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Review Article

Trafficking of DNA Repair Factors into Mitochondria for the Repair of Oxidative Lesions on mtDNA

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• APE1; Mitochondria; mtDNA; Oxidative damages; Tim40/MIA import pathway

Abstract

Cells become cancerous after being faced with several cellular insults, including DNA damages caused by oxidative stresses. Mitochondria are the main endogenous source of reactive oxygen species (ROS) because most ROS are generated by the oxidative phosphorylation processes that occur in mitochondria. ROS induce oxidative lesions and base alkylation in mtDNA that are thought to be repaired via BER pathway. However, the DNA repair proteins and other components/factors of BER pathway are not produced in the mitochondria but are translocated from cytoplasm or nucleus. This review explicitly presents the molecular, cellular and structural basis for trafficking of DNA repair proteins/factors, in particular APE1 from the cytoplasm to the mitochondria.

INTRODUCTION

Alterations in DNA and protein sequences have a severe implication in the development and progression of many diseases, such as cancers and neurodegenarative disorders [1-7]. Apurinic/apyrimidinic endonuclease 1 (APE1) is an essential protein that possesses DNA repair activity and redox activity. As such APE1 is known to interact with a number of proteins as presented in Figure 1 [8], and therefore, is implicated in a number of diseases (Figure 2 & 3) [9]. APE1 has a redox active Cysteine (Cys65) through which it maintains reduced state of several redox-regulated transcription factors, such as AP-1, NFκB and p53. This reduced state is required for facilitating their binding onto the promoter of the target gene so as to turn on their gene expression. The DNA repair and redox activities of APE1 reside within distinct domains of the protein. APE1 is involved in the base excision repair (BER) pathway, which repairs oxidative base damage caused by various oxidative and alkylating agents, both of endogenous and exogenous nature. The BER pathway includes four distinct steps: (i) recognition and removal of the modified base by a DNA glycosylase and generation of apurinic/apyrimidinic (AP) sites; (ii) processing of the generated AP site by APE1 protein; (iii) incorporation of the correct nucleotide(s) through the activity of the polymerase γ; and (iv) nick sealing catalyzed by the DNA ligase III. Although much is known about how APE1 proteins are targeted to and imported into nucleus, very little is known about how APE1 gets targeted and translocated into mitochondria and recruited to sites of DNA lesions. Li [10], demonstrated that the mitochondrial targeting signal (MTS) of APE1 resides within residues 289-318 of the C-terminus, which is masked by the NLS domain at N-terminal (residues 1-39), therefore suggesting the occurrence of specific and regulated mechanisms of protein unfolding-refolding to ensure proper APE1 localization. Deletion of the N-terminal NLS (NΔ33) redirects the truncated protein (NΔ33 APE1) towards non-canonical subcellular compartments such as mitochondria [11].

Researchers found that APE1 cause deregulated expression of a pro-inflammatory gene, *IL8* [12]. A novel APE1 interacting partner, peroxiredoxine 1 (PRDX1), is involved in cellular redox regulation. PRDX1 knockdown in HepG2 cells causes increased expression of a pro-inflammatory gene, *IL8*. For this, reduction of cysteine in NFκB is required. APE1 interacts with PRDX1 in the nucleus and in absence of this interaction (as in case of PRDX1 knockdown), APE1 interact NFκB and causes reduction of its cysteine. PRDX1 interacts with APE1 in nucleus in such a way that PRDX1 sequesters APE1 redox activity, thereby keeps a check on APE1 from reducing NFκB. Based on results presented by Cesaratto and Xie [13,14], from several such experiments for instance, ELISA assay, Western blots, Immunofluorescence microscopy, Microarray analysis, and qPCR, it can be interpreted that PRDX1 interaction with APE1 represents a cellular defensive mechanism in which the APE1-PRDX1 interaction keeps a check on APE1 redox activity from activating superfluous *IL8* expression, which otherwise could increase the susceptibility of pro-inflammatory response and cancer metastasis.

