frontiers in
MOLECULAR NEUROSCIENCE

REVIEW ARTICLE
published: 10 April 2012
doi: 10.3389/fnmol.2012.00040

Acetylcholinesterase involvement in apoptosis

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To date, more than 40 different types of cells from primary cultures or cell lines have shown AChE expression during apoptosis and after the induction apoptosis by different stimuli. It has been well-established that increased AChE expression or activity is detected in apoptotic cells after apoptotic stimuli *in vitro* and *in vivo*, and AChE could be therefore used as a marker of apoptosis. AChE is not an apoptosis initiator, but the cells in which AChE is overexpressed undergo apoptosis more easily than controls. Interestingly, cells with downregulated levels of AChE are not sensitive to apoptosis induction and AChE deficiency can protect against apoptosis. Some tumor cells do not express AChE, but when AChE is introduced into a tumor cell, the cells cease to proliferate and undergo apoptosis more readily. Therefore, AChE can be classified as a tumor suppressor gene. AChE plays a pivotal role in apoptosome formation, and silencing of the AChE gene prevents caspase-9 activation, with consequent decreased cell viability, nuclear condensation, and poly (adenosine diphosphate-ribose) polymerase cleavage. AChE is translocated into the nucleus, which may be an important event during apoptosis. Several questions still need to be addressed, and further studies that address the non-classical function of AChE in apoptosis are needed.

Keywords: acetylcholinesterase, apoptosis, tumor

The human gene that encodes acetylcholinesterase (AChE) is located on chromosome 7q22 (Ehrlich et al., 1992; Getman et al., 1992). Acetylcholinesterase is a type B carboxylesterase that rapidly hydrolyzes the neurotransmitter acetylcholine (ACh) at cholinergic synapses, as well as at neuromuscular junctions (Grisaru et al., 1999; Sperling et al., 2008). Additional splice variants involving both the 5′ and 3′ ends of the gene have also been reported. Among these splice variants are three distinct AChE variants, each with a different carboxyterminal sequence, called the "synaptic" (S), "erythrocytic" (E), and "readthrough" (R) AChE isoforms (Soreq and Seidman, 2001; Meshorer and Soreq, 2006). "Synaptic" AChE-S constitutes the principal multimeric enzyme in brain and muscle; soluble, monomeric "readthrough" AChE-R has been reported in embryonic and tumor cells and is induced under psychological, chemical, and physical stress; and finally, glypiated dimers of erythrocytic AChE-E are associated with red blood cell membranes (Grisaru et al., 1999). In addition to its catalytic function of the hydrolysis of acetylcholine, AChE has been shown to be involved in many non-cholinergic functions, such as cell growth, stem cell differentiation (Sperling et al., 2008), neuritogenesis, cell adhesion (Paraoanu and Layer, 2008), synaptogenesis, activation of dopamine neurons, tumorigenesis, amyloid fiber assembly (Inestrosa et al., 1996; Alvarez et al., 1997), hematopoiesis, and thrombopoiesis (Greenfield, 1996; Layer, 1996; Small et al., 1996; Soreq and Seidman, 2001). Almost a decade ago, work by our group revealed that AChE-S expression can be induced during apoptosis in various cell types, including cells that did not originate from the nervous or hematopoietic system (Zhang et al., 2002). It has also been shown that AChE exerts its pivotal role in apoptosis by its participation in the formation of the apoptosome (Park et al., 2004). In this review we will summarize the functional evidence for the role of AChE during apoptosis, despite the fact that our understanding of the apoptotic role of AChE is still not complete.

Abbreviations: AChE, acetylcholinesterase; AChE-E, erythrocytic acetylcholinesterase; AChE-S, synaptic acetylcholinesterase; AChE-R, readthrough acetylcholinesterase; AML, acute myeloid leukemia; BuChE, butyrylcholinesterase; HCC, human colorectal carcinoma; Icv, intracerebroventricular; RA, refractory anemia; TGF-β, transforming growth factor-beta; TGF-α, transforming growth factor-alpha; TgS mice, transgenic mice overexpressing AChE-S.

