Chromatin and Other Obstacles to Base Excision Repair: Potential Roles in Carcinogenesis

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Abstract

DNA is comprised of chemically reactive nucleobases that exist under a constant barrage from damaging agents. Failure to repair chemical modifications to these nucleobases can result in mutations that can cause various diseases, including cancer. Fortunately, the base excision repair (BER) pathway can repair modified nucleobases and prevent these deleterious mutations. However, this pathway can be hindered through several mechanisms. For instance, mutations to the enzymes in the BER pathway have been identified in cancers. Biochemical characterization of these mutants has elucidated various mechanisms that inhibit their activity. Furthermore, the packaging of DNA into chromatin poses another obstacle to the ability of BER enzymes to function properly. Investigations of BER in the base unit of chromatin, the nucleosome core particle (NCP), have revealed that the NCP acts as a complex substrate for BER enzymes. The constituent proteins of the NCP, the histones, also have variants that can further impact the structure of the NCP and may modulate access of enzymes to the packaged DNA. These histone variants have also displayed significant clinical effects both in carcinogenesis and patient prognosis. This review focuses on the underlying molecular mechanisms that present obstacles to BER and the relationship of these obstacles to cancer. In addition, several chemotherapeutics induce DNA damage that can be repaired by the BER pathway and understanding obstacles to BER can inform how resistance and/or sensitivity to these therapies may occur. With the understanding of these molecular mechanisms, current chemotherapeutic treatment regiments may be improved, and future therapies developed.

Introduction

While the preservation of the code of life is essential, DNA is chemically reactive and susceptible to modification and degradation (1,2). DNA is inundated by damaging agents from both endogenous and exogenous sources (3,4). The damage resultant from these agents includes single- and double-strand breaks, inter- and intra-strand crosslinks, abasic sites, and modification of the nucleobases (5).

There are a variety of cellular mechanisms that allow for the repair of DNA damage (6). The base excision repair (BER) pathway is responsible for the repair of modified nucleobases, referred to here as lesions (Fig. 1). This pathway is initiated by a glycosylase, which is responsible for the recognition and excision of the lesion by cleavage of the glycosidic bond. Each of the 11 human glycosylases recognizes specific lesions that together span a variety of modifications (Table I). Glycosylases may be classified as either bifunctional or monofunctional. Bifunctional glycosylases first remove the lesion, resulting in an apurinic/apyrimidinic (AP) site, and then catalyze a strand break 3′ to the AP site. Monofunctional glycosylases only excise the lesion, resulting in an AP site that is further processed by AP endonuclease 1 (APE1), which incises the backbone to create a nick with 3'-OH and 5'-deoxyribose phosphate (5′-dRP) termini. Polymerase β (pol β) removes the 5'-dRP and catalyzes nucleotide incorporation at the 3'-OH. Finally, the repair is completed by a DNA ligase that seals the nick in the backbone (5).

Clinical Ramifications of BER

Due to the essential role of BER in maintaining genomic integrity, a multitude of ramifications for human health result when any part of the pathway is deficient or aberrant. These errors in BER can occur at any step in the pathway, from the initiation of the repair event

by a glycosylase to the final sealing of the nick by DNA ligase. The relationship between errors in the BER pathway and the development and prognosis of cancer have been investigated extensively. A discussion of all the errors that have been observed in the BER pathway is beyond the scope of this review. Here, we highlight several mutant enzymes which demonstrate how BER can go awry and lead to cancer.

