

Dynamic Regulation of APE1/Ref-1 as a Therapeutic Target Protein

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Apurinic/aprimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multifunctional protein that plays a central role in the cellular response to DNA damage and redox regulation against oxidative stress. APE1/Ref-1 functions in the DNA base excision repair pathway, the redox regulation of several transcription factors, and the control of intracellular redox status through the inhibition of reactive oxygen species (ROS) production. APE1/Ref-1 is predominantly localized in the nucleus; however, its subcellular localization is dynamically regulated and it may be found in the mitochondria or elsewhere in the cytoplasm. Studies have identified a nuclear localization signal and a mitochondrial target sequence in APE1/Ref-1, as well as the involvement of the nuclear export system, as determinants of APE1/Ref-1 subcellular distribution. Recently, it was shown that APE1/Ref-1 is secreted in response to hyperacetylation at specific lysine residues. Additionally, post-translational modifications such as phosphorylation, S-nitrosation, and ubiquitination appear to play a role in fine-tuning the activities and subcellular localization of APE1/Ref-1. In this review, we will introduce the multifunctional role of APE1/Ref-1 and its potential usefulness as a therapeutic target in cancer and cardiovascular disease.

Key Words: DNA-(apurinic or apyrimidinic site) lyase; Protein processing, post-translational; Oxidation-reduction; Biomarkers

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INTRODUCTION

Apurinic/aprimidinic endonuclease 1/redox factor-1 (APE1/Ref-1), also known as APEX1, is the mammalian ortholog of *Escherichia coli* Xth (exonuclease III). APE1/Ref-1 functions as an apurinic/aprimidinic endonuclease in the DNA base repair pathway. Oxidative DNA lesions, including apurinic/aprimidinic (AP) sites, have been reported as occurring at an estimated rate of 1.5×10^5 residues/cell/day.¹ APE1/Ref-1 also modulates the redox status, and thereby the activity of several transcription factors such as activator protein-1 (AP-1), nuclear factor kappa B (NF- κ B), p53, the cAMP response element binding protein (CREB), and hypoxia-inducible factor-1- α (HIF-1 α). The formation of disulfide bonds is thought to be a necessary step for the redox activities of APE1/Ref-1, with cysteine residues C65, C93, and C99 playing important roles in the thiol-mediated redox reactions.²⁻⁴

APE1/Ref-1 is a relatively abundant protein ($\sim 10^4$ - 10^5 copies/cell) with a long half-life.⁵ The human APE1/Ref-1

gene is approximately 3 kb in length; it consists of four introns and five exons and is located on chromosome 14q11.2-12.⁶ The human APE1/Ref-1 cDNA is approximately 1.4 kb in length and the mature 954-nucleotide sequence encodes a protein of 318 amino acids, with a molecular weight of 36.5 kDa.⁷ BLAST multiple alignment of the human APE1/Ref-1 amino acid sequence with sequences from different mammalian species demonstrated that the C-terminus is conserved more than the N-terminus. This comparison also revealed a high degree of identity (93-99%) between the protein sequence from human APE1/Ref-1 and the protein sequences from other mammals: rat (*Rattus norvegicus*, 93%), mouse (*Mus musculus*, 94%), cow (*Bos taurus*, 94%), dog (*Canis lupus familiaris*, 95%), and chimpanzee (*Pan troglodytes*, 99%). Interestingly, there is only a single difference between the human and chimpanzee sequences, with the aspartic acid (D) at position 148 changed to glutamic acid (E).

SUBCELLULAR LOCALIZATION OF APE1/Ref-1

APE1/Ref-1 is predominantly localized in the nucleus where it performs DNA repair and transcriptional regulatory activities. These nuclear functions of APE1/Ref-1 are presumed to be essential for cell viability and genomic stability. Despite the presence of strong nuclear targeting elements within the APE1/Ref-1 sequence, cytoplasmic and mitochondrial localizations of the protein have also been reported in some cell types, such as those with high metabolic or proliferative rates.^{4,8,9} Mainly, the cytoplasmic or mixed cytoplasmic and nuclear staining pattern of APE1/Ref-1 in some cell populations is unexpected for a presumably nuclear protein¹⁰ and suggests multiple potential functions for APE1/Ref-1.⁹

