BAOJ Cancer Research & Therapy

Anthony J Berdis , BAOJ Cancer Res Ther 2017, 3: 4
3: 043

Review

Is Inhibiting Translation DNA Synthesis a Viable Therapeutic Option in Oncology

Anthony J Berdis[1,2,3*]

[1]*Department of Chemistry Cleveland State University, 2351 Euclid Avenue, Cleveland, OH 44115, USA*

[2]*The Center for Gene Regulation in Health and Disease, Cleveland State University, 2351 Euclid Avenue, Cleveland, OH 44115, USA*

[3]*Case Comprehensive Cancer Center, 11000 Euclid Avenue, Cleveland, OH 44106, USA*

Abstract

Statement of Clinical Problem

Certain DNA damaging agents including chlorambucil, temozolomide, cyclophosphamide, and cisplatin function as anti-cancer agents by indirectly inhibiting DNA synthesis. At the molecular level, these agents hinder DNA synthesis by altering the composition and structure of nucleic acid. Unfortunately, the effectiveness of these agents can be compromised by the ability of various DNA polymerases to replicate the formed DNA lesions in a process termed translesion DNA synthesis.

Aim of Work

This review briefly describes clinical data that highlight complications associated with translesion DNA synthesis activity during chemotherapy. The biochemical and cellular mechanisms accounting for how translesion DNA synthesis is normally regulated are discussed. Particular emphasis is placed on describing how dysfunctional activity of DNA polymerases that catalyze translesion DNA synthesis contributes to oncogenesis and drug resistance. Final, discussions are provided on recent efforts to develop new therapeutic agents that specifically target the DNA polymerases involved in translesion DNA synthesis.

Conclusions

Translesion DNA synthesis is now recognized as an important biological process that plays critical roles cancer initiation, progression, and response to treatments. The process of translesion DNA synthesis is complicated due to the vast array of DNA lesions that can form inside a cell coupled with the large number of DNA polymerases that can misreplicate these lesions. However, significant progress is being made toward developing the chemical tools necessary to inhibit the activity of DNA polymerases that perform translesion DNA synthesis.

Background

DNA Damaging Agents as Therapeutic Agents

Approximately 10 million cancer patients in the United States receive DNA damaging agents such as cisplatin, temozolomide, and ionizing radiation as part of their therapy [1]. The primary cytotoxic effect of these agents lies in their ability to alter the structure of nucleic acid so that it is no longer a usable substrate for efficient chromosomal replication (Figure 1). For example, anti-cancer agents such as cisplatin and chlorambucil create crosslinked lesions in DNA which hinder the movement of DNA polymerases [2,3]. By blocking DNA replication during S-phase, these DNA lesions subsequently induce cell death [4-6]. Other agents such as temozolomide and cyclophosphamide also react with functional groups present on DNA to alter the hydrogen-bonding coding potential of the nucleobase [7]. These modifications can enhance the frequency of misincorporation events and increase the amount of "mismatched" DNA formed inside a cancer cell. The formed mismatches are good substrates for DNA repair enzymes, and their activity can either correct

***Corresponding Author:** Anthony J Berdis, Cleveland State University, 2351 Euclid Avenue, Cleveland, OH 44115, USA, Fax: (216) 687-9298, Tel: (216) 687-2454; E-mail: a.berdis@csuohio.edu

Sub Date: October 9th, 2017, **Acc Date:** October 25th, 2017, **Pub Date:** October 30th, 2017.

Citation: Anthony J Berdis (2017) Is Inhibiting Translation DNA Synthesis a Viable Therapeutic Option in Oncology?. BAOJ Cancer Res Ther 3: 043.

the lesion or induce cell death. An excellent example of this phenomenon is temozolomide, a monofunctional DNA damaging agent that non-enzymatically alkylates several functional groups on guanine and on adenine in DNA [8]. Simple alkylation of the O^6 position of guanine changes the hydrogen-bonding capabilities of the natural base, resulting in the frequent misinsertion of dTMP opposite O^6-methylguanine which then activates the mismatch DNA repair pathway [9] .

