

Review Article

# The Role of Hax-1 in Neuronal Death

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### Abstract

HS-1 associated protein X-1 (Hax-1), an intracellular protein, with molecular weight of 35-kDa is encoded by the Hax-1 gene. It was first discovered as a binding partner of hematopoietic cell-specific protein 1 (HS-1), a protein involved in the maturation of T cells, which indicates involvement of Hax-1 in immune response. In addition to its involvement in immune response, Hax-1 is recently found to be involved in cell apoptosis. Hax-1 interacts with a number of intracellular substances including caspase-3, caspase-9 and the mitochondrial proteases to inhibit apoptosis and promote cell survival and proliferation. It has been found that Hax-1 plays an important role in the development of the central nervous system and the pathophysiology of some neurological diseases. Thus, this molecule may represent a new target for therapy of some neurological diseases with Hax-1 dysfunction. However, the mechanism of action of Hax-1 in these processes remains unclear. This review aims to summarize the current knowledge regarding the role of Hax-1 in neuronal death.

**Keywords:** Hax-1; Ischemia; Apoptosis; Caspase; Traumatic brain injury; Epilepticus

## Introduction

Hax-1 was firstly identified in B cell signal transduction by a yeast two-hybrid assay [1-3]. The prototypical Hax-1 is a 35kDa protein, but its size varies from ~26 to ~35kDa due to alternative splicing patterns of the Hax-1 gene [1,4]. Hax-1 protein contains two putative Bcl-2 homology (BH1 and BH2) domains, a PEST motif (proline, glutamic acid, serine, threonine), and a COOH-terminal transmembrane (TM) domain, which contains several protein-binding regions. The NH2-terminal of Hax-1 has an acid box (amino acids 30-41) with unknown function, composed mostly of glutamic and aspartic acids [4]. The putative PEST sequence of Hax-1 (amino acids 104-117) suggests its rapid degradation (Figure 1). Due to its homology and structural similarities to the Bcl-2, it was thought as a novel protein that regulates apoptosis and promotes cell survival [5,6]. The Hax-1 is mainly localized in mitochondria, the endoplasmic reticulum and the nuclear envelope [3], which indicates different functions of Hax-1. The studies have found that Hax-1 interacts with various proteins to regulate cell apoptosis [7,8], Ca<sup>2+</sup> homeostasis [5,9], cell mobility, migration [10,11], transcript stability and transport [12-14]. Although Hax-1 plays key roles in a variety of cellular functions mentioned above, in this review, we focus on the anti-apoptotic property of Hax-1 and its involvement in neuronal degeneration and apoptosis.

## Expression of Hax-1

Hax-1 is ubiquitously but differentially expressed among human and rodent tissues [15]. The mRNA of Hax-1 is highly expressed in the skeletal muscles, heart, colon and testis, whereas relatively low expressed in the kidney, liver, brain, pancreas, lung, and placenta in human [15,16]. Mouse Hax-1 shares about 86% identities to human Hax-1 [17] suggesting a similar functional importance in human. However, in contrast to humans, testis has the highest Hax-1 mRNA level compared to all other tissues in rat [1]. The Hax-1 mRNA levels are about 15 times higher in rat testis than that of the lungs, brain in rat, whereas there are intermediate levels of mRNA observed in rat

liver, heart, and skeletal muscle [1]. Similar to rat, the highest levels of Hax-1 mRNA are found in mouse testis, followed by kidney and liver, while moderate levels are found in the mouse's brain and heart, and the lowest levels are in mouse's lung, spleen, and skeletal muscle [17]. Notably, there is a discrepancy in Hax-1 expression between the amounts of Hax-1 mRNA and its corresponding protein levels in rat or in mouse. For example, although Hax-1 mRNA levels are significantly different among testis, liver, and brain in rat, protein analysis assays indicated that protein levels of Hax-1 were approximately same in all three tissues [1]. Discrepancies in Hax-1 expression were also found in mouse's organs [17]. Mouse heart showed high expression of Hax-1 mRNA, whereas protein expression of Hax-1 is only on a comparable level to its level in spleen and lung. In mouse brain, the mRNA levels of Hax-1 are moderate, whereas the protein levels of Hax-1 are considerably high, and high level of Hax-1 mRNA in mouse testis does not match the moderate levels of its protein [17]. These discrepancies suggest that there exists a tissue-specific post-transcriptional or post-translational regulation of the Hax-1 gene, such as transcript stability, translation efficiency and protein stability, in rats and mice [4,15]. We anticipate these discrepancies also exist in human in a similar or different pattern. There are some studies on the expression of Hax-1 in rodent central nervous system. Most of these studies indicate that Hax-1 is primarily expressed by neurons [18,19]. Shi et al. reported that Hax-1 was expressed by neurons, but was undetectable in astrocytes and microglia [19], and similarly, Rami et al. also found Hax-1 in neurons and only very low levels of Hax-1 in astrocytes and microglia [18]. In consistent with previous studies, we found that Hax-1 was highly expressed in neurons of mouse cortex,