Nuclear localization of APE1/Ref-1 was significantly decreased by the deletion of 20 N-terminal amino acids, suggesting that this sequence contains a nuclear localization signal (NLS). Furthermore, fusion of these 20 N-terminal residues to EGFP resulted in it being localized to the nucleus.¹¹ The nuclear translocation of APE1/Ref-1 is also supported by the interaction of APE1/Ref-1 with karyopherin α , an adaptor protein that recognizes the proteins containing the NLS and plays an important role in the import of nuclear proteins.^{11,12}

The role of the nuclear export system (NES) in the subcellular localization of APE1/Ref-1 has been previously investigated. S-nitrosoglutathione (GSNO), an S-nitrosating agent, induces cytoplasmic translocation of APE1/Ref-1 in an exportin-1-independent manner. Exportin-1, also known as chromosome region maintenance 1 (CRM1), is a eukaryotic protein that mediates the nuclear export of proteins. Mutation analyses demonstrate that the point mutation of S-nitrosation sites, such as Cys93 and Cys310, inhibits the cytoplasmic redistribution of APE1/Ref-1, indicating that the cytoplasmic translocation is dependent on S-nitrosation.¹³ The inhibition of NO-induced APE1/Ref-1 nuclear export through the overexpression of histone deacetylase2 and p50 suggests the involvement of an endogenous regulator of APE1/Ref-1 in nuclear export. Deletion of amino acids 64 to 80 of APE1/Ref-1 resulted in a complete loss of NO-induced APE1/Ref-1 nuclear export, suggests the region containing 64-80 amino acids might serve as NES.¹³ An aberrant tumor suppressor p53 may also result in the cytoplasmic overexpression of APE1/Ref-1 and a subsequent increase in the nuclear export of APE1/Ref-1 via S-nitrosation.^{13,14}

APE1/Ref-1 has also been shown to have a mitochondrial targeting sequence (MTS). Reactive oxygen species (ROS) are a major source of mitochondrial DNA damage. In a rat thyroid cell line, APE1/Ref-1 was found located in the mitochondria.¹⁵ The translocation of APE1/Ref-1 to the mitochondria in HeLa cells has also been observed following oxidative stress induced by hydrogen peroxide, menadione, or hypoxia.¹⁶ Mutation analyses have confirmed that the MTS of APE1/Ref-1 is located in the region containing the 289 to 318 residues of the C-terminus. The MTS se-

quence is normally masked by the N-terminal structure and this masking prevents interaction between MTS and the mitochondrial transporter, TOM protein.¹⁶ Recent data demonstrated that APE1/Ref-1 interacts with Mia40, a mitochondrial import protein, and that this interaction is responsible for the trafficking of APE1/Ref-1 into the mitochondria.¹⁷ APE1/Ref-1-deficient A549 cells displayed mitochondrial membrane depolarization and increased ROS production. Silencing of the APE1/Ref-1 gene results in increased apoptosis through the mitochondrial pathway following photodynamic therapy.¹⁸ The cellular transfection of MTS-fused APE1/Ref-1 results in significantly more effective suppression of mitochondrial dysfunctions by protein kinase C activation in endothelial cells than wild-type APE1/Ref-1.¹⁹

EXTRACELLULARLY SECRETED APE1/Ref-1 AS A POTENTIAL BIOMARKER

APE1/Ref-1 secretome analysis using Secretome 2.0 identified APE1/Ref-1 as a potential non-classically secreted protein.²⁰ We first demonstrated the presence of the APE1/Ref-1 protein in the plasma of endotoxemic rats as a 37 kDa immunoreactive band that was then identified as *Rattus norvegicus* APE1/Ref-1 (accession no. AAG49922.1) by liquid chromatography/tandem mass spectrometry. This suggests that plasma APE1/Ref-1 can be used as a serological biomarker for endotoxemia.²⁰ APE1/Ref-1 can be secreted in response to hyperacetylation by aspirin or a histone deacetylase inhibitor, and the acetylation of specific lysine residues K6 and K7 of APE1/Ref-1 acts as a key molecular mechanism of the extracellular secretion of APE1/Ref-1.^{21,22} Serum APE1/Ref-1 autoantibodies have been detected in lung cancer patients at significantly higher levels than in healthy controls, suggesting that these may be used as a potential marker for lung cancer.²³ Recently, APE1/Ref-1 levels in biological samples such as serum and urine have been reported using a newly developed, sandwich enzyme-linked immunosorbent assay. Jin et al found that the levels of APE1/Ref-1 were higher in serum from patients with coronary artery disease than in the serum from control patients.²⁴ Recently, it has been reported that plasma or urine APE1/Ref-1 levels significantly increase in bladder cancer patients; the area under the curve analysis showed diagnostic values with high specificity and sensitivity.^{25,26} Although the underlying mechanisms for secreted APE1/Ref-1 are yet to be fully delineated, a few reports have been able to provide some insight into its biological functions. Recombinant wild-type APE1/Ref-1 displayed anti-inflammatory activity in endothelial cells²⁰ and acetylated APE1/Ref-1 was found to be able to bind to the receptor for advanced glycation end products, which resulted in apoptosis in cells.²² During hyperacetylation in culture, a time-dependent increase in secreted APE1/Ref-1 has been confirmed. Recombinant human APE1/Ref-1 with reducing activity was also shown to induce a conformational change in recombinant human tu-