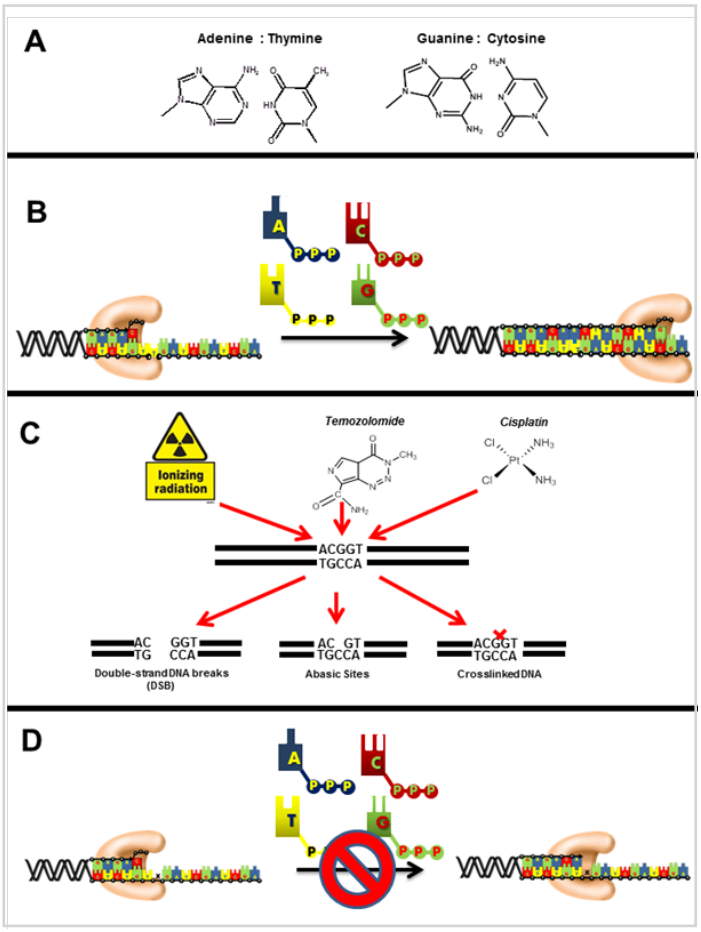

Figure 1. General strategy for using DNA damaging agents as therapeutic agents against cancer. (A) Chemical structures for correct Watson-Crick base pairs. The efficiency and fidelity of DNA polymerization depends upon the formation of base pairing information present on the incoming nucleotide with its templating partner. (B) DNA replication is the process of duplicating DNA to generate two copies of an organism's genetic information. This complex biological reaction is catalyzed by DNA polymerases that add mononucleotides into a growing primer using nucleic acid templates to guide each incorporation event. (C) Commonly used chemotherapeutic agents cause cell death by inflicting DNA damage. Although DNA is the common target, these modalities create distinct DNA lesions that affect the process of DNA synthesis. (D) The ultimate goal of generating DNA lesions is to inhibit the ability of a cancer cell to efficiently complete DNA synthesis. This inhibition produces both cytostatic and cytotoxic effects.

Complications Associated with DNA Damaging Agents

Although DNA damaging agents are at the forefront of therapeutic intervention for many cancers, these agents are not panaceas since they possess a number of complications. Major complications include adverse side effects such as anemia, thrombocytopenia, immunosuppression, and gastrointestinal ailments that arise from the non-selective killing of heathy cells. Another major complication is the development of resistance that is caused by the ability of DNA polymerases to misreplicate DNA lesions generated by these anti-cancer drugs [10-12]. This activity, termed translesion DNA synthesis (TLS), can produce several negative effects in patients receiving DNA damaging agents (Figure 2). First, TLS activity allows for DNA lesions to be by-passed and thus reduces the overall effectiveness of important modalities such as ionizing radiation, cisplatin, and temozolomide. In addition, TLS activity is highly pro-mutagenic. As a result, TLS activity can produce more mutations in a cancer to generate more aggressive malignacies [13]. For example, recent data from Johnson et al. provide a definitive role for TLS activity in generating resistance to temozolomide in glioblastoma patients [14]. In this study, tumors isolated from patients treated with temozolomide showed significantly higher mutation rates (~90 mutations/Mb) compared to tumor biopsies obtained prior to treatment (<4 mutations/Mb). Furthermore, temozolomide-resistant tumors displayed acquired somatic mutations in genes associated with DNA mismatch repair, retinoblastoma, and mammalian target of rapamycin (mTOR). Lastly, the inappropriate replication of DNA lesions formed by certain DNA damaging agents is linked with a phenomenon known as treatment-related cancers [15] .