Figure 1: Domain structure of human Hax-1 protein.

striatum, brain stem and cerebellum, and there were no differences in the protein level of Hax-1 among these areas [20]. The widely and highly expressions of Hax-1 in brain may suggest its important role in regulation of neuron functions.

## The Molecular Targets Involved in Anti-Apoptotic Function of Hax-1

### Caspase-9 and caspase-3

Caspase-9 is an important initiator caspase, and activation of caspase-9 promotes the activation of death effectors caspase-3, -6 and -7 leading to cell apoptosis. Recent studies have found that Hax-1 executes its anti-apoptotic function by interacting directly with caspase-9 in the mitochondria at an early stage of apoptosis [6,21]. In a cell free caspase activation system, recombinant Hax-1 showed inhibitory effect on the processing of both caspase-9 and caspase-3 in a dose-dependent manner [6]. Both caspase-9 activation and cell death induced by hypoxia/reoxygenation (H/R) were significantly attenuated by overexpression of Hax-1, and knockdown of Hax-1 gene with Hax-1 siRNA dramatically enhanced cell death under H/R condition [6]. Furthermore, the subcellular locations of these two proteins were changed after H/R: there existed enhanced colocalization of the Hax-1 and caspase-9 in mitochondria, and partial translocation of caspase-9 [6]. These results suggest that interaction between Hax-1 and caspase-9 in mitochondria inhibits caspase-9 activation, which promotes cell survival. The interaction between caspase-9 and Hax-1 is through direct binding of caspase-9 to its binding domain, which is the amino acid sequence of 175 to 206 in Hax-1 [6]. This is an important finding because the inhibitor of apoptosis proteins (IAPs) was previously thought the only class of protein to directly inhibit caspase-9 [21]. Additionally, it was found that caspase-3 cleaves Hax-1 at Asp<sup>127</sup> to execute apoptosis, and overexpression of Hax-1 significantly inhibited the activity of caspase-3 and therefore suppressed cell apoptosis [8].

### High temperature-regulated A2 (HtrA2) and X-linked inhibitor of apoptosis (XIAP)

HtrA2, also known as Omi, is a mitochondrial serine protease. HtrA2 processing in mitochondria is required for its normal function. Upon apoptosis, HtrA2 is released to cytosol and then binds and degrades IAPs to execute pro-apoptotic effect [22]. Association between Hax-1 and HtrA2 has been demonstrated to be involved in apoptosis [7,23]. Cilenti et al. reported that Hax-1 was degraded by HtrA2 in mitochondria upon apoptosis stimuli, and Hax-1 expression was reduced significantly during apoptosis [23]. In the presence of inhibitor of HtrA2, cell death and Hax-1 degradation were dramatically suppressed. Furthermore, cell apoptosis was significantly reduced and no Hax-1 degradation was observed in HtrA2 mutant cells. All these results indicate that the pro-apoptotic property of HtrA2 causes Hax-1 degradation leads to suppress the anti-apoptotic effect of Hax-1. Conversely, Chao et al. demonstrated Hax-1 is essential to inhibit apoptosis in lymphocytes and neurons by interacting with HtrA2 [7]. The interaction between Hax-1 and mitochondrial proteases, Parl (presenilin-associated, rhomboid-like) and HtrA2, is required to suppress apoptosis [7]. This interaction allows Hax-1 to present HtrA2 to Parl, and thereby facilitates the processing of HtrA2 to the active protease. Once processed, the mature HtrA2 is released into the intermembrane space of mitochondria and

provides protection from apoptosis by inhibiting the accumulation of pro-apoptotic Bax. Furthermore, XIAP is a protein of IAP family, which prevents apoptosis by interacting and inhibiting caspase-3, -7 and -9. Kang et al. identified XIAP as an interactor of Hax-1, and also determined the binding domain and binding affinity of Hax-1 to XIAP. Hax-1 formed a complex with XIAP and significantly inhibited ubiquitination of XIAP, which increases XIAP level. Hax-1-XIAP complex significantly enhanced cell survival in the HEK 293T cells [24]. Therefore, inhibition of XIAP degradation is one of the mechanisms in Hax-1-mediated anti-apoptosis. In addition to interacting with above mentioned molecules, Hax-1 also inhibits cell apoptosis and promotes cell survival by stabilizing mitochondrial membrane potential [25] and interacting with other partner like PrP [26]. Recent reports show that Hax-1 also regulates contractility and calcium cycling in the cardiac myocytes [9] implicating its importance in calcium homeostasis in cell survival. Although these mechanisms were not described in neurons, they give us a clue to understand the effect of Hax-1 in neuronal apoptosis.

