What does acetylcholinesterase do in hematopoietic cells?
Review Article

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Summary

Acetylcholinesterase (AChE) is an essential component of cholinergic synapses since it hydrolyzes acetylcholine released from presynaptic nerve terminals. However, it is well documented that AChE is also expressed in a variety of non-cholinergic tissues including hematopoietic cells. Despite the recent progress made in our understanding of the molecular mechanisms regulating expression of AChE, our knowledge of the precise function of this enzyme in hematopoietic cells still remains limited. Previous work has led to the notion that AChE may be involved in myelodysplastic syndromes as well as acute myeloid leukaemias since it may regulate hematopoiesis by acting as a tumor suppressor gene. In addition, recent studies have further demonstrated the involvement of AChE in the proliferation of multipotent stem cells, as well as in the mechanisms leading to apoptosis in cells undergoing erythroid and megakaryocytic differentiation. In this review, we first present an overview of the cellular and molecular biology of AChE and then, focus more specifically on the expression of AChE in hematopoietic cells. Finally, we also discuss the recent evidence linking AChE expression and the proliferative capacity of these cells. A better understanding of the functional significance of AChE in hematopoietic cells may be relevant for the future design of novel therapeutic strategies against proliferative disorders of hematopoietic tissues.

I. Introduction

Acetylcholinesterase (AChE; EC 3.1.1.7) is an essential component of cholinergic synapses in both central and peripheral nervous systems. Within these specialized structures, AChE is responsible for the rapid hydrolysis of acetylcholine released from presynaptic nerve terminals thereby ensuring precise temporal control of synaptic transmission (see for review Massoulié et al., 1993; Taylor and Radic, 1994). However, it is well documented that AChE is also expressed in a variety of non-cholinergic tissues. For example, non-cholinergic regions of the brain such as the hippocampus and cerebellum express large amounts of AChE (see for example Landwehrmeyer et al., 1993; Legay et al., 1993a; Hammond et al., 1994 and refs therein). Furthermore, AChE has been shown to be homologous to the cell adhesion molecules glutactin and neurotactin (Krejci et al., 1991) as well as to neuroligins which are neuronal cell surface proteins (Ichtchenko et al., 1996). Such findings have led to the suggestion that AChE may perform additional, non-classical function in the nervous system (Robertson and Yu, 1993; Greenfield, 1995; Layer and Willbold, 1995).

Homomeric Forms :

Heteromeric Forms :

Figure 1. Quaternary structures of AChE molecular forms. Homomeric forms consist of monomer G_1, dimer G_2, tetramer G_4 and a glycophospholipid (GPI)-linked dimer. Heteromeric forms consist of the hydrophobic-tailed G_4 form and the asymmetric forms containing a collagenic structural subunit. Only the asymmetric form A_{12} is shown.
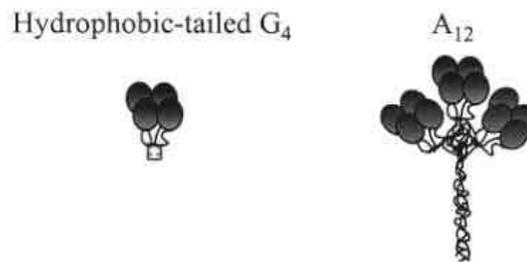
Interestingly, AChE is also abundantly expressed in hematopoietic cells where its expression is even more puzzling. Although the presence of AChE in erythrocytes was detected more than 70 years ago (see Lawson and Barr, 1987), its role in blood cell physiology still remains unclear. In recent years however, there has been considerable interest in this issue, and there is now increasing evidence suggesting the existence of a link between AChE expression and the proliferation and differentiation of hematopoietic cells. In this brief review, we initially describe the cellular and molecular biology of AChE and then focus more specifically on the putative involvement of this enzyme in the development of blood cells and elements. Our main objective is to highlight some of the latest findings which should prove useful to design further experimentation dealing with the regulation and functional significance of AChE during hematopoiesis under normal and pathological conditions.

II.The AChE molecular forms and splice variants

AChE exists as a family of molecular forms which differ in their structures and hydrodynamic characteristics while displaying similar catalytic properties (Taylor, 1991; see Massoulié et al., 1993). The molecular forms may be classified as homomeric or heteromeric on the basis of their association with specialized structural subunits (**Figure 1**).

Homomeric forms include monomer G_1, dimer G_2 and tetramer G_4 as well as a glycophospholipid-linked (GPI) dimer. Heteromers on the other hand, consist of: (**i**) amphiphilic tetramers G_4 linked to a 20 kDa hydrophobic anchor; and (**ii**) the asymmetric forms A_4, A_8 or A_{12} in which 1, 2 or 3 soluble tetramers attach to a collagenic subunit, respectively. The functional significance of such polymorphism remains to be established yet, it has been proposed that it allows the placement of AChE molecules at distinct cellular locations where they can assume site-specific functions.