Improper functioning of the 11 human glycosylases has been observed in various cancer phenotypes. This improper functioning can be caused by mutations and/or dysregulated expression. Abnormally low expression of oxoguanine glycosylase 1 (OGG1), which excises the prototypic oxidative lesion 8-oxo-7, 8-dihydroguanine (8-oxoG), has been linked to gastric cancer (7), aggressive breast cancer (8), ovarian cancer (9), and lung cancer (10). Mutations in MutY DNA glycosylase (MUTYH), which excises adenine (A) when paired with 8-oxoG, are observed in colorectal cancer (11-13) and cholangiocarcinoma (14). Overexpression of endonuclease-like protein 1 (NTH1) has been implicated in the development of cancer by promoting genetic instability (15) and mutants have been observed in colorectal cancer (16,17). Deletion of both NTH1 and endonuclease VIII-like 1 (NEIL1) leads to the development of pulmonary and hepatocellular tumors in mice (18). Mutations in NEIL1 have also been identified in patients with cholangiocarcinoma (14). Functionally impaired mutants of NEIL2 have been implicated in an increased risk of lung cancer (19). Mutations in the uracil superfamily DNA glycosylases uracil DNA glycosylase (UNG) and thymine DNA glycosylase (TDG) have been implicated in familial colorectal cancer (20). Combined knockout of single-strand selective monofunctional uracil glycosylase 1 (SMUG1) and UNG leads to increased risk of cancer in mice (21). Deficiency in SMUG1 expression has also been identified in highly aggressive breast cancers (22). Conversely, a specific polymorphism in the gene encoding UNG confers a

decreased risk of esophageal cancer (23). The overexpression of alkyladenine DNA glycosylase (AAG) has been associated with development of breast cancer (24). Surprisingly, while AAG-deficient mice exhibit greater sensitivity to alkylation damage, they do not show overt phenotypic abnormalities or an increased propensity for developing cancer (25,26).

Mutations and/or deficiencies in BER enzymes that act downstream of the glycosylase have also been implicated in the development and prognosis of cancer. Abnormalities in the second enzyme in the BER pathway, APE1, have been hypothesized to play a role in the development of prostate cancer (27), lung cancer (28), gastric cancer (29), and ovarian cancer (30). Likewise, pol β has been implicated in cancers, with approximately 30% of tumors expressing pol β variants (31). These variants have been observed in gastric cancer (32,33), ovarian cancer (34), and esophageal carcinoma (35). However, for the last step in BER it has been reported that DNA ligase I polymorphisms either shown no association with lung cancer (36) or a weak association with upper aerodigestive tract cancers and moderate association with lung cancer (37). These associations between BER enzymes and cancer have led to numerous studies to understand the biochemical processes underlying these phenomena.

Biochemical Studies of BER Enzymes Implicated in Carcinogenesis

Biochemical studies have strived to elucidate how mutations in glycosylases can interfere with substrate binding and/or catalytic activity and thereby promote carcinogenesis. Many cancers are associated with oxidative damage, and therefore studies have focused on the glycosylases responsible for repair of oxidatively damaged DNA. NEIL1 G83D, a mutant identified in cholangiocarcinoma, can bind its substrate but has impaired catalytic ability and can inhibit wild-type (WT) NEIL1 binding (38,39). Several MUTYH mutants have been associated with cancer including Y165C, G382D, and D222N (40,41). Biochemical analysis revealed that

these mutants exhibit minimal glycosylase activity (42). The D222N mutant is an active site mutant that cannot perform chemistry. Meanwhile, Y165C and G382D mutants have decreased binding capacity. Another mechanism for diminished glycosylase activity is observed for OGG1 S326C, known to be associated with lung cancer (43,44) and a lower ability to prevent 8-oxoG-induced mutagenesis (45). While this mutant can bind and excise 8-oxoG, it has a diminished specificity, indicated by a value for k_{cat}/K_m that is half of that of WT (46). OGG1 R154H is another mutant associated with the development of kidney carcinomas (47) and gastric cancer (48). Biochemical studies of R154H revealed substantially lower excision of 8-oxoG compared to WT. The loss of excision activity was due to lower substrate specificity caused by a relaxed active site pocket which manifested in a lower k_{cat}/K_m value relative to WT (49). The OGG1 and MUTYH mutants are the best characterized and less information is available for the other glycosylase mutants associated with cancer. However, other studies have focused on downstream BER enzymes' roles in carcinogenesis.