mor necrosis factor- α (TNF- α) receptor type 1 by thiol-disulfide exchange. The anti-inflammatory effects produced by acetylation in TNF- α -stimulated endothelial cells are tightly linked to secreted APE1/Ref-1.²⁷

POST-TRANSLATIONAL MODIFICATIONS OF APE1/Ref-1

Post-translational modifications (PTM) are covalent and enzymatic modifications of proteins that occur after translation, and are important for forming the mature protein products and for cellular signaling pathways. Phosphorylation, a type of PTM, is a common mechanism for the regulation of enzyme activity in target proteins. APE1/Ref-1 is a target substrate for phosphorylation by serine/threonine casein kinases and protein kinase C.^{28,29} The phosphorylation of APE1/Ref-1 has been shown to abolish its DNA repair activity³⁰ and to increase the redox activity of transcription factor AP-1 in response to ROS.^{28,31} The S-nitrosation of cysteine residues is another important PTM. The S-nitrosation of APE1/Ref-1 induced by exposure to GSNO results in the cytoplasmic localization of the protein, either by stimulating nuclear export or by inhibiting nuclear import.¹³ Acetylation of target proteins is yet another important PTM. APE1/Ref-1 can be acetylated by histone acetyltransferase p300.³² Histone acetyltransferase p300 is activated by intracellular calcium and the acetylation of APE1/Ref-1 at K6 or K7 enhances its binding to negative calcium response elements. APE1/Ref-1 is also known to bind with silent mating type information regulation 2 homolog 1 (Sirtuin 1, SIRT1), which deacetylates the K6 and K7 lysine residues.³³ The acetylation of residues K6 and K7 of APE1/Ref-1 is known to be a key step for its secretion in cultured cells.²¹ Finally, APE1/Ref-1 is also subjected to PTM through ubiquitination by E3 ubiquitin ligase at multiple lysine residues near its N-terminus.³⁴ The degradation of APE1/Ref-1 is mediated by mouse double minute 2 (MDM2), which is involved in ubiquitination.³⁵ In contrast to the predominantly nuclear localization of APE1/Ref-1, the ubiquitinated APE1/Ref-1 protein is clearly localized in the cytoplasm,³⁵ suggesting underlying mechanisms for the cytoplasmic localization of APE1/Ref-1.

APE1/Ref-1 AS A TARGET MOLECULE IN CANCER AND CARDIOVASCULAR DISEASES

Evidence is accumulating for the alteration of APE1/Ref-1 in cancer etiology. APE1/Ref-1 regulates genomic stability against oxidative stress and any mutations in APE1/Ref-1 would be expected to contribute to carcinogenesis. The high-energy expenditure of cancer cells and disturbances to their antioxidant systems can generate high levels of ROS, which may involve APE1/Ref-1. Several cancer cells and tissues show high nuclear expression or cytoplasmic localization of APE1/Ref-1 in response to increased cytoplasmic and/or mitochondrial ROS. An alteration in the

subcellular localization of APE1/Ref-1 has been associated with drug resistance, tumor aggressiveness, and poor prognosis.³⁶ In lung cancer patients, nuclear and cytoplasmic APE1/Ref-1 expression markedly increases in the tissues from tumor regions. An alteration to cellular antioxidant systems, which leads to enhanced superoxide production and lipid peroxidation, is observed in the tumor regions of lung cancers.³⁷ The transcription of APE1/Ref-1 can be abrogated by p53, which inhibits Sp1 binding to the APE1/Ref-1 promoter.³⁸ p53 functions as a tumor suppressor, and plays an important role in apoptosis and genomic stability. Therefore, understanding the crosstalk between p53 and APE1/Ref-1 may help in the design and development of anti-cancer drugs.

