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The multifunctional APE1 DNA repair–redox signaling protein as a drug target in human disease

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Apurinic/apyrimidinic (AP) endonuclease–reduction/oxidation factor 1 (APE1/Ref-1, also called APE1) is a multifunctional enzyme with crucial roles in DNA repair and reduction/oxidation (redox) signaling. APE1 was originally described as an endonuclease in the Base Excision Repair (BER) pathway. Further study revealed it to be a redox signaling hub regulating critical transcription factors (TFs). Although a significant amount of focus has been on the role of APE1 in cancer, recent findings support APE1 as a target in other indications, including ocular diseases [diabetic retinopathy (DR), diabetic macular edema (DME), and age-related macular degeneration (AMD)], inflammatory bowel disease (IBD) and others, where APE1 regulation of crucial TFs impacts important pathways in these diseases. The central responsibilities of APE1 in DNA repair and redox signaling make it an attractive therapeutic target for cancer and other diseases.

Introduction

APE1/Ref-1 (also called APE1) is a multifunctional enzyme with crucial roles in DNA repair and reduction/oxidation (redox) signaling. APE1 was originally described as an endonuclease in the BER pathway as reviewed elsewhere [1]. Further study revealed APE1 to be a redox signaling hub that regulated TFs [1]. The central responsibilities of APE1 in DNA repair and redox signaling make it an attractive therapeutic target for cancer and other diseases (Fig. 1).

APE1 in DNA repair

The functions of APE1 within the BER pathway include repair of nondistorting DNA lesions, such as oxidized, alkylated, and deaminated bases. The BER pathway comprises two branches: short- and long-patch BER [2] (Fig. 1). The endonuclease function of APE1 is essential for both branches of BER. During BER, a glycosylase first removes the lesioned base by cleaving its *N*-glycosidic bond, leaving an apurinic/apyrimidinic site (AP) site. APE1 then nicks the phosphodiester backbone of the DNA to create a 3′-hydroxyl group termini and a 5′-deoxyribose moiety, allowing a DNA polymerase to enter the DNA helix and incorporate one or more nucleotides. A ligase completes the repair.

APE1 is also a part of nucleotide incision repair (NIR), a glycosylase-independent pathway [3]. In NIR, an endonuclease incises at a damage base site, allowing DNA synthesis to begin. This incision is the initial step in NIR, rather than an abasic site or an incision, as in BER. APE1 is reported to recognize and incise at particular base damages, specifically 5,6-dihydro-2′-deoxyuridine (DHU), 5,6-dihydrothymidine (DHT), 5-hydroxy-2-deoxyuridine (5OHU), and α-2′-deoxynucleotide. These incisions generate single-strand break ends with a 3′-hydroxyl and a 5′-dangling modified nucleotide [4]. The active site of endonuclease activity is the same for BER and NIR [5].

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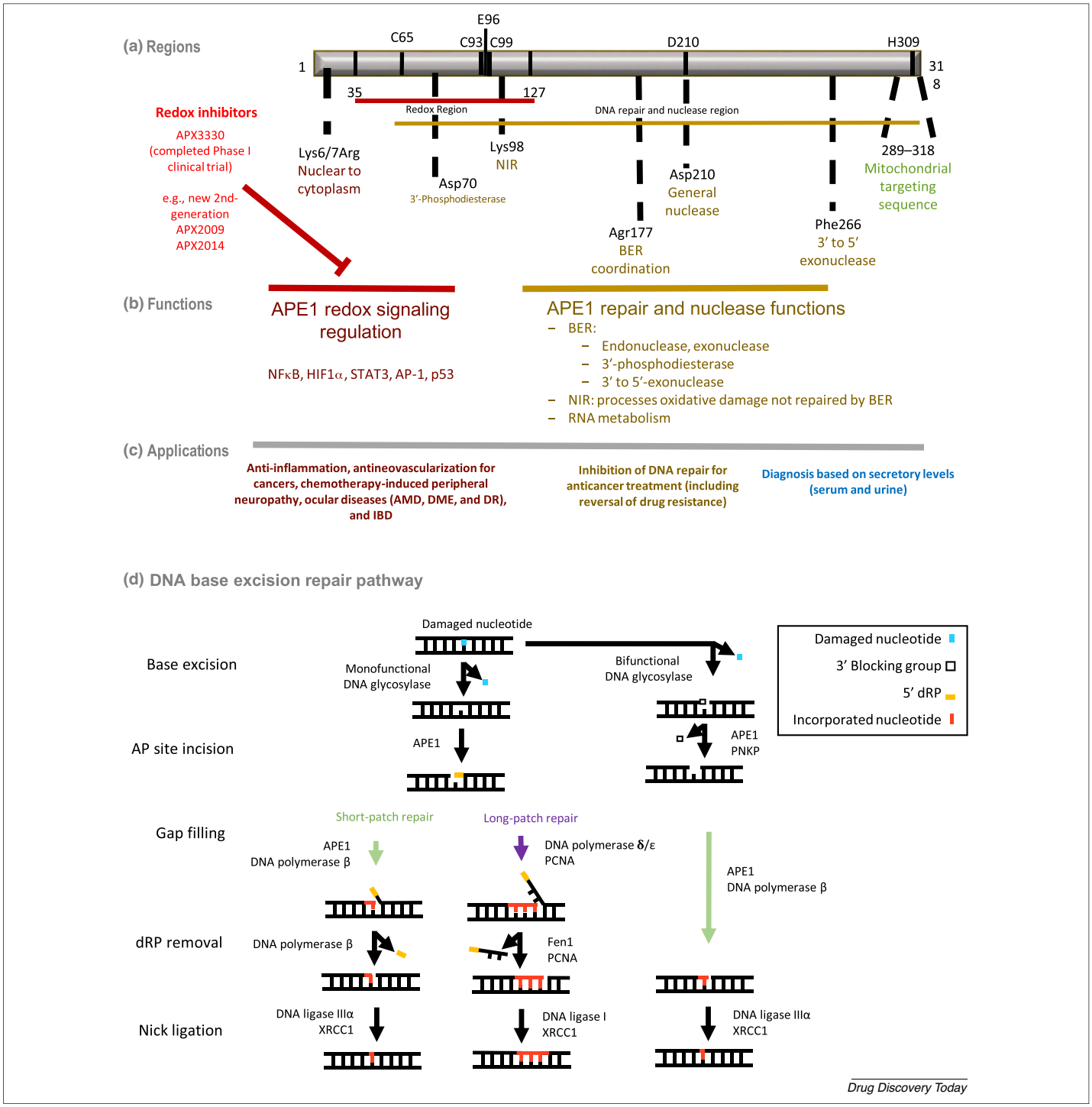

