

The Function of Ubiquitin Protein Ligase E3A and its Roles in Human Diseases

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Received: January 8, 2015

Revised: January 23, 2015

Accepted: January 25, 2015

Published online: March 30, 2015

ABSTRACT

UBE3A (ubiquitin protein ligase E3A), also called as human papillomavirus E6 related protein (E6-AP), functions as a co-activation factor as well as an E3 ubiquitin ligase. Recent studies show that it plays important roles in the pathogenesis of genetic diseases, infectious diseases, cardiomyopathy, and cancers. Here, we reviewed the structure and function of UBE3A and its relation with human diseases.

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Key words: UBE3A; Ubiquitin; Ubiquitination; Human diseases

Zhou XF, Jafari N, Deng SS, Huang C. The Function of Ubiquitin Protein Ligase E3A and its Roles in Human Diseases. *Journal of Biochemistry and Molecular Biology Research* 2015; 1(1): 14-18 Available from: URL: <http://www.ghrnet.org/index.php/jbmr/article/view/1011>

INTRODUCTION

UBE3A (ubiquitin protein ligase E3A), which was also named

human papillomavirus E6 related protein (E6-AP), is one of the important members in the ubiquitin proteasome system. The UBE3A gene is located on chromosome 15, encoding a 100 kDa protein^[1]. The protein UBE3A exists in both the nucleus and cytoplasm. It was detected in many normal human tissues or organs, including prostate, testicles, ovaries, uterus, breast, and brain tissue^[2,3]. UBE3A plays an important role in the biological function of normal cells as well as the development of many human diseases. In this review, we summarize the structure and function of UBE3A and its relation to human diseases.

THE CHARACTERS OF UBE3A GENE

UBE3A gene is located on chromosome 15q11-13 in human being, while it is on chromosome 7 in mice^[1,3]. *UBE3A* coding area is about 60 kb, and the length of mRNA is about 5 kb. As a result of alternative splicing, there are several different *UBE3A* transcripts in cells, and these transcripts encode different proteins. So far, three different UBE3A protein isoforms have been identified. They were named as isoform I, II and III, respectively, and the isoform I is also called as E6-AP. Compared to isoform I, the isoform II and III have 20 and 23 additional amino acids in their N-terminus, respectively. These additional N-terminal amino acids are not essential for the functions of UBE3A. Thus, these different isoforms should possess the same functions^[1].

It was reported that there are two UBE3A-homologous pseudogenes, UBE3AP1 and UBE3AP2 in cells. The nucleotide sequences of these genes are 90-95% identical with that of UBE3A. UBE3AP1 and UBE3AP2 are located on human chromosome 2 and 21 respectively, and their functions remain to be understood^[4].

THE FUNCTIONS OF UBE3A PROTEIN

UBE3A/E6-AP is composed of 865 amino acid residues, with apparent molecular weight about 100 kDa. UBE3A/E6-AP protein has five functional domains, including a HETC domain, an E6 binding domain, a P53 binding domain, three nuclear receptor

response domains and an activation domain^[5-7]. In eukaryotic cells, UBE3A/E6-AP has two functions: hormone-dependent co-activation and ubiquitin protein ligase. The two functions of UBE3A are relatively independent. In another word, its function as a nuclear hormone receptor activator is disposable for its ubiquitin protein ligase activity^[7].

The function of UBE3A/E6-AP as a co-activation factor

It is reported that UBE3A plays an important role in gonadal development, sexual maturation and tumorigenesis. UBE3A is an important co-activator of steroid hormone receptors, such as androgen receptors (AR), estrogen receptors (ER) and some auxiliary regulatory proteins. UBE3A interacts with WW domain binding protein 2 and enhances the activation to AR and ER^[8]. UBE3A regulates the activation of steroid hormone receptors and enhances the activity of the hormone-dependent receptors through its activation domain at the amino acid terminus^[7,9].

In prostate cells, UBE3A regulates PI3K-Akt signaling pathway by co-activation of steroid hormone receptors, promoting cell growth and proliferation while inhibiting apoptosis^[10]. Although the over-expression of Akt or PI3K does not result in tumor formation, they play a good supplementary role for carcinogenic factors associated with tumorigenesis. PI3K or Akt assists tumor growth by inhibiting apoptosis and enhancing the tolerance ability to low oxygen and nutrient deficiency. Thus, UBE3A functions as a co-activation factor to regulate PI3K-Akt signaling pathway, thus participating in tumorigenesis^[9].

