Published in final edited form as:

Neurobiol Aging. 2016 February ; 38: 11–13. doi:10.1016/j.neurobiolaging.2015.10.027.

Abeta 1-40 Enhances the Proliferation of Human Diploid Fibroblasts

Lindsey Theda^a, Michelle K. Drews^{a,b}, Galynn Zitnik^a, Junko Oshima^{a,c}, and George M. Martin^{a,*}

^aDepartment of Pathology, University of Washington, Seattle, WA, USA

^cDepartment of Medicine Chiba University, Japan

Abstract

There is a vast literature on the role of beta amyloid (A β) peptides in the pathogenesis of Alzheimer's disease. There is a paucity of research, however, on potential physiological functions of these evolutionarily conserved products of the β amyloid precursor protein (APP). Based on previous studies in neuroblastoma cells (Zitnik, et al., 2007), we hypothesized that A β may contribute to the proliferation of somatic cells. We present evidence supporting this hypothesis for the case of cultured hTERT immortalized human skin fibroblasts. Optimal concentrations ranged from 100 pM – 10 nM, depending upon the nature of the assay.

Keywords

Beta-amyloid; Alzheimer's disease; Cell culture

1. Introduction

One of the classical pathological hallmarks of advanced Alzheimer's disease (AD) is the widespread deposition of plaques consisting of beta-amyloid (A β) (Vinters, 2015). A β , a 4 KD protein fragment formed from the beta amyloid precursor protein (APP) by proteolytic cleavage by β and γ -secretases, has been extensively studied in the context of Alzheimer's disease pathology (Selkoe, 2011). In particular, oligomers of the 42 amino acid A β 1-42 isoform are neurotoxic and promote amyloid deposition, while A β 1-40 peptides inhibit amyloid deposition (Selkoe, 2011).

From an evolutionary perspective, A β likely evolved as the result of its enhancement of one or more physiological functions (Rajasekhar, et al., 2015). Postulated normative functions of A β 1-42 include the support of long-term potentiation and synaptic plasticity (Garcia-Osta and Alberini, 2009, Puzzo, et al., 2008). Several lines of evidence also support roles for APP

*Corresponding author: George M. Martin, M.D., Box357470, Rm HSB-K543, 1959 NE Pacific St, Seattle WA 98105. gmmartin@u.washington.edu.

^bCurrent address: Stanford University School of Medicine, Stanford, CA, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and A β in mitosis, proliferation, and differentiation (Lopez-Sanchez, et al., 2005). It has been postulated that A β -mediated cell cycle re-entry may be the first step leading to aberrant cell cycle progression and neuronal death (Seward, et al., 2013). Several recent reports have also emphasized antioxidant activities for A β via the sequestration of metals such as copper and zinc. (Rajasekhar, et al., 2015).

Our interest in this subject was prompted by the surprising localizations of APP epitopes within the mitotic apparatus (Zitnik, et al., 2007). We therefore sought evidence of the potential roles of A β peptides in the proliferation of normal diploid somatic cells. The present report lends support for this hypothesis for the case of normal human skin fibroblasts.

2. Materials and Methods

2.1. Cells, reagents, and Western analysis

An hTERT immortalized human fibroblast line, 82-6pBlox, was maintained in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) at 37° C in 5% CO₂ and ambient oxygen (see Supplemental Methods for details) (Huang, et al., 2005).

Embryonic stem cell media (ES media) was prepared by adding 10% heat-inactivated "ES cell" qualified serum (Stem Cell Technologies, Vancouver, BC, Canada), 20 μ g/mL recombinant human EGF (Stem Cell Technologies), 1 μ L/mL 0.2% heparin (Stem Cell Technologies), and 10 μ g/mL recombinant human basic EGF (Stem Cell Technologies) (Zitnik, et al., 2007). ES media does not contain any detectible peptide with immunoreactivity to A β epitopes (Zitnik, et al., 2007). Detailed information on how ES media is made was not available. A β 1-40 peptide (A1075, Sigma Aldrich, St Louis, MO) was reconstituted in DMSO, and then diluted with PBS before being added to ES media to the desired final concentration. The reverse peptide, A β 40-1 (REV), was similarly prepared.

Western analyses of A β were performed as described previously (Zitnik, et al., 2007). Briefly, FBS or ES serum was immunoprecipitated with either anti- A β antibody 4G8 or anti-actin antibody (Signet Laboratories, Dedham, MA), and were subjected to western analysis using anti-A β antibody 6E10 (Signet Laboratories) (see Supplemental Methods) (Zitnik, et al., 2007).

