International Journal of Biology and Life Sciences
ISSN: 2957-9511 | Vol. 3, No. 3, 2023

The Progress of Research in Molecular Structure and Function of Histone Deacetylase 6

Xiaoyan Zhu, Wenke Zhou *

School of Environmental Ecology and Biological Engineering, Wuhan Institute of Technology, Wuhan, Hubei 430205, China
*** Corresponding author:** Wenke Zhou (Email: zhouwkwit@sina.cn)

Abstract: Histone deacetylases (HDACs) family play an important role in the regulation of cell expression. Histone deacetylase (HDAC) 6 is a special member of the family of histone deacetylases proteins. The specific molecular structure of histone deacetylase 6 determines that HDAC6 can specifically deacetylate non-histone proteins and their substrates, and regulate cell growth, metastasis and apoptosis. However, numerous studies have shown that HDAC6 does have a significant impact on certain diseases, such as cancer, central nervous system diseases, etc. Some studies have even found that HDAC6 may extend life span. This paper mainly talks about the structure and function of HDAC6 from several aspects, including the molecular structure of histone deacetylase 6, the effect of HDAC6 inhibitor, the effect of histone deacetylase 6 on Drosophila longevity and the effect of HDAC6 on nervous diseases. Hoping the introduction of this paper can let people understand HDAC6 so that more researchers can pay attention to HDAC6 and do some studies about it, and then lead to more and better research results.

Keywords: Histone Deacetylase 6; HDAC6 Inhibitor; Drosophila Longevity; Tumor.

1. Introduction

In eukaryotes, histone is one of the important components of chromosomes in the cell nucleus. Acetylation and deacetylation of histone are important regulation methods in the process of gene expression and play a key role in gene transcription [1]. Histone deacetylases (HDACs) are a group of enzymes that regulate gene expression, cell differentiation and survival by affecting the intracellular protein. Histone deacetylase is one of the main regulatory enzymes in the deacetylation of lysine, and deacetylation of lysine is a reversible post-translational modification process, which can affect signal transmission, RNA splicing, chromatin remodeling, protein stability and so on. Histone deacetylases can regulate gene expression through histone deacetylation, and some Histone deacetylases subtypes can also affect the function of non-histone and regulate various cellular pathways. Histone deacetylases inhibitors can affect a variety of cellular effects, like inhibit angiogenesis and induce apoptosis, which has attracted wide attention as an anti-tumor agent [2]. It is known that there are eleven kinds of histone deacetylases in human body, all of which are nuclear proteins except histone deacetylase 6. histone deacetylase 6 (HDAC6) exists in cytoplasm which is well known as an important epigenetic modification protein and it has a special structure and many unique biological functions. It has been proved that histone deacetylase 6 can specifically deacetylate the non-histones and their corresponding substrates, and it also can regulate the cell growth, metastasis and apoptosis through deacetylase-dependent and deacetylase-independent mechanisms [3]. At present, it is known that histone deacetylase 6 is highly expressed in a variety of tumors, and tumors are a major disease which may endanger human life and health. Aim to find a way to treat these diseases, some researchers focus on histone deacetylase 6 gene and expect to find some things helpful. Fortunately, hard work pays off, and a large number of studies have found that histone deacetylase 6 is indeed helpful for the treatment of tumors, cancers and other diseases, and is a potential therapeutic target. A large number of studies on the function of histone deacetylase 6 gene have been carried out through various biological experiments, most of which are animals with homology to human histone deacetylase 6 gene, such as mice, Drosophila and so on. Among them, Drosophila is the most frequently used experimental animal, because compared with other model organisms, Drosophila has the advantages of easy breeding, simple genome chromosome composition, clear genetic background, easy analysis, and strong reproductive ability, low cost of training advantages. And because of these unique advantages, many well-known experimental results in fruit flies have been revealed, such as embryonic development, circadian rhythm, innate immunity and other achievements have been Nobel Prize in Physiology or Medicine [4]. This is because the proteins that carry out functions during Drosophila growth and development share about 61% homology with human proteins, including the HDACs gene. Therefore, studying the structure and function of HDAC6 gene in drosophila can not only save the research cost, but also improve the research efficiency. In particular, recent studies on Drosophila longevity have found that the histone deacetylase 6 gene may be associated with longevity, some researchers have used CRISPR/Cas9, miRNA and other gene editing techniques to knock out the histone deacetylase 6 gene in Drosophila and found that knocking out the histone deacetylase 6 gene in drosophila can significantly extend the lifespan of Drosophila. Other studies have found that HDAC6 has an effect on nerve cells and has a significant effect on the treatment of diseases of the nervous system. Because the research's on HDAC6 are numerous and miscellaneous, in order to facilitate people to understand HDAC6 more specifically, this paper summarizes some existing research results of HDAC6, in order to help the people to understand the HDAC6 and hope that this paper can be helpful for researchers in following-up research on HDAC6.