Cancer-related APE1 mutants have also demonstrated impaired enzymatic activity. The APE1 mutants L104R and E126D have decreased activity due to diminished DNA binding capacity. In contrast, molecular modeling predicts that the R237A mutant has an altered structure that potentially affects overall folding and/or solubility (50). The mutants D148E and G241R have no effect on the repair ability as defined by binding and ability to perform chemistry. Significantly, despite this retained repair ability, APE1 D148E has been implicated in susceptibility to lung cancer (51,52). These associations again highlight the complexity of understanding the relationship between APE1 mutants, enzymatic activity, and carcinogenesis.

Extensive work has also been conducted with pol β mutants to understand their causative relationship to human cancers. Mutations of pol β are observed in 30% of tumors, indicating a

strong link between pol β and carcinogenesis (31). These mutations also lead to notable clinical outcomes and the putative mechanism for dysfunction has been described for several mutants. Pol β P242R, for example, is associated with a poorer prognosis for lung cancer (53) and lymphoma (54). Biochemical experiments with pol β P242R have revealed DNA binding similar to WT, but a slower rate of DNA synthesis (55). This slower rate may contribute to accumulation of single strand breaks (SSBs) which is supported by the increased instances of SSBs in cells with pol β P242R subsequent to treatment with methyl methanesulfonate (MMS) (55). Another pol β mutant, I260M, has been associated with prostate cancer (56). It has been shown to have tighter DNA binding, lower fidelity, and a slower rate of synthesis compared to wild-type pol β (57). The mutation is in the hinge region of the polymerase which causes a greater degree of freedom for positioning the incoming dNTP in the active site and incorporation of the incorrect dNTP and loss of fidelity (58). Fidelity loss from a slower rate of synthesis has also been observed in the colorectal cancer associated mutant pol β S229L (59). Pol β E288K is another mutant which lacks fidelity, but as a result of defective product release (60). Pol β K289M has been associated with colorectal cancer (61) and causes mutations *in vivo* (62). This mutation is caused by lower fidelity resulting from a shift in the rate determining step from product release to initial dNTP binding (63). A final mechanism that may contribute to the biological effects of pol β mutants is the loss of dRP lyase activity, as observed for pol β L22P (64). These results indicate the vast array of mechanisms by which polymerase function can be disrupted and lead to a variety of cancers.

Studies using duplex DNA not incorporated into chromatin have allowed the detailed characterizations of the entire BER pathway. This includes, but is not limited to, the identification of enzymes and substrates involved; the structure of these enzymes; the important

residues involved in binding and performing chemistry; and the coordination of the entire pathway. These biochemical studies have contributed to our understanding of several mechanisms relating mutant BER enzymes and carcinogenesis. However, it is unclear from these experiments on unincorporated duplex DNA why other mutations observed in cancers may be problematic. The decreased capacity for these BER mutants to bind their substrates is a recurring mechanism observed in enzymes throughout the BER pathway. This consideration of binding is particularly important because while DNA is sometimes physically accessible in cells, it exists in a packaged environment for most of the cell cycle. Therefore, the effect on substrate binding when DNA is packaged needs to be considered and can be accomplished using biochemical studies of BER in the minimal unit of DNA packaging in chromatin, the nucleosome core particle (NCP). Furthermore, variants of the histone proteins that comprise the core of NCP have strong associations with cancer that will be discussed in more detail below.

Initiation of BER in Chromatin

The NCP is the basic unit of packaging in eukaryotic chromatin. The NCP is comprised of 145-147 base pairs of DNA wrapped approximately 1.7 times around an octamer core of histone proteins (65) and contains a 2-fold axis of pseudosymmetry known as the dyad axis (Fig. 2). The octamer core is comprised of two copies of each histone protein: H2A, H2B, H3, and H4 (66), each containing a globular core region and a disordered tail (65). Within an NCP, any given nucleobase is present within a unique local structural architecture that governs how it may be accessed by enzymes. When considering the activity of a glycosylase in an NCP, a variety of factors must be considered. First and foremost, the nature of the lesion's unique location and environment within the NCP must be considered. The location of any position can be defined by two parameters: (1) its rotational positioning, i.e. orientation in the DNA helix facing inwards