AChE LEVELS ARE INCREASED IN APOPTOTIC CELLS AFTER APOPTOTIC STIMULI BOTH *IN VITRO* AND *IN VIVO*

AChE is expressed in cholinergic neurons, neuromuscular junctions, hematopoietic cells, including erythrocytes (Grisaru et al., 1999) and megakaryocytes (Paulus et al., 1981; Tranumjensen and Behnke, 1981), as well tissues that are not innervated by cholinergic neurons (Small et al., 1996). There is mounting evidence, however, that AChE expression is induced during apoptosis in a variety of other cell types (Zhang et al., 2002). Human lung fibroblast (HLF) cells and rat kidney (NRK) cells do not normally express AChE, but have been shown to express high levels of AChE when undergoing apoptosis or when moving from an inactive to an active state (Zhang et al., 2002; Jin et al., 2004). In general, cells that normally express low levels of AChE, such as PC-12, showed increased levels of AChE and AChE activity whilst undergoing apoptosis (Yang et al., 2002; Jing et al., 2008). Steinritz et al. (2007) reported that

increased AChE activity was found in sulfur mustard (SM)-treated A549 cell culture, as determined by Ellman assay and Western blotting. Interestingly, AChE activity showed a strong correlation with the number of TUNEL-positive cells. Furthermore, AChE activity may be a potential marker of apoptosis in A549 cells after SM injury (Steinritz et al., 2007). Other diseases with a possible apoptotic role in disease pathogenesis such as Alzheimer's disease and diabetes also show cholinesterase involvement. Allam et al. observed that butyrylcholinesterase and AChE-related proteins were found to be common to both Alzheimer's disease and diabetes; these proteins may play an etiological role via their influence on insulin resistance and lipid metabolism (Allam et al., 2006). Toiber et al. (2008) showed that in transfected primary brain cultures, an alternative N terminal extended transcript of AChE (N-AChE-S) induced cell death, morphological impairments and caspase-3 activation. In addition in cortical tissues from AD patients, N-AChE-S overexpression coincides with Tau hyper-phosphorylation. In other pathologies, Hu et al. (2009) showed increased expression levels of AChE and caspase-3 in the brain and peripheral immune system of focal cerebral ischemic rats. Xiao et al. (2006) utilized AChE with Bax, c-fos, and p53 genes as markers of apoptosis. Huang et al. (2005) have developed a novel assay that utilizes fluorochrome-tagged physostigmine (Ph-F) *in situ* to detect active AChE that has been induced during apoptosis. Cells that show an increase in AChE activity during apoptosis are summarized in **Table 1**.

CELL SENSITIVITY TO APOPTOSIS IS RELATED TO AChE EXPRESSION LEVELS

AChE is not a universal activators of apoptosis as it has been well-established that cholinergic neurons and neuromuscular junctions that express AChE do not initiate apoptosis under normal conditions without an apoptotic stimulus. However, AChE-positive cells, such as PC-12 (Yang et al., 2002; Jing et al., 2008) as well as neurons have been shown to respond more sensitively to apoptotic stimuli than AChE-negative cells (Jin et al., 2002, 2004). To investigate this, a stable cell line that overexpressed AChE was established from NRK cells. Overexpression of AChE was shown to inhibit cell proliferation and promote apoptosis in these cells. Transgenic mice which overexpress catalytically active AChE-S and have a higher levels of AChE hydrolytic activity than control mice, show increased levels of cell labeling by both bromodeoxyuridine and caspase-3; which reflects increased neural apoptosis (Cohen et al., 2008) though these transgenic mice also showed increased neuronal survival through an as yet unidentified compensatory mechanism. AChE was also shown to suppress cell proliferation via catalytic hydrolysis of acetylcholine in human colorectal carcinoma (HCC) (Montenegro et al., 2006).