2. The Molecular Structure of HDAC6

HDACs proteins are divided into two major classes, class I

and Class II, in which class I HDACs are proteins that localize only to the nucleus, whereas Class II HDACs are larger proteins that shuttle between the cytoplasm and the nucleus. Histone deacetylase 6 is a member of the HDACs II family which predominantly localized in the cytoplasm of various cell types and is the only HDAC subtype with two tandem catalytic domains. HDAC6 contains two N-terminal deacetylation domains, a c-terminal ubiquitin-binding domain, a strong nuclear output signal, and eight consecutive repeats of tetradecapeptide [5]. HDAC6 is predominantly present in the cytoplasm, figure I is the result of nuclear export signals and the SE14 motif, and when cell proliferation is arrested, a portion of the protein translocates into the nucleus due to its nuclear localization signal at the N terminus [6].The histone deacetylase 6 gene was located in the region of p11.22-23 on X chromosome, with a length of 21923 bp. Encoded by 28 exons ranging from 41 bp to 677 bp, an open reading frame at the 5' end of the gene reveals a promoter of Tata and CCAAT cassette (absent) , which contains the long CpG island of 1 kb. In terms of protein structure, HDAC6 has two unique catalytic regions: the first catalytic region is H216A which starts at 215 amino acids, and the second catalytic region-H611A which starts at 610 amino acids. Both catalytic regions are highly homologous and contain a zinc finger structure bound to ubiquitin [7]. the c-terminal zinc finger region can bind to both polyubiquitinated misfolded proteins and actin. There is a repeat sequence of a tetradecapeptide between the c'-terminal second catalytic region-H611A-and the carboxy-terminal ubiquitin-binding region, a unique structure that determines the deacetylation-targeted localization of HDAC6 to stabilize HDAC6 in the cytoplasm [1,8]. The active site of deacetylation in vitro is mainly located in the second catalytic region of C' terminal, namely H611A. In vivo, HDAC6 was mainly distributed in cytoplasm, perinuclear structure and 1eadingedge, and highly expressed in heart, liver, kidney and other internal organs [9].

Fig 1. Schematic representation and functional domains of human HDAC6. HDAC6 is the only HDAC with two tandem deacetylase domains (DD1 and DD2) including catalytic activity. A nuclear export signal (NES) prevents the accumulation of the protein in the nucleus and the Ser-Glu-containing tetrapeptide (SE14) region ensures stable anchorage of the enzyme in the cytoplasm. The nuclear localization signal (NLS) translocates HDAC6 into nucleus. The linker (dynein motor binding, DMB) between both CATs can bind to dynein and the high affifinity ubiquitin-binding zinc fifinger domain (BUZ).