towards the histones (IN), outwards towards solution (OUT), or somewhere in between (MID) and (2) its translational position defined as its distance from the dyad axis, i.e. located more internally in the NCP or closer to the DNA ends. Another consideration with regards to translational positioning is the observed phenomenon of DNA ends near the exit/entry region spontaneously and transiently dissociating from the histone core (Fig. 3). This dynamic unwrapping of the DNA ends has been observed by FRET experiments (67-70) and by increased accessibility of these sites to restriction enzymes (71). Furthermore, the periodicity of the DNA within the NCP varies depending on its translational position (72). Additional factors, such as the presence of superhelices and the histone tails also create further nuances in local NCP microenvironments. Foundational studies also demonstrated that damage formation in the NCP occurs differentially depending on rotational position (73) and strength of association of the DNA to the octamer core (74). Taken together, these factors illustrate that damage located within the NCP is a complex substrate for DNA repair. Early studies demonstrated that the NCP inhibits the activity of UNG and SMUG1 compared to unpackaged DNA at certain locations in the NCP (75). Further the repair of alkylation damage in regions occupied by nucleosomes exhibited slower rates of repair when compared to regions between nucleosomes (76,77). Understanding the effects of DNA packaging on initiation of BER by glycosylases can contribute to our understanding of the links between DNA damage repair and carcinogenesis.

DNA repair within the NCP has been most extensively studied in the dyad region, a location where there is minimal transient unwrapping of the DNA. The dyad region also lacks adjacent DNA superhelices. To characterize initiation of BER in the dyad region, our laboratory investigated the activity of several glycosylases working under identical reaction conditions (78). Specifically, we investigated the activity of the OGG1 glycosylase excising 8-oxoG, UDG

excising U, and AAG excising εA. The rotational orientation for each target lesion was varied to face OUT, MID, or IN. Both AAG and UDG exhibited activity that correlated with rotational orientation, with the OUT lesion being most readily excised, and the IN lesion the least, a result which was observed by others for UNG (79). Significantly, two kinetic phases can be resolved for UDG acting on MID and IN sites, and it has been proposed by us (80) and others (81) that this biphasic kinetics reflects conformational changes within the NCP that expose occluded sites. In contrast to AAG and UDG, OGG1 had limited excision activity, regardless of rotational orientation of the lesion. Significantly, we have shown that the OGG1 activity is inhibited in the dyad region regardless of rotational positioning (80). Furthermore, others have shown that activity of NTH1 removing thymine glycol (Tg) is lower in the dyad region than an area located towards the DNA entry/exit area for an IN lesion (82). Decreased NCP dynamics and the unique periodicity of DNA near the dyad may explain the inhibition of certain glycosylases in this region of the NCP.

With its own unique architecture that lacks the dynamics observed at sites closer to the entry/exit region, the dyad region is not representative of the DNA throughout the entire NCP. We therefore expanded our investigations of rotational positioning to a region close to the DNA entry/exit region with a focus on the excision activity of OGG1 and UDG (83). The target lesions, 8-oxoG and U, were located approximately 20 bp from the end of the DNA. In contrast to the dyad region, glycosylase activity was observed at all rotational positions, albeit to varying degrees. Additionally, biphasic kinetics were observed for all rotational positions, reflecting that conformational changes in the NCP may modulate access to lesions toward the DNA ends of the NCP due to transient unwrapping. SMUG1 and UNG have been reported to experience fewer translational effects when comparing the entry/exit region and the dyad (75). However, reduction

of unwrapping via crosslinking of the DNA to the histone octamer reduces repair by UNG and UDG (84,85). Furthermore, enhanced repair activity closer to the DNA ends was previously observed with UDG (86), consistent with our results showing greater repair of the DNA closer to the entry/exit region. Additionally, both NTH1 and NEIL1 have shown enhanced excision activity on Tg lesions in the DNA entry/exit region (87). Lesion exposure rates have also been shown to be rate limiting for NTH1, further solidifying the importance of transient unwrapping in facilitating BER initiation (88). Experiments in yeast provide *in vivo* evidence that BER enzymes may exploit unwrapping. Treatment with MMS reveals all nucleobase positions throughout the NCP are susceptible to damage. However, after a period of recovery time there is persistent damage in the dyad region, indicative of hindered repair, which subsequently leads to mutations (89). Further evidence demonstrates somatic and germline mutations follow the periodicity in the NCP (90). Specifically, mutations are observed in areas of the genome packaged IN towards the histones. These observed mutational hotspots are hypothesized to be a result of hindered repair at these sites (90). Taken together, these results show that DNA lesions that are located IN and in the dyad region are less likely to be repaired, perpetuating mutagenic and cytotoxic effects.