FIGURE 1
Apurinic/apyrimidinic endonuclease 1 (APE1) protein regions, functions, and applications. Amino acids (AAs) indicated in **(a)** are the major AAs that are vital for the APE1 functions shown in **(b)** [102]. The redox signaling region spans AA 35–12,7 whereas the DNA repair region includes AA ~65–318. Lys at position 6 and 7 affects the movement of APE1 from the nucleus to cytoplasm. Asp70 is involved in the 3′-phosphodiesterase activity of APE1, Lys98 is implicated in nucleotide incision repair (NIR), Arg 177 for base excision repair (BER) coordination, Asp210 for general nuclease function, and Phe266 for 3′ to 5′-exonuclease. Cys involved in APE1 redox activity are Cys65, 93, and 99. The mitochondrial targeting sequence has been localized to AA 289–318. Maroon color reflects regions of APE1 functions and applications involved in redox signaling; gold indicates APE1 repair functions, nuclease functions and applications; green indicates mitochondrial targeting sequences; and blue indicates potential diagnosis applications. Red indicates redox inhibitors that have either reached clinical trials (APX3330) or are in the pre-investigation new drug (IND)-enabling stage for future clinical trials [1,102,103]. **(c)** Applications that have been related to APE1 and potential indications for therapeutics or diagnosis of a variety of diseases. **(d)** Details of the DNA BER pathway and the role of APE1. Abbreviations: AMD, age-related macular degeneration; DME, diabetic macular edema; DR, diabetic retinopathy; IBD, irritable bowel disease.

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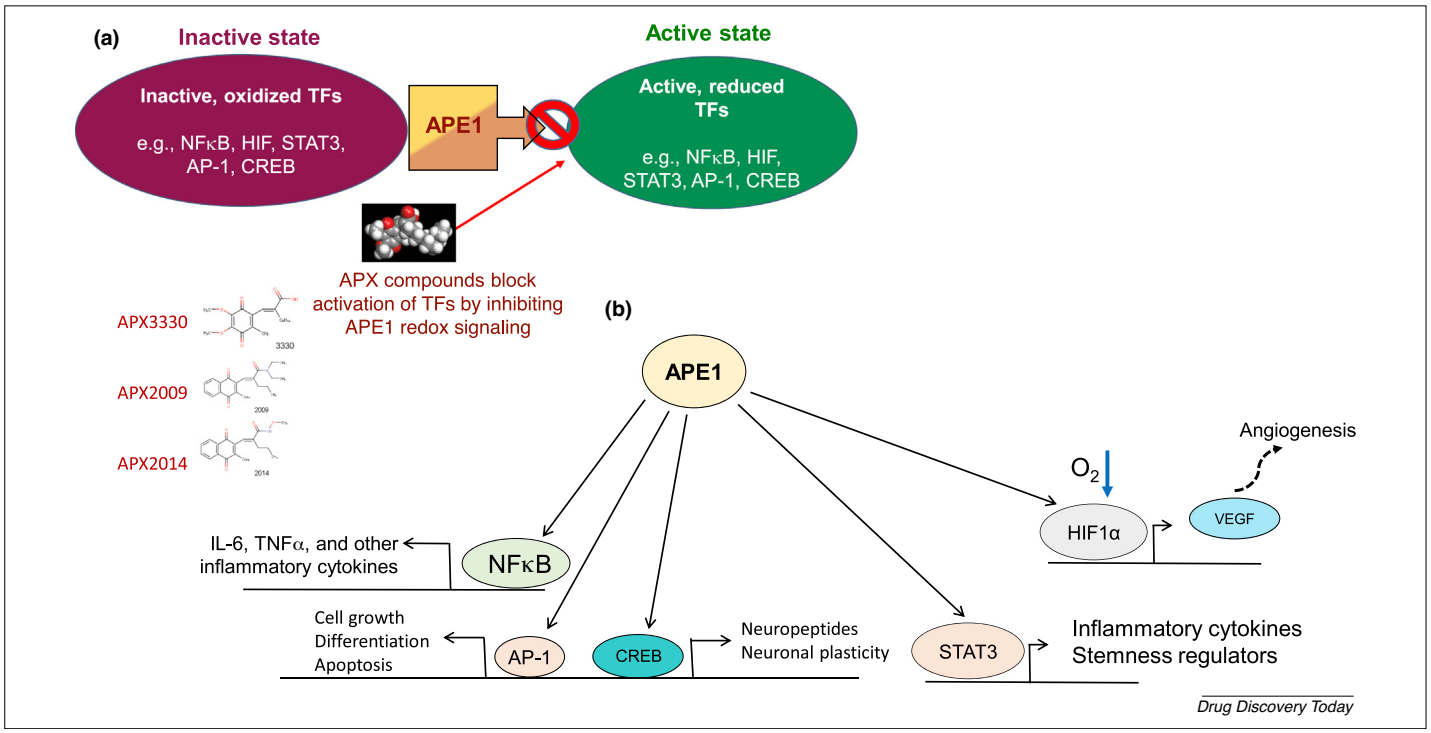