3. Action of Histone Deacetylase 6 Inhibitors

The degradation of proteins is one of the important functions of cells, mainly occurring in proteasomes and sedimentary bodies. The accumulation of misfolded abnormal proteins is an important cause of cell apoptosis [2]. In normal cells, misfolded proteins are labeled by ubiquitin and then degraded in proteasomes, while ubiquitinated proteins that escape degradation will aggregate in the sediment for degradation, and this process that cannot be separated from the role of histone deacetylase 6. This is due to the unique structure of the HDAC6, which has a ubiquitin binding domain on its C-terminal that can bind to the misfolded protein, then transfer and accumulate misfolded proteins to the sediment and respond to misfolded proteins to prevent cess apoptosis [2,10]. And because of its unique structure and function, HDAC6 has received more and more attention and has become a potential therapeutic target for a variety of diseases. To put it bluntiy that the main concern is its role in tumors and the central nervous system. The histone deacetylase 6 inhibitors can reduce the activity of sediment through various pathways, mainly used in the treatment of tumor and protection of the central nervous system protection [11]. At present, the histone deacetylase 6 inhibitors found in research mainly include ACY-1215, Tubacin, and Tubastatin A and so on [12]. ACY-1215 can be used to treat multiple myeloma (MM). Combined with bortezomib, it can trigger synergistic anti MM effects and significantly reduce the viability of tumor cells [13]. The second one, Tubacin is a highly selective HDAC6 inhibitor with the chemical formula $C_{41}H_{43}N_3O_7S$ and relative molecular weight 721.86 [14]. Due to its high selectivity for HDAC6, it can block sphingomyelin synthesis and inhibit HDAC6 mediated α- Deacetylation of tubulin [15]. Tubacin can slow down the proliferation of tumor cells by blocking the action of autophagosomes, and can improve the sensitivity of tumor cells to other therapeutic drugs, enhancing the therapeutic effect [16]. Currently, Tubacin is mainly used for the treatment of malignant glioma, acute lymphoblastic leukemia (ALL), EB virus associated Burkitt lymphoma, and other tumors [2]. Tubacin has an inhibitory effect on the growth of glioma cells, can significantly inhibit the proliferation and movement of U251 cells, and increase the apoptosis rate of tumor cells. Tubacin has a good anti glioma effect by inducing the increase of ROS concentration in malignant glioma cells and killing malignant glioma cells [17]. Tubacin can also inhibit the proliferation of pre B cells and T cells, and inhibit the growth of ALL cells in a dose dependent manner, and play a role under IC_{50}. Tubacin can chelate with zinc ions at the catalyst site to inhibit the activity of HDAC6, destroy the molecular complex of HDAC6 and kinetin, and enhance the acetylation of α-tubulin, in collaboration with bortezomib, can also specifically induce apoptosis in ALL cells without affecting normal T lymphocytes [18]. Furthermore, Tubacin combined with vincristine or bortezomib can further weaken the viability of tumor cells and enhance the efficacy of other anti-tumor drugs. The third one, Tubastatin A has a high selectivity for HDAC6, its chemical formula is $C_{20}H_{21}N_3O_2$, and its relative molecular weight is 335.4. Tubastatin A has higher specificity and lower lipophilicity than Tubastatin [2]. Tubastatin A inhibits NF around the infarct- κB nuclear transfer, and it affects the expression of TNF-α and IL-6, while also preventing neuronal degeneration, stimulating axonal growth, and having a protective effect on the nervous system. Tubastatin A has anti-inflammatory and anti-rheumatic effects by inhibiting IL-6 and TNF- α. The release of inflammatory cytokines plays an anti-inflammatory and anti-rheumatic role [19]. HDAC6 can affect the expression of primary cilia, induce massive proliferation of cholangiocarcinoma cells, and promote the occurrence and development of cholangiocarcinoma. Tubastatin A, as a specific HDAC6 inhibitor, has a good effect on inhibiting the growth of cholangiocarcinoma [20]. In the immune function of the body, Tubastatin A can regulate the percentage of granulocytes and lymphocytes in the blood, reduce the level of local and systemic proinflammatory

cytokines, reduce the occurrence of acute liver injury and macrophage apoptosis, increase the number of circulating monocytes, enhance the phagocytosis of phagocytes, and repair immune function. Generally speaking, HDAC6 inhibitors have good application prospect and research and development potential in the treatment of tumor and central nervous system diseases due to the special structure of HDAC6 and its important roal in cell expression.