## The Role of Hax-1 in Neuronal Death

### Congenital neutropenia

The defects and dysfunctions caused by deficiency of Hax-1 mainly appear in the immune system and central nerve system [7]. Till recent years, studies indicate that Hax-1 plays an important role in the development of the nervous system and pathophysiology of some neurological diseases. Hax-1 deficiency has been identified as a cause of severe congenital neutropenia (SCN), which is a rare disease of myelopoiesis resulting in life-threatening infections due to a lack of neutrophils [27-29]. Notably, in addition to myelopoiesis the SCN patients also have epilepsy and severe neurodevelopmental delay such as delay of motor, cognitive, and intellectual development [29,30]. In animal studies, the Hax-1 knock-out mice failed to survive longer than 14 weeks. Death was caused by failure of motor coordination and functions, ultimately leading to failure to eat or drink. Histological analysis showed that there existed extensive apoptosis of neurons and astrocyte infiltration in the striatum and the cerebellum in Hax-1 knock-out mice [7]. Extensive astrocyte infiltration observed in the Hax-1 knockout mouse brain suggests an inhibitory role of Hax-1 in neuroinflammation. Above studies suggest that Hax-1 play an important role in neural development and normal neuronal functions.

### Epilepticus

Accumulation of cytochrome-c and activation of caspase-3 and -9, which lead to cell apoptosis, were observed in the seizures-induced hippocampal injury rat model. Protein level of Hax-1 was dramatically increased at 6h post-injury, but there were no significant change at 12h and 24h after injury. Significantly increased levels of HtrA2 were also detected at 6, 12, and 24h post-seizure. In the colocalization experiments, the researchers identified two groups of Hax-1 expressing cells: caspase-3 free and caspase-3 positive. Both of the cell populations are Hax-1 and HtrA2 positive cells but their fates are different, which is the first cell population should be survived cells and the second cell population underwent apoptosis. These observations suggest interactions between Hax-1 and HtrA2 may have different outcomes by activation different signaling pathways. In caspase-3 positive cells, HtrA2 cleaved and degraded Hax-1,

inhibiting anti-apoptotic effect of Hax-1 and leading to cell apoptosis. However, in caspase-negative cells, Hax-1 facilitates processing of HtrA2 by recruiting Parl and processed HtrA2 inhibits pro-apoptotic Bax-1 accumulation to promote cell survival [31]. These results suggested that Hax-1 is involved in the pathophysiology of cell death induced by epilepsy.

### Traumatic brain injury(TBI) and cerebral ischemia

Shi et al. found that progressive upregulation of Hax-1 in rat brain from 12h, and its level was reached to the peak at day 3 after traumatic injury and then slowly reduced to normal level. Immunostaining of Hax-1 showed that neurons around the lesion site highly expressed Hax-1 at day-3 after TBI. At the same time, significant enhanced neuron apoptosis was identified with extensive activation of caspase-3 and decreased level of anti-apoptotic protein Bcl-2 in rats with TBI. All these results suggested that Hax-1 may be involved in the pathophysiology of TBI. Up-regulation of Hax-1 may protect neurons from death after TBI possibly through inhibition of caspase-3 activity [19]. However, another study by Rami et al. showed that Hax-1 expression was dramatically decreased at 6,24, and 48h post-ischemia in mice, and caspase-3 was also significantly activated from 6h and accumulation of cleaved caspase-3 was observed at 24h and 48h after ischemia. Notably, the HtrA2/Hax-1 ratio positively correlated to cell injury in ischemic brain, which HtrA2/Hax-1 ratio and cell apoptosis both reached the peak at 24 h after ischemia. Furthermore, Hax-1 negative or Hax-1 moderately positive cells were caspase-3 positive and TUNEL positive cells; most Hax-1 positive cells were TUNEL-negative and caspase-3 free. These results suggested that Hax-1 level correlates well to cell survival [18]. In consistent with Rami's study, our previous study also indicated that Hax-1 level was significantly reduced at 24h after ischemia in the ischemic hemisphere. There was a reverse correlation between Hax-1 expression and infarct size. We also performed in-vitro experiments using oxygen/glucose deprivation (OGD) condition. OGD induced cell apoptosis and down-regulation of Hax-1 in both brain endothelial cell line (bEnd5 cells) and neuronal cell line (neuro-2a cells) [20]. Above studies indicate that Hax-1 is involved in progress of neuron apoptosis in cerebral ischemia and TBI, however, further study will be needed to elucidate the mechanism involved in Hax-1 protection against brain injury.