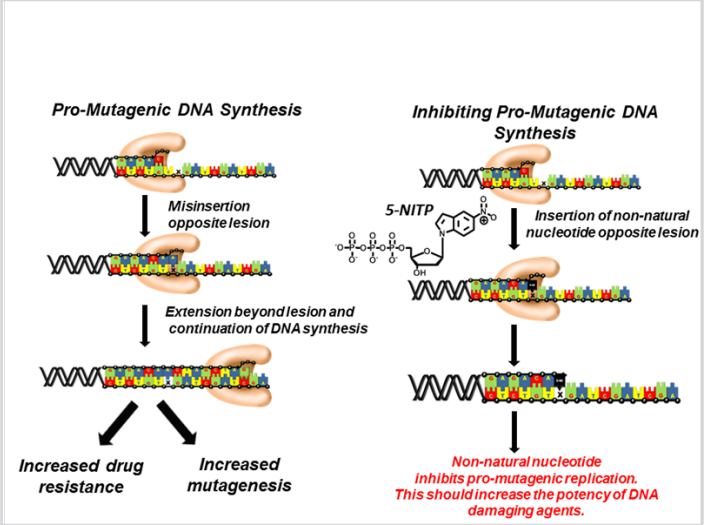

Figure 2. Complications associated with DNA damaging agents and a proposed strategy to combat these complications. Anti-cancer agents such as temozolomide, cisplatin, and ionizing radiation produce DNA lesions that can induce apoptosis. Unfortunately, these DNA lesions can by misreplicated by various DNA polymerases. The replication of damaged DNA can cause mutagenesis and drive drug resistance. To combat these problems, we propose a strategy that applies artificial nucleotides to selectively inhibit DNA polymerases involved in replicating damaged DNA generated by these types of anti-cancer agents.

Coordination of DNA Polymerase Activity During Translesion DNA Synthesis

As expected, the coordination of TLS activity at the cellular level is remarkably complex. Much of this complexity arises from the sheer number of DNA polymerases in human cells that process chromosomal and mitochondrial DNA. In particular, humans possess at least 15 different DNA polymerases that in many cases have overlapping roles in DNA replication, DNA repair, DNA recombination, and translesion DNA synthesis (Figure 3) [16,17]. Of these 15 DNA polymerases, there are five (pol α, pol δ, pol ε, pol γ, and telomerase) that are intimately involved in chromosomal and mitochondrial DNA synthesis. These polymerases are referred to as "classical" DNA polymerases as they rely almost exclusively on canonical Watson–Crick base pairing rules to catalyze efficient and faithful DNA polymerization. In addition, there are several DNA polymerases that are essential for efficient DNA repair in humans. These include pol β that participates in base excision (BER) and nucleotide excision repair (NER) as well as pol λ and pol μ which function during non-homologous end joining, a mechanism for repairing double-strand DNA breaks. In addition, lymphoid tissue express a unique DNA polymerase, denoted as terminal deoxynucleotidyl transferase (TdT), that functions during V(D)J recombination to promote immunological diversity [18]. In contrast to replicative DNA polymerases, the polymerases involved in DNA repair do not possess a 5'→3' exonuclease "proofreading" activity. The final group of DNA polymerases are termed "specialized" polymerases as they can efficiently replicate a number of structurally distinct DNA lesions. Members of this family include pol η, pol ι, pol κ, pol θ, pol φ, pol σ, pol ζ, and Rev1. These specialized DNA polymerases also lack 5'→3' exonuclease activity and are thus highly error-prone.

The most widely accepted model explaining the proper coordination of these various DNA polymerases during TLS involves initial stalling of a replicative polymerase before or at the site of DNA damage. This stalling event triggers a signal that recruits one or more specialized DNA polymerases to the DNA lesion. Once at the lesion, the specialized polymerase incorporates a dNTP and then, depending upon the nature of the damaged DNA, can either dissociate from the lesion or extend beyond it. In the former instance, an "extender" DNA polymerase such as pol ζ is often required for elongation beyond the damaged DNA. In either case, a replicative DNA polymerase displaces the extender DNA polymerase once the lesion is by-passed. This allows DNA synthesis on the remainder of the undamaged DNA to continue with minimal disruption to the overall continuity of leading and lagging strand DNA synthesis.

