HHS Public Access

Author manuscript

DNA Repair (Amst). Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

DNA Repair (Amst). 2020 September ; 93: 102920. doi:10.1016/j.dnarep.2020.102920.

NTHL1 in Genomic Integrity, Aging and Cancer

Lipsa Das, **Victoria G. Quintana**, **Joann B. Sweasy***

Department of Cellular and Molecular Medicine and University of Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ 85724

Abstract

Efficient DNA repair is essential to maintain genomic integrity. An average of 30,000 base lesions per cell are removed daily by the DNA glycosylases of the base excision repair machinery. With the advent of whole genome sequencing, many germline mutations in these DNA glycosylases have been identified and associated with various diseases, including cancer. In this graphical review, we discuss the function of the NTHL1 DNA glycosylase and how genomic mutations and altered function of this protein contributes to cancer and aging. We highlight its role in a rare tumor syndrome, NTHL1-associated polyposis (NAP), and summarize various other polymorphisms in NTHL1 that can induce early hallmarks of cancer, including genomic instability and cellular transformation.

Introduction

DNA repair mechanisms are critical in maintaining genomic stability and efficient cellular replication. Specifically, the base excision repair (BER) pathway processes about 30,000 damaged base lesions per cell per day [1]. The key players for the initiation of BER, DNA glycosylases, recognize and remove specific base damages, such as oxidized or alkylated bases resulting in an apurinic/apyrimidinic site. In the case of monofunctional glycosylases, apurinic/apyrimidinic endonuclease 1 (APE1) cuts the DNA backbone 5' to the abasic site, leaving a gap with a 5'deoxyribose phosphate (5'dRP) (Fig. 1A). DNA polymerase β (Pol β) removes the 5'dRP and fills in the single nucleotide gap. Bifunctional glycosylases, including NTHL1, remove the damaged base, cleave the DNA backbone and create a single-strand gap harboring modified 3'ends, which require remodeling by enzymes including APE1 and polynucleotide kinase (PNK) phosphatase. Pol β fills the gap and the nick is sealed in combination with ligase IIIα-X-ray cross-complementing protein (XRCC1) [1]. Bifunctional glycosylases (NTHL1, OGG1, NIEL1/2/3) can perform both glycosylase and lyase functions unlike monofunctional glycosylases (MBD4, MYTYH, MPG, SMUG1, TDG1, UNG) that contain only glycosylase activity [1].

*Corresponding author: Joann B. Sweasy, University of Arizona Cancer Center, 1515 N Campbell Avenue, Room 4963C, Tucson, AZ 85724-5024, Phone: 520-626-5549, FAX: 520-626-6898, jsweasy@email.arizona.edu.

Defects in DNA repair pathways have been associated with various diseases, including cancer [1]. Cancer progression is a multistep process from tumor initiation, due to mutations and genetic alteration, to cellular transformation leading to tumor growth and metastasis. DNA glycosylases are the first line of defense in the BER pathway to prevent cellular mutations. If mutations occur in these glycosylases, it may lead to increased mutagenesis in the cells and possibly genomic alterations. Hence, the need to understand the implications of BER proteins in disease progression is widely appreciated and requires further investigation. Here, we focus on the bifunctional glycosylase NTHL1, and review its function in BER, aging and cancer.

NTHL1 and its importance

NTHL1, a bifunctional DNA glycosylase, belongs to the endonuclease III family, and is evolutionarily conserved from *Escherichia coli* (Nth-Eco) to *Saccharomyces sp*. (Nth-Spo), and mammals including mice and humans (NTHL1) (Fig. 1B) [2, 3]. Each of these NTHL1 homologues is comprised of an evolutionarily conserved catalytic EndoIII structure in the C-terminal tail which contains a helix-hairpin-helix (HhH) motif and an iron-sulfur binding cluster (FES). Interestingly, the mammalian NTHL1 contains additional residues in the N-terminal tail, and is thought to have a regulatory function in mouse and humans [4]. Though not fully characterized, the N-terminal region of human NTHL1 is less conserved compared to mice (53% identity, 68% similarity) and is structurally disordered in comparison to the rest of the protein (84% identity, 92% similarity) [5].

In human and mouse, the N-terminal tail has three clusters of basic residues that harbor the nuclear localization signal (NLS) and one mitochondrial transit peptide (MTP) sequence. Several studies have indicated localization differences between the mouse and human NTHL1. Ikeda *et al*. have shown that human NTHL1 exclusively localizes to the nucleus, and mouse NTHL1 localizes to the mitochondria, while others have seen that both have dual transport abilities to either organelle [3, 6]. Although not fully understood, this region is probably important in regulating protein transport and may provide additional regulatory mechanisms in the complex BER pathway of evolutionarily advanced life forms.

