence for a minor DNA ligase I-dependent subpathway of alt NHEJ (71).

DNA ligase inhibitors

Attempts to identify DNA ligase inhibitors by screening of chemical libraries have been met with limited success (74–76). With the elucidation of the atomic resolution structure of human DNA ligase I complexed with nicked DNA in 2004 (4), it became possible to use a computer-aided drug design approach to identify DNA ligase inhibitors (Figure 4). A large database of small molecules was screened in silico for molecules that were predicted to bind in a pocket formed by residues His337, Arg449 and Gly453 on the DNA binding surface of the DBD of human DNA ligase I identified in the DNA ligase I-nicked DNA crystal structure (77,78). Of the approximately 200 compounds identified by the *in silico* screen, 10 inhibited human DNA ligase I but not T4 DNA ligase, an ATP-dependent bacteriophage DNA ligase that lacks a DBD. As expected based on the design of the screen, almost all of the compounds were competitive inhibitors with respect to nicked DNA (77,78). The exception was L82, which is an uncompetitive inhibitor of DNA ligase I. The inhibitors also exhibited activity against DNA ligases III and IV, presumably reflecting the conserved structure of the DBDs of the human DNA ligases. A DNA ligase I-specific inhibitor (L82) and inhibitors that inhibited DNA ligases I and III (L67) and all three DNA ligases (L189) were characterized further (77). These inhibitors retained their specificity in cell extract assays of DNA repair and also inhibited the growth of the human cells in culture. Although L67 and L189 are cytotoxic (77), the relative contributions of inhibition the function of DNA ligase IIIα in mitochondrial DNA replication and repair and inhibition of the function of both DNA ligase I and DNA ligase IIIα in nuclear DNA replication and repair to cell death have not been determined. Unexpectedly, subtoxic levels of L67 and L189 preferentially sensitized cancer cell lines to the cytotoxic effects of DNA agents (77), providing the first evidence that the DNA ligases inhibitors may have utility in the development of therapeutic strategies that target abnormalities in the DNA damage response of tumor cells.

In addition to their potential as cancer therapeutics, DNA ligase inhibitors can be used as probes to provide insights into the catalytic mechanisms and cellular functions of these enzymes but this will require the identification of more specific inhibitors. In a recent study, Srivatava *et al*. built a homology-based model of the DNA ligase IV DBD and used this to guide the selection of derivatives of L189 that may be more specific for DNA ligase IV (79). This screen identified a compound, SCR7, that inhibited DNA ligases III and IV but not DNA ligase I (79). Since atomic resolution structures are now available for DBDs of all the human DNA ligases (Figure 2B), more refined modeling approaches will likely lead to the identification of specific inhibitors of each of the human DNA ligases.

DNA ligases as biomarkers of abnormal DNA repair

The observation that DNA ligase inhibitors preferentially sensitize cancer cells to the cytotoxic effects of DNA damaging agents prompted an examination of the levels of human DNA ligases in cancer cell lines (77). As expected, the steady state levels of DNA ligase I were elevated in cancer cells lines compared with normal cells, presumably due to the increased proliferative activity of cancer cells (80). Strikingly, a pattern of elevated levels of DNA ligase IIIα and reduced levels of DNA ligase IV was observed in a significant fraction of cancer cell lines (77). Rassool and colleagues showed that this altered expression of DNA ligases IIIα and IV underlies the abnormal repair of DSBs in chronic myeloid leukemia (CML) cell lines expressing BCR-ABL1 (81,82). Specifically, the activity of DNA ligase IIIα-dependent alt NHEJ was significantly higher in these cell lines compared with normal myeloid cells. Interestingly, the steady levels of DNA ligases IIIα and PARP1, another alt NHEJ protein, and alt NHEJ activity itself were even higher in BCR-ABL1-postive CML cell lines that had acquired resistant to the ABL-specific inhibitor imatinib (82). A similar dysregulation of DSB repair occurs in cell lines expressing internal tandem duplication (ITD) mutations of the FMS-like tyrosine kinase-3 (FLT3) receptor that are present in a subset of acute myeloid leukemia patients with poor prognosis (83) and in breast cancer cell lines with acquired or intrinsic resistance to anti-estrogen therapies (84). Importantly, altered expression of DNA ligase IIIα and other proteins involved in the repair of DSBs by NHEJ have been detected in bone marrow samples from CML patients and tumor biopsies from breast cancer patients (82,84). Thus, the expression levels of DNA ligases IIIα and IV serve as biomarkers to identify cancers with abnormal repair of DSBs by alt NHEJ.