FIGURE 2
Redox signaling function of apurinic/apyrimidinic endonuclease 1 (APE1). **(a)** APE1 converts inactive, oxidized transcription factors (TFs) to active, reduced TFs through protein–protein interactions with APE1. These TFs include, but are not limited to nuclear factor (NF)-κB, hypoxia-inducible factor (HIF)-1α, signal transducer and activator of transcription 3 (STAT3), Activator protein 1 (AP-1), and cAMP-response element binding protein (CREB). APX compounds, such as APX3330, which has completed Phase I clinical trials, or next-generation compounds APX2009 and APX2014, block the ability of APE1 to perform this redox signaling activity. **(b)** Cartoon of APE1 regulation of various TFs regulated by APE1 redox signaling. Inhibition of NFκB leads to a reduction in IL-6, TNFα, and other inflammatory agents. HIF-1α inhibition leads to a reduction in the production of vascular endothelial growth factor (VEGF), carbonic anhydrase 9 (CA9), and other angiogenesis-related genes. STAT3 inhibition results in a decrease in inflammatory cytokines as well as stemness regulators. AP-1, a dimer of c-Fos and c-Jun, regulates the expression of genes involved in cell growth, differentiation, and apoptosis. Finally, CREB is a TF that has been implicated in the expression of neuropeptides and genes involved in neuronal plasticity [1].

APE1 in redox signaling

The redox function of APE1 is unique to mammals [1]. APE1 redox signaling is a controlled process that directs the activity of diverse TFs, which subsequently affect gene expression and protein production (Fig. 2). The redox function of APE1 depends primarily on a buried cystine residue, Cys65, which lies within its N-terminal tail and is independent from the DNA repair portion of APE1 (Fig. 1) [1]. Cys93 and Cys99 are also implicated in the redox function of APE1 [6]. It is necessary for APE1 to be in a reduced state to activate TFs. APE1 reduction occurs through a thiol/sulfide exchange with thioredoxin. Once reduced, activated TFs bind to DNA [1]. TFs regulated by APE1 include signal transducer and activator of transcription 3 (STAT3), Activator protein 1 (AP-1), hypoxia-inducible factor (HIF)-1α, p53, nuclear factor (NF)κB, and others (Table 1). These TFs are involved in many cellular processes, including growth, inflammation, and angiogenesis [7,8]. APE1 overexpression alters its regulation of TFs as well as their downstream effectors, which, clinically, can include tumorigenic transformation, migration, and drug resistance [7].

Additional functions of APE1

RNA processing

RNA, as well as DNA, can become damaged [9]. In fact, RNA is more susceptible to base oxidation than is DNA because its bases are not protected by hydrogen bonding [10]. Damaged mRNA can lead to defective or mutant proteins. Oxidized and abasic RNAs have inhibitory effects on reverse transcriptase activity and decrease fidelity [11]. However, oxidized mRNA can have a regulatory role in the cell [12].

APE1 can cleave small interfering (si)RNA using its endonuclease activity, leading to RNA degradation [13]. Therefore, APE1 is a potential regulator of the mRNA pool, ensuring that lesioned RNA is not transcribed. APE1 binds to RNA with its 33-nucleotide N-terminal tail and acts upon RNA using its 3′-ribophosphate and 3′-exoribonuclease activity [13]. *In vitro*, APE1 can regulate cMyc mRNA levels via degradation [13]. Knockdown of APE1 results in an increased number of oxidized mRNAs, implying that APE1 is involved in mRNA cleansing [13].

Relatedly, APE1 enhances post-translational maturation of miRNA-221/222, an RNA that promotes the Akt pathway and, therefore, cell survival and proliferation [14]. miR221/222 is oncogenic when upregulated, causing thyroid papillary carcinomas, glioblastomas, prostate cancer (PCa), and gastric carcinoma [15].

Role of APE1 in mitochondria and metabolism

APE1 is necessary for mitochondrial BER [16]. The mitochondrial-targeting sequence at the C terminus of APE1 interacts with mitochondrial import and assembly protein Mia40, allowing APE1 to accumulate in the inner membrane space to further participate in mitochondrial BER and stabilize the mitochondrial genome [17]. Moreover, inhibition of the endonuclease function

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TABLE 1
APE1 redox-controlled TFs