Zhang et al. (2002) showed that the downregulation of AChE expression with antisense inhibited apoptosis, and they suggest that AChE is potentially a marker and a regulator of apoptosis. Park et al. (2004) explored the role of AChE in apoptosis by silencing the *AChE* gene. Silencing of *AChE* abolished the expression of AChE and prevented caspase-9 activation, decreased cell viability, nuclear condensation, and poly(adenosine diphosphate-ribose) polymerase cleavage. Animal models of degenerative diseases were found to be more easily established in $AChE^{+/+}$ mice than $AChE^{+/-}$ mice. Espallergues et al. (2010) showed that the intracerebroventricular injection of amyloid-beta (25–35) failed to induce learning deficits in $AChE^{+/-}$ mice, but impaired learning in $AChE^{+/+}$ controls. The peptide showed reduced toxicity in the forebrain structures of $AChE^{+/-}$ mice, as an increase in lipid peroxidation levels was measured in the hippocampus of $AChE^{+/+}$ but not $AChE^{+/-}$ mice. They concluded that the increase in cholinergic tonus observed in $AChE^{+/-}$ mice did not result in increased memory functions but allowed a significant prevention of the deleterious effects of muscarinic blockade or amyloid toxicity. Ye et al. (2010) recently reported that AChE is expressed during ischemia–reperfusion (I/R)-induced apoptosis *in vivo*. Their study demonstrated that AChE may be a pro-apoptotic factor and that the inhibition of AChE reduces the degree of renal I/R injury.

AChE AND TUMOR SUPPRESSION

Papers reporting the presence of AChE in tumors have first been reported almost 40 years ago (Gearhart and Mintz, 1974). The exact role of AChE in oncogenesis or tumor progression is still unclear and beyond the scope of this review. However a number of studies (Gearhart and Mintz, 1974; Takahashi et al., 1995; Johnson and Cotter, 1997) have shown an genetic alterations in the long arm of chromosome 7 the genetic locus of the AChE gene, in several tumors. Furthermore, the agricultural use of AChE inhibitors is known to induce several types of tumors (Dich et al., 1997; Cabello et al., 2001; Abou-Donia, 2003). Stephenson et al. (1996) investigated gene copy-number alterations at these genes in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). These data raise the intriguing possibility that the proaptotic role of AChE could play a role in tumor suppressor. (Stephenson et al., 1996).

Cholinesterases are downregulated in HCC (Montenegro et al., 2006), squamous cell carcinoma, and retinoblastoma (Qavi and Al-Rajhi, 2009). Zhao et al. (2011) demonstrated that AChE was significantly downregulated in the cancer tissues of 69.2% of HCC patients, and the low level of AChE expression in HCC was correlated with tumor aggressiveness, the elevated risk of postoperative recurrence, and a low survival rate. Both the recombinant AChE protein and the enhanced expression of AChE significantly inhibited HCC cell growth *in vitro* and tumorigenicity *in vivo*. The studies describe the function of AChE as a tumor growth suppressor that acts in the regulation of cell proliferation, relevant signaling pathways, and the chemosensitivity of HCC cells. AChE is a promising independent prognostic predictor for HCC recurrence and the survival of HCC patients (Montenegro et al., 2006). AChE-R was shown to increases germ cell apoptosis (Mor et al., 2008).

LOCALIZATION OF AChE TO THE CELL NUCLEUS AND APOPTOTIC BODIES MAY PLAY A ROLE IN APOPTOSIS INDUCTION

Zhang et al. (2002) have shown by cytochemical staining and immunohistochemistry that the presence of AChE is not homogeneous in either the cytoplasm or in the nucleus. To establish whether this heterogeneity is due to the stage of

Table 1 | Summarized cell lines in which AChE expression or activity were increased by the apoptotic stimuli with various apoptotic inducers.