4. Effect of HDAC6 Gene Knockout on Drosophila Melanogaster

It is known that HDAC6 which plays an important role in the process of biological immune regulation and gene expression modification actually affects the life and health of organisms. Based on this proposition, a research team used Drosophila melanogaster as experimental material to study the effect of HDAC6 on the life span of old Drosophila melanogaster. Because HDAC6 gene exists in all organisms, the research group observed its influence on Drosophila by knocking out or knocking down HDAC6 in Drosophila. Their experiment found that knocking out the HDAC6 gene of Drosophila melanogaster would induce its overall change. When the HDAC6 of Drosophila melanogaster is systematically knocked down, compared with normal Drosophila melanogaster, it can be observed that the whole life span of Drosophila melanogaster with systemic HDAC6 knock-down is longer. Specific knockdown of HDAC6 in the brain of Drosophila melanogaster can further prolong the life span of Drosophila melanogaster compared with systemic knockdown of HDAC6, especially in aging Drosophila melanogaster, which significantly prolongs the life span of Drosophila melanogaster [21]. The tube climbing experiment is a classic method to measure the locomotion ability of fruit flies. After all the fruit flies are knocked off the bottom of the tube, the crawling time for the top five of each strain to climb to the 6 cm scale line is recorded. The shorter the climbing tube, the better the motor ability of fruit flies. Through the tube climbing experiment, it was found that the whole body knocking down HDAC6 can obviously improve the motor ability of Drosophila melanogaster, and the motor ability of Drosophila melanogaster with brain-specific knocking down HDAC6 is stronger[22].In this experiment, the HDAC6 of old Drosophila melanogaster was analyzed by gene transcriptome, and the changes of gene transcription during the aging process of normal Drosophila melanogaster in control group and Drosophila melanogaster in experimental group were explored. In the control group, with the aging of Drosophila melanogaster, most of the up-regulated genes are related to innate immune and inflammatory response functions, while most of the down-regulated genes are related to mitochondrial function and chitin metabolism. The researchers found that in the Drosophila group, knocking down HDAC6 can significantly down-regulate the transcription expression of innate immune and inflammatory response genes in normal Drosophila, thus reducing the inflammatory response in Drosophila head. Researchers believe that HDAC6 can positively regulate immune-related genes during the aging process of Drosophila, while knocking down HDAC6 can significantly inhibit the over-activation of innate immune and inflammatory response genes. It is speculated that HDAC6 may be a direct target to regulate healthy life span, especially in brain and other nerve tissues, and play an anti-aging role by inhibiting the expression of inflammatory genes. However, it is still unknown which target genes in immune and inflammatory signaling pathways HDAC6 regulates life span and whether it plays a similar role in other tissues, which requires further research by researchers.

5. Effects of HDAC6 on Neurological Diseases

Prions virus are a class of hydrophobic proteins that infect animals and have no immunity in host cells, which can cause central nervous system diseases. The biggest threat to humans is that prions virus cause degeneration of the central nervous system in humans and livestock, which can eventually lead to death. The World Health Organization has named prion virus diseases and AIDS as the century`s most dangerous diseases. In order to solve these diseases, the scientists carried out a series of experiments. There have been experimental findings that HDAC6 may be a therapeutic target for prion diseases and other neurodegenerative disorders. Because HDAC6 contains a ZnF-UBP domain in addition to its deacetylase domains, allowing it to bind to and facilitate the transportation of ubiquitinated misfolded proteins aggregated at the perinuclear region of cells to form aggresomes which are considered to be key organelles in the clearance of cytotoxic protein aggregates associated with neurodegenerative diseases [23]. HDAC6 promotes the fusion of autophagosomes and lysosomes. Many studies have demonstrated that HDAC6 is required for autophagic degradation of misfolded proteins in neurodegenerative diseases, and HDAC6 influences both recruitment of autophagyspecifific proteins to the aggregates and the lysosomal dynamics. The autophagy is essential for maintaining the health and function of normal cells, but the importance of this process is even more evident in neurodegenerative disorders [24]. the research shows that HDAC6 can protect neurons from PrP106-126 toxicity by regulating the autophagy system and the PI3K- Akt-mammalian target of rapamycin (mTOR) axis. PrP106-126 alters the expression and location of HDAC6. PrP106-126 treatment induced a rapid increase of HDAC6 and stimulated the relocation of HDAC6 from the cytoplasm to aggregate at the perinuclear region which might facilitate the clearance of toxic peptide as HDAC6 was co-localized with FITCPrP106-126.Overexpression of HDAC6 attenuated the cytotoxicity of PrP106-126, whereas that blockage of HDAC6 activity or expression exacerbated the cytotoxicity of PrP106- 126. HDAC6 can control autophagosome maturation essential for ubiquitin-selective quality-control autophagy, and that HDAC6 deficiency results in impaired autophagic flflux and accumulation of autophagosomes. overexpression of HDAC6 alone did not induce autophagy, whereas overexpression of HDAC6 signifificantly induces autophagy in neurons exposed to PrP106-126 stimulation. This suggests that HDAC6 may act as a stress regulator to protect neuronal cells via autophagy when exposed to PrP106-126 or similar insults and/or stressors.[24] we showed that overexpression of HDAC6 in neurons slightly reduced the phosphorylated mTORC1 and phosphorylated p70S6K in response to PrP106-126 stimulation, and conversely, knockdown of HDAC6 significantly increased these molecules. Consistent with this, the autophagy marker LC3-II increased when HDAC6 was overexpressed in neurons, but LC3-II protein levels signififi-cantly decreased in HDAC6-defificient