Activity of DNA ligase inhibitors in preclinical models of human cancer and patient samples

The increased activity of alt NHEJ in leukemia and breast cancer cell lines suggested that these cells may be preferentially sensitive to agents that inhibit alt NHEJ and induce DSBs. Notably, cell lines with increased alt NHEJ were hypersensitive to combination of a PARP inhibitor and the DNA ligase inhibitor L67 that inhibited alt NHEJ (82,84). Since knockdown of DNA ligase IIIα by RNAi had essentially the same effect as L67 in combination with the PARP inhibitor, it appears that L67 exerts its effect by inhibiting DNA ligase IIIα rather than DNA ligase I (82,84). Notably, studies with bone marrow samples from CML patients showed that sensitivity to the repair inhibitor combination correlated with the expression levels of both DNA ligase IIIα and PARP-1 (82). Thus, it appears that alt NHEJ is a valid therapeutic target in certain tyrosine kinase-activated leukemias and breast cancers and, more importantly, it appears to occur more frequently in forms of these diseases that are resistant to frontline therapies.

A recent study with SCR7, an inhibitor of DNA ligases IIIα and IV, suggested that the major DNA ligase IV-dependent NHEJ pathway may also be a therapeutic target in certain cancers (79). Specifically, SCR7 reduced cell proliferation in a DNA ligase IV-dependent manner and its effect on the growth of cancer cell lines appeared to correlate with the DNA ligase IV expression levels (79). In addition, SCR7 reduced the growth of tumors formed by several human cancer cell lines in mouse xenografts and, in tumors that did not respond to SCR7, the presence of SCR7 increased the tumor-inhibitory effects of agents that cause DSBs (79).

Conclusions

The requirement for DNA ligation in DNA replication and almost all DNA repair pathways makes DNA ligases an attractive target for the development of inhibitors that will potentiate

the activity of genotoxic agents used to treat cancer. This effort is complicated by the presence of multiple species of DNA ligase in human cells with distinct but overlapping cellular functions and the essential roles of DNA ligases in the maintenance and replication of nuclear and mitochondrial genomes. Nonetheless, DNA ligase inhibitors identified by rational structure-based approaches have produced promising results in cell culture and mouse models of human cancer. In one of these examples, the DNA ligase inhibitor L67 was used in combination with a PARP inhibitor to selectively target a DNA repair abnormality in cancer cell lines and clinical samples from patients with leukemia that can be identified based upon altered expression of the human DNA ligases. Thus, the results of these preclinical studies support the further development of DNA ligase inhibitors as therapeutic agents for the selective targeting of DNA repair defects in cancer cells.