Inhibition of BER in certain NCP locations may further explain how obstacles to glycosylase activity lead to carcinogenesis. DNA packaging in NCPs leads to differential repair based on the unique NCP architecture location of the lesion, with some sites being easier to access by glycosylases than others. Therefore, certain types of damage in certain locations within the NCP are far less likely to be removed than others. However, even for damage that is recognized and removed by a glycosylase, the downstream BER processes still need to be

completed. Enzymes that act downstream in the BER pathway also must overcome the obstacles presented by the NCP.

Downstream BER Enzymatic Activity in the NCP

If a glycosylase excises its target lesion from an NCP, a potentially more deleterious AP site is left in its place. These AP sites are known to react with the lysine-rich histone tails of NCPs to promote strand cleavage (91,92). Intentional strand breaks are introduced by APE1, but this deleterious event is regulated by tightly coupled coordination between BER enzymes. Specifically, the BER downstream pathway has been described as a "passing of the baton" that allows for sequestering of these AP sites until pol β activity (93). It is therefore essential to understand how AP site processing can occur within the packaged and lysine-rich environment of the NCP.

APE1: There have been several studies of APE1 activity on NCPs which reveal that incision depends on the rotational position of the AP site. In comparison to unincorporated duplex, there is a 10-fold reduction in APE1 incision at AP sites facing IN, while AP sites facing OUT are fully incised (94). A MID site showed intermediate levels of incision. Electrophoretic mobility shift assay (EMSA) experiments showed that the reduced incision activity for MID and IN sites is a result of a lower binding affinity for the NCP substrate compared to unincorporated duplex DNA. While WT APE1 is inhibited at certain locations within the NCP, the cancer-associated mutants of APE1, R237C and G241R, are even more substantially inhibited by the NCP architecture; interestingly, these mutants showed no reduction in incision activity in unincorporated duplex DNA (95). The presence of other nuclear factors that may modulate interactions between APE1 and the histone octamer core adds additional complexity in understanding APE1 activity on the NCP. While purified APE1 demonstrates inhibition at

certain sites in the NCP, a nuclear extract from CHO-K1 cells alleviates inhibition (96). These results suggest how tumor-associated mutants of APE1 may lead to altered repair in NCPs that could lead to deleterious mutations and carcinogenesis.

Pol β: Several studies of the complete BER pathway on the NCP reported that the limiting step in the pathway is strong inhibition of single nucleotide insertion by pol β on gaps facing inwards towards the histone octamer (75,79,97-99). This inhibition of pol β activity was also observed for multi-nucleotide, strand-displacement synthesis (99). Furthermore, while the acetylation of pol β has shown to improve strand-displacement synthesis in duplex, this effect is not observed in NCPs (100). The dRP lyase activity of pol β has also been investigated in NCPs and intriguingly there was enhancement of lyase activity at the DNA ends in NCPs relative to duplex (101), which may be due to a contribution of lysine from the histone core promoting AP site cleavage. Further studies on the searching mechanism of pol β suggest that in duplex DNA it can hop from site to site but this processive nature is abolished in NCPs (102). These results indicate a strong inhibition of pol β nucleotide insertion in NCPs but little effect on its lyase activity. It is currently unknown how the cancer associated pol β mutants behave in NCPs.