Human NTHL1 is known to excise oxidized pyrimidine lesions, including thymine glycol (Tg), 5-hydroxycytosine (5-OH-Cyt), 5-hydroxy-6-hydrothymine (5-OH-6-HThy), 5-hydroxycytosine (5-OH-Cyt), 5,6-dihydrouracil (5,6-DHU), and purine derived 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy) and 5-hydroxyuracil (5-OH-U), indicating the importance of this enzyme in the removal of oxidative pyrimidine damage (Fig. 1C) [2, 5, 7]. Interestingly, *in vivo* studies found that NTHL1-deficient mice lacked the ability to remove Tg but not 5-OH-Cyt or 5-OH-6-HThy, indicating that compensatory activity from other glycosylases may serve as an additional defense to maintain genomic integrity [7].

NTHL1-Associated Polyposis and cancer

Recently, a germline variant of NTHL1, p.Q90*, has been associated with a rare colorectal cancer (CRC) syndrome called NTHL1-associated polyposis (NAP) (Fig 2). This variant is

rare in the European population (Mutant Allele Frequency= 0.0023) shown by the Exome Aggregation Consortium (ExAC) database [8]. This pathogenic variant has a nonsense mutation, changing a glutamine to a premature stop codon [9]. The p.Q90* variant is predicted to lack a DNA binding domain and glycosylase activity. This extremely rare syndrome (0.014%) was initially identified in the germlines of seven patients [8]. Interestingly, all individuals developed adenomatous polyps which has been a key feature of this tumor syndrome. Since these initial studies, other families have been observed to carry this mutation with either a homozygous p.Q90* mutation or heterozygous mutations (p.Q90*/c.709+1G>A and p.Q90*/p.Q287*) [8]. From these studies, the p.Q90* NTHL1 variant is now known to have a highly-penetrant predisposition to adenomatous polyposis while also having a high risk for developing colorectal cancer (CRC), breast cancer, and various other malignancies [8, 10]. To date, NAP is associated with a spectrum of benign and malignant tumors but further investigation into this syndrome is needed to accurately define the cancer risk and susceptibility.

In mice harboring an NTHL1 targeted deletion ($NTHL^{-/-}$), there was no evidence of any gross phenotypic abnormalities, including spontaneous development of tumors. However, they did have a deficiency in the genomic repair of pyrimidine oxidative lesions, as expected, with increased endogenous levels of Fapy in the liver [7, 11, 12]. The Tg levels however remained below detection level in both wild-type and $NTHL^{-/-}$ mice. Other studies in human organoids have shown that a deficiency in NTHL1 results in a unique mutational signature (signature 30 characterized by C->T transition) [13]. This unique signature was also observed in a breast cancer patient harboring another germline nonsense mutation of NTHL1, p.Q287*, indicating that an NTHL1 deficiency can lead to a multi-tumor phenotype [8, 10]. These findings highlight the importance of germline DNA repair defects and their consequences in cancer.

NTHL1 variants and cancer

With the advent of genomic sequencing, many single nucleotide polymorphisms (SNPs) in *NTHL1* and other BER genes have been catalogued in the human population. These have been mapped to both exonic and intronic sequences, often resulting in functional variants of NTHL1 protein [8, 14, 15]. However, as these germline SNP alleles are rare, their correlation to human diseases has remained elusive due to inadequate statistical power. The cumulative contribution of these rare variants is expected to be more pronounced in the population in determining susceptibility to human diseases. Alternate approaches using molecular techniques will be important to define these variants in the etiology of cancer and other diseases.

Specific cellular and biochemical studies have identified key amino acids in NTHL1, which if mutated, can alter its enzymatic activity, lesion recognition or DNA binding abilities. Our work on two different SNPs in NTHL1 has shown that these induce cellular transformation (Fig. 3A) [14, 15]. SNP rs3087468 is found in 6.2% of population, results in a single amino acid substitution variant of NTHL1, D239Y, which renders the glycosylase inactive [14]. Expression of this variant in non-transformed mammary epithelial cells led to genomic instability, a hallmark of cancer. The inability of D239Y to repair DNA lesions resulted in

increased double stranded breaks (DSBs) and chromosomal aberrations. These defects accumulated in S and G2/M phases, which was perhaps a consequence of replication fork collapse at unrepaired DNA lesions. Within 2–3 passages, we observed an increase in anchorage independent growth, focus formation and cell invasion indicating that cellular transformation had occurred.

Another rare SNP (rs2302172) resulting in a missense mutation, R33K, had no effect on the enzymatic activity or substrate specificity of NTHL1, but led to similar cellular transformation [15]. How a conservative arginine-to-lysine mutation induces cellular transformation without altering enzymatic activity remains an interesting question. R33K is located in the disordered N-terminal region, unique to mammalian cells. This region has been previously shown to negatively regulate NTHL1 activity by inhibiting product release [4]. Conversely, it can also stimulate NTHL1 activity by mediating homodimerization of NTHL1 or interaction with transcription factor Y box-binding protein 1 (YBX-1) [16, 17]. Interestingly, the lysine 63 residue in the N-terminal tail is a site for ubiquitination by TRIM26, hence regulating its degradation [18]. These data suggest that the N-terminal tail of NTHL1 has evolved as a critical regulatory mechanism in complex DNA repair pathways of advanced life forms. Mutations in this region may interfere with this tight regulation and lead to instability.

