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Expression and Function of APP and its Metabolites Outside the Central Nervous System

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Abstract

Amyloid precursor protein (APP) derived amyloid beta (Aβ) peptides have been extensively investigated in Alzheimer's disease pathology of the brain. However, the function of full length APP in the central nervous system remains unclear. Even less is known about the function of this ubiquitously expressed protein and it metabolites outside of the central nervous system. This review summarizes key aspects of the current understanding of the expression and function of APP and its proteolytic fragments in specific non-neuronal tissues.

Keywords

beta amyloid; alzheimer; amyloid precursor protein; adipose tissue; epidermis; intestine; muscle

1. Introduction

Much of the study of amyloid precursor protein (APP) has focused on changes in the central nervous system during Alzheimer's disease (AD) pathology. As previously reviewed (Zheng and Koo, 2011, Zhang, et al., 2012), in the central nervous system (CNS) full length APP has been suggested to function as a cell surface receptor contributing to cell adhesion and cell-cell interactions via its extracellular domain possibly through trans-dimerization. The C-terminus of APP contains a YENPTY sequence between residues 682 and 687 that is a consensus sequence for a phosphotyrosine binding domain interaction. The N-terminal fragment, sAPP-α, is neuroprotective, promotes neurite outgrowth and synaptogenesis, facilitates learning and memory, acts as a growth factor and regulates cell adhesion. On the other hand, the N-terminal sAPP-β fragment can stimulate axonal pruning and neuronal cell death. APP cleavage to generate the amyloid beta (Aβ) peptide can lead to peptide-mediated neurotoxicity, neurofibrillary tangle formation and synaptic loss. The APP intracellular domain (AICD) fragment that is often generated during proteolytic processing is capable of behaving as a transcription factor, controlling cell death and neprilysin-mediated Aβ degradation, altering calcium and ATP homeostasis, and regulating intracellular trafficking and cytoskeletal dynamics. However, considerably less work has been published describing

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the function and expression of full-length APP or its cleavage products outside the central nervous system in spite of the well-recognized ubiquitous expression pattern.

2. APP expression, processing, and structure

Amyloid precursor protein (APP) is a type I integral membrane protein with a large extracellular N-terminal domain, a hydrophobic transmembrane domain, and a short C-terminus intracellular domain (Kang, et al., 1987, Dyrks, et al., 1988). There are three major isoforms of the protein derived from alternative splicing, APP751, APP770, APP695, with APP695 demonstrating highest levels of expression in brain (Ponte, et al., 1988, Tanaka, et al., 1988, Tanaka, et al., 1989). APP processing has recently been extensively described elsewhere (Zheng and Koo, 2011, Zhang, et al., 2012) and will, therefore, not be heavily detailed here. Briefly, APP undergoes post-translational processing via two pathways, the non-amyloidogenic and the amyloidogenic. In the non-amyloidogenic pathway, APP is sequentially cleaved first by α-secretase (ADAMs) to generate an N-terminal sAPP-α fragment. The remaining C83 C-terminal fragment is further cleaved by γ-secretase (Presenilin1 or Presenilin2, Presenilin enhancer 2 (PEN2), Anterior pharynx-defective 1 (APH1) and Nicastrin) to release a P3 peptide and its C-terminal intracellular counterpart, the APP intracellular domain (AICD). In the amyloidogenic processing pathway, APP is again sequentially cleaved but first by the β-secretase (BACE1) to now yield the N-terminal sAPP-β fragment. The remaining C99 C-terminal fragment undergoes ε and γ cleavages by γ-secretase to release the amyloid beta (Aβ) 1–40/42 peptides and an AICD. A characteristic accumulation of Aβ peptides in the brains of AD patients has helped to draw research focus to these particular peptide metabolites of APP. Finally, APP can also be processed by caspase 3 to release cytotoxic C31 and Jcasp fragments. Amyloid precursor protein and mRNA have been shown to be expressed in the brain, thymus, heart, muscle, lung, kidney, adipose tissue, liver, spleen, skin, and intestine (Selkoe, et al., 1988, Joachim, et al., 1989, Yamada, et al., 1989, Sandbrink, et al., 1994, Akaaboune, et al., 2000, Herzog, et al., 2004, Galloway, et al., 2007, Lee, et al., 2008). Therefore, even though the function and processing of APP in neurons may appear particularly relevant to the study of AD, the wide-spread expression of the protein suggests it has a broader role in both normal and disease physiology.