Previous studies have shown that AChE is encoded by a single gene (Rotundo et al., 1988; Maulet et al., 1990; Soreq et al., 1990; Li et al., 1993a; Chan and Jasmin, 1995). Although only one copy of the gene exists, several transcripts are produced by alternative splicing (Sikorav et al., 1987, 1988; Schumacher et al., 1988; Maulet et al., 1990) (**Figure 2**). In mammals, exon 1 is untranslated while exons 2, 3 and part of exon 4 appear in all AChE transcripts since they encode the common catalytic domain of the mature protein (Li et al., 1991, 1993a). The C-terminal region of the protein is variable and is encoded either by the rest of exon 4 (called R for readthrough), or by alternatively spliced exons 5 or 6 to yield the H (hydrophobic) or T (tail) transcript, respectively. Two polyadenylation signals have been identified which in the case of the T transcript for

example, give rise to two mRNAs of 2.4 and 3.2 kb (Rachinsky et al., 1990; Legay et al., 1993a).

The H transcript encodes the AChE catalytic subunit that contains the signal for GPI addition (Li et al., 1991) which ultimately leads to the formation of GPI-linked dimers (Li et al., 1991; Legay et al., 1993b; Michaelson et al., 1994; Coussen et al., 1995). This GPI-linked AChE dimer is expressed in mature erythrocytes and T lymphocytes (Ott et al., 1982; Szelenyi et al., 1982; Rosenberry and Scoggin, 1984; Roberts et al., 1987). By contrast, the T transcript can give rise to all other AChE molecular forms when co-expressed with appropriate structural subunits (Duval et al., 1992). The T transcript is abundantly expressed in muscle and neuron where it accounts therefore, for the multiplicity of molecular forms found in these cell types (Li et al., 1991; Duval et al., 1992; Li et al., 1993a; Legay et al., 1993a). Finally, the R transcript encodes a secreted AChE monomer whose expression may be developmentally regulated (Li et al., 1993b; Legay et al., 1995; Chan et al., 1998).

III. Expression of AChE in hematopoietic cells

Previous studies have shown that T lymphocytes (Szelenyi et al., 1982), platelets (Schukla, 1986; Koekebakker and Barr, 1988; Sánchez-Yagüe et al., 1990) and erythrocytes (Low and Finean, 1977; Massoulié and Bon, 1982; Toutant et al., 1989) express significant levels of AChE. Interestingly, there are notable differences between species concerning the distribution of the enzyme in blood cell lineages. Biochemical analysis of AChE in erythrocytes has shown that humans have the highest levels of enzyme activity while cats have none (Zajicek, 1957). However, platelets and megakaryocytes from cats contain large amounts of AChE whereas in humans, these cells express only low levels of enzyme activity. Other species such as guinea pigs, horses, rabbits, and rodents, fall in between these two extremes, but AChE activity tends to typically be found predominantly in one cell lineage (Zajicek, 1957). The significance of this variability between species and cell types remains currently unclear.

Amongst blood cell lineages, erythrocytes have been the most thoroughly studied in terms of AChE expression. Mammalian erythrocytes express GPI-linked AChE dimers on the extracellular surface of their plasma membrane (Ott et al., 1982; Rosenberry and Scoggin, 1984) and it has been shown that the GPI moiety is particularly important for anchoring AChE molecules onto the cell membrane (Incardona and Rosenberry, 1996). Interestingly, a certain degree of variation has been observed in the structure of the inositol ring within the glycolipid anchor. For example, AChE has been shown to be released readily by phosphatidylinositol-specific phospholipase C (PI-PLC) in

porcine, bovine and rat erythrocytes, but not from human or murine erythrocytes. The presence of an additional acyl chain on the inositol ring in the latter two species is thought to confer the resistance of the GPI-linked AChE dimer to PI-PLC treatment (Roberts et al., 1988a,b). Such addition effectively prevents the formation of a cyclic myo-inositol 1:2-monophosphate which is an intermediate product of the PI-PLC cleavage reaction (Wilson et al., 1985). Consequently, deacylation of AChE in human and murine erythrocytes by alkaline hydroxylamine treatment renders the enzyme susceptible again to cleavage by PI-PLC (Toutant et al., 1989; 1991). By contrast to the information already available on the expression of AChE in erythrocytes, only a few studies have examined AChE in lymphocytes and platelets most likely because of their limited quantities in circulating blood (Méflah et al., 1984; Bartha et al., 1987; Richier et al., 1992).