The functions of UBE3A as an ubiquitin ligase

UBE3A is an important member of ubiquitin proteasome system (UPS). The key functions of UBE3A as an ubiquitin ligase is outlined in figure 1. UBE3A interacts with the E6 protein of the high-risk human papillomaviruses (HPVs). The E6/UBE3A complex targets

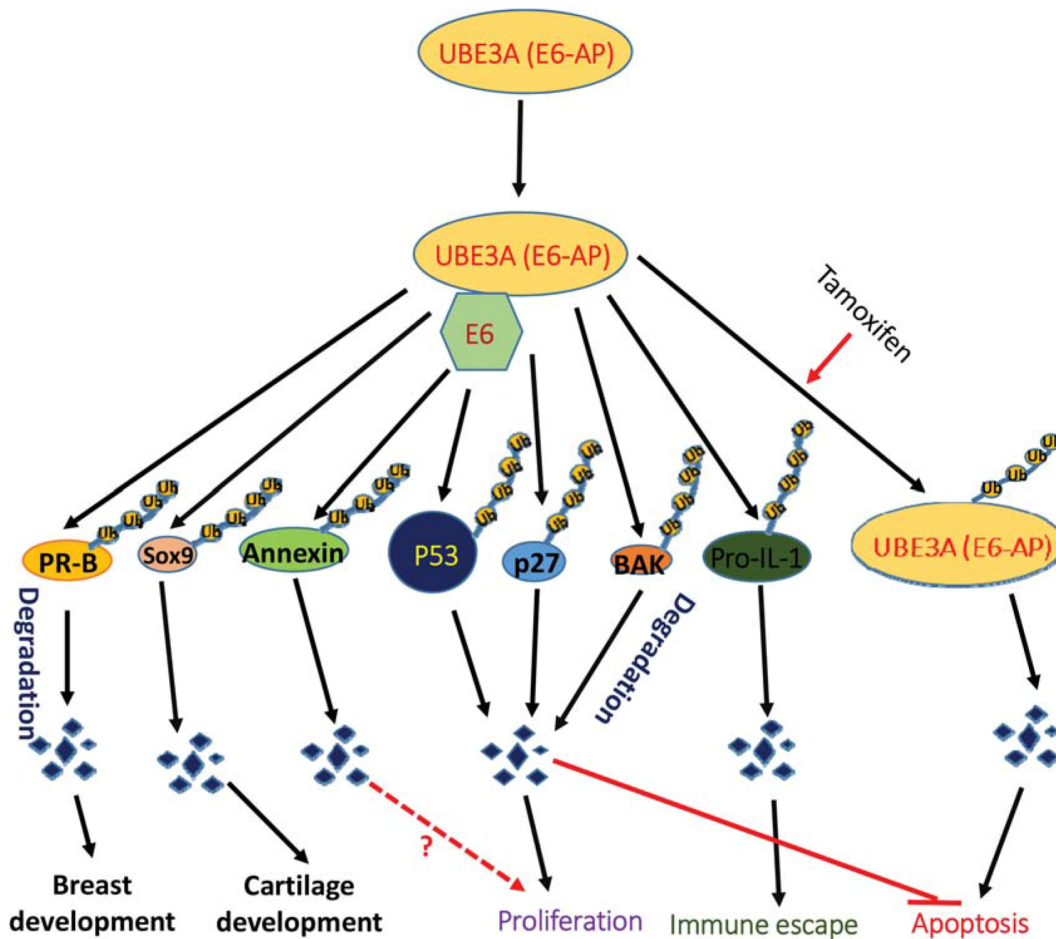


Figure 1 UBE3A regulates proliferation, apoptosis, immune response, and breast and cartilage development through its function as an E3 ubiquitin ligase. The E6 protein activates UBE3A, which in turn ubiquitinates p53, p27 and BAK, respectively, resulting in their degradation, consequently inhibiting apoptosis and promoting cell proliferation. UBE3A undergoes auto-ubiquitination, which is promoted by tamoxifen. UBE3A ubiquitinates pro-interleukin 1 (Pro-IL-1) to escape the immune response. UBE3A also ubiquitinates Annexin, probably regulating cell apoptosis or proliferation. The ubiquitination and degradation of PR-B and Sox9 regulates the development of breast and cartilage.

p53 in cervical cancer, resulting in ubiquitination and degradation of p53^[11]. It was reported that abnormal expression of UBE3A was found in a variety of tumors, such as prostate cancer, cervical cancer and breast cancer^[5], but the specific relationship between the abnormality of UBE3A and the diseases is poorly understood.

UBE3A is regulated by its autoubiquitination and degradation, which is in turn governed by its interaction with the E6 protein^[12]. Over-expression of E6 protein in cells results in a 3-fold decrease in the half-life of E6AP. Also, the half-life of E6AP is shorter in HPV-positive cervical cancer cells than in HPV-negative cervical cancer cells. Tamoxifen (Tam), an ER antagonist widely used for treatment of hormone-responsive breast cancer, promotes UBE3A auto-ubiquitination and degradation^[13]. Tam-mediated E6AP autoubiquitination is required for Tam-mediated anti-breast cancer effect. It is generally thought that the ubiquitin ligase activity plays more important role in the biological function of UBE3A than its co-activation factor activity. For example, UBE3A-mediated ubiquitination and degradation of progesterone receptors (PR-B) is involved in the development of the normal mammary gland; Dysregulation of UBE3A leads to the occurrence and development of breast tumor^[8]. Thus, E6AP is a potential therapeutic target in breast cancer.