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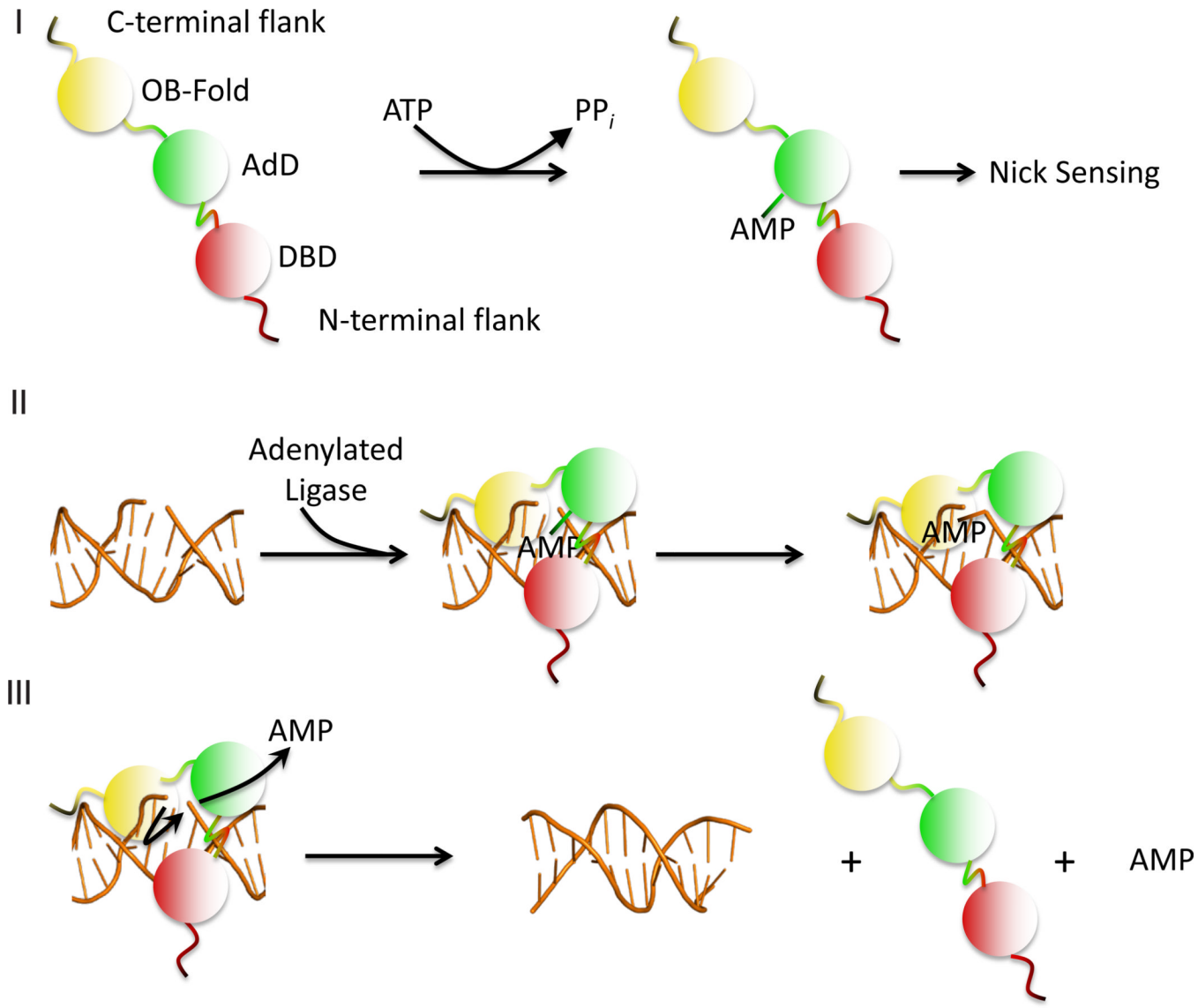

Figure 1.

Steps in the DNA ligation reaction. (I) The catalytic region of the DNA ligase consisting of the DNA binding domain (DBD, red), adenylation domain (AdD, green) and oligonucleotide/oligosaccharide binding-fold (OB-Fold, yellow), interacts with ATP to adenylate an active site lysine within the adenylation domain (AdD, green), releasing pyrophosphate; (II) When the adenylated ligase recognizes and binds to a DNA nick, it undergoes a conformational change such that the DBD, AdD and OB-fold encircle the nick. Within this compact structure, the AMP moiety is transferred from the ligase polypeptide to the 5' phosphate of the nick; (III) The non-adenylated ligase polypeptide utilizes the 3' hydroxyl terminus of the nick as a nucleophile to attack the 5' DNA-adenylate, resulting in phosphodiester bond formation and the release of the ligase polypeptide and AMP