TF	Function	Type of cancer regulated by APE1	Refs
AP-1	Proliferation, differentiation, apoptosis	Pancreatic, BC, CRC	[1]
ATF	Stress response	*In vitro*	[1]
c-Myb	Cell cycle regulation	*In vitro*	[1]
CREB	Neuroplasticity, memory formation	*In vitro*	[1]
Egr-1	Differentiation, mitogenesis	Bone, colon, HeLa	[80]
HIF-1	Glycolysis, angiogenesis	Pancreatic	[7,8]
NFκB	Proliferation, migration, invasion	Pancreatic, BCa	[8]
NY-F	Chromatin accessibility, transcription start site selection	HeLa	[81]
P53	DNA repair, cell-cycle arrest, apoptosis	Colon, liver	[1,18]
PAX5/8	Differentiation	Lymphocyte	[81]
PEBP2	Hematopoiesis	*In vitro*	[81]
PTEN	Cell-cycle regulation	Bone, colon, HeLa	[80]
STAT3	Proliferation, invasion, immunosuppression	Pancreatic, liver, BCa	[1]
TTF-1	Thyroid and lung gene activation	HeLa	[82]

of APE1, but not its redox function or interaction with NPM1, results in increased expression of P53, decreased cellular metabolism, reduced mitochondrial function, and greater sensitivity to genotoxicity in colon cells [18]. Research has suggested that both the DNA repair and redox functions of APE1 are essential to maintain the integrity of mitochondria [18].

Nucleophosmin/nucleoplasmin and APE1 interaction

Nucleophosmin/nucleoplasmin (NPM1) is a protein involved in histone chaperones, DNA repair, regulation of ARF-p53, apoptosis inhibition, ribosome formation, and transport of basic proteins to the nucleolus [19,20]. It is found primarily in the granular nucleolus, but is also shuttled to the nucleus and cytoplasm [19,20]. NPM1 binds preferentially to G-quadruplex-forming nucleotides and can bind both double and single-stranded DNA [19,20].

NPM1 controls the localization of BER proteins, including APE1 [21]. Binding of APE1 to NPM1 is necessary for APE1 to be translocated to the nucleolus [21]. A mutant of NMP1 (NPM1c+) causes acute myeloid leukemia, a disease in which NPM1 is translocated to the cytoplasm, bringing APE1 to the cytoplasm [22–24]. The localization of APE1 to the cytoplasm reduces its ability to function in BER, as well as inhibiting NPM1 [22–24]. In a study of triple-negative breast cancer (TNBC) cell lines, treatment with platinum-based compounds caused NMP1 and APE1 to be shuttled out of the nucleolus and into the nucleoplasm [25]. This change in localization sensitized the cells to other cytotoxicity agents [25].

APE1 as a therapeutic opportunity for various cancers

APE1 is one among many deregulated proteins involved in cancer survival signaling pathways [26]. Its multifunctional role in DNA repair, TF redox signaling, miRNA metabolism, and RNA quality control makes it a significant therapeutic target in cancers of pancreas, bladder, colon, rectum, blood, peripheral neurons, lung, breast, prostate, liver, ovary, bone, cervix, skin, glial cells, and head and neck [1]. Its aberrant expression levels in many of these cancers are associated with chemo and radioresistance. As evidenced by current studies, combinatorial synthetic lethal targeting of APE1 in conjunction with metabolic or hypoxic pathway regulators might be an effective strategy for improving the response rate to treatment. A brief discussion of APE1 in cancer is provided herein, with additional listings in Table 2.

Overexpression

APE1 is overexpressed in nonsmall cell lung cancer (NSCLC) tumor tissues, with patients having lower APE1 levels showing significantly longer progression-free survival and longer overall survival [27]. In TNBC, APE1 overexpression correlated with malignant clinical manifestation (median survival: 82.0 months for low versus 66.9 months for high expression) [28]. High-grade PCa tissues and prostatic intraepithelial neoplasia (PIN) show elevated APE1 levels compared with benign hypertrophy (BPH) and normal prostate sections, which was strongly associated with tumor grade, tumor stage, and early recurrence [29]. Its interactions with STAT3 under oxidative stress appear to drive PCa progression [30].

APE1 is also constitutively overexpressed in esophageal adenocarcinoma (EAC), in which its redox function via the epidermal growth factor receptor (EGFR)–STAT3 axis, Cyclooxygenase-2–vascular endothelial growth factor (COX2/VEGF) regulation, activation of NFκB–p65, as well as the repair function of APE1 are actively involved [31]. APE1 expression is high in human bladder cancer (BCa) tissue relative to benign urothelium. Fishel *et al.* demonstrated that *in vitro* and *in vivo* treatment with novel redox selective APE1 inhibitors effectively reduced BCa tumor proliferation and growth and, in combination with standard-of care chemotherapy cisplatin, could be more effective than cisplatin alone [32]. Studies also demonstrated that urinary APE1 levels could be a reliable diagnostic biomarker for BCa (area under the curve 0.83) because APE1 levels were associated with tumor grade stage, muscle invasion, and disease recurrence [33–35].

The repair function of APE1 is associated with telomere maintenance. A deficiency of APE1 [APE1 short hairpin (sh)RNA] controls cellular fate in an hTERT-dependent manner. Li *et al.* [36] demonstrated that APE1 mediated senescence in hTERT-negative BJ cells while promoting apoptosis in hTERT-positive BJ cells. This effect could be the result of the dislocation of shelterin (telomere binding protein complex) proteins, such as TRF1, TRF2, and POT1, from telomeres [37].