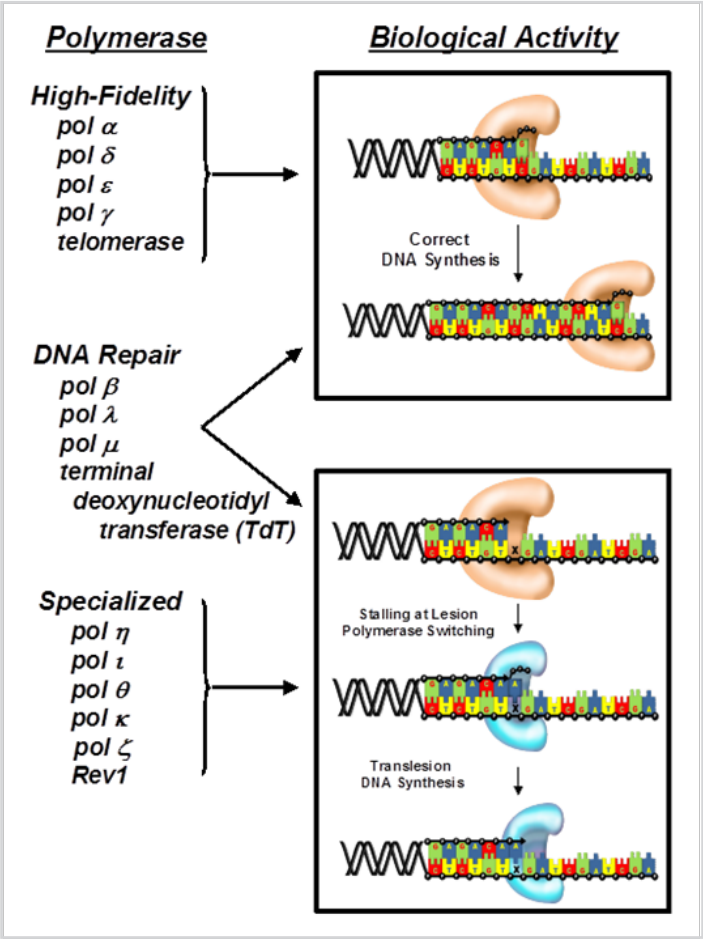

Figure 3. Summary of human DNA polymerases and their involvement in DNA replication, DNA repair, DNA recombination, and translesion DNA synthesis. Humans possess at 15 different DNA polymerases that have overlapping roles in processing normal and damaged DNA. "Classical" DNA polymerases such as pol α, pol δ, pol ε, pol γ, and telomerase are involved in chromosomal and mitochondrial DNA synthesis. DNA polymerases including pol β, pol λ, pol μ, and TdT are involved in DNA repair and DNA recombination. "Specialized" polymerases including pol η, pol ι, pol κ, pol θ, pol φ, pol σ, pol ζ, and Rev1 are unique in their ability to efficiently replicate a number of structurally distinct DNA lesions.

Dysfunctional Translesion DNA Synthesis in Cancer

Under normal cellular conditions, the process of translation DNA synthesis is tightly coordinated to prevent the induction of mutagenic events. This regulation is necessary as specialized DNA polymerases may erroneously replicate undamaged DNA. Indeed, there is a significant amount of pre-clinical and clinical data indicating that de-regulation in the activity of certain specialized DNA polymerases is a common event associated with the development of resistance to many chemotherapeutic agents. For example, pol η can extend beyond cisplatin-DNA lesions, and over expression of this specialized DNA polymerases causes resistance to

cisplatin in cancer cell lines whereas down regulation causes increased cellular sensitivity to cisplatin [19]. In human studies, higher mRNA expression of pol η correlates with poor outcomes in patients with non-small-cell lung cancer and is also associated with shorter survival times in patients receiving platinum drugs [20]. Similar observations are seen with other specialized DNA polymerases such as polι which is over expressed in breast cancer cells and found to be upregulated in ~30% of glioma tumors [21]. The over expression of pol ι is appears to be clinically relevant as patients with pol ι–positive gliomas had shorter survival rates [21].

Current Strategies to Inhibit Translesion DNA Synthesis

These examples provide the basis for a provocative question. That is, can selectively inhibiting one or more specialized DNA polymerases provide an effective strategy to combat clinical complications associated with unregulated TLS activity? Before delving into this question, we must first ask about the potential benefits and risk associated with this strategy. One potential benefit of inhibiting TLS activity would be to increase the cytotoxic effects of DNA damaging agents to potentiate their effectiveness. This potentiation would benefit cancer patients receiving chemotherapy as lower doses of DNA damaging agents could be administered, thus reducing the risk of potential side effects. In addition, inhibiting TLS activity would combat drug resistance caused by the replication of damaged DNA. Finally, preventing pro-mutagenic DNA synthesis could hinder cancer recurrence caused by mutagenesis.