PRDX1 knockdown or loss causes protein hyperoxidation in HepG2, HeLa cells, MDA-MB-231 and other cell lines [13,15]. PRDX1 interacts with RAD51 under oxidative conditions

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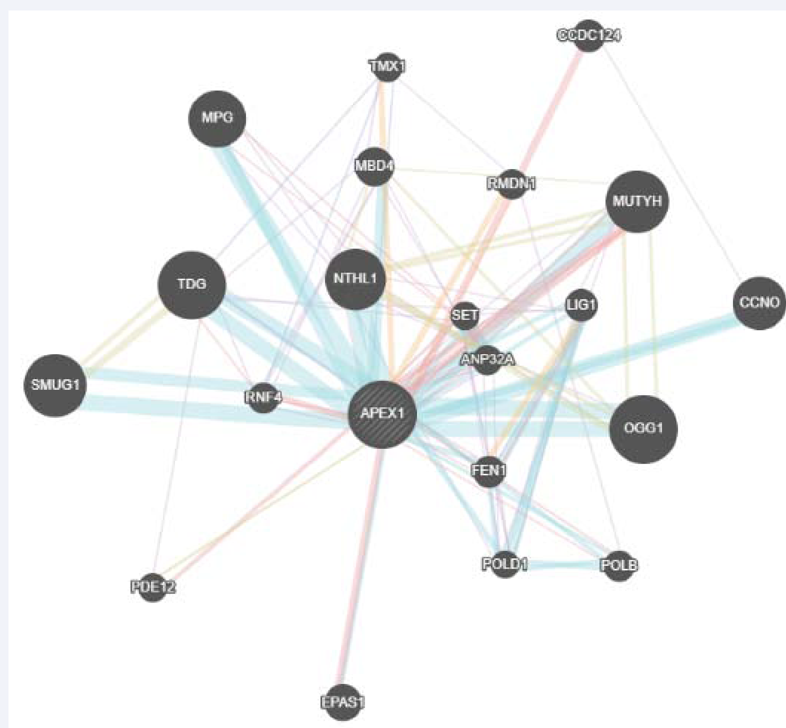

Figure 1 Experimentally verified interacting partners of APE1.

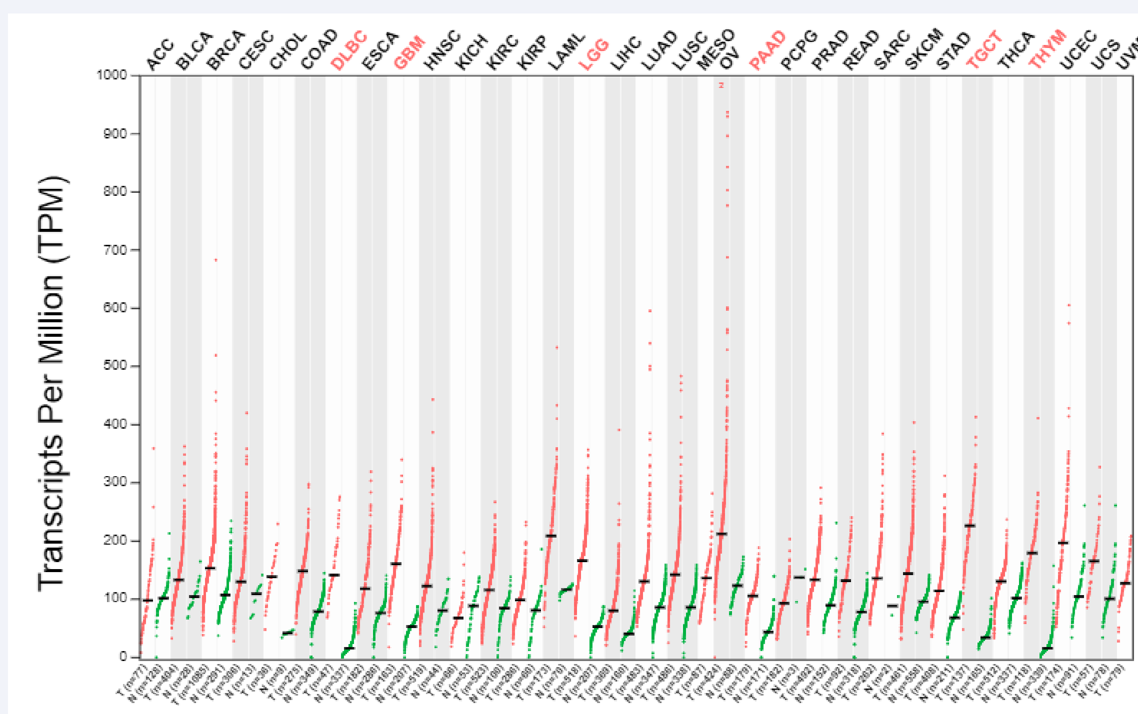

Figure 2 The gene expression profile across all tumor samples and paired normal tissues represented in the form of a Dot Plot. Each dot represent expression of samples.