3. APP function and Aβ pathology in skin

APP expression in the mammalian epidermis is predominantly in basal keratinocytes, but can also be found in the melanotyes and in melanoma cells (Hoffmann, et al., 2000, Herzog, et al., 2004). APP is also expressed *in vitro* in the immortalized human keratinocyte cell line, HaCaT, as well as in proliferating primary keratinocytes (Herzog, et al., 2004). Aβ deposits have been reported beneath the epidermal/dermal junction as well as near small blood vessels and glandular structures in human tissue (Joachim, et al., 1989).

Aspects of APP function in the epidermis have already been reviewed (Herzog, et al., 2004) and all of these will, therefore, not be extensively repeated. However, several key points will be re-emphasized. It appears that APP promotes cell adhesion to several components of the extracellular matrix based upon its *in vitro* interactions with perlecan, laminin, collagen type IV, and entactin. Studies using wild type and $APP^{-/-}$ mice demonstrate that APP may also function as a membrane receptor and regulate behavior of the axonal transport protein, kinesin-I, which mediates movement of membrane-bound compartments such as melanosomes along microtubules. Interestingly, APP also appears to regulate copper homeostasis as suggested by its *in vitro* ability to reduce Cu(II) to Cu(I) leading to oxidative stress and apoptosis in the basal epidermis in human keratinocytes.

In addition to these functions attributed to full length protein, the APP fragment, sAPPα, promotes human keratinocyte proliferation and migration and regulates melanocyte function *in vitro*. This is consistent with the fact that $APP^{-/-}$ keratinocytes have reduced proliferative and cell substrate adhesion potential. These findings suggest that sAPPα belongs to a family of structurally similar cysteine-rich growth factors for epidermal keratinocytes involved in growth, differentiation and wound repair.

APP or its metabolites may also play some role in epidermal pathology. For instance, APP levels are upregulated in keratinocytes in psoriasis, a very common chronic inflammatory human skin disease in which keratinocyte proliferation and differentiation are perturbed leading to alteration in epidermal thickness and composition (Romanowska, et al., 2009). Processing of APP to Aβ *in vitro* in human psoriasis patient keratinocytes increases transcription of kynureninase, which can induce an inflammatory skin reaction (Romanowska, et al., 2009). Increased APP expression also correlates with advanced melanoma progression in human tissues and downregulation by RNA interference *in vitro* in human melanoma cell lines results in terminal and irreversible differentiation (Botelho, et al., 2010).

APP processing and Aβ release has also been shown to be regulated by protein kinase C (PKC) activity in cultured skin fibroblasts from familial Alzheimer's disease (FAD) patients, in which phorbol ester stimulation of PKC-α activity increases sAPP-α and decreases Aβ secretion (Gasparini, et al., 1998). Aβ secretion is basally elevated in cultured skin fibroblasts from FAD patients, suggesting that FAD has a deficit in PKC activity. FAD cultured fibroblasts also have increased total membrane bound calcium with attenuated calcium uptake as well as increased lactate production and altered glucose utilization compared to non-AD controls. This suggests that APP may be involved in regulating a disease phenotype in FAD epidermis (Gasparini, et al., 1998).

Collectively, these findings from the skin indicate that APP and its metabolites have a role in regulating epidermal cell phenotypes with a significant ability to modulate proliferative and migratory behavior. Disease-associated changes in expression or processing of APP correlate with perturbations of these behaviors in a variety of diseases.

4. APP function and Aβ pathology in adipose tissue

APP and its Aβ fragments are also expressed in human adipose tissue adipocytes and macrophages (Lee, et al., 2008). Obesity upregulates APP levels *in vivo* in human adipose tissue, which correlates with insulin resistance, hyperinsulinemia, and an increase in the expression profile of the proinflammatory genes, monocyte chemotactic protein -1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and interleukin-6 (IL-6), in human adipocytes *in vitro* (Lee, et al., 2008). Elevated human adipocyte APP gene expression *in vivo* also correlates with increased plasma Aβ40 levels (Lee, et al., 2009). Tumor necrosis factor α (TNFα) stimulation increases APP protein levels *in vitro* in 3T3-L1 adipocytes in a dose-dependent manner (Sommer, et al., 2009). This correlates with an observed increase in levels of adipose tissue TNFα and APP in a murine model of diet-induced obesity although agonist antibody stimulation of APP does not alter *in vitro* murine adipocyte viability, proinflammatory TNFα secretion, or differentiation state (Puig, et al., 2012). These data from both rodent and human studies indicate that APP expression in adipose tissue appears to be correlatively up-regulated during proinflammatory conditions, such as obesity, and is positively regulated by direct proinflammatory stimulation of adipocytes. However, the function of APP or its metabolites basally or during proinflammatory upregulation in adipose tissue remains unclear.