Ligase: The final step in the BER pathway involves the sealing of the nick in the phosphate backbone by a ligase. Surprisingly, despite the need for ligase I/III to encircle its substrate, there have been reports of ligase activity in the NCP (103-105). Specifically, ligase I was shown to repair nicks at the dyad and closer to the DNA entry/exit region without alteration of the NCP structure (103). However, the histone tails were shown to inhibit ligase activity in shorter DNA but no inhibition was observed when longer DNA ends were present to mimic linker DNA (103). This effect was attributed to binding of the DNA by the histone tails in the longer DNA ends. While ligase I activity was not a result of altered NCP structure, ligase IIIα

was shown to associate with XRCC1 and promote disruption of the NCP structure that enhanced both its activity and the activity of pol β (105). Furthermore, while lesion orientation was important for other BER enzymes, it has no substantial effect on ligation. It is worth noting, however, this ligase activity was only seen in a biologically-derived NCP positioning DNA sequence (105). However, while there was little effect due to rotational orientation for ligase IIIα, there was significant inhibition as the nick was moved closer to the dyad. This result indicates that ligase activity may be opportunistic, taking advantage of the transient unwrapping of DNA that occurs in the NCP (104). These results again show the complexity of the NCP substrate and the multitude of ways in which the NCP can interfere with BER.

These results demonstrate that packaging of DNA in an NCP can inhibit various steps of the BER pathway, albeit to varying degrees and with a dependence on the location of the substrate within the NCP. Understanding how this inhibition is overcome, or not overcome, in the cell may help elucidate how cancerous mutations occur. However, while these results have been informative, they have mostly been concerned with NCPs assembled with canonical histone proteins. It has been observed that the histones themselves can vary as well, adding another layer of complexity to this system.

Histone variants

Variant histones are histone proteins that can be substituted for the canonical proteins in the NCP and can confer unique function and structure. The histones that possess the most variants are H2A and H3. H2A variants include H2A.X, H2A.Z, macroH2A, and H2A.Bbd which have specific localization that implicates their role(s) in defined biological processes. For example, H2A.X is distributed throughout the genome and is associated with double-strand break repair (106). H2A.Z is found in euchromatin and is associated with gene silencing (107).

MacroH2A is localized to the centrosome of the inactive X chromosome during late G1 and has been observed to repress transcription (108). Finally, H2A.Bbd co-localizes with hyperacetylated H4 and is associated with open chromatin and DNA synthesis (109). The histone variants also demonstrate appreciable clinical significance to carcinogenesis and cancer prognosis. The relationship between cancer biology and histone variants is extensive and has been reviewed elsewhere (110) but will be briefly covered here. The variant H2A.Z is overexpressed in colorectal cancer (111), breast cancer (112), and melanoma (113), while a causative role for overexpression has been identified for certain breast cancers (114). In contrast to the positive association between cancer and H2A.Z expression, lower levels of macroH2A have been observed in highly aggressive cancers (115-118), lung cancer (119), melanoma (113), testicular, lung, bladder, cervical, breast, colon, ovarian, and endometrial cancers (120). Loss of a single H2A.X allele leads to cancer susceptibility (121) and H2A.X maps to a cytogenetic region that is frequently mutated in cancers (122). Higher levels of CENP-A, an H3 variant, are observed in breast cancer (118), colorectal cancer (115), and testicular cancer (116). Furthermore, CENP-A overexpression has been observed with ectopic localization to regions of high histone turnover (115,123).

Variant histones impart a unique architecture to the NCP through alterations in DNA-histone contacts and changes in histone size (Fig. 4). For example, incorporation of CENP-A imparts a significant compaction of the DNA around the dyad relative to canonical NCPs (124). Heterotypic nucleosomes containing both H3.3 and CENP-A have been observed (123) and exhibit a hybrid NCP structure with separate regions defined by CENP-A and H3.3 (125). MacroH2A is almost three times the size of canonical H2A due to a 30 kDa macro domain connected via a lysine-rich linker to the histone core domain (126). The macro H2A-containing

octamer is more stable in lower salt conditions (127) and DNA in the dyad region is more resistant to nuclease digestion compared to canonical NCPs (128). H2A.Z has subtler differences than CENP-A and macroH2A with no changes to the histone-DNA contacts, but minor changes to histone-histone interactions (129). However, these changes to a single NCP structure do not necessarily reflect the effects these variants have on higher-order packaging. Overall, these variants impart unique structural properties to nucleosomes that may help explain their observed biological functions.