neurons exposed to PrP106- 126. The study showed that overexpression of HDAC6 and the subsequent activation of autophagy may partially involve the downregulation of phosphorylated mTOR, but the negative regulation of phosphorylated mTORC1 by HDAC6 is possibly essential for autophagy under PrP106-126 stimulation.[24] These results indicate that PrP106-126 reduced phosphorylated Akt, however, overexpression of HDAC6 could restore phosphorylated-Akt. In addition, knockdown of endogenous HDAC6 aggravated the reduction of phosphorylated Akt and cell viability under PrP106-126 stimulation, but treatment with IGF-1, a physiology activator of Akt, prevented PrP106-126- induced neuronal cell death, indicating a critical role of HDAC6 in the activation of Akt and the importance of activated Akt combating the cytotoxicity of the prion peptide [25].Collectively, these results demonstrate that in addition to activation of autophagy, HDAC6 protects neurons from PrP106- 126- induced neuronal death through the activation of Akt.

6. Conclusion

In this thesis, we focus on the specific molecular structure of HDAC6, the effect of HDAC6 inhibitor, the effect of HDAC6 knockout on Drosophila and the effect of HDAC6 on neurological diseases, the important function of HDAC6 in the process of cell expression modification and the effect of HDAC6 inhibitors on the medical and health fields of tumor and other diseases were introduced, effects of HDAC6 gene on life span and exercise capacity of Drosophila melanogaster and effects of HDAC6 on biological central nerve cells. It is known that HDAC6 is involved in the regulation of gene transcription and other important cell pathways. The inhibitors of HDAC6 can be used in the treatment of tumor and cancer. Histone deacetylase 6 may be an important drug target, in the near future, HDAC6 gene knockout may extend the life span of Drosophila melanogaster, or it may be possible to realize the extension of life span of other organisms and even the dream of longevity of human beings. Histone deacetylase 6 also has a roal in neurological diseases, and may have an effect on the treatment of mental diseases such as Alzheimer's disease. All these results indicate that HDAC6 is of great research value and significance, and we believe that with the further study of HDAC6, tumors will be completely conquered in the near future, and cancer will no longer be a threat to human beings, we will have a longer life, a healthier body, a longer time to achieve the meaning and value of life.

References

[1] Li Bin, Wang Jiming. Histone deacetylase 6 (HDAC6) and tumor. Journal of Clinical and Experimental Medicine, 2006, 5(4).

[2] Shi Xuhan, Wei Wenbin. Characteristics and application of HDAC6 inhibitors. Chinese Journal of Medical Frontiers, 2017,9(2).

[3] Chen Guozhen, Tian Jie. Histone acetylase and deacetylase and embryonic development. International Journal of Reproductive Health, 2008.