A large number of proteins, including BAK (Bcl-2 homologous antagonist killer), c-MYC, Mcm-7 (minichromosome maintenance protein 7), PR-B (progesterone receptor-B protein), were found to be substrates of UBE3A^[14-18]. Ubiquitination of these substrates by UBE3A results in their degradation by the UPS. In recent years, some new substrates of UBE3A were identified, including p27, annexins I, IL- β 1 and SOX9.

P27, an important cell cycle protein kinase inhibitor, plays a crucial role in the regulation of mammalian cell cycle progression^[19]. Carrano *et al.* found that UBE3A interacted with p27 through its C-terminal end and caused p27 ubiquitination, resulting in the p27 degradation by the UPS in cells^[20]. Depletion of UBE3A by shRNA led to an increase in the level of p27, whereas over-expression of UBE3A resulted in depression of p27. These results indicate that UBE3A mediates p27 ubiquitination and degradation^[21].

Annexin A1, a member of annexin family, is associated with the cellular immune response, cell signaling transmission, cell differentiation, cell proliferation and apoptosis, and even tumorigenesis^[22,23]. It was reported that UBE3A ubiquitinated annexin A1 by a Ca²⁺-dependent manner, resulting in degradation of annexin A1 by the UPS^[24]. Annexin A2, another member of annexin family, is abundantly expressed in many cancer tissues and plays an important role in tumorigenesis and cancer progression. Annexin A2 was over-expressed and ubiquitinated in breast cancer tissues^[25], and degradation of annexin A2 is also associated with the UPS in vascular endothelial cells^[26]. However, it is unclear whether degradation of annexin A2 is mediated by UBE3A.

Interleukin 1-beta (IL-1 β), a cytokine generated by mononuclear-macrophages, plays important roles in information transmission, activation, proliferation and differentiation in T and B cells, as well as inflammatory reaction. It was reported that pro-IL-1 β was ubiquitinated and degraded by the UPS in an E6/E6-AP complex-dependent manner, whereas knockdown of E6-AP restored the levels of pro-IL-1 β . Down-regulation of IL-1 by E6-AP could be a key mechanism for the immune escape and consequential tumorigenesis of HPV-infected host cells^[27].

Sox9 (SRY-related high mobility group box gene of 9), a gene involved in early embryonic development, was identified as a testis-determining factor^[28]. Sox9 encodes a key transcription factor

that regulates cartilage formation, especially in collagen protein synthesis^[29]. It positively regulated collagen synthesis in the process of development and maturation of cartilages^[30]. It has been shown that Sox9 interacts with the HECT domain of UBE3A through its HMG domain, and that Sox9 was ubiquitinated in a UBE3A-dependent manner and consequently degraded by the UPS. Also, the level of Sox9 in process of cartilage development was regulated by the ubiquitin-protein ligase function of UBE3A; Abnormal regulation of UBE3A may lead to the occurrence of cartilage diseases^[31].

THE ROLES OF UBE3A IN HUMAN DISEASES

Angelman syndrome

UBE3A is important to maintain the physiological functions of cells^[5], whereas abnormality of UBE3A may contribute to the development of diseases. For example, the mutation and deletion of maternal UBE3A gene can cause Angelman syndrome. The patients with Angelman syndrome are characterized as severe exercise, mental retardation, hypotonia, ataxia, epilepsy, language barriers and special face with great characteristics of mandible and stick out one's tongue, which morbidity is about 1/15,000^[32]. The animal experiments showed that topoisomerase inhibitors unsilenced the paternal UBE3A allele, providing a potential strategy to treat Angelman syndrome.

Prader-Willi syndrome

Prader-Willi syndrome, similar with Angel syndrome, is a neurodevelopmental disorder^[33]. Prader-Willi syndrome is caused by defects or mutations of important fragments of paternal chromosome 15. Unlike Angel' syndrome, which is caused by the loss of function of UBE3A gene, Prader-Willi syndrome is considered a contiguous gene disorder. Although Prader-Willi syndrome is caused by defects in multiple genes, the imprinting of paternal UBE3A by UBE3A antisense transcript (UBE3A-ATS) is involved in the pathological process of this disease^[34]. UBE3A imprinting by UBE3A-ATS inhibits the expression of UBE3A in neurons, and UBE3A imprinting in neurons only requires UBE3A-ATS expression, indicating a role of UBE3A in Prader-Willi syndrome^[35].