Various other mutations in NTHL1 catalogued in the database of single nucleotide polymorphisms (dbSNP) have been functionally characterized. Using biochemical assays, four NTHL1 variants, Q90X, Y130X, R153X, Q287X, were found to be defective in repairing exogenously induced 5-OH-U lesions [19]. This led to an increased accumulation of somatic mutations, particularly C->T, in human cells. Taken together, above mentioned studies have identified many variants of NTHL1 with a capacity to induce mutagenesis and cellular transformation. Whether the mechanism relies on the differences in the interacting proteome of NTHL1 variants and their altered regulation in BER pathways requires further investigation.

In addition to mutations, NTHL1 is reported to be upregulated in cancer. Among various BER genes, NTHL1 was overexpressed in Non-small cell lung cancer (NSCLC) [20]. In a clinical study of urothelial cancer patients, high NTHL1 expression negatively correlated with disease-free survival characterized by local recurrence of resected tumor or metastasis [21]. However, the overall NTHL1 expression remained insignificant in prognosis of grade or overall survival. In cellular models, overexpression of NTHL1 led to replication stress and cellular transformation (Fig. 3B) [20]. Overexpression of a catalytically inactive NTHL1 also resulted in similar effects. Hence, the resultant phenotypes were observed irrespective of its enzymatic activity, suggesting potential contributions from interacting proteins. One possible mechanism was attributed to its interaction with XPG, a critical player in homologous recombination (HR) repair of DSBs. The authors showed that NTHL1 overexpression decreased HR, possibly by sequestering XPG, and a switch to the error prone non-homologous end joining (NHEJ) pathway [20]. This study revealed a balance between NTHL1 expression and other DNA repair pathways, and that dysregulation can potentially impact the progression of cancer.

NTHL1 in ageing

Increased oxidative stress and defects in DNA repair pathways have been long associated with accelerated ageing. Telomeres are classically known for maintenance of genomic stability and a marker for ageing, as the telomere length decreases with each cell cycle. Telomeric DNA is susceptible to damage by oxidative agents and can induce a persistent DNA damage response [22]. In a striking study, Vallabhaneni *et al.* discovered that NTHL1$^{-/-}$ mice significantly compromised telomeric integrity (Fig.4) [12]. *NTHL*$^{-/-}$ MEFs isolated from these mice accumulated DNA lesions in telomeric sequences compared to non-telomeric loci. Various telomeric defects were evident, including fragile telomeres, 53BP1 positive foci and chromatid exchange. Overall, telomere length was not affected spontaneously, but the exogeneous addition of pro-inflammatory IL-6 induced significant attrition. In a telomerase null background, NTHL1 deletion led to shortening of telomeres and the activation of Chk1/2 regulated DNA damage response. No follow-up studies are yet reported testing if NTHL1 associates with telomeric sequences or the proteins responsible for telomeric maintenance.

In vitro studies revealed no significant NTHL1 activity in repairing the telomeric Tg lesion, unlike NEIL1 and NEIL3 [23]. It is important to note that these experiments were performed on telomeric sequences assembled into quadruplex DNA *in vitro* and may be limited in identifying the role of NTHL1 in telomeric maintenance in whole organism. This is reflected in significant associations of NTHL1 with human longevity, discovered in two independent studies. In a case controlled 11-year longitudinal study in the Danish population, a rare allelic variant of NTHL1, rs3211994, was discovered to be significantly associated with longer lifespan, especially males [24]. In another study on genotyped participants of Framingham Heart Study cohort, out of 550,000 SNPs, a rare SNP in the intronic sequence of NTHL1 (rs2516739) emerged among the top 40 candidates significantly associated with longevity [25]. These results are very promising and warrant future investigation into the function of NTHL1 in maintaining telomeric integrity and ageing.

Conclusion

We conclude that genomic polymorphisms in NTHL1 and its altered function compromise genomic integrity and induce cancer-associated phenotypes. However, future investigations are necessary to determine the underlying mechanisms and explore the potential to confer individual susceptibility to cancer.

References

[1]. Wallace SS, Murphy DL, Sweasy JB, Base excision repair and cancer, Cancer Lett, 327 (2012) 73–89. [PubMed: 22252118]

[2]. Dizdaroglu M, Karahalil B, Senturker S, Buckley TJ, Roldan-Arjona T, Excision of products of oxidative DNA base damage by human NTH1 protein, Biochemistry, 38 (1999) 243–246. [PubMed: 9890904]

[3]. Ikeda S, Kohmoto T, Tabata R, Seki Y, Differential intracellular localization of the human and mouse endonuclease III homologs and analysis of the sorting signals, DNA Repair (Amst), 1 (2002) 847–854. [PubMed: 12531031]

[4]. Liu X, Roy R, Truncation of amino-terminal tail stimulates activity of human endonuclease III (hNTH1), Journal of molecular biology, 321 (2002) 265–276. [PubMed: 12144783]