Histochemical studies of early hematopoietic cells have revealed that AChE is present in several distinct subcellular compartments. In human bone marrow cultures, AChE is detected in both the nucleus and cytoplasm of erythroblasts (Koekebakker and Barr, 1988), as well as in the nucleus of immature megakaryocytes (Lev-Lehman et al., 1997). A close examination of AChE expression during murine erythroid cell maturation further indicates that the enzyme is widely distributed in the nuclear membrane, endoplasmic reticulum and Golgi apparatus at early stages of development, and that it becomes confined to the Golgi apparatus in orthochromatic nucleated red blood cells in accordance with the end of AChE biosynthesis at this particular stage of cellular differentiation (Keyhani and Maigne, 1981). In addition, it appears that AChE is also secreted from normoblasts (Keyhani and Maigne, 1981) and megakaryocytes (Paulus et al., 1981). Taken together, these results suggest that the subcellular distribution of AChE as well as the species of molecular forms that are expressed, vary with the stage of hematopoietic cell differentiation. Accordingly, these changes in AChE localization and expression may therefore reflect distinct roles for the different molecular forms at specific stages of cell maturation (see Chan et al., 1998).

Previous studies have also determined the species of mRNAs expressed in various hematopoietic tissues. Analysis of rat fetal liver and spleen has shown for example that all three splice variants are present in these hematopoietic organs (Legay et al., 1993b). Studies using adult mouse bone marrow (Li et al., 1993a) and murine erythroleukemia (MEL) cells (Chan et al., 1998) have also revealed a similar pattern of expression thereby suggesting that hematopoietic cells from both embryonic and adult tissues are capable of expressing, albeit at different levels, the R, H and T transcripts of AChE.

Figure 2. Structure of the mammalian AChE gene and alternative splicing of AChE mRNAs. The promoter (P), exons (dark boxes), introns (light boxes) and 3'UTR (hatched box) containing two polyA+ signals (A) are shown. 4' denotes part of exon 4 that is retained together with intron 4 in the splicing of the R transcript. Note that splicing from exon 4 to either exon 5 or 6 generate the H and T transcripts, respectively.
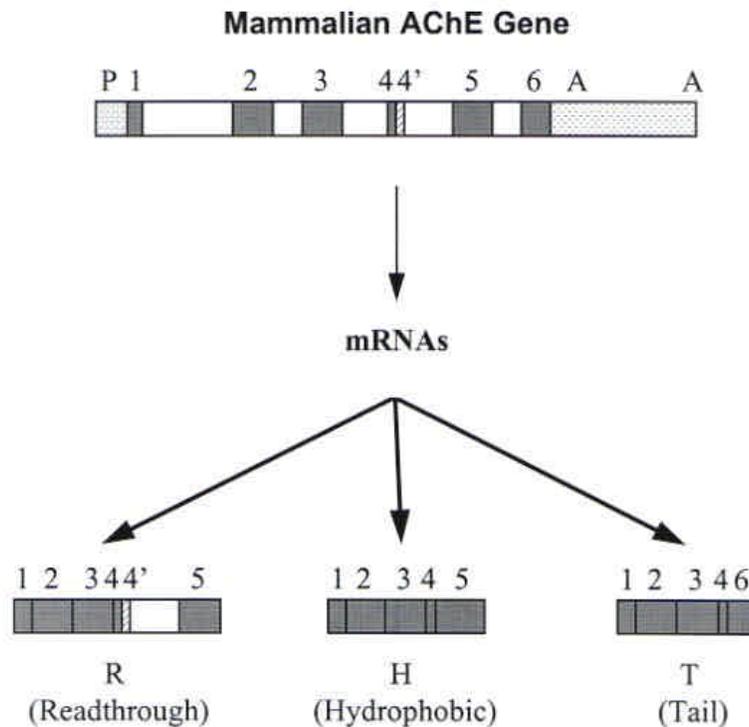
Since in our recent studies we have observed a preponderance of R and T transcripts in MEL cells (Chan et al., 1998; see also Li et al., 1993a) which basically correspond to erythroblasts and normoblasts (Friend et al., 1971), and since mature hematopoietic cells express significant amounts of GPI-linked dimers that are encoded by the H transcript (see **Figure 2**), it may thus be hypothesized that the splicing pattern of immature AChE mRNA changes during differentiation of hematopoietic cells hence, further supporting the notion that different AChE molecular forms, originating from different transcripts (see above), are required at distinct stages of hematopoiesis (Chan et al., 1998).

IV. Function of AChE in hematopoietic cells

The hypothesis that AChE is involved in physiological functions other than the termination of neurotransmission has received considerable attention particularly in the nervous system where these putative additional roles are collectively referred to as the non-cholinergic functions of AChE (Robertson and Yu, 1993; Greenfield, 1995; Layer and

Willbold, 1995). In this context, there has been an increasing number of reports that have recently demonstrated that AChE can in fact regulate neuronal morphogenesis and differentiation independently of its catalytic activity (see for example Layer et al., 1993; Jones et al., 1995; Small et al., 1995, Dupree and Bigbee, 1996; Inestrosa et al., 1996; Beeri et al., 1997; Holmes et al., 1997; Koenigsberger et al., 1997; Robitzki et al., 1997; Srivatsan and Peretz, 1997; Sternfeld et al., 1998).