The extranuclear functions of APE1/Ref-1 are being uncovered, especially the cellular defense mechanism against oxidative stress.³⁹ APE1/Ref-1 provides cellular protection against DNA damage or oxidative stress.⁴⁰ In global cerebral ischemia animal models, APE1/Ref-1 expression decreases in neuronal cells, and downregulation of its expression is closely related with neuronal apoptosis.^{41,42} Cell growth requires the maintenance of an adequate blood supply. APE1/Ref-1 shows cytoprotective activity in normal endothelial cells.⁴³ APE1/Ref-1 is known to interact with transacting factors such as HIF-1- α , which is known to bind to the HIF-1 DNA recognition site. Overexpression of APE1/Ref-1 increases hypoxia-induced proteins containing an HIF-1- α binding site.⁴⁴ Therefore, APE1/Ref-1 acts as a transcriptional modulator for hypoxia-induced binding of transcription proteins to HIF-1.

The antioxidant activity of APE1/Ref-1 is closely linked to its extranuclear functions.⁴⁵ APE1/Ref-1 controls intracellular ROS via the inhibition of rac1, a subunit of the NADPH oxidase system, and increases the bioavailability of nitric oxide by enhancing the activity of endothelial nitric oxide synthase.^{43,46} APE1/Ref-1 has been found to suppress cytokine-induced monocyte adhesion as well as the expression of vascular cell adhesion molecules in endothelial cells.^{47,48} In endothelial cells, oxidized low-density lipoprotein or PKC β II induces serine 36 phosphorylation of p66shc, which is inhibited by APE1/Ref-1 overexpression.⁴⁹ Recently, an NLS-deletion mutant of APE1/Ref-1, which is exclusively localized in the cytoplasm, has been found to inhibit TNF- α -induced VCAM-1 expression in the cultured endothelial cells, suggesting an anti-inflammatory function for cytoplasmic APE1/Ref-1.⁴

APE1/Ref-1 may also be involved in the regulation of blood pressure. Heterozygous APE1/Ref-1(+/-) mice are hypertensive and display impaired endothelium-dependent vasorelaxation and reduced vascular NO levels.⁴³ Furthermore, APE1/Ref-1 protein expression levels are elevated in renin-dependent, aortic coarctation hypertensive rat models.⁵⁰ An association between hypertension and APE1/Ref-1 gene polymorphism has been observed in a human study and hence, the alterations in the APE1/Ref-1 gene can be an important target for essential hypertension.⁵¹ Current knowledge provides an insight into the mechanisms under-

lying the hypertension observed in heterozygous APE1/Ref-1 mice. APE1/Ref-1 is involved in calcium-mediated renin suppression. Increased intracellular calcium enhances the association of APE1/Ref-1 with the HDAC1 co-repressor, which remains bound to the renin enhancer. Furthermore, renin expression is increased through APE1/Ref-1 knock-down.⁵² Collective data with increased renin expression and higher plasma-renin activity in APE1/Ref-1 heterozygous mice indicates that APE1/Ref-1 plays the role of negative regulator of renin expression.⁵³ The role of APE1/Ref-1 as a transcriptional repressor for aldosterone synthase gene (CYP11B2) was also investigated. APE1/Ref-1 overexpression was found to induce reduction of transcription of CYP11B2. In contrast, gene silencing of APE1/Ref-1 led to increased transcription. Therefore, APE1/Ref-1 is a novel transcriptional repressor of CYP11B2.⁵⁴ Systemic hypertension in heterozygous APE1/Ref-1 mice may therefore be due to increased renin and aldosterone production; however, this requires further investigation.