Co-Administration of Gemcitabine with DNA Damaging Agents

Is there clinical evidence that supports this therapeutic strategy? The short answer is yes. In fact, the nucleoside analog gemcitabine (Gemzar) is frequently combined with platinum drugs such as cisplatin and oxaliplatin to treat ovarian and pancreatic cancer [22-25]. Several pre-clinical studies have examined the ability of gemcitabine to synergize the cytotoxic effects of platinum-based drugs and these results suggest a direct effect against the activity of certain specialized DNA polymerases [26-28]. For example, cancer cells that are deficient in pol η are more sensitive to gemcitabine and cisplatin compared to normal human fibroblast cells that possess pol η. More importantly, these pol η deficient cells are ∼10-fold more sensitive to the combined treatment of gemcitabine and cisplatin.

Co-Administration of Purine Nucleosides with DNA Damaging Agents

Unfortunately, there are also clinical findings that highlight the risks associated with combining certain nucleoside analogs with other DNA damaging agents. For example, attempts to combine nucleoside analogs such as fludarabine (Fludara) and cladrabine (Leustatin) with DNA damaging agents have proven unsuccessful. Indeed, a study performed by Rai et al. was discontinued since patients receiving fludarabine and chlorambucil showed evidence for excessive hematological toxicity with no improvement in overall response compared to fludarabine monotherapy [29]. A similar study using chlorambucil with escalating doses of fludarabine in patients with chronic lymphoblastic leukemia (CLL) also showed high levels of hematological toxicity [30]. Identical complications were experienced in patients receiving the combination of cladribine and chlorambucil [31]. The reason for the onset of hematological toxicities may reflect a lack of selectivity exhibited by these purine nucleosides. In fact, in vitro studies demonstrate that the triphosphate form of fludarabine (F-ara-ATP) inhibits pol α and pol ε potently with IC_{50} values of 1.6 μM and 1.3 μM, respectively, while other polymerases such as pol β and pol γ are ∼10-fold less sensitive [32,33]. In this case, the higher potency of fludarabine against replicative DNA polymerases likely places a high burden on DNA replication and DNA repair in healthy cells, and this ultimately causes non-specific killing.

Artificial Nucleosides that Selectively Target Damaged DNA

In an attempt to achieve more selective cell-killing effects, recent efforts have focused on developing artificial nucleosides that are efficiently utilized by specialized DNA polymerases during the replication of lesions produced by DNA damaging agents. One therapeutically important DNA lesion is the abasic site. This non-instructional DNA lesion is non-enzymatically formed by anti-cancer agents such as temozolomide and cyclophosphamide [34]. We developed an artificial nucleotide designated 3-ethynyl-5-nitroindolyl-2'-deoxyriboside triphosphate (3-Eth-5-NITP) that functions as an efficient surrogate for the natural nucleotide, dATP, that is preferentially utilized during TLS [35] (Figure 4) provides a structural comparison between dATP and 3-Eth-5-NITP. In vitro kinetic approaches compared the ability of pol η, the high-fidelity polymerase involved in chromosomal replication and pol δ, a specialized DNA polymerase, to incorporate dATP and 3-Eth-5-NITP opposite an a basic site [36] . Our studies show that pol η, incorporates dATP opposite the non-instructional lesion 500-fold more efficiently than the high-fidelity polymerase, pol δ. This large difference verifies that the specialized polymerase, pol η, likely contributes to the error-prone replication of this lesion inside cells. More importantly, we demonstrated that pol η utilizes 3-Eth-5-NITP ~30-fold more efficiently than dATP when replicating an abasic site. Furthermore, this artificial analog blocks extension beyond the lesion and terminates pro-mutagenic DNA synthesis. Finally, these studies showed that 3-Eth-5-NITP is highly selective for damaged DNA as it is not incorporated opposite un-damaged DNA.

Based on these encouraging in vitro data, we next performed cell-based studies to evaluate if the corresponding artificial nucleoside, 3-Eth-5-NIdR, could potentiate the effects of certain DNA damaging agents [36]. Using acute lymphoblastic leukemia (ALL) as the model, we showed that co-treating ALL cells with temozolomide and sub-lethal doses of 3-Eth-5-NIdR resulted in a synergistic increase in cell death. This synergism in apoptotic cell death was caused by inhibiting TLS activity, and this

was confirmed by demonstrating that the amount of 3-Eth-5-NITP incorporated into genomic DNA increased with the addition of the DNA damaging agent. Current efforts are underway to test the efficacy of 3-Eth-5-NIdR when combined with temozolomide using xenograft mouse models of several human cancers to demonstrate proof-of-concept for this strategy. The preliminary data from these in vivo studies look very promising, and the combined diagnostic and therapeutic activities of this artificial nucleoside could represent a new paradigm in personalized medicine.