[4] Han Yuting, Xu Bowen, Li Yutong, et al. Frontier Techniques for Gene Regulation of Model Animal Drosophila. Genetics, 2022,44(1).

[5] Deng Fangjing, Zhu Jeff, Wu Xiongfei. Inhibition of HDAC6 can enhance autophagy and alleviate renal ischemia reperfusion injury. Journal of Medical Research, 2022,52(10).

[6] Yingxiu Li,Donghee Shin,So Hee Kwon.Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes [J]. FEBS Journal, 2012.

[7] Wendy, Yu Xiujun, Guo Yansu. Histone deacetylase and autophagy [J]. Shandong Pharmaceutical, 2011.

[8] Bertos NR, Gilquin B, Chan GK, et al.Role of the tetradecapeptide repeat domain of human histone deacetylase6 in cytoplasmic retention. J Biol Chem,2004,279(46).

[9] Hideshima T,Bradner JE,Wong J,et al.Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor actitumor activity in multiple myeloma. Prac Natl Acad Sci U S A,2005,102(24).

[10] Kawaguchi Y, Kovacs JJ, Mclaurin A,et al.The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress.Cell, 2003, 115 (6).

[11] Nawrocki ST,Carew JS,Pion MS,et al.Aggresome disruption:a noval strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells.Cancer Res,2006,66(7).

[12] Gong Shuwen, Shen Shuijuan, Zhang Sumei, et al. Study on the inhibitory effect of HDAC6 inhibitors on lupus nephritis in mice. Modern Practical Medicine, 2019.

[13] Wang N, Bartlow P, Ouyang Q,et al.Recent advances in antimultiple myeloma drug development.Pharm Pat Anal, 2014, 3(3).

[14] Haggarty SJ, Koeller KM, Wong JC,et al.Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediate dtubulin deacetylation. Proc Natl Acad Sci USA, 2003,100(8).

[15] Butler KV, Kalin J, Brochier C, et al.Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A.J Am Chem Soc,2010,132(31).

[16] Watson GW,Wickramasekara S,FangY,et al.HDAC6 activity is not required for basal autophagic flux in metastatic prostate cancer cells.Exp Biol Med(Maywood),2015,241(11).

[17] Ling Xiaozheng, Lu Haozhong, Li Qifeng, et al. Experimental study on the inhibitory effect of Tubacin on the growth of glioma cells. Chinese Journal of Neurosurgery, 2016,32(5).

[18] Zhang Wenbin, Wang Yao, Jiao Fangzhou, et al. Research progress of histone deacetylase 6 in related diseases. Journal of Difficult Diseases, 2019.

[19] Ma Lin, Liu Wei. The therapeutic effect of effective ingredients and their compatibility of traditional Chinese medicine on experimental rheumatoid arthritis. Liaoning Journal of Traditional Chinese Medicine, 2015.

[20] Zhou Yongbin, Zhang Yanzhong, Chen Boyi, et al. Progress in epigenetics of cholangiocarcinoma. World's Latest Medical Information Digest, 2017.

[21] Yu Wei, Han Jingdong. Immunosuppression induced by brain-specific HDAC6 knockdown improves aging performance in Drosophila melanogaster. Phenomics, 2022.

[22] Qi Jiancheng. The effect of salicin on the lifespan of Drosophila melanogaster and its mechanism exploration. Master's thesis of Sichuan Agricultural University, 2019.

[23] Nawrocki ST, Carew JS, Maclean KH, et al. Myc regulates aggresome formation, the induction of Noxa, and apoptosis in response to the combination of bortezomib and SAHA. Blood,2008.

[24] Ting Zhu, Deming Zhao,Zhiqi Song,et al.HDAC6 alleviates prion peptide- mediated neuronal death via modulating PI3K-Akt-mTOR pathway. Neurobiology of Aging,2015.

[25] Simon D,Herva ME, Benitez MJ,et al.Dysfunction of the PI3K-Akt-GSK-3 pathway is a common feature in cell culture and in vivo models of prion disease.Neuropathol Appl Neurobiol, 2014.