Functional significance

Reduction in APE1 levels increase breast cancer (BC) susceptibility to olaparib treatment. APE1 and its regulator NPM1 rescue TNBC cells from platinating compound cytotoxicity [25]. Xie *et al.* observed elevated levels of secreted APE1 within the serum of patients

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TABLE 2

Role of APE1 in cancers[a]

Cancer type	APE1 contribution	Refs
PDAC	Overexpression: chemoresistance, radioresistance Redox function: chemoresistance, angiogenesis Repair function: gemcitabine resistance	[26,32,1]
BCa	Overexpression Urinary levels as diagnostic marker High serum levels	[32]
CRC	Overexpression Redox function: chemoresistance Asp148 Glu polymorphism increases risk	[18,83]
Leukemia	Loss of stability causes apoptosis Redox function Endonuclease activity	[40]
MPNST	Overexpression: redox function	[70]
Lung	Overexpression and chemoresistance Ile64Val and Asp148Glu promoter variants increase risk	[27,83]
BC	Overexpression Asp148Glu polymorphism protects against acute adverse effects after radiotherapy Asp148Glu polymorphism might contribute to ionizing radiation hypersensitivity and cancer susceptibility Hyperacetylation resulting in secretion causes apoptosis in TNBC [low expression might select for aggressive ER-positive cancers; high expression in Luminal A subtype (ER positive)[b]]	[46,84–89]
PCa	Overexpression Redox function Asp148Glu polymorphism contributes to increased cancer risk	[30,32]
Liver	Overexpression Subcellular localization contributes to cancer properties Secretion into serum enhances inflammatory response	[39]
EAC	Overexpression Repair function protects against acidic bile salts (ABS)-induced DNA damage Redox function via EGFR–STAT3 axis, COX2/VEGF regulation, and activation of NFκB-p65	[31]
Cervical	Overexpression Cytoplasmic overexpression: radioresistance	[90]
Head and neck	Nuclear localization protects HNSCCs from oxidative stress, thus evading death Asp148Glu polymorphism associated with smoking and/or tobacco chewing increases risk	[83]
Oral	Overexpression and high serum levels Asp148Glu polymorphism associated with malignant transformation	[83,38]
GBM	Overexpression Repair activity: chemoresistance Redox function: resistance to TMZ	[41]
Ovarian	Overexpression and chemoresistance Cytoplasmic localization associated with tumor progression and low survival Interaction with NPM1 through BER and rRNA activities enhances cancer aggressiveness Redox function	[32]
Osteosarcoma	Overexpression Cytoplasmic Overexpression – Platin Resistance Induces angiogenesis	[92]
Melanoma	Overexpression Overexpression in MiTF-positive melanoma cells Repair activity inhibition targets melanoma Loss of APE1 through Wnt antagonist, sFRP2, renders melanoma sensitive to vemurafenib resistance	[93]

[a] Abbreviations: ER, estrogen receptor; HNSCC, head and neck squamous cell carcinoma; MiTF, melanocyte inducing transcription factor; sFRP2, secreted frizzled related protein 2.
[b] Contradictory results.

with oral squamous cell carcinoma (OSCC) and proposed that this might serve as a diagnostic biomarker for OSCC. Indeed, lower levels of APE1 were associated with longer disease-free survival after cisplatin therapy [38]. Redox inhibition of APE1 using APX3330 (E3330) in combination with 5-FU resulted in enhanced colorectal cancer (CRC) tumor regression. In contrast, inhibition of the endonuclease activity of APE1, but not the redox function or the interaction with NPM1, triggers p53-mediated effects on cell metabolism in colon cancer cell lines, sensitizing them to genotoxic treatment [18]. In hepatocellular carcinoma (HCC), upregulation of APE1 mRNA is linked with disease progression [39].

Logsdon *et al.* demonstrated effective blockade of the APE1–HIF1–CA9 pathway, creating a 'drug-synthetic lethality', which further resulted in enhanced inhibition of 3D pancreatic ductal adenocarcinoma (PDAC) tumor growth [26]. Exploring a different synthetic lethality, Cardoso *et al.* demonstrated that the redox

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TABLE 3

Inhibitors of APE1 DNA repair and redox signaling

Compound	Function inhibited	Refs
3-Benzylcarbamoyl-2-methoxybenzoic acid inhibitors	Repair	[69]
5-(Hydroxymethyl)-2-furfural,	Antioxidant	[69]
6-Hydroxy-dl-DOPA	Repair	[69]
9-Aminoellipticine aminoellipticine 1, isopropyl-oxazolopyridocarbazole	Repair	[69]
AJAY -1, -2, -3, -4	Repair	[65]
APE1-endonuclease inhibitor (Compound 3)	Repair	[66]
APX2007, APX2009, APX2014, APX2032	Redox	[60,94]
APX3330	Redox	[1,71,95]
ARO1, ARO3, ARO6	Repair	[96]
AT-101	Repair	[69]
Aurintricarboxylic acid inhibitors	Repair	[69]
Bis-naphthalene macrocycles	Repair	[69]
CRT0044876	Repair	[69]
Fiduxosin	Repair	[97]
Heterodimeric compounds	Repair	[69]
Hycanthone	Repair	[69]
Lucanthone	Repair	[69]
MC043, MC047, MC042, and MC018	Repair	[67]
Methoxyamine	Repair	[69]
Methyl-3,4-dephostatin	Repair	[69]
MGAP-9	Repair	[98]
Mitoxantrone	Repair	[69]
MLS001196838, MLS000587064, MLS000737267, and MLS000090966	Repair	[69]
Myricetin	Repair	[69]
N-(3-(Benzo[d]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)acetamide	Repair	[69]
NCI-13,755, NCI-13793	Repair	[99]
Pharmacophore model inhibitors	Repair	[100]
PNRI-299	Weak redox	[69]
Reactive Blue 2	Repair	[69]
SB206553	Mild repair	[97]
Spiclomazine	Repair	[97]
Tanshinone IIA	Weak redox	[69]
Thiolactomycin	Repair	[69]
Troglitazone	Mild repair	[97]
Tyrphostin AG 538	Repair	[69]
Zinc69565400/related compounds	Repair	[101]