Infectious diseases

E6-AP binds to the substrate binding domain of Hsp70/Hsc70 chaperones and enhances the degradation of chaperone bound proteins^[36]. Stresses induce a dramatical increase in the expression of E6-AP, while overexpression of E6-AP protects against endoplasmic reticulum stress-induced cell death. Furthermore, UBE3A was redistributed in microtubule, resulting in inhibition of apoptosis induced by stress. Interestingly, hepatitis c virus (HCV) infection induces UBE3A-mediated ubiquitination and degradation of target proteins, such as HCV core protein. HCV core protein plays an important role in tumorigenesis and progress of liver cancer related with HCV^[37-40].

Cardiomyopathy

Cardiomyopathy, a progressive disease, results from changes of ventricular structure and impairing of cardiac function, which leads to a high mortality. However, the underlying mechanisms of cardiomyopathy remain to be elucidated. Some evidence suggests that cardiomyopathies might be involved in cell apoptosis. It is reported that the transcriptional and translational levels of both UBE3A and p53 significantly increase in response to H₂O₂ treatment. This result suggests a potential role of UBE3A and p53 in myocardial cell apoptosis^[39].

UBE3A PLAYS AN IMPORTANT ROLE IN CANCERS

Cervical cancer

UBE3A is tightly related with tumors. It has been shown that expression of UBE3A increases in cervical cancer tissues, and the level of UBE3A positively correlates with the malignant phenotypes of cervical cancer. Knock down of UBE3A results in suppression in the proliferation of cervical cancer cells while promotion of apoptosis^[40]. Also, p53 was accumulated in HPV-positive cervical cancer cells upon UBE3A knockdown, accompanying with growth inhibition of both HeLa and SiHa lines. These results indicated that high level of UBE3A in cervical cancer cells might trigger tumor progression^[41]. It has been reported that GRIM-19, a cell death regulatory protein, prevent E6/E6-AP complexes from degrading p53, resulting in the accumulation of p53 and consequently promoting apoptosis of cancer cells^[42]. Thus, E6-AP might be a potential therapeutic molecular target in cervical intraepithelial neoplasia^[43].

Prostate cancer

Transfection of LnCap prostate cancer cells with plasmid expressing E6-AP by electroporation promoted the abilities of migration and invasion of prostate cancer cells, indicating the role of E6-AP in the invasion and migration of prostate cancer cells. Over-expression of UBE3A also strengthened the proliferation of prostate cancer cells with more cells in S phase, and enhanced the size of prostate cancer cells^[10]. In prostate cancer tissues, the level of UBE3A is negatively correlated with those of androgen receptors (AR). Also, the interaction of UBE3A with AR and the increase in AR protein level in the prostate glands of UBE3A null mice suggest the role of UBE3A in regulating AR degradation^[44].

Breast cancer

It is well known that UBE3A regulates a variety of biological process, such as transcription, signal transduction, cell survival, cell cycle control, and DNA repair^[45]. We have showed that the expression levels of UBE3A in breast cancer tissue samples are 2-fold higher than those in the adjacent normal tissues, as determined by restriction fragment differential display polymerase chain reaction (RFDD-PCR) and matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF-TOF MS) combined with two-dimensional polyacrylamide gel electrophoresis. UBE3A was differently expressed in human breast cancer tissues, which was expressed in root distribution-like breast cancer cells more than nest-like cancer cells^[46]. Ramamoorthy *et al.* also confirmed that abnormal expression of UBE3A could lead to occurrence of breast cancer^[47]. Furthermore, the level of UBE3A negatively correlates with the expression of ER in breast cancer tissues, probably because of the ubiquitination of ER by UBE3A. Tamoxifen treatment reduced expression level of UBE3A, while enhanced that of some relevant factors with UBE3A, including cytochrome c, Bax and p21, suggesting that UBE3A could be one of therapeutic targets for breast cancers^[45].

CONCLUSIONS

UBE3A regulates cell proliferation, apoptosis, immune escape and the development of breast and cartilage through ubiquitinating a variety of substrate proteins, such as p53, p27, BAK, pro-IL-1, PR-B and Sox9, as well as its function as a steroid hormone receptor coactivation factor.

Initially, it was found to be related with cervical cancer involved in HPV, but more and more evidence show that it is associated with many human diseases, including Angel's syndrome, Prader-Willi syndrome, infectious diseases, cardiomyopathy, cervical cancer, prostate cancer and breast cancer. UBE3A could be a potential therapeutic target for some diseases, such as cancers. However, detailed molecular mechanisms and its precise roles in human diseases remain to be defined.

ACKNOWLEDGEMENTS

This work is supported by an American Cancer Society Research Scholar Grant (RSG-13-184-01-CSM), and a start-up fund from Markey Cancer Center, University of Kentucky (to C.H.). S.D. is a visiting scholar from North Sichuan Medical College supported by China Scholarship council.

CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

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