5. APP function and Aβ pathology in intestine

APP is expressed in the intestine and is localized to enterocytes, neurons, and smooth muscle of the muscualaris externa in mice (Puig, et al., 2011). APP and Aβ levels are increased in absorptive columnar epithelial cells in mice fed a high fat diet that is enriched in saturated fat and cholesterol. However, Aβ levels are attenuated by fasting for 65hr suggesting that APP or its metabolites may regulate chylomicron biosynthesis (Galloway, et al., 2007). Aβ immunoreactivity colocalizes with apo B in small intestine enterocytes along the lengths of the villi and Aβ levels are attenuated in mice fed a diet free of saturated fat but supplemented with cholesterol, again supporting the idea that Aβ is involved in chylomicron biosynthesis (Galloway, et al., 2009, Pallebage-Gamarallage, et al., 2009). Enterocyte Aβ immunostaining localizes to perinuclear regions suggesting a location within the golgi apparatus or rough endoplasmic reticulum (Galloway, et al., 2007). Comparing wild type and $APP^{-/-}$ mice demonstrates that APP expression also regulates the behavior of enteric neurons, macrophages and epithelial cells to modulate motility and absorption as well as barrier integrity (Puig, et al., 2011). $APP^{-/-}$ mice also have an attenuated intestinal inflammatory profile suggesting APP may regulate host-microbe interaction or susceptibility to gastrointestinal inflammatory disease (Puig, et al., 2011). There may also be some contribution of APP or its metabolites to traditionally non-intestine related disease. For instance, Aβ immunoreactive plaques are detectable in the intestines of AD patients (Joachim, et al., 1989). These findings indicate that APP expression and metabolite generation are not limited to a particular cell type in the digestive system with a myriad set of functions attributed to these proteins ranging from specific absorption and gut motility to immune response.

6. APP function and Aβ pathology in muscle

APP is also present in the pre and post synaptic compartments of mouse skeletal muscle and in cultured murine myogenic cells (Akaaboune, et al., 2000). APP is detected as early as murine embryonic day 16 (E16) in the cytoplasm of developing muscle fibers. By E18 APP distributes throughout the muscle fiber sarcoplam and at birth (P0) APP immunoreactivity begins localizing to the neuromuscular junctions (NMJs). By postnatal day 5 (P5), APP immunoreactivity restricts to NMJs where it colocalizes with acetylcholine receptors (AChRs). In this NMJ localization, APP expression continually increases into adulthood (Akaaboune, et al., 2000). Not surprisingly, APP expression is required for NMJ synapse formation of murine motor neurons (Wang, et al., 2005). In the absence of APP, murine NMJs demonstrate aberrant localization of presynaptic proteins with postsynaptic AChR clusters and decreased synaptic vesicles at presynaptic terminals and a significant increase in synaptic dysfunction (Wang, et al., 2005).

These critical roles of APP in NMJ function suggest that altered expression or behavior of APP or its metabolites may be involved in disease. Muscle fibers of patients with the debilitating skeletal muscle disorder, inclusion-body myositis (IBM), have intrafiber “plaque-like” accumulation of intracellular Aβ which is preferentially greater for Aβ42 than Aβ40 (Askanas and Engel, 2006). Accordingly, APP and Aβ are suggested to accumulate in skeletal muscle of IBM patients where they are hypothesized to act through a variety of mechanisms to promote myofiber degeneration, atrophy, and death (Greenberg, 2010). In addition, Aβ40 and Aβ42 levels are elevated in the temporalis muscles of AD vs. nondemented individuals suggesting some perturbation of neuromuscular function (Kuo, et al., 2000).

These data from muscle indicate that APP and its metabolites have not only a developmental role in NMJ formation but also may be critically involved in regulating normal transmission

at this specialized form of synapse. Moreover, altered expression or accumulation of APP or its breakdown products in muscle, at least, correlates with diverse disease conditions but may also play a role in neuromuscular pathology.