function of APE1 directly regulates STAT3 transcriptional activity and, hence, concurrent blockade of STAT3 and APE1 redox function synergistically can inhibit PDAC growth [32]. In myeloid leukemia cell lines, redox inhibition of APE1 using APX3330 synergized with retinoic acid (RA) mediated differentiation (shift of population to positive CD11b marker) [32] and triggered apoptosis of leukemia T cells [including drug-resistant, relapsed (T cell acute lymphoblastic leukemia (T-ALL) cells] by downregulating the transcription of the prosurvival genes *Bcl-xL* and *Survivin* [40].

In patients with glioblastoma (GBM), oxidative stress elevated APE1 levels, thus increasing the repair of abasic sites resulting in enhanced drug resistance. The redox as well as repair function of APE1 also contribute to temozolomide (TMZ) resistance [41]. Malignant peripheral nerve sheath tumor (MPNST), a soft-tissue sarcoma arising from peripheral nerve sheath, which is common among patients with neurofibromatosis I, has considerably limited treatment options [42]. The STAT3–HIF1α–VEGF-A signaling axis drives MPNST progression and suggests targeting of APE1 as a viable treatment strategy [43].

Polymorphisms

Emerging evidence suggests that certain APE1 polymorphisms are clinically relevant and contribute to cancer pathology [44]. L104R, R237C, D148E, and D283G are the most common substitution mutations among many found in APE1 [45]. They are linked with acute adverse effects to radiotherapy and chemoresistance in BC and CRC [46–50]. Most reported of the polymorphisms is Asp148Glu, the presence of which reduces the ability of APE1 to communicate with its BER partners [51,52]. In bladder cancer, APE1 is reported to be present as four variants: one with a single substitution (D148E) and three with double substitution mutations (I64V/D148E, W67R/D148E, and E86G/D148E). APE1 with D148E or I64V/D148E or E86G/D148E substitutions show expression levels and nuclear localization similar to WT APE1. However, APE1 with the W67R/D148E double substitution mutation was expressed in lower amounts and localized to the nucleolus. Also, increased secretion of APE1 D148E might result from the increased sensitivity of intracellular acetylation, as demonstrated using Trichostatin A (TSA, an HDAC inhibitor) [34]. These single nucleotide polymorphism (SNP) variants increase the risk of developing cancers of the lung (Ile64Val and Asp141Glu) [53,54] and prostate [in combination with other gene variants, such as XRCC1 Arg399Gln (a key protein in DNA repair)] [55,56]. Thus, it is important to further understand all functional variants of APE1 to define a link between its genetic variation, repair and/or redox function, and disease susceptibility.

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The contribution of APE1 also extends to other disease pathologies

APE1 has emerged as a therapeutic target for treating neovascular AMD and other neovascular diseases. The redox function of APE1 is required for normal retinal angiogenesis. APX3330 inhibited the proliferation, migration, and tube formation of mouse retinal vascular endothelial cells (RVECs) [57]. It also regulated the response of retinal pigment epithelium (RPEs) cells to oxidative stress by reducing NFκB and HIF1α activity [58,59]. APX2009 and APX2014, which are second-generation APX3330 analogs, further blocked endothelial cell proliferation, tube formation, migration, and NFκB activity under *in vitro* conditions and blocked angiogenesis *ex vivo* [60]. A Phase II trial using APX3330 to treat DR and DME is planned to start in early 2021.

IBD is characterized by the loss of redox homeostasis resulting in enteric nervous system (ENS) damage and gastrointestinal (GI) dysfunction. IBD has no cure, defies early detection, and lacks reliable biomarkers, thereby increases the risk of colon cancer. Crohn's disease and ulcerative colitis (UC) are its principal forms [61]. Responsible factors include NFκB, AP-1, HIF, and STAT3, TFs that are regulated by APE1. In a recently published study, inhibition of APE1 redox function by APX3330 reduced enteric neuropathy and intestinal dysfunction [62]. Additionally, polymorphisms of APE1 (Asp148Glu) and XRCC-1 (Arg399Gln) have also been found to be associated with UC [63]. Given these results, targeting APE1 redox signaling appears to be an attractive avenue of translational research in this area.

Inhibitors of APE1 DNA repair or redox signaling

APE1 has been implicated in resistance to DNA interactive drugs and cancer pathogenesis. Therefore, APE1 is a crucial node to target for cancer therapeutics. For example, inhibition of the DNA repair function of APE1 sensitized resistant tumors to DNA interactive drugs (bleomycin, carmustine, TMZ, and gemcitabine) and increased levels of apoptosis [1]. Inhibition of the redox function of APE1 prevented DNA binding of cancer-associated TFs and subsequently their effects on the transcriptome [1]. Thus, there is a vital need to identify effective inhibitors for both the repair and redox functions of APE1. Several studies have investigated both DNA repair and redox inhibition through various screening paradigms. A list of APE1 inhibitors at all stages of study are listed in Table 3.