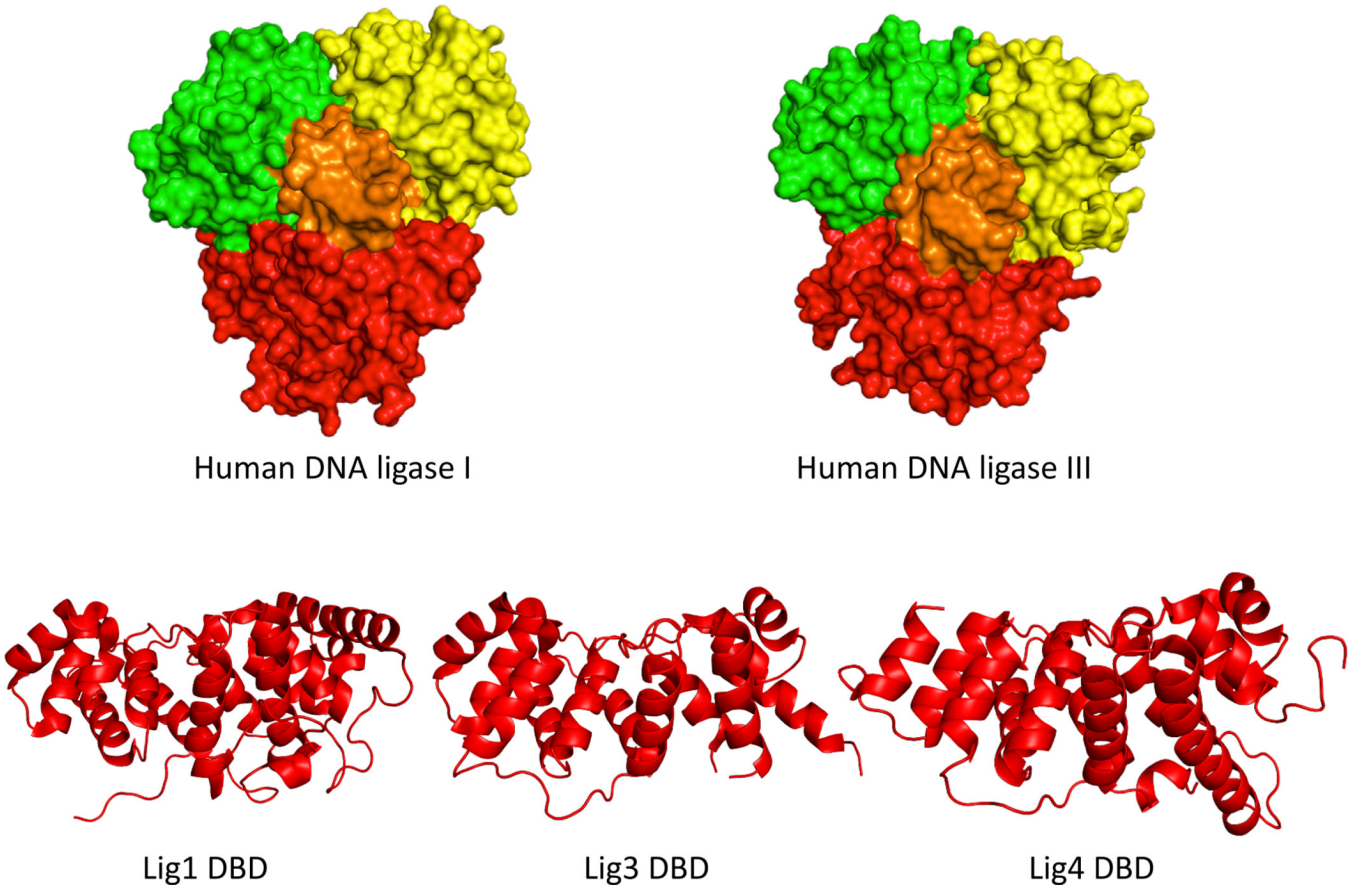

Figure 2.

Structures of human DNA ligases. A. Comparison of space-filling representations of the catalytic regions of buman DNA ligase I (Left panel) and human DNA ligase III (Right panel) bound to nicked DNA. The DNA binding domain is indicated in red, the adenylation domain in green, the oligonucleotide/oligosaccharide binding-fold domain in yellow and the nicked DNA in orange; B. Ribbon diagrams showing the secondary structures of the DNA binding domains of human DNA ligases I, III and IV