Cell type	Apoptotic inducer	Increase of AChE expression or activity	Reference
A549	Sulfur mustard	Yes	Steinritz et al. (2007)
Animal brain	HBP hypobaric hypoxia (HBH)	Yes	Muthuraju et al. (2009)
Brain tissue of rats	Ischemia	Yes	Hu et al. (2009)
BRL	Detachment	Yes	Zhang et al. (2002)
C57BL/6J beta cells	Streptozotocin	Yes	Zhang et al. (2012)
Dami	TGFβ	Yes	Zhang et al. (2002)
EC prim. bovine endothelial cell	TGFβ	Yes	Zhang et al. (2002)
EC prim. swine endothelial cell	Detachment	Yes	Zhang et al. (2002)
HEK293T	H_2O_2, cisplatin	Yes	Zhang et al. (2008), Gong et al. (2009)
HEL	Huangqi (Hex)	Yes	Cheng et al. (2004)
HeLa	A23187, thapsigargin, topotecan, H_2O_2, TNFα + CHX	Yes	Zhang et al. (2002), Huang et al. (2005); Gao et al. (2009), Zhu et al. (2007a,b,c)
HFL-1	Aging	Yes	Zhang et al. (2002)
HL-60	Topotecan, H_2O_2, Daunorubicin	Yes	Zhang et al. (2002), Huang et al. (2005)
HLF	Aging	Yes	Zhang et al. (2002), Jin et al. (2002)
HOS	TGFβ	Yes	Zhang et al. (2002)
HT-29	Etoposide	Yes	Park et al. (2004, 2008)
HUVEC	Serum-starved medium	Yes	Xie et al. (2011)
IL-3-deprived (murine) bone marrow derived mast cells	A nitric oxide donor	Yes	Park et al. (2008)
Jarkat Cell	Topotecan, H_2O_2	Yes	Huang et al. (2005)
K562	Huangqi (Hex)	Yes	Cheng et al. (2004)
Lymphocytes	Post partum	Yes	Pick et al. (2004)
M07e	Minus GM-CSF	Yes	Zhang et al. (2002)
Malme-3M	Etoposide	Yes	Park et al. (2008)
MDA-MB-435s	A23187, thapsigargin	Yes	Zhu et al. (2007c)
Meg-01	TGFβ	Yes	Zhang et al. (2002)
Mouse hippocampal granule cells from Tgs mice	Tgs	Yes	Cohen et al. (2008)
Myoblast	Staurosporine	AChE-R	Pegan et al. (2010)
NIH/3T3	Detachment	Yes	Zhang et al. (2002)
MIN6 cells	Streptozotocin	Yes	Zhang et al. (2012)
NRK	G418	Yes	Jin et al. (2004)
Osteoblast	Long-term culture	Yes	Gu et al. (2002)
PC-12	H_2O_2, A23187, thapsigargin	Yes	Jing et al. (2008), Jiang et al. (2007)
PC-3	TGFβ	Yes	Zhang et al. (2002)
Primary cortical neuron	Sodium selenite	Yes	Xiao et al. (2006)
Primary cultured rat articular chondrocytes	Infected with 100 MOI adenoviral TRAIL	Yes	Park et al. (2008)
Rat kidney	Ischemia/reperfusion	Yes	Ye et al. (2010)
Rat smooth muscle	TGF-β	Yes	Zhang et al. (2002)
Raw264.7	SIN-1, a nitric oxide donor	Yes	Park et al. (2008)
Retinal	Light-induced retinal damage	Yes	Kehat et al. (2007)
SH-SY5Y	Tertiary butylhydroperoxide	N-AChE	Kehat et al. (2007)
SK-MEL-5	Etoposide	Yes	Park et al. (2008)
SK-N-SH	TNFα + CHX	Yes	Zhang et al. (2002)

(Continued)

Table 1 | Continued

Cell type	Apoptotic inducer	Increase of AChE expression or activity	Reference
SW620 cells	Etoposide, excisanin A	Yes	Deng et al. (2006)
TE671	Etoposide	Yes	Park et al. (2008)
U373MG	Etoposide	Yes	Park et al. (2008)