Future Directions

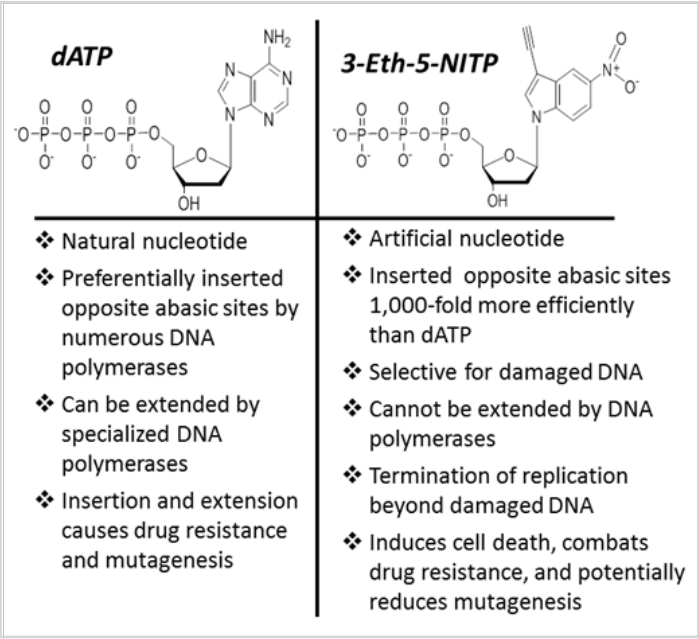

Figure 4. Structural and functional comparisons of the natural nucleotide, dATP, versus the artificial nucleotide, 3-Eth-5-NITP. 3-ethynyl-5-nitroindolyl-2'-deoxyriboside triphosphate (3-Eth-5-NITP) represents a prototypical artificial nucleotide that is efficiently and selectively inserted opposite an abasic site, a non-instructional DNA lesion commonly formed by several anti-cancer agents. See text for further details.

"Custom Designing" Nucleoside Analogs for DNA Lesions. A final question to consider is how applicable this strategy will be toward treating various types of cancer. On one hand, nucleoside analogs are widely used in oncology and are the largest class of antineoplastic agents used clinically [37-39]. In fact, there are eleven FDA approved nucleoside analogs that account for ~20% of all drugs used in chemotherapy [40]. Thus, past success with developing and applying new nucleoside analogs predicts an easy route for their development. However, the key issue is the intrinsic difficulty in creating an artificial nucleoside analog that is a specific partner for a therapeutically relevant DNA lesion and/or that functions as a selective substrate for a particular specialized DNA polymerase. This is especially important since there are a large number of therapeutic modalities that generate anti-cancer effects by creating specific forms of DNA damage. For example, ionizing radiation, which is used in greater than 50% of all cancer patients, exerts its anti-cancer effects by generating double strand DNA breaks (DSBs). Cisplatin, another widely used therapeutic agent, kills cancer cells by producing intra- and interstand DNA crosslinks. In general, the diversity in DNA lesions produced by these modalities will require sophisticated efforts to customize an artificial nucleotide in order to make it exquisitely specific for a particular DNA lesion. At face value, this appears to be a remarkably daunting challenge. However, rational drug design and computational approaches have been used to create artificial nucleotides that are efficiently and selectively incorporated opposite therapeutically important DNA lesions such as O^6-methyguanine, 8-oxoguanine, and DSBs [41-43]. While the therapeutic activity of these artificial nucleoside analogs has not yet been fully explored, it is easy to envision that these analogs (or similar derivatives) may be useful in the treatment of cancer or as cellular probes to further understand the clinical importance of TLS activity in response to cancer chemotherapy.

Conclusion

The process of replicating DNA lesions plays several important roles in the initiation and progression of cancer as well as in the response to several chemotherapeutic agents including cisplatin, cyclophosphamide, and temozolomide. An approach to inhibit the process of translesion DNA synthesis is to co-administer nucleoside analogs that prevent the ability of certain DNA polymerases to replicate these DNA lesions. Progress in this area is evident in the development of artificial nucleoside analogs that are selective for specific DNA lesions such as abasic sites and double strand DNA breaks, two lesions commonly formed by agents such as cyclophosphamide, temozolomide, and ionizing radiation.