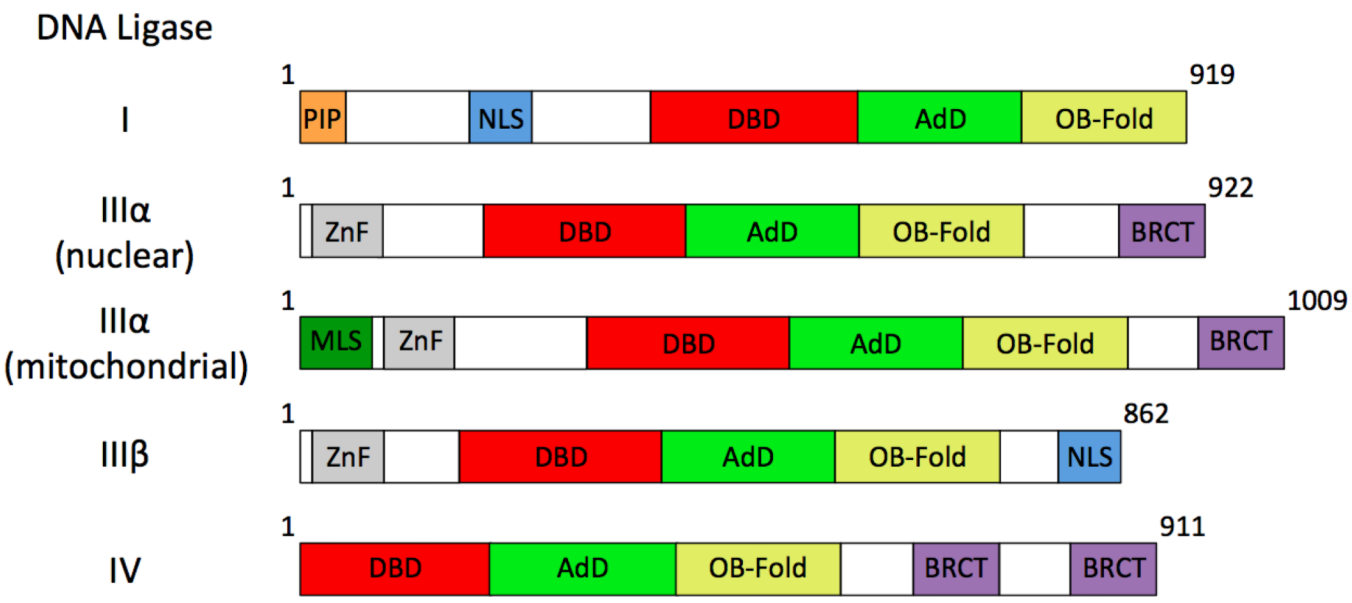

Figure 3.

DNA ligase polypeptides encoded by the human *LIG* genes. All DNA ligases contain a conserved catalytic region consisting of a DNA-binding domain (DBD, red), an adenylation domain (AdD, green) and an oligosaccharide/oligonucleotide binding-fold (OB-Fold, yellow) domain. The *LIG1* gene encodes a single polypeptide with a nuclear localization signal (NLS, blue) and proliferating cell nuclear antigen interacting box (PIP, orange) motif within a non-catalytic N-terminal region. The *LIG3* gene encodes multiple polypeptides, each of which contain an N-terminal zinc-finger (ZnF, grey). Mitochondrial and nuclear DNA ligase IIIα are generated by alternative translation initiation with the mitochondrial version having an N-terminal mitochondrial localization signal (MLS, dark green). Both of the DNA ligase IIIα polypeptides have a C-terminal breast and ovarian cancer susceptibility protein 1 C-terminal (BRCT, purple) domain. An alternative splicing event in male germ cells generates DNA ligase IIIβ which has a C-terminal nuclear localization signal (NLS, blue) in place of the BRCT domain. The *LIG4* gene encodes a single polypeptide that contains two C-terminal BRCT domains separated by a linker region