Histone variants modulate DNA repair, with their structural alterations playing a key role for specific variants. H2A.Z mediates an essential chromatin remodeling step for the repair of DSBs (130), an observation reinforced by compaction of nuclei in yeast lacking H2A.Z (131). The macro domain of macroH2A reduces the recruitment of DNA repair machinery while also compacting chromatin following poly(ADP-ribose) polymerase 1 (PARP1) activation (132). Similarly, CENP-A interacts with PARP2 (133) and CENP-A is found in nuclear foci and depletion of CENP-A results in increased apoptosis following irradiation (134). CENP-A recruitment is also observed upon induction of DSBs in both human and mouse cells (135).

While there is little known about the relationship between the H2A variants and BER, CENP-A appears to interact with the BER machinery. CENP-A co-localizes with UNG in cells (136). Furthermore, lower UNG expression in human cells leads to a reduction in CENP-A assembly; conversely, overexpression increases CENP-A assembly (136). A further connection between the BER pathway and histone variants is the observation that depletion of macroH2A results in sensitivity to MMS treatment, which is known to create lesions repaired by BER (137). The understanding of the relationship between BER, NCPs, and histone variants in DNA repair can not only inform potential carcinogenic mechanisms but also potential treatments.

Chemotherapy and BER

Chemotherapeutic approaches generally target DNA replication due to the high levels of synthesis in cancerous cells. However, patients receiving chemotherapy can develop resistance to previously-effective drugs (138). One mechanism of resistance involves DNA repair and several chemotherapeutic agents directly alter nucleobases and produce lesions that can be excised by glycosylases. Many review articles have address the connections between BER and cancer therapy (139-143); here we touch on a few examples where BER initiation may be implicated in chemotherapeutic response.

The chemotherapeutic agent 5-fluorouracil (5-FU) works via several mechanisms. It inhibits thymidylate synthase (TS) and, as a result, dUMP levels increase 100-fold within 3 h of treatment in cancer cells (144). This high level of dUMP may contribute to lethality via misincorporation of U into DNA. Despite these high levels of U, UNG knockout does not confer any altered responsiveness to 5-FU (145). However, 5-FU can be incorporated into DNA (146) and its excision by SMUG1 confers resistance to cells (147). Furthermore, 5-FU is a substrate for UNG, TDG, and SMUG1 in an overlapping system that may create a complex response to 5-FU treatment. Taken together, these results demonstrate that 5-FU efficacy could be affected by the UDG superfamily of glycosylases, either through removal of 5-FU directly or U incorporated following TS inhibition.

The chemotherapeutic temozolomide (TMZ) is an alkylating agent that produces lesions that are recognized by glycosylases. With regard to the BER pathway, the majority of alkylation damage is excised by AAG, including lesions created by TMZ. Decreased sensitivity to a variety of chemotherapeutic agents was observed in mouse embryonic stem cells overexpressing AAG, which was postulated to be derived from an enhanced ability to repair damaged bases (148-150).

Other work has shown that overexpression of AAG in breast cancer cells increases sensitivity to chemotherapeutics, including TMZ, which was attributed to increased strand breaks via generation of AP sites (151). Furthermore, overexpression of AAG has been demonstrated to result in microsatellite instability, frameshift mutagenesis, and increased strand breaks upon exposure to alkylating agents (152). Knockdown of AAG in human carcinoma cells showed enhanced sensitivity to chemotherapeutics, including TMZ in direct contradiction to the results seen in breast cancer cells (153). AAG-deficient mice showed enhanced resistance to some alkylating therapeutics but demonstrated susceptibility to others (154,155). It is apparent from these studies that the relationship between AAG and chemotherapeutic response remains to be fully elucidated.

There is still much to be learned about how to harness BER to produce favorable clinical outcomes. While there is evidence of the involvement of BER in chemotherapeutic resistance and sensitivity the exact relationships remain unclear. Future studies investigating BER in packaged DNA may help elucidate new targets. A particularly attractive area is understanding potential interplay between the histone variants and BER, given the significant clinical outcomes associated with some variants. A holistic approach to understanding BER as it may occur in a cellular environment will strengthen understanding of potential carcinogenic mechanisms and inform future therapies.