and protects the functially important cys319 residue in RAD51 [14]. PRDX1 catalyzes the reduction of H2O2 into H2O. The knockdown of PRDX1 is also expected to cause the hyperoxidation of cell organelles, especially mitochondria which can be easily detected and visualized by immunofluorescence imaging with mitochondria specific red-fluorescent dye, such as MITOTRACKER. Besides this, PRDX1 knockdown, indeed, may cause upregulation of mitochondrial respiration and which in-

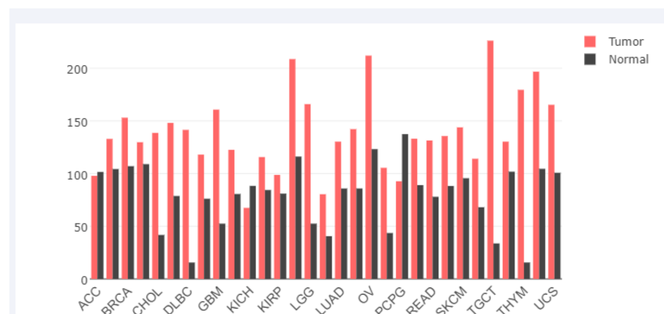

Figure 3 The gene expression profile across all tumor samples and paired normal tissues represented in the form of a Bar plot in which the height of bar represents the median expression of certain tumor type or normal tissue.

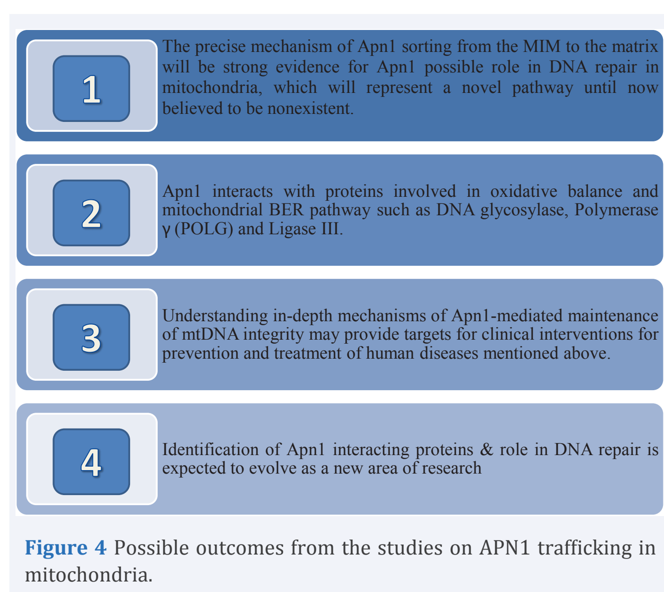

Figure 4 Possible outcomes from the studies on APN1 trafficking in mitochondria.

turn many elevates glucose uptake by the cells, both contributed to increased proliferation of PRDX1 knockdown cells as compared to wild-type cells indicating that PRDX1 knockdown may render cells go cancerous.

APE1 TRAFFICKING IN MITOCHONDRIA IS VIA TIM, TOM AND MIA IMPORT PATHWAY

Researchers previously demonstrated that proteins such as Mdm35, which possesses a twin CX_9C motif, act as a preferable substrate for the Tim40/MIA import pathway [15-17]. Basically, proteins with α-helical hairpin structure bridged by two intramolecular disulfides are trapped by a Mia40-dependent oxidative process [18]. The APE1 trafficking via Mia40 involves a redox-assisted mechanism, dependent on the disulfide transfer system. The MIA system is composed of two essential proteins: Mia40/Tim40 and Erv1/ALR. Mia40 is the oxidoreductase that catalyzes the formation of the disulfide bonds in the substrate, while ALR reoxidizes Mia40 for turn-over of the system. In light of available information, I want to present the cellular, molecular and structural basis of APE1 trafficking from the mitochondrial inner membrane (MIM) to the matrix for the repair of oxidative lesions on mtDNA.