APE1 DNA repair function inhibitors

Methoxyamine (MX), one of the first studied molecules affecting APE1 repair activities, is an alkoxyamine derivative that acts as an indirect inhibitor of the repair function of APE1 [64]. MX reacts with the open-ring sugar in the DNA after a damaged nucleotide has been removed via glycosylase, thereby blocking APE1 endonuclease activity. In xenograft models of colon cancer, the addition of MX increased the cytotoxicity of TMZ, a DNA interactive drug [64]. However, MX is not a direct inhibitor of the DNA repair activity of APE1, binding to AP sites and preventing APE1 cleavage. MX advanced to clinical trials, with eight trials attempted. In a Phase II trial completed in 2019 the response criteria was not met (NCT02395692). A current Phase I/II trial initiated in 2013 for relapsed solid tumors and lymphoma is estimated to complete in 2021 with 140 patients (NCT01851369). To date, clinical studies have not shown success with MX.

A 5-point pharmacophore model for APE1 small-molecule inhibitors was used to identify new compounds [65]. The authors conducted an *in silico* screen of 10,159 compounds and ten were identified as potential APE1 endonuclease inhibitors based on the quality of their virtual docking. Further analysis was performed on four compounds with a 2-methyl-4-amino-6,7-dioxoloquinoline core (AJAY 1–4). AJAY-4 exhibited the lowest overall median growth inhibition concentration (~4 μM) in an NCI-60 cell line panel. The mechanism of action is suggested to be the build-up of AP sites, which activates PARP and PARP cleavage, subsequently triggering caspase-3 and caspase-7-associated apoptosis. However, none of these compounds have advanced to significant *in vivo* studies or clinical trials.

A fluorescence-based quantitative high-throughput screen (HTS) of 352 489 small molecules from the NIH Molecular Libraries Small Molecule Repository was performed [66]. Compound 3, with a chemical structure of *N*-(3-(benzo[*d*]thiazol-2-yl)-6-isopropyl-4,5,6,7-tetrahydrothieno [2,3-*c*]pyridin-2-yl)acetamide, was identified to inhibit APE1 endonuclease activity at low micromolar concentrations in HeLa cells. From whole-cell extract assays, compound 3 potentiated cytotoxicity of methyl methanesulfonate (MMS) and TMZ and caused hyperaccumulation of AP sites in HeLa cells when combined with MMS [66]. Nevertheless, this compound has not significantly advanced beyond *in vitro* studies.

In silico analysis of a library of 15- to 16-membered macrocycles from the Boston University Center for Molecular Diversity (BU-CMD) screening collection, led to selection of 66 potential inhibitors of APE1 with favorable preliminary docking results [67]. From the 66 compounds assayed, four exhibited concentration-dependent inhibition of APE1 endonuclease activity (MC043, MC047, MC042, and MC018). The chemotype was further explored; three second-generation macrocyclic lactams 13, 21, 24 assessed by comet analysis following treatment with MMS exhibited potent inhibition of APE1 endonuclease activity. These results suggest further development of these chemotypes as APE1 repair inhibitors.

There are numerous other one-off studies of reported APE1 repair inhibitors, such as 7-nitroindole-2-carboxylic acid (NCA) and CRT0044876. However, for multiple reasons, including lack of favorable drug-like properties or of *in vivo* success, these compounds did not progress to lead optimization [1,68,69]. Additional repair inhibitors are listed in Table 3.

APE1 redox inhibitors

Although there have been several studies looking at APE1 redox inhibition, including several compounds from natural sources, none of these natural products have demonstrated specific or direct inhibition of redox signaling associated with APE1 [1]. An example of these include resveratrol, which has varied bioavailability, sporadic *in vivo* efficacy and low molecular specificity [68]. Soy isoflavones, such as genistein and daidzein, have been suggested to be inhibitors of APE1 redox function; however, they do not directly inhibit the redox function of APE1, have significant off target effects, and have not proved valid as APE1 redox inhibitors [1]. Another low-specificity natural product, curcumin, has significant off target effects [1].

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By contrast, a series of compounds have been developed that are specifically targeted to APE1 redox signaling inhibition and blocking activation of downstream TFs, such as HIF-1α, NFκB, STAT3 and others [1]. APX3330 is the only APE1 redox inhibitor, or any APE1 inhibitor, to enter and complete Phase I clinical trials (NCT03375086). It is a dimethoxy benzoquinone specific for the redox signaling function of APE1 without negatively altering the DNA repair function of APE1 [1]. The nucleophilic Cys65 residue responsible for redox signaling in APE1 is blocked by APX3330 and prevents the ability of APE1 to act as a reducing agent on the downstream TFs. Numerous studies have demonstrated the specific inhibition of APE1 redox signaling function by APX3330 in numerous models both *in vitro* and *in vivo*, including pancreatic cancer, BCa, PCa, leukemia [1] and MPNSTs [70]. Furthermore, second-generation APX compounds, such as APX2007, APX2009, APX2014, and APX2032, are naphthoquinones, in which adjacent dimethoxy moieties in APX3330 are replaced by a second aromatic ring. The lipophilic side chain is shorter and carboxamide is added to replace carboxylic acid, which removes the negative charge present in APX3330. These alterations demonstrated increased efficacy in tumor studies as well as in other disease models reliant on NFκB, HIF-1α, or STAT3, such as inflammation and angiogenesis-driven retinal diseases and IBD [1,62]. Protective effects on dorsal root ganglion and enteric neurons for chemotherapy-induced peripheral neuropathy (CIPN) and IBD have also been observed with APX compounds [1].