[5]. Luna L, Bjørås M, Hoff E, Rognes T, Seeberg E, Cell-cycle regulation, intracellular sorting and induced overexpression of the human NTH1 DNA glycosylase involved in removal of formamidopyrimidine residues from DNA, Mutat Res, 460 (2000) 95–104. [PubMed: 10882850]

[6]. Takao M, Aburatani H, Kobayashi K, Yasui A, Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage, Nucleic Acids Res, 26 (1998) 2917–2922. [PubMed: 9611236]

[7]. Takao M, Kanno S, Kobayashi K, Zhang QM, Yonei S, van der Horst GT, Yasui A, A back-up glycosylase in Nth1 knock-out mice is a functional Nei (endonuclease VIII) homologue, J Biol Chem, 277 (2002) 42205–42213. [PubMed: 12200441]

[8]. Weren RD, Ligtenberg MJ, Geurts van Kessel A, De Voer RM, Hoogerbrugge N, Kuiper RP, NTHL1 and MUTYH polyposis syndromes: two sides of the same coin?, J Pathol, 244 (2018) 135–142. [PubMed: 29105096]

[9]. Weren RD, Ligtenberg MJ, Kets CM, de Voer RM, Verwiel ET, Spruijt L, van Zelst-Stams WA, Jongmans MC, Gilissen C, Hehir-Kwa JY, Hoischen A, Shendure J, Boyle EA, Kamping EJ, Nagtegaal ID, Tops BB, Nagengast FM, Geurts van Kessel A, van Krieken JH, Kuiper RP, Hoogerbrugge N, A germline homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer, Nature genetics, 47 (2015) 668–671. [PubMed: 25938944]

[10]. Grolleman JE, de Voer RM, Elsayed FA, Nielsen M, Weren RDA, Palles C, Ligtenberg MJL, Vos JR, Ten Broeke SW, de Miranda N, Kuiper RA, Kamping EJ, Jansen EAM, Vink-Borger ME, Popp I, Lang A, Spier I, Huneburg R, James PA, Li N, Staninova M, Lindsay H, Cockburn D, Spasic-Boskovic O, Clendenning M, Sweet K, Capella G, Sjursen W, Hoberg-Vetti H, Jongmans MC, Neveling K, Geurts van Kessel A, Morreau H, Hes FJ, Sijmons RH, Schackert HK, Ruiz-Ponte C, Dymerska D, Lubinski J, Rivera B, Foulkes WD, Tomlinson IP, Valle L, Buchanan DD, Kenwrick S, Adlard J, Dimovski AJ, Campbell IG, Aretz S, Schindler D, van Wezel T, Hoogerbrugge N, Kuiper RP, Mutational Signature Analysis Reveals NTHL1 Deficiency to Cause a Multi-tumor Phenotype, Cancer cell, 35 (2019) 256–266 e255. [PubMed: 30753826]

[11]. Chan MK, Ocampo-Hafalla MT, Vartanian V, Jaruga P, Kirkali G, Koenig KL, Brown S, Lloyd RS, Dizdaroglu M, Teebor GW, Targeted deletion of the genes encoding NTH1 and NEIL1 DNA N-glycosylases reveals the existence of novel carcinogenic oxidative damage to DNA, DNA Repair (Amst), 8 (2009) 786–794. [PubMed: 19346169]

[12]. Vallabhaneni H, O'Callaghan N, Sidorova J, Liu Y, Defective repair of oxidative base lesions by the DNA glycosylase Nth1 associates with multiple telomere defects, PLoS Genet, 9 (2013) e1003639. [PubMed: 23874233]

[13]. Drost J, van Boxtel R, Blokzijl F, Mizutani T, Sasaki N, Sasselli V, de Ligt J, Behjati S, Grolleman JE, van Wezel T, Nik-Zainal S, Kuiper RP, Cuppen E, Clevers H, Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer, Science, 358 (2017) 234–238. [PubMed: 28912133]

[14]. Galick HA, Kathe S, Liu M, Robey-Bond S, Kidane D, Wallace SS, Sweasy JB, Germline variant of human NTH1 DNA glycosylase induces genomic instability and cellular transformation, Proceedings of the National Academy of Sciences of the United States of America, 110 (2013) 14314–14319. [PubMed: 23940330]

[15]. Marsden CG, Jaruga P, Coskun E, Maher RL, Pederson DS, Dizdaroglu M, Sweasy JB, Expression of a germline variant in the N-terminal domain of the human DNA glycosylase NTHL1 induces cellular transformation without impairing enzymatic function or substrate specificity, Oncotarget, In Press (2020).