CELLULAR AND MOLECULAR BASIS FOR APE1 TRAFFICKING

Over 99% of proteins required for mitochondrial functioning are encoded by the nuclear genome [19,20], and transported into the mitochondria after synthesis in the cytosol. Such proteins use the TOM (translocase of outer mitochondrial membrane) and TIM (translocase of inner mitochondrial membrane) translocation machineries in the mitochondrial outer membrane (MOM) and MIM, respectively, for their transport into mitochondrial matrix [21]. APE1 is an essential component of the BER pathway in mitochondria and is translocated from cytosol to mitochondria using similar mechanism. Recently, Barchiesi [16], demonstrated that APE1 interacts with the mitochondrial import and assembly protein Mia40 in the intermembrane space (IMS) and that suggested that Mia40 is responsible for APE1 trafficking into the MIM. However, further translocation of APE1 from the MIM to the matrix is still not clear. The repair synthesis by APE1 is critical for maintenance of mitochondrial DNA stability and integrity because unrepaired mtDNA could potentially increase the susceptibility to mitochondrial and other diseases such as cancer, cardio-vascular, and neuro-degenerative disorders.

APPROACH FOR TESTING AND CONFIRM APE1 TRAFFICKING IN MITOCHONDRIA

APE1 has a yeast counterpart, Apn1, in *Saccharomyces cerevisiae* which can be efficiently used for elucidating the mechanism of Apn1 sorting from the IMS to the matrix for repairing lesions of mtDNA. For this, an *in vitro* import assays for radioisotope-labelled Apn1 need to establish. It would be essential to test and confirm that the Apn1 uses the MIA system to reach the IMS by using the mutants of Mia40 and Erv1 (Figure 4). Further, optimization of the assay condition to achieve efficient sorting of the Apn1 to the matrix, not the IMS is required. There exists some translocators such as the TIM23 complex and TIM22 complex and their possible involvement in Apn1 trafficking in mitochondria is also required to be checked by using their temperature-sensitive (ts) mutant mitochondria. Test of requirement of energy like the membrane potential across the IM and ATP hydrolysis by matrix Hsp70 can also be checked. One can also use crosslinking approaches or affinity co-purification to look for transiently interacting partner proteins for Apn1 sorting. If one could successfully find candidates for transiently interacting partner proteins of Apn1, one can scale up the system by using recombinant Apn1 as a substrate and identify the interacting proteins by mass spectrometry (MS) analyses. In parallel, one can also try to identify functional partner proteins for Apn1 that interact with Apn1 in the matrix by expressing Apn1 with an affinity tag in yeast cells, and purify protein complexes involving Apn1 by affinity purification for the affinity tag after isolation and solubilization of mitochondria. After purifying the Apn1 interacting protein complex from the matrix, one can conduct biophysical/structural analyses on the Apn1 complex with other DNA repair proteins/factors that are involved in either Apn1 trafficking from MIM to matrix or are functioning as active players in BER pathway. The structural/ biophysical characterization of Apn1-interacting protein complex will be done using XRD. Using the AP endonuclease assay (an *in vitro* functional assays for APE1), the function of the Apn1 partner proteins can be tested.

CONCLUSION

The mitochondrial genome typically encodes only a small number of proteins, most of which are polytopic membrane proteins of the respiratory chain complexes. It is assumed that in the event of oxidative and alkylated base damages, APE1 and some other repair proteins of BER pathway are transported into the mitochondrial matrix to perform DNA repair synthesis process. However, little is known about how APE1 and other DNA repair proteins are targeted to mitochondria and sorted to matrix. This review explicitly presented the molecular, cellular and structural basis for APE1 trafficking from cytoplasm to mitochondria. The structure based mechanistic details of proteins/pathways involved in Apn1 trafficking from the MIM to matrix and in mitochondrial DNA repair are not yet well understood, in particular owing to unavailability of structures at atomic resolution (of Apn1 complex with interacting proteins).

REFERENCES

1. Dakal TC, Kala D, Dhiman G, Yadav V, Krokhotin A, Dokholyan NV. Predicting the functional consequences of non-synonymous single nucleotide polymorphisms in IL8 gene. Sci Rep. 2017; **7**: 6525.

2. Dakal TC. Methodologies for ascertaining the anti-cancer potential of DNA G-quadruplex stabilizers. J Bioinform, Genomics Proteomics. 2018; 3: 1028.