7. Conclusions

The structure and processing of APP is well described even though the function of the full length protein and its many metabolites remains unclear. This is compounded by the fact that the biology of this protein may be different depending upon the cell type or tissue involved. Nevertheless, defining the function of APP and its proteolytic fragments in a cell-by-cell fashion will likely provide insight not only into Alzheimer's disease but myriad disease conditions such as psoriasis, obesity, gut inflammatory disease, inclusion-body myositis and other diseases of peripheral tissues. In fact, determining the behavior of APP and its products in peripheral cells may, by comparison, better define their normal and disease actions in neurons or within the brain since even this behavior remains unclear. For instance, appreciating the peripheral distribution and processing of APP may provide insight into mechanisms for increases in circulating Aβ levels which could cross the blood-brain-barrier and contribute to brain parenchymal Aβ load during AD (Mackic, et al., 2002). More importantly, there exists the tantalizing possibility that targeting generation or function of APP and its breakdown products might be therapeutically applicable to conditions beyond AD.

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Abbreviations

AChRs	acetylcholine receptors
ADAM	a disintegrin and metalloprotease domain
AD	Alzheimer's disease
APP	amyloid precursor protein
Aβ	amyloid beta
APH1	Anterior pharynx-defective 1
AICD	APP intracellular domain
BACE1	β-secretase
CNS	central nervous system
FAD	familial Alzheimer's disease
IBM	inclusion-body myositis
IL-6	interleukin-6
MIP-1α	macrophage inflammatory protein-1α
MCP-1	monocyte chemotactic protein -1
NMJs	neuromuscular junctions
PEN2	Presenilin enhancer 2
PKC	protein kinase C

TNFα tumor necrosis factor α

References

Akaaboune M, Allinquant B, Farza H, Roy K, Magoul R, Fiszman M, Festoff BW, Hantai D. Developmental regulation of amyloid precursor protein at the neuromuscular junction in mouse skeletal muscle. Mol Cell Neurosci. 2000; 15:355–367. [PubMed: 10845772]

Askanas V, Engel WK. Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. Neurology. 2006; 66:S39–S48. [PubMed: 16432144]

Botelho MG, Wang X, Arndt-Jovin DJ, Becker D, Jovin TM. Induction of terminal differentiation in melanoma cells on downregulation of beta-amyloid precursor protein. J Invest Dermatol. 2010; 130:1400–1410. [PubMed: 19759550]

Dyrks T, Weidemann A, Multhaup G, Salbaum JM, Lemaire HG, Kang J, Muller-Hill B, Masters CL, Beyreuther K. Identification, transmembrane orientation and biogenesis of the amyloid A4 precursor of Alzheimer's disease. Embo J. 1988; 7:949–957. [PubMed: 2900137]

Galloway S, Jian L, Johnsen R, Chew S, Mamo JC. beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. J Nutr Biochem. 2007; 18:279–284. [PubMed: 16962759]

Galloway S, Takechi R, Pallebage-Gamarallage MM, Dhaliwal SS, Mamo JC. Amyloid-beta colocalizes with apolipoprotein B in absorptive cells of the small intestine. Lipids Health Dis. 2009; 8:46. [PubMed: 19845970]

Gasparini L, Racchi M, Binetti G, Trabucchi M, Solerte SB, Alkon D, Etcheberrigaray R, Gibson G, Blass J, Paoletti R, Govoni S. Peripheral markers in testing pathophysiological hypotheses and diagnosing Alzheimer's disease. Faseb J. 1998; 12:17–34. [PubMed: 9438407]

Greenberg SA. Theories of the pathogenesis of inclusion body myositis. Curr Rheumatol Rep. 2010; 12:221–228. [PubMed: 20425523]

Herzog V, Kirfel G, Siemes C, Schmitz A. Biological roles of APP in the epidermis. Eur J Cell Biol. 2004; 83:613–624. [PubMed: 15679106]

Hoffmann J, Twiesselmann C, Kummer MP, Romagnoli P, Herzog V. A possible role for the Alzheimer amyloid precursor protein in the regulation of epidermal basal cell proliferation. Eur J Cell Biol. 2000; 79:905–914. [PubMed: 11152291]

Joachim CL, Mori H, Selkoe DJ. Amyloid beta-protein deposition in tissues other than brain in Alzheimer's disease. Nature. 1989; 341:226–230. [PubMed: 2528696]

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature. 1987; 325:733–736. [PubMed: 2881207]

Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE. Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. Am J Pathol. 2000; 156:797–805. [PubMed: 10702395]

Lee YH, Martin JM, Maple RL, Tharp WG, Pratley RE. Plasma amyloid-beta peptide levels correlate with adipocyte amyloid precursor protein gene expression in obese individuals. Neuroendocrinology. 2009; 90:383–390. [PubMed: 19672057]

Lee YH, Tharp WG, Maple RL, Nair S, Permana PA, Pratley RE. Amyloid precursor protein expression is upregulated in adipocytes in obesity. Obesity (Silver Spring). 2008; 16:1493–1500. [PubMed: 18483477]

Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B, Zlokovic BV. Circulating amyloid-beta peptide crosses the blood-brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. Vascul Pharmacol. 2002; 38:303–313. [PubMed: 12529925]

Pallebage-Gamarallage MM, Galloway S, Johnsen R, Jian L, Dhaliwal S, Mamo JC. The effect of exogenous cholesterol and lipid-modulating agents on enterocytic amyloid-beta abundance. Br J Nutr. 2009; 101:340–347. [PubMed: 18631412]

Ponte P, Gonzalez-DeWhitt P, Schilling J, Miller J, Hsu D, Greenberg B, Davis K, Wallace W, Lieberburg I, Fuller F. A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. Nature. 1988; 331:525–527. [PubMed: 2893289]

Puig KL, Swigost AJ, Zhou X, Sens MA, Combs CK. Amyloid precursor protein expression modulates intestine immune phenotype. J Neuroimmune Pharmacol. 2011; 7:215–230. [PubMed: 22124967]

Puig KL, Floden AM, Adhikari R, Golovko MY, Combs CK. Amyloid precursor protein and proinflammatory changes are regulated in brain and adipose tissue in a murine model of high fat diet-induced obesity. PLoS One. 2012; 7:e30378. [PubMed: 22276186]

Romanowska M, Evans A, Kellock D, Bray SE, McLean K, Donandt S, Foerster J. Wnt5a exhibits layer-specific expression in adult skin, is upregulated in psoriasis, and synergizes with type 1 interferon. PLoS One. 2009; 4:e5354. [PubMed: 19399181]

Sandbrink R, Masters CL, Beyreuther K. Beta A4-amyloid protein precursor mRNA isoforms without exon 15 are ubiquitously expressed in rat tissues including brain, but not in neurons. J Biol Chem. 1994; 269:1510–1517. [PubMed: 8288617]

Selkoe DJ, Podlisny MB, Joachim CL, Vickers EA, Lee G, Fritz LC, Oltersdorf T. Beta-amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc Natl Acad Sci U S A. 1988; 85:7341–7345. [PubMed: 3140239]

Sommer G, Kralisch S, Lipfert J, Weise S, Krause K, Jessnitzer B, Lossner U, Bluher M, Stumvoll M, Fasshauer M. Amyloid precursor protein expression is induced by tumor necrosis factor alpha in 3T3-L1 adipocytes. J Cell Biochem. 2009; 108:1418–1422. [PubMed: 19862700]

Tanaka S, Nakamura S, Ueda K, Kameyama M, Shiojiri S, Takahashi Y, Kitaguchi N, Ito H. Three types of amyloid protein precursor mRNA in human brain: their differential expression in Alzheimer's disease. Biochem Biophys Res Commun. 1988; 157:472–479. [PubMed: 3144271]

Tanaka S, Shiojiri S, Takahashi Y, Kitaguchi N, Ito H, Kameyama M, Kimura J, Nakamura S, Ueda K. Tissue-specific expression of three types of beta-protein precursor mRNA: enhancement of protease inhibitor-harboring types in Alzheimer's disease brain. Biochem Biophys Res Commun. 1989; 165:1406–1414. [PubMed: 2514687]

Wang P, Yang G, Mosier DR, Chang P, Zaidi T, Gong YD, Zhao NM, Dominguez B, Lee KF, Gan WB, Zheng H. Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-Like protein 2. J Neurosci. 2005; 25:1219–1225. [PubMed: 15689559]

Yamada T, Sasaki H, Dohura K, Goto I, Sakaki Y. Structure and expression of the alternatively-spliced forms of mRNA for the mouse homolog of Alzheimer's disease amyloid beta protein precursor. Biochem Biophys Res Commun. 1989; 158:906–912. [PubMed: 2493250]

Zhang H, Ma Q, Zhang YW, Xu H. Proteolytic processing of Alzheimer's beta-amyloid precursor protein. J Neurochem. 2012; 120(Suppl 1):9–21. [PubMed: 22122372]

Zheng H, Koo EH. Biology and pathophysiology of the amyloid precursor protein. Mol Neurodegener. 2011; 6:27. [PubMed: 21527012]

Highlights

Expression and processing of amyloid precursor protein is reviewed.

Functions of amyloid precursor protein and proteolytic fragments in non-neural tissue are reviewed.

Possible roles of amyloid precursor protein fragments in disease conditions other than Alzheimer's disease are reviewed.