[16]. Liu X, Choudhury S, Roy R, In vitro and in vivo dimerization of human endonuclease III stimulates its activity, J Biol Chem, 278 (2003) 50061–50069. [PubMed: 14522981]

[17]. Marenstein DR, Ocampo MT, Chan MK, Altamirano A, Basu AK, Boorstein RJ, Cunningham RP, Teebor GW, Stimulation of human endonuclease III by Y box-binding protein 1 (DNA-binding protein B). Interaction between a base excision repair enzyme and a transcription factor, J Biol Chem, 276 (2001) 21242–21249. [PubMed: 11287425]

[18]. Williams SC, Parsons JL, NTH1 Is a New Target for Ubiquitylation-Dependent Regulation by TRIM26 Required for the Cellular Response to Oxidative Stress, Mol Cell Biol, 38 (2018).

[19]. Shinmura K, Kato H, Kawanishi Y, Goto M, Tao H, Yoshimura K, Nakamura S, Misawa K, Sugimura H, Defective repair capacity of variant proteins of the DNA glycosylase NTHL1 for 5-hydroxyuracil, an oxidation product of cytosine, Free radical biology & medicine, 131 (2019) 264–273. [PubMed: 30552997]

[20]. Limpose KL, Trego KS, Li Z, Leung SW, Sarker AH, Shah JA, Ramalingam SS, Werner EM, Dynan WS, Cooper PK, Corbett AH, Doetsch PW, Overexpression of the base excision repair NTHL1 glycosylase causes genomic instability and early cellular hallmarks of cancer, Nucleic Acids Res, 46 (2018) 4515–4532. [PubMed: 29522130]

[21]. Wang LA, Yang B, Rao W, Xiao H, Wang D, Jiang J, The correlation of BER protein, IRF3 with CD8+ T cell and their prognostic significance in upper tract urothelial carcinoma, OncoTargets and therapy, 12 (2019) 7725–7735. [PubMed: 31576137]

[22]. Blasco MA, Telomeres and human disease: ageing, cancer and beyond, Nature reviews. Genetics, 6 (2005) 611–622.

[23]. Zhou J, Liu M, Fleming AM, Burrows CJ, Wallace SS, Neil3 and NEIL1 DNA glycosylases remove oxidative damages from quadruplex DNA and exhibit preferences for lesions in the telomeric sequence context, J Biol Chem, 288 (2013) 27263–27272. [PubMed: 23926102]

[24]. Soerensen M, Dato S, Tan Q, Thinggaard M, Kleindorp R, Beekman M, Jacobsen R, Suchiman HE, de Craen AJ, Westendorp RG, Schreiber S, Stevnsner T, Bohr VA, Slagboom PE, Nebel A, Vaupel JW, Christensen K, McGue M, Christiansen L, Human longevity and variation in GH/IGF-1/insulin signaling, DNA damage signaling and repair and pro/antioxidant pathway genes: cross sectional and longitudinal studies, Experimental gerontology, 47 (2012) 379–387. [PubMed: 22406557]

[25]. Yashin AI, Wu D, Arbeev KG, Ukraintseva SV, Joint influence of small-effect genetic variants on human longevity, Aging, 2 (2010) 612–620. [PubMed: 20834067]