In hematopoietic cells, the presence of AChE remains an enigma but there is nonetheless, considerable interest in identifying the physiological role of AChE in these cells particularly since the AChE gene maps to 7q22 (Getman et al., 1992) which is considered a critical region of the genome involved in the development of myelodysplastic syndromes and acute myeloid leukemias (Kere et al., 1989; Baranger et al., 1990; Mufti, 1992; Green, 1993). Additional clinical observations have further supported a link between aberrations in the AChE gene and severe hematological disorders. For example, the AChE gene frequently undergoes incomplete somatic amplification (Lapidot-Lifson et al., 1989) and mutation (Zakut et al., 1992) in hematological proliferation disorders such as megakaryocytopoiesis and

thrombopoiesis. Furthermore, organophosphates, which are potent inhibitors of AChE and key components of pesticides, are believed to represent causative agents in various forms of leukemias (Brown et al., 1990). Although the role of AChE in hematopoietic cells is still unclear, the location of the AChE gene in a region which may contain a novel myeloid-specific tumor suppressor gene (Le Beau et al., 1986; Neuman et al., 1992; Johansson et al., 1993; Rodrigues et al., 1996; Le Beau et al., 1996), has led to the suggestion that AChE may in fact function as a tumor suppressor by regulating proliferation, differentiation and apoptotic events during normal hematopoietic cell development (Soreq et al., 1994; Stephenson et al., 1996).

Over the last two decades, several laboratories have directly examined the role of AChE in hematopoietic cells by using distinct experimental approaches. Treatment of mice with the AChE inhibitor neostigmine, resulted in significant increases in colony forming unit-megakaryocytes in the humerus as well as in the percentage of progenitor cells undergoing DNA synthesis (Burnstein et al., 1980). Similarly, suppression of AChE expression using sequence-specific antisense oligonucleotides in cultures from mouse bone marrow cells led to enhanced proliferation of pluripotent stem cells committed to erythropoiesis, megakaryocytopoiesis and macrophage production (Soreq et al., 1994). Interestingly, and of particular relevance, normal apoptosis in these cells was significantly reduced in comparison to untreated cell cultures (Soreq et al., 1994). Based on these latter studies, it appears therefore that the functional role of AChE is to limit the proliferation of hematopoietic stem cells since its function is expected to be inversely related to the effects of the AChE antisense oligonucleotides.

Additional studies performed by other laboratories including ours, have also examined the relationship that appears to exist between AChE and the proliferative capacity of hematopoietic cells. Using MEL cells in culture for example, we have demonstrated a large increase in both intracellular and secreted AChE activity during cellular differentiation which coincides with hemoglobinization and the concomitant loss of their proliferative capacity (Chan et al., 1998). Paoletti and co-workers have found that fast-growing MEL cell clones express consistently lower levels of AChE enzyme activity as compared to slow-growing ones (Paoletti et al., 1992). In addition, treatment of these cells with exogenous AChE has been shown to lead to a marked decrease in cell growth (Paoletti et al., 1992). In our experiments, we have also recently observed following AChE addition to the growth media, significantly more cell death in MEL cells already committed to the differentiation program most likely as a result of apoptotic events (unpublished observation). Together these results suggest therefore that AChE can act as a negative regulator of cellular replication along the differentiation program of hematopoietic stem cells. Further confirmation of the role of AChE in regulating apoptosis in these cells may lead to the identification of additional regulatory mechanisms controlling programmed cell death in hematopoietic tissues and that the loss of this regulatory step may in fact be involved in the etiology of hematological disorders including leukemias.

V. Conclusions and perspectives

The notion that AChE fulfils additional, non-cholinergic functions has received an increasing amount of attention. In this context, hematopoietic cells are of considerable interest since there is now ample evidence showing that AChE is expressed both in early hematopoietic progenitors as well as in mature blood cells and elements. Although the specific function of AChE in hematopoietic cells remains obscure, converging lines of evidence suggest the existence of a link between AChE levels and the proliferative capacity of these cells. Future experiments will therefore prove useful not only to further test this hypothesis directly, but also, to begin delineating the splice variants, the regions of the AChE molecule as well as the signal transduction pathways that may be involved in mediating these effects. Because of the postulated clinical relationship between AChE expression and hematological disorders, it may also be envisaged that studies focusing on the regulation and functional significance of AChE expression in hematopoietic cells may ultimately lead to the design of novel therapeutic strategies.

Acknowledgement

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