APE1/Ref-1 INHIBITORS AND THEIR CLINICAL APPLICATION

E3330 ([[(2E)-3-[5-(2,3dimethoxy-6-methyl-1,4-benzoquinoly)]-2-nonyl-2-propenoic acid]) is a quinone compound that functions as a redox inhibitor of APE1/Ref-1 by increasing disulfide bond formation with Cys 65 and Cys 93 residues of APE1/Ref-1.⁵⁵ E3330 suppresses the inflammatory response in activated macrophages and in tumor-associated macrophages.⁵⁶ E3330 also inhibits the in vitro growth of endothelial progenitor cells and VEGF secretion.^{57,58} Recently, Jiang et al.⁵⁹ reported that APE1/Ref-1 regulated tumor angiogenesis through a TGF- β -dependent pathway, as indicated by inhibition of endothelial transwell migration and tube formation in the APE1/Ref-1-siRNA transfected group. In addition, siRNA for APE1/Ref-1 was found to suppress tumor angiogenesis and growth in vivo in a xenograft model.^{59,60} AR03 ([2,4,9-trimethylbenzo[b][1,8]-naphthyridin-5-amine) is an inhibitor of APE1/Ref-1-mediated repair and blocks the cleavage of AP sites with micromolar efficiency. AR03 is thought to have potential as a therapeutic drug against glioblastoma and other cancers.⁶¹ In general, APE1/Ref-1 overexpression is associated with increased survival of cancer cells, resistance to therapy, and poor prognosis. Therefore, APE1/Ref-1 inhibitors are being studied in order to identify a compound that could have a beneficial role in the treatment or co-treatment of several cancers. Methoxyamine (TRC102) is an inhibitor of the DNA repair activity of APE1/Ref-1, and Phase I and Phase II clinical trials are being conducted for this compound on patients with advanced solid tumors (Clinical Trials Identifier, NCT00892385). Similarly, lucanthone is a direct inhibitor of DNA repair activity of APE1/Ref-1 and is currently in a Phase II clinical trial on patients with brain metastasis from lung cancers (Clinical Trials Identifier, NCT02014545).

CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, APE1/Ref-1 is known as a multifunctional protein with an important role in DNA repair and redox regulation, as well as antioxidant activities. The intracellular expression and subcellular localization of APE1/Ref-1 are strictly controlled by several localization signals in response to stimuli and post-translational modifications. Genome-wide analysis of human samples is expected to help identify the pathogenesis underlying a wide range of diseases, including cancer, ischemic reperfusion injury, hypertension, and inflammatory diseases. Proteomic analyses of biological samples such as tissue and blood would be helpful to further understand the multifunctional role of APE1/Ref-1. The recent finding that the APE1/Ref-1 secreted into the extracellular domain of inflammatory cytokine receptors possesses redox inhibitory activity illustrates the potential of APE1/Ref-1 as a biomarker for several kinds of disorders. Recombinant APE1/Ref-1 proteins could be used as reducing modifiers, alone or in combination with treatment regimens against inflammatory disorders. The development of a conditional APE1/Ref-1 knockout system is essential to overcoming the embryonic lethality of APE1/Ref-1 knockout and thereby provide a model for better understanding of the biological and tissue-specific functions of APE1/Ref-1.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Beckman KB, Ames BN. Oxidative decay of DNA. *J Biol Chem* 1997;272:19633-6.
2. Georgiadis MM, Luo M, Gaur RK, Delaplane S, Li X, Kelley MR. Evolution of the redox function in mammalian apurinic/apyrimidinic endonuclease. *Mutat Res* 2008;643:54-63.
3. Luo M, Zhang J, He H, Su D, Chen Q, Gross ML, et al. Characterization of the redox activity and disulfide bond formation in apurinic/apyrimidinic endonuclease. *Biochemistry* 2012;51:695-705.
4. Park MS, Kim CS, Joo HK, Lee YR, Kang G, Kim SJ, et al. Cytoplasmic localization and redox cysteine residue of APE1/Ref-1 are associated with its anti-inflammatory activity in cultured endothelial cells. *Mol Cells* 2013;36:439-45.
5. Tell G, Quadrioglio F, Tiribelli C, Kelley MR. The many functions of APE1/Ref-1: not only a DNA repair enzyme. *Antioxid Redox Signal* 2009;11:601-20.
6. Harrison L, Ascione G, Menninger JC, Ward DC, Demple B.