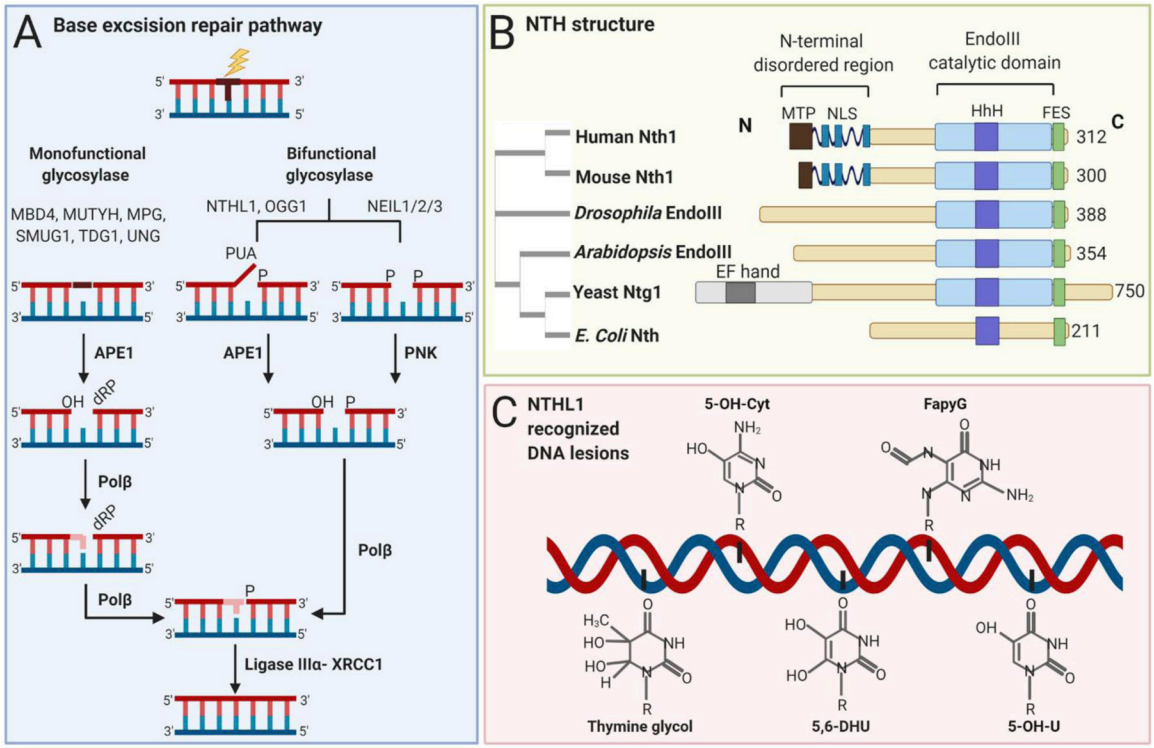

Figure 1.

NTHL1 structure and function. (A) The first step in BER is the removal of the damaged base by a DNA glycosylase [1]. Upon removal of the damaged base by a monofunctional DNA glycosylase, an endonuclease (APE1) cleaves the DNA phosphate backbone to the abasic site, creating a single nucleotide gap. Pol β then fills in the gap. Bifunctional DNA glycosylases, including NTHL1, remove the damaged base, cleave the DNA phosphate backbone, and create a gap with modified 3' ends of phosphate (P) or phospho-α,β-unsaturated aldehyde (PUA), then remodeled by enzymes APE1 or PNK. This is followed by gap filling by Pol β. The XRCC1 ligase then seals the nick. (B) NTHL1 is evolutionarily conserved between human, mouse, yeast, *Drosophila*, plant species like *Arabidopsis sp.* and the *E. coli* endonuclease Nth. Each of these homologues is composed of a conserved catalytic EndoIII structure in the C-terminal which contains a helix-hairpin-helix (HhH) motif and the iron-sulfur binding cluster (FES). The N-terminus is less conserved, is structurally disordered and not completely characterized in these homologs. The nuclear localization signal (NLS) and mitochondrial transit peptide (MTP) are present in both human and mouse species. Yeast Nth1 has a longer N-terminal tail that is reported to contain a calcium binding EF hand motif. (C) NTHL1 has DNA glycosylase activity primarily on oxidized pyrimidine lesions such as Tg, 5-OH-Cyt, 5-OH-6-HThy, 5-OH-Cyt, 5,6-DHU, FapyG and 5-OH-U [2, 5, 7].

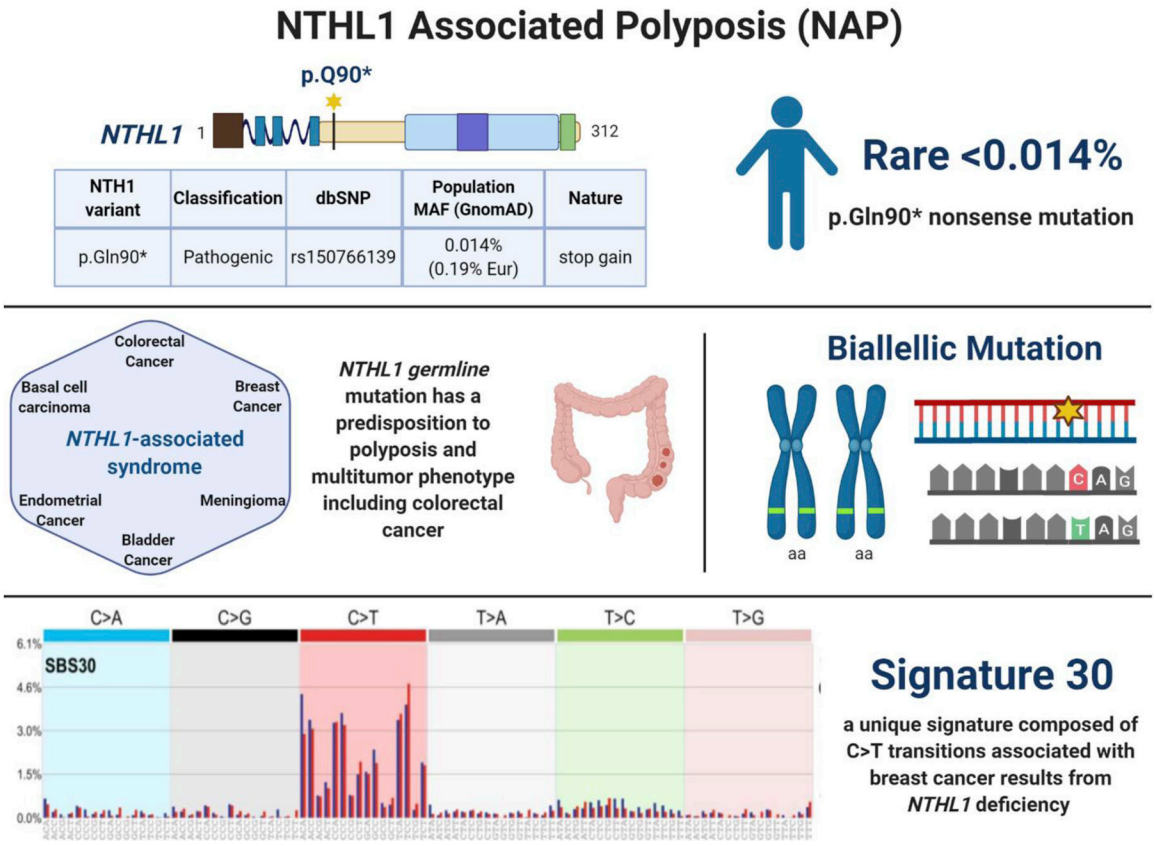

Figure 2.