### Conclusion

The fate of the cells under disease conditions is determined by the balance between pro- and anti-apoptotic signaling pathways. Similarity of Hax-1 to anti-apoptotic protein Bcl-2 leads to a number of studies, which investigated the role of Hax-1 in regulating cell apoptosis. Hax-1 undergoes subcellular translocation to bind to its partners in response to a variety of cellular stimuli and inhibits both the death receptor- and mitochondria-mediated apoptosis pathways [6,23,32]. To date, Hax-1 has been known as an anti-apoptotic protein expressed in the various tissues and regulates cell survival and apoptosis in various disease conditions. Down-regulation of Hax-1 will shift the balance to the pro-apoptosis leading to the cell death in some conditions. Although Hax-1 was discovered 10 years ago and has been intensively studied, the role of Hax-1 in neurological diseases is not well defined. More efforts should be made to investigate the molecular mechanisms underlying Hax-1 anti-apoptotic action

in neurological diseases, which will help us to better understand the pathophysiology of neurodegeneration, and may find a new target to treat these diseases.

### References

- Grzybowska EA, Sarnowska E, Konopiński R, Wilczyńska A, Sarnowski TJ, Siedlecki JA. Identification and expression analysis of alternative splice variants of the rat Hax-1 gene. *Gene*. 2006; 371: 84-92.
- Lees DM, Hart IR, Marshall JF. Existence of multiple isoforms of HS1-associated protein X-1 in murine and human tissues. *J Mol Biol*. 2008; 379: 645-655.
- Suzuki Y, Demoliere C, Kitamura D, Takeshita H, Deuschle U, Watanabe T. HAX-1, a novel intracellular protein, localized on mitochondria, directly associates with HS, a substrate of Src family tyrosine kinases. *J Immunol*. 1997; 158: 2736-2744.
- Fadeel B, Grzybowska E. HAX-1: a multifunctional protein with emerging roles in human disease. *Biochim Biophys Acta*. 2009; 1790: 1139-1148.
- Vafiadaki E, Sanoudou D, Arvanitis DA, Catino DH, Kranias EG, Kontrogianni-Konstantopoulos A. Phospholamban interacts with HAX-, a mitochondrial protein with anti-apoptotic function. *J Mol Biol*. 2007; 367: 65-79.
- Han Y, Chen YS, Liu Z, Bodyak N, Rigor D, Bisping E, et al. Overexpression of HAX-1 protects cardiac myocytes from apoptosis through caspase-9 inhibition. *Circ Res*. 2006; 99: 415-423.
- Chao JR, Parganas E, Boyd K, Hong CY, Opferman JT, Ihle JN. Hax1-mediated processing of HtrA2 by Parl allows survival of lymphocytes and neurons. *Nature*. 2008; 452: 98-102.
- Lee AY, Lee Y, Park YK, Bae KH, Cho S, Lee do H, et al. HS 1-associated protein X-1 is cleaved by caspase-3 during apoptosis. *Mol Cells*. 2008; 25: 86-90.
- Zhao W, Waggoner JR, Zhang ZG, Lam CK, Han P, Qian J, et al. The anti-apoptotic protein HAX-1 is a regulator of cardiac function. *Proc Natl Acad Sci U S A*. 2009; 106: 20776-20781.
- Radhika V, Onesime D, Ha JH, Dhanasekaran N. Galpho13 stimulates cell migration through cortactin-interacting protein Hax-1. *J Biol Chem*. 2004; 279: 49406-49413.
- Gallagher AR, Cedzich A, Gretz N, Somlo S, Witzgall R. The polycystic kidney disease protein PKD2 interacts with Hax-, a protein associated with the actin cytoskeleton. *Proc Natl Acad Sci U S A*. 2000; 97: 4017-4022.
- Al-Maghrebi M, Brulé H, Padkina M, Allen C, Holmes WM, Zehner ZE. The 3' untranslated region of human vimentin mRNA interacts with protein complexes containing eEF-1gamma and HAX-1. *Nucleic Acids Res*. 2002; 30: 5017-5028.
- Szwarc M, Sarnowska E, Grzybowska EA. [HAX-1 protein: multifunctional factor involved in apoptosis, cell migration, endocytosis and mRNA transport]. *Postepy Biochem*. 2007; 53: 218-227.
- Modem S, Reddy TR. An anti-apoptotic protein, Hax-, inhibits the HIV-1 rev function by altering its sub-cellular localization. *J Cell Physiol*. 2008; 214: 14-19.
- Yap SV, Koontz JM, Kontrogianni-Konstantopoulos A. HAX-1: a family of apoptotic regulators in health and disease. *J Cell Physiol*. 2011; 226: 2752-2761.
- Mirmohammadsadegh A, Tartler U, Michel G, Baer A, Walz M, Wolf R, et al. HAX-1, identified by differential display reverse transcription polymerase chain reaction, is overexpressed in lesional psoriasis. *J Invest Dermatol*. 2003; 120: 1045-1051.
- Hippe A, Bylaite M, Chen M, von Mikecz A, Wolf R, Ruzicka T, et al. Expression and tissue distribution of mouse Hax1. *Gene*. 2006; 379: 116-126.
- Rami A, Langhagen A. Specific alterations of the HtrA2/HAX-1 ratio in the penumbra upon focal cerebral ischemia in mice. *Neurochem Res*. 2012; 37: 548-556.