- Human apurinic endonuclease gene (APE): structure and genomic mapping (chromosome 14q11.2-12). *Hum Mol Genet* 1992;1:677-80.
7. Seki S, Takata A, Nakamura T, Akiyama K, Watanabe S. A possible cause of heterogeneity of mammalian apurinic/apurimidinic endonuclease. *Int J Biochem* 1993;25:53-9.
 8. Tell G, Damante G, Caldwell D, Kelley MR. The intracellular localization of APE1/Ref-1: more than a passive phenomenon? *Antioxid Redox Signal* 2005;7:367-84.
 9. Kakolyris S, Kaklamanis L, Giatromanolaki A, Koukourakis M, Hickson ID, Barzilay G, et al. Expression and subcellular localization of human AP endonuclease 1 (HAP1/Ref-1) protein: a basis for its role in human disease. *Histopathology* 1998;33:561-9.
 10. Duguid JR, Eble JN, Wilson TM, Kelley MR. Differential cellular and subcellular expression of the human multifunctional apurinic/apurimidinic endonuclease (APE/ref-1) DNA repair enzyme. *Cancer Res* 1995;55:6097-102.
 11. Jackson EB, Theriot CA, Chattopadhyay R, Mitra S, Izumi T. Analysis of nuclear transport signals in the human apurinic/apurimidinic endonuclease (APE1/Ref1). *Nucleic Acids Res* 2005;33:3303-12.
 12. Pemberton LF, Blobel G, Rosenblum JS. Transport routes through the nuclear pore complex. *Curr Opin Cell Biol* 1998;10:392-9.
 13. Qu J, Liu GH, Huang B, Chen C. Nitric oxide controls nuclear export of APE1/Ref-1 through S-nitrosation of cysteines 93 and 310. *Nucleic Acids Res* 2007;35:2522-32.
 14. Wu HH, Chu YC, Wang L, Tsai LH, Lee MC, Chen CY, et al. Cytoplasmic Ape1 expression elevated by p53 aberration may predict survival and relapse in resected non-small cell lung cancer. *Ann Surg Oncol* 2013;20 Suppl 3:S336-47.
 15. Tell G, Crivellato E, Pines A, Paron I, Pucillo C, Manzini G, et al. Mitochondrial localization of APE/Ref-1 in thyroid cells. *Mutat Res* 2001;485:143-52.
 16. Li M, Zhong Z, Zhu J, Xiang D, Dai N, Cao X, et al. Identification and characterization of mitochondrial targeting sequence of human apurinic/apurimidinic endonuclease 1. *J Biol Chem* 2010;285:14871-81.
 17. Barchiesi A, Wasilewski M, Chacinska A, Tell G, Vascotto C. Mitochondrial translocation of APE1 relies on the MIA pathway. *Nucleic Acids Res* 2015;43:5451-64.
 18. Li MX, Shan JL, Wang D, He Y, Zhou Q, Xia L, et al. Human apurinic/apurimidinic endonuclease 1 translocalizes to mitochondria after photodynamic therapy and protects cells from apoptosis. *Cancer Sci* 2012;103:882-8.
 19. Joo HK, Lee YR, Park MS, Choi S, Park K, Lee SK, et al. Mitochondrial APE1/Ref-1 suppressed protein kinase C-induced mitochondrial dysfunction in mouse endothelial cells. *Mitochondrion* 2014;17:42-9.
 20. Park MS, Lee YR, Choi S, Joo HK, Cho EJ, Kim CS, et al. Identification of plasma APE1/Ref-1 in lipopolysaccharide-induced endotoxemic rats: implication of serological biomarker for an endotoxemia. *Biochem Biophys Res Commun* 2013;435:621-6.
 21. Choi S, Lee YR, Park MS, Joo HK, Cho EJ, Kim HS, et al. Histone deacetylases inhibitor trichostatin A modulates the extracellular release of APE1/Ref-1. *Biochem Biophys Res Commun* 2013;435:403-7.