HUVEC, human umbilical vein endothelial cells.

apoptosis induction, the authors examined the time course of AChE expression in the leukemic Meg-01 cell line after treatment with transforming growth factor (TGF)-β. Cytochemical staining showed AChE initially appears in the cytosol and afterward in the nucleus. AChE-positive staining follows the nuclear morphological changes such as condensation and fragmentation. Eventually, when the cell membrane ruptures, AChE is released. They also examined the expression of AChE by transmission electron microscopy. At 2 h after TGF-β treatment, AChE was observed only in the cytosol but by 4 h had moved entirely to the nucleus. Therefore, during the induction of apoptosis, AChE is first synthesized in the cytosol and then accumulates in the nucleus. In general AChE is present in the nucleus at the mid- and late-stages of apoptosis (Yang et al., 2002; Jin et al., 2004; Xie et al., 2011). Santos et al. (2007) initially investigated the expression and subcellular localization of acetylcholinesterase (AChE) in endothelial cells and showed the expression of a 70-kDa AChE in both the cytoplasmic and nuclear compartments. The levels of this novel isoform are decreased in response to vascular endothelial growth factor via the proteasome pathway. They found that AChE was downregulated in human leukemic T cells compared with normal T cells, which suggested that the decreased expression of the 55-kDa AChE protein may contribute to an angiogenic response and be associated with tumorigenesis. Importantly, nuclear expression is not specific to endothelial cells, but is also present in non-neuronal and neuronal cells. Xie et al. (2011) showed that the 55-kDa AChE protein was induced during apoptosis and was negatively regulated by the Akt pathway. PC-12 express AChE at basal levels in normal cells, but a 55-kDa AChE protein can be found during the progression of apoptosis. This 55 kDa AChE protein was temporally expressed after the activation of caspases and was caspase-dependent. The inhibition of protein synthesis with CHX indicated that the 55-kDa AChE protein resulted from the cleavage of the 68-kDa AChE (Xie et al., 2011). There are still questions regarding AChE that need to be addressed, including its function when it enters the nucleus. Furthermore, whether AChE entry into the nucleus is an active or passive event, how is it transported and what is the signal that allows it to enter the nucleus are all questions which still need to be resolved.

AChE COULD PLAYS A PIVOTAL ROLE IN THE FORMATION OF APOPTOSOMES

The role of AChE in apoptosis is a key question in this field. Park et al. (2004) showed that silencing of the *AChE* gene inhibited the interaction between Apaf-1 and cytochrome *c*. They concluded that AChE plays an important role in apoptosome formation. Their data showed that an antisense AChE oligonucleotide showed a similar effect by silencing the *AChE* gene. Small interfering RNA (siRNA) against the cytochrome *c* gene blocked the interaction of AChE with Apaf-1, whereas siRNA targeting the *Apaf-1* gene did not block the interaction of AChE with cytochrome *c*. These findings indicated that the interaction of AChE with cytochrome *c* is required for the interaction between cytochrome *c* and protease-activating factor-1. Park et al. (2008) also showed that the interactions of AChE with caveolin-1 and, subsequently with cytochrome *c*, appear to be indispensable for the formation of apoptosomes. To date, however, these findings have only been shown in one study and more evidence is required to confirm how AChE participates in the formation of the apoptosome.

ACKNOWLEDGMENTS

The author is grateful to Hermona Soreq, Jerusalem, for critical evaluation of this manuscript. This work was supported in part by grants from the National Natural Science Foundation of China (Nos. 30971481 and 31071213).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 January 2012; accepted: 18 March 2012; published online: 10 April 2012.

*Citation: Zhang X-J and Greenberg DS (2012) Acetylcholinesterase involvement in apoptosis. Front. Mol. Neurosci. **5**:40. doi: 10.3389/fnmol.2012.00040*