NTHL1-associated polyposis. A germline NTHL1 p.Q90* variant is associated with a rare colorectal cancer (CRC) syndrome called NTHL1-associated polyposis (NAP) affecting 0.014% of the population. The NAP syndrome is associated with various other cancers as well, such as breast, bladder, endometrial cancers, meningioma, and basal cell carcinoma. Studies in human organoids and breast cancer patients have shown that a deficiency in NTHL1 leads to development of a unique mutational signature (signature 30, characterized by C->T transition) in normal cells [13].

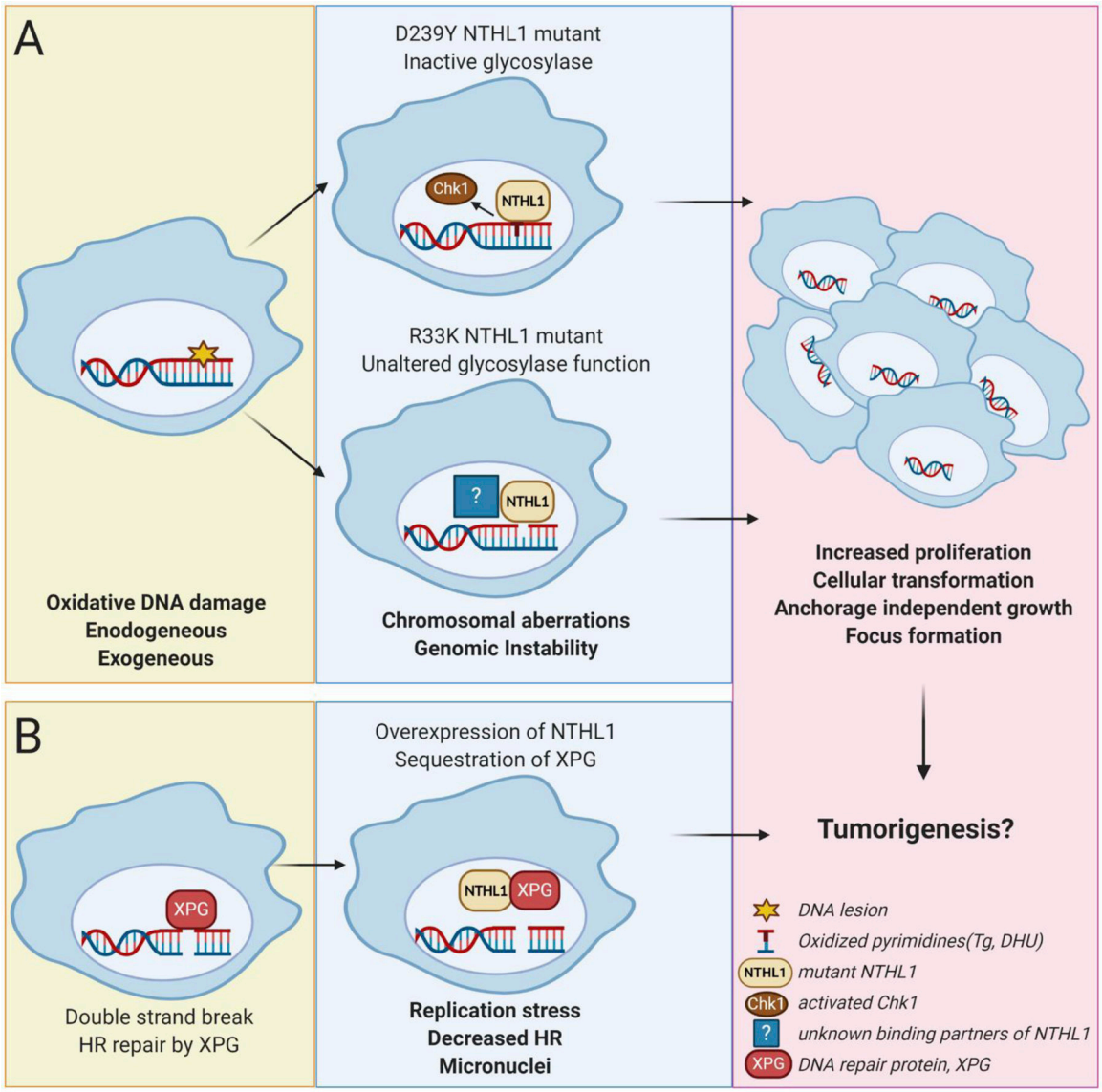

Figure 3.