 22. Lee YR, Kim KM, Jeon BH, Choi S. Extracellularly secreted APE1/Ref-1 triggers apoptosis in triple-negative breast cancer cells via RAGE binding, which is mediated through acetylation. *Oncotarget* 2015;6:23383-98.
 23. Dai N, Cao XJ, Li MX, Qing Y, Liao L, Lu XF, et al. Serum APE1 autoantibodies: a novel potential tumor marker and predictor of chemotherapeutic efficacy in non-small cell lung cancer. *PLoS One* 2013;8:e58001.
 24. Jin SA, Seo HJ, Kim SK, Lee YR, Choi S, Ahn KT, et al. Elevation of the serum Apurinic/Apyrimidinic endonuclease 1/Redox factor-1 in coronary artery disease. *Korean Circ J* 2015;45:364-71.
 25. Shin JH, Choi S, Lee YR, Park MS, Na YG, Irani K, et al. APE1/Ref-1 as a Serological Biomarker for the Detection of Bladder Cancer. *Cancer Res Treat* 2015;47:823-33.
 26. Choi S, Shin JH, Lee YR, Joo HK, Song KH, Na YG, et al. Urinary APE1/Ref-1: A Potential Bladder Cancer Biomarker. *Dis Markers* 2016;2016:7276502.
 27. Park MS, Choi S, Lee YR, Joo HK, Kang G, Kim CS, et al. Secreted APE1/Ref-1 inhibits TNF- α -stimulated endothelial inflammation via thiol-disulfide exchange in TNF receptor. *Sci Rep* 2016;6:23015.
 28. Hsieh MM, Hegde V, Kelley MR, Deutsch WA. Activation of APE/Ref-1 redox activity is mediated by reactive oxygen species and PKC phosphorylation. *Nucleic Acids Res* 2001;29:3116-22.
 29. Pines A, Perrone L, Bivi N, Romanello M, Damante G, Gulisano M, et al. Activation of APE1/Ref-1 is dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP. *Nucleic Acids Res* 2005;33:4379-94.
 30. Yacoub A, Kelley MR, Deutsch WA. The DNA repair activity of human redox/repair protein APE/Ref-1 is inactivated by phosphorylation. *Cancer Res* 1997;57:5457-9.
 31. Fritz G, Kaina B. Phosphorylation of the DNA repair protein APE/REF-1 by CKII affects redox regulation of AP-1. *Oncogene* 1999;18:1033-40.
 32. Bhakat KK, Izumi T, Yang SH, Hazra TK, Mitra S. Role of acetylated human AP-endonuclease (APE1/Ref-1) in regulation of the parathyroid hormone gene. *EMBO J* 2003;22:6299-309.
 33. Yamamori T, DeRico J, Naqvi A, Hoffman TA, Mattagajasingh I, Kasuno K, et al. SIRT1 deacetylates APE1 and regulates cellular base excision repair. *Nucleic Acids Res* 2010;38:832-45.
 34. Meisenberg C, Tait PS, Dianova II, Wright K, Edelmann MJ, Ternette N, et al. Ubiquitin ligase UBR3 regulates cellular levels of the essential DNA repair protein APE1 and is required for genome stability. *Nucleic Acids Res* 2012;40:701-11.
 35. Busso CS, Iwakuma T, Izumi T. Ubiquitination of mammalian AP endonuclease (APE1) regulated by the p53-MDM2 signaling pathway. *Oncogene* 2009;28:1616-25.
 36. Li M, Wilson DM 3rd. Human apurinic/apurimidinic endonuclease 1. *Antioxid Redox Signal* 2014;20:678-707.
 37. Yoo DG, Song YJ, Cho EJ, Lee SK, Park JB, Yu JH, et al. Alteration of APE1/ref-1 expression in non-small cell lung cancer: the implications of impaired extracellular superoxide dismutase and catalase antioxidant systems. *Lung Cancer* 2008;60:277-84.
 38. Zaky A, Busso C, Izumi T, Chattopadhyay R, Bassiouny A, Mitra S, et al. Regulation of the human AP-endonuclease (APE1/Ref-1) expression by the tumor suppressor p53 in response to DNA damage. *Nucleic Acids Res* 2008;36:1555-66.