References

1. Miller KD, Siegel RL, Lin, CC, Mariotto AB, Kramer JL, et al. (2016) Cancer treatment and survivorship statistics. CA, A Cancer Journal for Clinicians 66(4): 271-289.

2. Fuertes MA, Castilla J, Alonso C, and Pérez JM (2003) Cisplatin biochemical mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. Curr Med Chem 10(3): 257-266.

3. Sanderson BJ, Shield AJ (1996) Mutagenic damage to mammalian cells by therapeutic alkylating agents. Mutat. Res 355(1-2): 41-57.

4. Chu, G (1994) Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J. Biol. Chem 269(2): 787-790.

5. Gonzalez VM, Fuertes MA, Alonso C, Perez JM (2001) Is cisplatin-

induced cell death always produced by apoptosis? Mol Pharmacol 59(4): 657-663.

6. Roos WP, and Kaina B (2013) DNA damage-induced cell death: from specific DNA lesions to the DNA damage response and apoptosis. Cancer Lett 332(2): 237-248.

7. Gates KS, Nooner T, Dutta S (2004) Biologically relevant chemical reactions of N7-alkylguanine residues in DNA. Chem Res Toxicol 17(7): 839-856.

8. Friedman HS, Kerby T, Calvert H (2000) Temozolomide and treatment of malignant glioma. Clin Cancer Res 6(7): 2585-2597.

9. Karran P, Macpherson P, Ceccotti S, Dogliotti E, Griffin S, et al. (1993) O6-methylguanine residues elicit DNA repair synthesis by human cell extracts. J Biol Chem 268(21): 15878-15886.

10. Tomicic MT, Aasland D, Naumann SC, Meise R, Barckhausen C, et al. (2014) Translesion polymerase η is upregulated by cancer therapeutics and confers anticancer drug resistance. Cancer Res 74(19): 5585-5596.

11. Peng C, Chen Z, Wang S, Wang HW, Qiu W, et al. (2016) The Error-Prone DNA Polymerase κ Promotes TemozolomideResistance in Glioblastoma through Rad17-Dependent Activation of ATR-Chk1 Signaling Cancer Res 76(8): 2340-2353.

12. Roy U , Schärer OD (2016) Involvement of translesion synthesis DNA polymerases in DNA interstrand crosslink repair. DNA Repair (Amst) 44: 33-41.

13. Pande P, Malik CK, Bose A, Jasti VP, Basu AK (2014) Mutational analysis of the C8-guanine adduct of the environmental carcinogen 3-nitrobenzanthrone in human cells: critical roles of DNA polymerases η and κ and Rev1 in error-prone translesion synthesis. Biochemistry 53(32): 5323-5331.

14. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, et al (2014) Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 343(6167):189-193.

15. Karp JE, Sarkodee-Adoo CB (2000) Therapy-related acute leukemia. Clin. Lab. Med 20(1): 71-81.

16. Shcherbakova PV, Bebenek K, Kunkel TA (2003) Functions of eukaryotic DNA polymerases. Sci Aging Knowledge Environ2003(8): RE3.

17. Hubscher U, Maga G, Spadari S (2002) Eukaryotic DNA polymerases. Annu Rev Biochem 71: 133–163.

18. Nivard MJ, Pastink A, Vogel EW (1996) Mutational spectra induced under distinct excision repair conditions by the 3 methylating agents N-methyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine and N-nitrosodimethylamine in postmeiotic male germ cells of Drosophila. Mutat Res 352(1-2): 97–115.

19. Chen Y W, Cleaver JE, Hanaoka F, Chang C F, Chou KM (2006) A novel role of DNA polymerase eta in modulating cellular sensitivity to chemotherapeutic agents. Mol. Cancer Res 4(4): 257–265.

20. Ceppi P, Novello S, Cambieri A, Longo M, Monica V (2009) Polymerase eta mRNA expression predicts survival of non-small cell lung cancer patients treated with platinum-based chemotherapy. Clin. Cancer Res 15(3): 1039–1045.

21. Yang J, Chen Z, Liu Y, Hickey R J, Malkas L H (2004) Altered DNA polymerase iota expression in breast cancer cells leads to a reduction in DNA replication fidelity and a higher rate of mutagenesis. Cancer Res 64(16): 5597–5607.