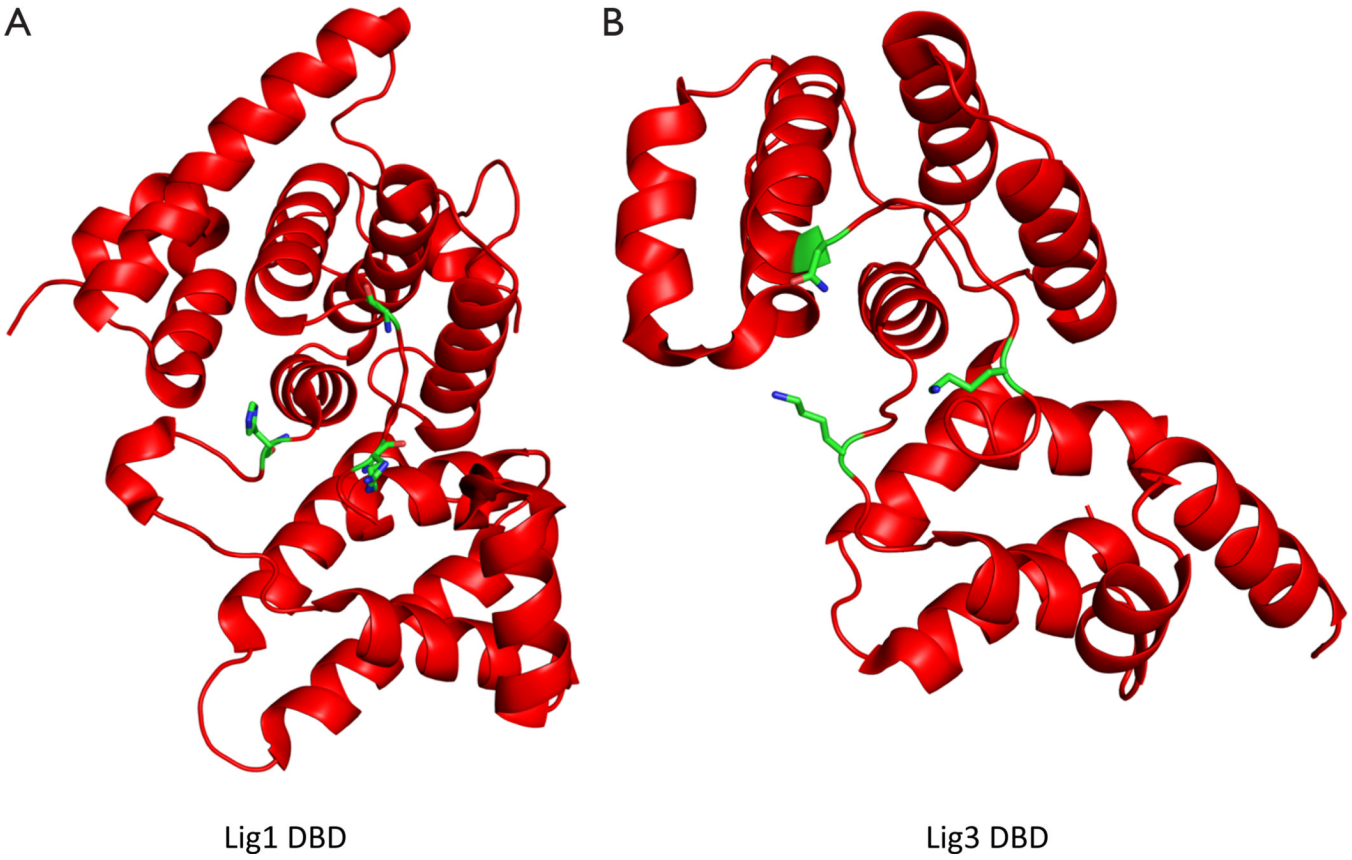

Figure 4.
DNA binding interface within the DNA binding domain targeted by computer-aided drug design. A. Residues forming the DNA binding pocket within the DNA binding domain (DBD) of human DNA ligase I that was targeted by *in silico* screening are indicated; B. A similar view of the comparable region within the DBD of human DNA ligase III

Table 1

Involvement of human DNA ligases in DNA replication and repair pathways

Nuclear DNA metabolism	
DNA republication	DNA ligase I DNA ligase III (backup)
Mismatch repair	Unknown
Base excision repair	DNA ligase III (short-patch) DNA ligase I (long-patch)
Nucleotide excision repair	DNA ligase III (constitutive) DNA ligase I (long-patch)
Single-strand break repair	DNA ligase III (short-patch) DNA ligase I (long-patch)
Homology-mediated double-strand break repair	Unknown
Non-homologous end joining double-strand break repair	DNA ligase IV DNA ligase III (alternative) DNA ligase I (alternative)
Mitochondrial DNA metabolism	
DNA replication	DNA ligase III
DNA repair	DNA ligase III