39. Jeon BH, Irani K. APE1/Ref-1: versatility in progress. *Antioxid Redox Signal* 2009;11:571-4.
40. Walker LJ, Craig RB, Harris AL, Hickson ID. A role for the human DNA repair enzyme HAP1 in cellular protection against DNA damaging agents and hypoxic stress. *Nucleic Acids Res* 1994;22:4884-9.
41. Walton M, Lawlor P, Sirimanne E, Williams C, Gluckman P, Dragunow M. Loss of Ref-1 protein expression precedes DNA fragmentation in apoptotic neurons. *Brain Res Mol Brain Res* 1997;44:167-70.
42. Kawase M, Fujimura M, Morita-Fujimura Y, Chan PH. Reduction of apurinic/apyrimidinic endonuclease expression after transient global cerebral ischemia in rats: implication of the failure of DNA repair in neuronal apoptosis. *Stroke* 1999;30:441-8; discussion 9.
43. Jeon BH, Gupta G, Park YC, Qi B, Haile A, Khanday FA, et al. Apurinic/apyrimidinic endonuclease 1 regulates endothelial NO production and vascular tone. *Circ Res* 2004;95:902-10.
44. Ziel KA, Campbell CC, Wilson GL, Gillespie MN. Ref-1/Ape is critical for formation of the hypoxia-inducible transcriptional complex on the hypoxic response element of the rat pulmonary artery endothelial cell VEGF gene. *FASEB J* 2004;18:986-8.
45. Angkeow P, Deshpande SS, Qi B, Liu YX, Park YC, Jeon BH, et al. Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. *Cell Death Differ* 2002;9:717-25.
46. Ozaki M, Suzuki S, Irani K. Redox factor-1/APE suppresses oxidative stress by inhibiting the rac1 GTPase. *FASEB J* 2002;16:889-90.
47. Kim CS, Son SJ, Kim EK, Kim SN, Yoo DG, Kim HS, et al. Apurinic/apyrimidinic endonuclease1/redox factor-1 inhibits monocyte adhesion in endothelial cells. *Cardiovasc Res* 2006;69:520-6.
48. Song YJ, Lee JY, Joo HK, Kim HS, Lee SK, Lee KH, et al. Tat-APE1/ref-1 protein inhibits TNF-alpha-induced endothelial cell activation. *Biochem Biophys Res Commun* 2008;368:68-73.
49. Lee SK, Chung JI, Park MS, Joo HK, Lee EJ, Cho EJ, et al. Apurinic/apyrimidinic endonuclease 1 inhibits protein kinase C-mediated p66shc phosphorylation and vasoconstriction. *Cardiovasc Res* 2011;91:502-9.
50. Song SH, Cho EJ, Park MS, Lee YR, Joo HK, Kang G, et al. Redox regulating protein APE1/Ref-1 expression is increased in abdominal aortic coarctation-induced hypertension rats. *J Korean Soc Hypertens* 2012;18:126-35.
51. Naganuma T, Nakayama T, Sato N, Fu Z, Soma M, Yamaguchi M, et al. Haplotype-based case-control study on human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 gene and essential hypertension. *Am J Hypertens* 2010;23:186-91.
52. Fuchs S, Philippe J, Corvol P, Pinet F. Implication of Ref-1 in the repression of renin gene transcription by intracellular calcium. *J Hypertens* 2003;21:327-35.
53. Sengupta S, Chattopadhyay R, Mantha AK, Mitra S, Bhakat KK. Regulation of mouse-renin gene by apurinic/apyrimidinic endonuclease 1 (APE1/Ref-1) via recruitment of histone deacetylase 1 corepressor complex. *J Hypertens* 2012;30:917-25.
54. McManus F, Sands W, Diver L, MacKenzie SM, Fraser R, Davies E, et al. APEX1 regulation of aldosterone synthase gene transcription is disrupted by a common polymorphism in humans. *Circ Res* 2012;111:212-9.
55. Su D, Delaplane S, Luo M, Rempel DL, Vu B, Kelley MR, et al. Interactions of apurinic/apyrimidinic endonuclease with a redox inhibitor: evidence for an alternate conformation of the enzyme. *Biochemistry* 2011;50:82-92.
56. Jedinak A, Dudhgaonkar S, Kelley MR, Sliva D. Apurinic/Apyrimidinic endonuclease 1 regulates inflammatory response in macrophages. *Anticancer Res* 2011;31:379-85.
57. Zou GM, Karikari C, Kabe Y, Handa H, Anders RA, Maitra A. The Ape-1/Ref-1 redox antagonist E3330 inhibits the growth of tumor endothelium and endothelial progenitor cells: therapeutic implications in tumor angiogenesis. *J Cell Physiol* 2009;219:209-18.
58. Jiang A, Gao H, Kelley MR, Qiao X. Inhibition of APE1/Ref-1 redox activity with APX3330 blocks retinal angiogenesis in vitro and in vivo. *Vision Res* 2011;51:93-100.
59. Jiang X, Shan J, Dai N, Zhong Z, Qing Y, Yang Y, et al. Apurinic/apyrimidinic endonuclease 1 regulates angiogenesis in a transforming growth factor β -dependent manner in human osteosarcoma. *Cancer Sci* 2015;106:1394-401.
60. Ren T, Qing Y, Dai N, Li M, Qian C, Yang Y, et al. Apurinic/apyrimidinic endonuclease 1 induced upregulation of fibroblast growth factor 2 and its receptor 3 induces angiogenesis in human osteosarcoma cells. *Cancer Sci* 2014;105:186-94.
61. Bapat A, Glass LS, Luo M, Fishel ML, Long EC, Georgiadis MM, et al. Novel small-molecule inhibitor of apurinic/apyrimidinic endonuclease 1 blocks proliferation and reduces viability of glioblastoma cells. *J Pharmacol Exp Ther* 2010;334:988-98.