19. Shi W, Zhao W, Shen A, Shao B, Wu X, Yang J, et al. Traumatic brain injury induces an up-regulation of Hs1-associated protein X-1 (Hax-1) in rat brain cortex. *Neurochem Res.* 2011; 36: 375-382.
20. Hu J, Mu C, Hao J. Cerebral ischemia reduces expression of Hs1-associated protein X-1 (Hax-1) in mouse brain. *Neurosci Lett.* 2013; 534: 338-343.
21. Shaw J, Kirshenbaum LA. HAX-1 represses postmitochondrial caspase-9 activation and cell death during hypoxia-reoxygenation. *Circ Res.* 2006; 99: 336-338.
22. Li W, Srinivasula SM, Chai J, Li P, Wu JW, Zhang Z, et al. Structural insights into the pro-apoptotic function of mitochondrial serine protease HtrA2/Omi. *Nat Struct Biol.* 2002; 9: 436-441.
23. Cilenti L, Soundarapandian MM, Kyriazis GA, Stratico V, Singh S, Gupta S, et al. Regulation of HAX-1 anti-apoptotic protein by Omi/HtrA2 protease during cell death. *J Biol Chem.* 2004; 279: 50295-50301.
24. Kang YJ, Jang M, Park YK, Kang S, Bae KH, Cho S, et al. Molecular interaction between HAX-1 and XIAP inhibits apoptosis. *Biochem Biophys Res Commun.* 2010; 393: 794-799.
25. Han J, Goldstein LA, Hou W, Froelich CJ, Watkins SC, Rabinowich H. Deregulation of mitochondrial membrane potential by mitochondrial insertion of granzyme B and direct Hax-1 cleavage. *J Biol Chem.* 2010; 285: 22461-22472.
26. Jing YY, Li XL, Shi Q, Wang ZY, Guo Y, Pan MM, et al. A novel PrP partner HS-1 associated protein X-1 (HAX-1) protected the cultured cells against the challenge of H<sub>2</sub>O<sub>2</sub>. *J Mol Neurosci.* 2011; 45: 216-228.
27. Carlsson G, van't Hooft I, Melin M, Entesarian M, Laurencikas E, Nennesmo I, et al. Central nervous system involvement in severe congenital neutropenia: neurological and neuropsychological abnormalities associated with specific HAX1 mutations. *J Intern Med.* 2008; 264: 388-400.
28. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nat Genet.* 2007; 39: 86-92.
29. Ishikawa N, Okada S, Miki M, Shirao K, Kihara H, Tsumura M, et al. Neurodevelopmental abnormalities associated with severe congenital neutropenia due to the R86X mutation in the HAX1 gene. *J Med Genet.* 2008; 45: 802-807.
30. Rezaei N, Chavoshzadeh Z, R Alaei O, Sandrock I, Klein C. Association of HAX1 deficiency with neurological disorder. *Neuropediatrics.* 2007; 38: 261-263.
31. Rami A, Kim M, Niquet J, Langhagen A. Alterations in the expression of the anti-apoptotic factor HAX-1 upon seizures-induced hippocampal injury in the neonatal rat brain. *Neurochem Res.* 2012; 37: 116-125.
32. Palmblad J, Papadaki HA. Chronic idiopathic neutropenias and severe congenital neutropenia. *Curr Opin Hematol.* 2008; 15: 8-14.