Altered NTHL1 function leads to genomic instability and cell transformation. (A) Germline variants of NTHL1 arising from rare SNPs found in the human population have altered function in DNA repair pathways. Cells expressing D239Y that renders the NTHL1 glycosylase inactive accumulate DSBs, chromatid breaks and fusions [14]. Similarly, another variant R33K, despite having unaltered function in lesion recognition and glycosylase/lyase activity, induces chromosomal aberrations [15]. The mechanism is not known but is hypothesized to be due to possible differences in the interacting protein partners of NTHL1, many of which remain unidentified. The expression of either the D239Y or R33K variant results in phenotypes associated with cancer, including increased proliferation, cellular transformation, and anchorage independent growth. (B) When NTHL1 is overexpressed, an increased replication stress is observed accompanied with a decrease in DSB repair by HR [20]. This is attributed to sequestration of a key HR associated protein, XPG, by NTHL1. NTHL1 overexpression ultimately results in micronuclei, a marker of

genetic instability and cellular transformation. Together, these defects in repair and genomic instability may induce tumorigenesis.

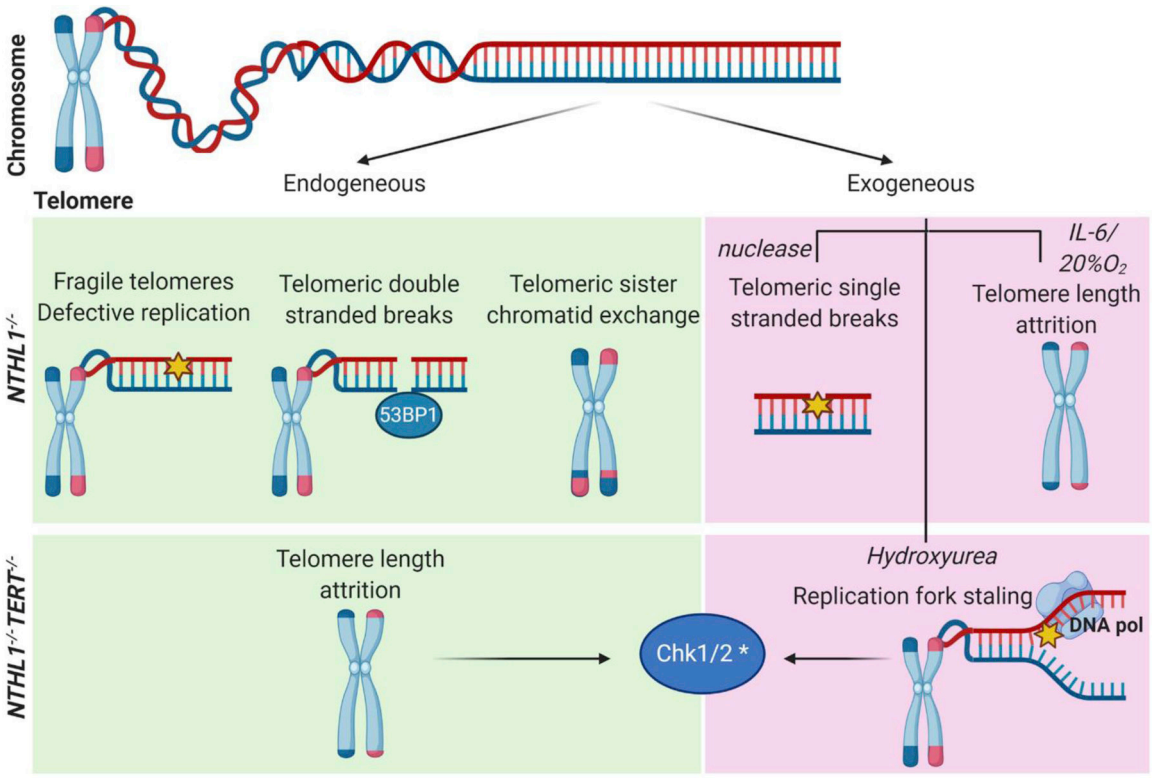

Figure 4.

NTHL1 maintains telomeric integrity. NTHL1 knockout mice (*NTH*$^{-/-}$) show multiple telomeric defects [12]. Mouse embryonic fibroblasts and bone marrow-derived cells exhibited a spontaneous increase in fragile telomeres, 53BP1 positive double stranded breaks in telomeric loci and sister chromatid exchange of telomeres. Upon dual knockout of *NTHL1* and telomerase reverse transcriptase (*TERT*) (*NTH*$^{-/-}$*TERT*$^{-/-}$), spontaneous telomere attrition was observed, significantly higher than *TERT*$^{-/-}$ mice. Application of exogenous oxidative stress also led to telomeric attrition, replication fork failure and activation of Chk1/2 resulting in apoptosis.