3. Dakal TC, Dhabhai B, Agarwal D, Gupta R, Nagda G, Meena AR, et al. Mechanistic basis of co-stimulatory CD40-CD40L ligation mediated regulation of immune responses in cancer and autoimmune disorders. Immunobiology (Accepted for publication). 2019a.

4. Dakal TC. Identification of BAP1-Associated MicroRNAs and Implications in Cancer Development. Int J Cancer Sci Ther. 2019b; 1: 1-4.

5. Sharma A, Dakal TC, Ludwig M, Fröhlich H, Mathur R, Reutter H. Towards a Central Role of ISL1 in the Bladder Exstrophy–Epispadias Complex (BEEC): Computational Characterization of Genetic Variants and Structural Modelling. Genes. 2018; 9: 609.

6. Sharma A, Osato N, Liu H, Asthana S, Dakal TC, Ambrosini G, et al. Common genetic variants associated with Parkinson's disease display widespread signature of epigenetic plasticity. Scientific Reports. 2019; 9: 1-9.

7. Jansen AML, Ghosh P, Dakal TC, Slavin TP, Boland CR, Goel A. Novel candidates in early-onset familial colorectal cancer. Fam Cancer. 2019; 1-10.

8. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 2010; 38: W214-220.

9. Zefang Tang, Boxi Kang, Chenwei Li, Tianxiang Chen, Zemin Zhang. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nuc Acids Res. 2019.

10. Li M, Zhong Z, Zhu J, Xiang D, Dai N, Cao X, et al. Identification and characterization of mitochondrial targeting sequence of human apurinic/apyrimidinic endonuclease 1. J Biol Chem. 2010; 285: 14871-14881.

11. Chattopadhyay R, Wiederhold L, Szczesny B, Boldogh I, Hazra TK, et al. Identification and characterization of mitochondrial abasic (AP)-endonuclease in mammalian cells. Nucleic Acids Res. 2006; 34: 2067-2076.

12. Cesaratto L, Codarin E, Vascotto C, Leonardi A, Kelley MR, Tiribelli C, et al. Specific inhibition of the redox activity of ape1/ref-1 by e3330 blocks tnf-α-induced activation of IL-8 production in liver cancer cell lines. PloS One. 2013; 8: e70909

13. Xie JY, Li MX, Xiang DB, Mou JH, Qing Y, Zeng LL, et al. Elevated expression of APE1/Ref-1 and its regulation on IL-6 and IL-8 in bone marrow stromal cells of multiple myeloma. Clin Lymphoma Myeloma Leuk. 2010; 10: 385-393.

14. Skoko J, Asan A, Woodcock C, Cao J. Loss of PRDX1 increases RAD51 Cys319 oxidation and decreases homologous recombination. Free Radic Biol Med. 2018; 128: S73-S74.

15. Endo T, Yamano K, Kawano S. Structural basis of for the disulfide relay system in the mitochondrial intermembrane space. Antioxid. Redox Signal. 2010; 13: 1359-1373.

16. Barchiesi A, Wasilewski M, Chacinska A, Tell G, Vascotto C. Mitochondrial translocation of APE1 relies on the MIA pathway. Nuc Acids Res. 2015; 43: 5451-5464.

17. Watanabe Y, Tamura Y, Kawano S, Endo T. Structural and mechanistic insights into phospholipid transfer by Ups1–Mdm35 in mitochondria. Nature Communications. 2015; 6: 7922.

18. Zhuang J, Wang PY, Huang X, Chen X, Kang JG, Hwang PM. Mitochondrial disulfide relay mediates translocation of p53 and partitions its subcellular activity. Proc Natl Acad Sci USA. 2013; 110: 17356-17361.

19. Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer HE, et al. The proteome of *Saccharomyces cerevisiae* mitochondria. Proc Natl Acad Sci USA. 2003; 100: 13207-13212.

20. Taylor SW, Fahy E, Zhang B, Glenn GM, Warnock DE, Wiley S, et al. Characterization of the human heart mitochondrial proteome. Nat Biotechnol. 2003; 21: 281-286.

21. Jensen RE, Dunn CD. Protein import into and across the mitochondrial inner membrane: role of the TIM23 and TIM22 translocons. Biochim Biophys Acta. 2002; 1592: 25-34.

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