Clinical trials

A Phase I clinical trial (NCT03375086) of APX3330 in patients with recently progressive advanced solid tumors was initiated in early 2018. Patients received oral doses of APX3330 on a twice-daily (b.i.d.) schedule during continuous 21-day (d) cycles until disease progression or drug intolerance. The study was initiated using single-patient cohorts until the occurrence of ≥G2 (grade 2) toxicity, at which time accrual proceeded using a standard 3 + 3 design. Additional patients per cohort were enrolled to obtain blood, circulating tumor cell (CTC), and biopsy samples as needed and to define the recommended Phase II study dose (RP2D). The study accrued 19 patients (13 males and 6 females; median age of 69 years). APX3330 was administered in escalating dose cohorts from 240 mg/d to 720 mg/d and was well tolerated through 600 mg/d, now defined as the RP2D [71,72]. The dose-limiting toxicity (at 720 mg/d) was a pruritic, diffuse macular rash that was spontaneously reversible.

Six of 19 patients had disease stabilization for ≥12 weeks; and 4 of 19 for ≥36 weeks, including 252 d (PCa), 337 d (melanoma), and 421 d (endometrial cancer). One partial response was noted in a patient with CRC on study for 357 d. Serum levels of APE1 decreased after administration of APX3330, as did CTCs in seven out of 16 patients evaluated. Tissue biopsy evaluations indicated reduced levels of transcription activity for regulators of cancer survival pathways (e.g., NFκB, HIF1, and STAT3), indicating that APX3330 mediates the redox activity of the APE1 protein target as expected [71,72].

Based upon these promising results, Apexian Pharmaceuticals (Indianapolis, IN, USA) is planning additional clinical trials in indications where APE1 functions to control the activity of NFκB, HIF, and STAT3, including a Phase II trial for patients with DME and DR as part of a licensing agreement with Ocuphire Pharma (Farmington Hill, MI). Additional indications are undergoing evaluation.

Future directions

With the surge in sequencing and big data platforms, several studies confirmed the known roles of APE1 in both tumor and normal cells as well as illuminating new pathways and interactions of APE1. The validation of these findings through drug screening and molecular studies will impact the way we understand the functions of APE1 within living cells and how we understand it as a target in cancer therapy. Here, we highlight recent relevant findings in which multi-omic approaches were used to better understand APE1 as a cancer target as well as pointing toward new clinical applications.

To study the effects of this important protein, APE1 overexpression and knockdown approaches were used in a variety of cell types with various global gene or protein expression analyses. The compilation of these studies clearly demonstrate that certain pathways repeatedly are affected. These include mitochondria-related processes, including oxidative phosphorylation and metabolism, transcriptional regulation, RNA metabolism, cell cycle, DNA repair, and stress responses, including oxidative stress signaling [12,73–76].

Previous preclinical and clinical studies suggest that there will not be one 'magic bullet' or singular target in hard-to-treat solid tumors and that multitargeted combination treatments that synergize will be more efficacious. Recent efforts have included trying to combine 'omics data with other publicly available databases, such as Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC), and Cancer Therapeutics Response Portal (CTRP), to determine which clinical agents will combine with APE1 inhibitors to create a drug-induced synthetic lethal event [77,78]. Using these tools to incorporate transcriptomic and drug sensitivity data of cancer cell lines from the public domain, predictions of which US Food and Drug Administration (FDA)-approved agents are likely to synergize with APX3330 are generated. Some classes of drug that are predicted to synergize with APE1 inhibition are receptor tyrosine kinases (RTKs), DNA-damaging agents and nucleoside analogs, and HDAC inhibitors. Combination drug screening is underway currently and the goal is to generate novel multitargeted drug combinations involving APE1 inhibition and improved response in *in vivo* and *in vitro* systems.

Pancreatic cancer studies combining APE1 silencing with single-cell RNA-sequencing led to elucidation of pathways including EIF2, mTOR, and protein kinase A (PKA). These pathways were significantly affected by APE1 signaling, had not previously been reported, and are related to translation as well as PKA, which might be contribute to drug resistance and/or the energy-sensing capabilities of the cell [74]. These pathway perturbations might be the result of the blockade of HIF1α signaling because APE1 regulates its DNA binding, and it is well known that these pathways can directly or indirectly influence HIF1α activity. There were also pathways in common with other previously published studies, including DNA repair, DNA damage, and apoptosis pathway perturbation. Within these low-passage patient-derived cells, 15% (3/20) of the significantly changed pathways were mitochondria-related processes.

Based on a clear demonstration that APE1 signaling affects mitochondria, the combination of metabolic inhibitors with

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APE1 inhibition has the potential to be therapeutically detrimental to cancer cells. There are many drugs that are in use in the clinic that target metabolism and could be repurposed or tested in combination with APE1 inhibitors [79]. Currently, it is not clear whether the redox activity or the DNA repair activity or both exert these effects on mitochondrial function. Data suggest that a large component of the effect on the TCA cycle results from the redox signaling activity of APE1. The effects of APE1 siRNA on the TCA cycle are similar to the reduction in function seen with APE1 redox inhibitor, APX2009 as assayed by gene expression as well as a mitochondria functional assay (Caston *et al.*, unpublished data, 2020).

Concluding remarks

In summary, as our understanding of the pathways in cancer and other diseases that intersect and depend upon APE1 signaling deepen, we will be able to design single-agent and combination therapies for patients. Although a significant amount of focus has been on the role of APE1 in cancer, recent findings support APE1 as a target in other indications, including ocular diseases (DME, DR, and AMD), IBD and other indications where APE1 regulation of TFs impacts important pathways in these diseases.

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