22. Richardson DL, Backes FJ, Seamon, LG, et al. (2008) Combination gemcitabine, platinum, bevacizumab for the treatment of recurrent ovarian cancer. Gynecol. Oncol 111(3): 461–466.

23. Ozols RF (2005) Gemcitabine and carboplatin in second-line ovarian cancer. Semin. Oncol 32(4suppl6): S4–S8.

24. Sehouli J (2005) Review of gemcitabine-based combinations for platinum-resistant ovarian cancer. Int. J. Gynecol. Cancer 15(1): 23–30.

25. Hoff PM, Fuchs CS (2003) The experience with oxaliplatin in the treatment of upper gastrointestinal carcinomas. Semin Oncol 30(15): 54–61.

26. Chua YJ, Cunningham D (2006) Chemotherapy for advanced pancreatic cancer. Best Pract. Res. Clin. Gastroenterol 20(2): 327–348.

27. Jensen PB, Holm B, Sorensen M, Christensen IJ, Sehested M (1997) In vitro cross-resistance and collateral sensitivity in seven resistant small-cell lung cancer cell lines: preclinical identification of suitable drug partners to taxotere, taxol, topotecan and gemcitabin. Br. J. Cancer 75(6): 869–877.

28. Chen YW, Cleaver JE, Hatahet Z (2008) DNA polymerase eta activity and translocation is regulated by phosphorylation. Proc Natl Acad Sci USA 105(43): 16578–16583.

29. Rai KR, Peterson BL, Appelbaum FR., Kolitz J, Elias L, et al.(2000) Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 343: 1750–1757.

30. Weiss M., Spiess T, Berman E, Kempin S (1994) Concomitant administration of chlorambucil limits dose intensity of fludarabine in previously treated patients with chronic lymphocytic leukemia. Leukemia 8(8): 1290–1293.

31. Tefferi A, Witzig TE, Reid JM, Li CY, Ames MM (1994) Phase I study of combined 2-chlorodeoxyadenosine and chlorambucil in chronic lymphoid leukemia and low-grade lymphoma. J Clin Oncol 12(3): 569–574.

32. Huang P, Chubb S, Plunkett W (1990) Termination of DNA synthesis by 9-beta-D-arabinofuranosyl-2-fluoroadenine. A mechanism for cytotoxicity. J Biol Chem 265(27): 16617-16625.

33. Gandhi V, Huang P, Chapman AJ, Chen F, Plunkett W (1997) Incorporation of fludarabine and 1-beta-D-arabinofuranosylcytosine 5'-triphosphates by DNA polymerase alpha: affinity, interaction, and consequences. Clin. Cancer Res 3(8): 1347-1355.

34. Taylor JS (2002) New structural and mechanistic insight into the A-rule and the instructional and non-instructional behavior of DNA photoproducts and other lesions. Mutat Res 510(1-2): 55-70.

35. Motea EA, Lee I, Berdis AJ (2012) Development of a 'clickable' non-natural nucleotide to visualize the replication of non-instructional DNA lesions. Nucleic Acids Res 40(5): 2357-2367.

36. Choi JS, Kim S, Motea E, Berdis A (2017) Inhibiting translesion DNA synthesis as an approach to combat drug resistance to DNA damaging agents. Oncotarget 8(25): 40804-40816.

37. Peters GJ, Schornagel JH, Milano GA (1993) Clinical pharmacokinetics of anti-metabolites. Cancer Surv 17: 123–156.

38. Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman AM (2000) Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol. Ther 87(2-3): 227–253.

39. García F, Fumero E, Gatell JM (2008) Immune modulators and treatment interruption. Curr. Opin. HIV AIDS 3(2): 124–130.

40. Parker WB (2009) Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chem Rev 109(7): 2880–2893.

41. Chavarria D, Ramos-Serrano A, Hirao I, Berdis AJ (2011) Exploring the roles of nucleobase desolvation and shape complementarity during the misreplication of O(6)-methylguanine. J Mol Biol 412(3): 325-339.

42. Choi JS, Dasari A, Hu P, Benkovic SJ, Berdis AJ (2016) The use of modified and non-natural nucleotides provide unique insights into pro-mutagenic replication catalyzed by polymerase eta. Nucleic Acids Res 44(3):1022-1035.

43. Berdis AJ, McCutcheon D (2007) The use of non-natural nucleotides to probe template-independent DNA synthesis. Chembiochem 8(12): 1399-1408.