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Legends to figures:

Figure 1. Overview of the BER pathway. A glycosylase recognizes and removes a modified nucleobase leaving an AP site. APE1 cleaves the DNA backbone, leaving a 3'-OH and 5'-dRP. Pol β removes the 5'-dRP and catalyzes nucleotide incorporation at the 3'-OH. Finally, the repair is completed by a DNA ligase that seals the nick in the backbone. The areas highlighted in red indicate where chemical changes catalyzed by BER enzymes have occurred.

Figure 2. Representation of the NCP with the dyad region indicated in red. This representation was created by merging crystal structures of an NCP with a histone octamer containing N-terminal tail regions (PDB entries 3lz0 and 1kx5, respectively). (A) Top view and (B) side view of the NCP with the rotational positions of nucleobases relative to the histone core indicated. Outward (blue), midway (purple), and inward (red) facing nucleobases are highlighted (only one DNA strand is shown for clarity).

Figure 3. Representation of the wrapped (left) and partially unwrapped (right) states of the DNA ends in the NCP.

Figure 4. Structures of canonical and histone variant-containing NCPs. The dyad region is highlighted in red, H3 variants in blue, and H2A variants in yellow. The canonical NCP (PDB 3lz0), CENP-A (PDB 3AN2), and H2A.Z (PDB 1F66) are shown. The macroH2A structure consists of an NCP with the histone domain (PDB 1U35), the macro domain (PDB 6FY5), and a

cartoon representation of the unstructured linker. Top panels represent top views and bottom panels represent side views.

Tables:

Table I. Table of Human Glycosylases and Major Substrates (156-159).

Glycosylase	Substrate
AAG (alkyladenine glycosylase)	3-meA, 7-meA, εA, Hx, X
UNG (Uracil DNA Glycosylase)	U; 5-FU
SMUG1 (Single Strand Selective Monofunctional Uracil Glycosylase 1)	U; hmU; 5-FU
TDG (Thymine DNA Glycosylase)	**T**:G; **U**:G; 5-caC; 5-fC; 5-FU
MBD4 (Methyl-CpG-Binding Domain 4)	**T**:G; **U**:G; **hmU**:G
MUTYH (MutY DNA Glycosylase)	**A**:8-oxoG
OGG1 (8-Oxoguanine DNA Glycosylase)	**8-oxoG**:C; FapyG
NTH1 (Endonuclease III-like Protein 1)	FapyA; FapyG; Tg; 5,6-DHU; 5-hC; 5-hU
NEIL1 (Endonuclease VIII-like Protein 1)	Sp; Gh; FapyG; FapyA; Tg; 5,6-DHT; 5,6-DHU
NEIL2 (Endonuclease VIII-like Protein 2)	Sp; Gh; 5-hU; 5,6-DHT; 5,6-DHU; 5-hC
NEIL3 (Endonuclease VIII-like Protein 3)	Sp; Gh; FapyG; FapyA; 5,6-DHT; 5,6-DHU

Bold indicates the lesion removed from a mispaired substrate. Abbreviations: 8-oxo-7,8-dihydroguanine (8-oxoG); thymine glycol (Tg); 5,6-dihydrouracil (5,6-DHU); hydroxymethyluracil (hmU); 5-hydroxycytosine (5-hC); 5-formylcytosine (5-fC); 5-carboxycytosine (5-caC); 5-fluorouracil (5-FU); 2,6-diamino-4-hydroxy-5-formamidopyrimidine G (FapyG); 4,6-diamino-5-formamidopyrimidine A (FapyA); guanidinohydantoin (Gh); spiroiminodihydantoin (Sp); 3-methyladenine (3-meA); 7-methylguanine (7-meG); 7-methyladenine (7-meA); 3-methylguanine (3-meG); 1,N^6-ethenoadenine (εA); uracil (U); xanthine (X); hypoxanthine (Hx); 5-hydroxyuracil (5-hU).