2.2. Proliferation assays and statistical analysis

Clone size distribution assays were determined three times by culturing the cells at clonal densities in 100-mm culture dishes in duplicate for 1 week, followed by staining with crystal violet and counting the number of cells within each clone as described previously (Huang, et al., 2005). The number of doublings was calculated as log₂. The 5-Bromo-2'-deoxyuridine (BrdU) incorporation assay was done three times by labeling for 30min on day 3 after cells were plated in various conditions (see Supplemental Methods for details). Cell growth curves were determined four times by plating cells at 4 $\times$ 10⁴ cells/35-mm dish in duplicate, and then manually counting cells with a hemocytometer every 24 hours.

Paired and two-sample unequal variance student t-tests were used for statistical analysis.

3. Results

We first confirmed that the majority of the reconstituted commercially obtained A β 1-40 was in the form of monomers (Fig. 1A). We also confirmed that FBS but not ES serum contains detectable levels of A β (Fig. 1A). It should be noted that the protein sequences of human A β and bovine A β are identical. We then used the clone size distribution assay to assess whether or not A β 1-40 impacts the proliferation of human diploid fibroblasts (Fig. 2A). There was substantial improvement in clonal proliferation in media with (FBS) vs. media without (ES or ES+REV) A β 1-40. Comparisons of the percentage cells dividing only once in FBS vs. ES media showed statistical significance with a p value of 0.015. The dose response data in ES media points to an optimum A β concentration of ~1nM.

The proliferative effects of A β 1-40 were then evaluated by BrdU incorporation assays (Fig. 2B). ES media and ES+A β 40-1 (REV) gave the lowest incorporations (11.0% and 14.4%, respectively) and FBS media provided the highest levels (28.9%). The highest BrdU-labeling index (19.1%) was at 100pM A β 1-40. When compared to ES media, these differences were statistically significant for FBS and 1nM A β 1-40 ($P = 0.012$ and 0.007 , respectively). Differences between BrdU incorporation in ES vs. REV and ES vs. A β 1-40 at concentrations of 100pM, 10nM, or 100nM were not statistically significant ($P = 0.068$, 0.102 , 0.082 , and 0.057 , respectively). A comparison between the combined data of all A β -treated samples (100pM, 1nM, 10nM and 100nM) and the combined data of all untreated samples (ES and Rev) showed a P value of 0.0049.

The above results were further supported by growth curve assays (Fig. 2C). Fibroblast proliferation on the first and third days with FBS or with ES media with 10nM A β 1-40 was significantly higher than those in ES media ($P = 0.014$ and 0.033 , respectively, for Day 1 and $P = 0.020$ and 0.006 , respectively, for Day 3). Proliferation in A β 40-1 (REV), 1nM A β 1-40, and 100nM A β 1-40 were not significantly different from that in ES media ($P = 0.053$, 0.066 and 0.075 , respectively, for Day 1 and $P = 0.080$, 0.056 and 0.116 , respectively, for Day 3). This indicates that under the experimental conditions used in these assays, 10nM of A β 1-40 provided optimal concentrations for proliferation. In contrast, concentrations of 100nM inhibited cell proliferation. Growth curves appeared to have leveled off by day 3 in all conditions probably because of contact inhibition and the fact that media with too low or too high A β 1-40 concentrations are not optimal for fibroblast growth and survival. Results of Day 2 did not rise to the statistical significance cutoff value of 0.05 probably because of a larger experimental variation between data points. We did not observe overt light microscopic abnormalities in cell morphologies in these experiments, although cells in slow-growing cultures tended to be flatter and larger (Fig. 1B).

4. Discussion

In this report, we have investigated the role of A β 1-40 upon the proliferation of a non-neuronal cell type. The results of three independent assays were consistent with this hypothesis and with a U shaped dose-response curve. Normal endogenous levels of A β are estimated to range from 200–1000 pM (Cirrito, et al., 2003). While pM concentrations of exogenous A β have been shown to have significant physiological effects (Garcia-Osta and

Alberini, 2009, Puzzo, et al., 2008), μM concentrations have been shown to create mitotic spindle abnormalities and aneuploidies in human and non-human somatic cells (Borysov, et al., 2011). Additional research will be required to determine optimal A β concentrations for the proliferation of a variety of cell types, certainly including astroglia and microglia. The proliferative effect of A β has also been shown in cerebral endothelial cells and in choroid plexus epithelial cells (Bolos, et al., 2013, Boscolo, et al., 2007). Additional research should also evaluate the degree to which aggregated moieties adhere to the various plastic or glass vehicles utilized for the preparation, storage and delivery of such peptides. Given the propensity of such aggregates to adhere to these vesicles (Lewczuk, et al., 2006), it is likely that our estimates of concentrations and those of other investigators are overestimates.

Mechanisms by which A β promotes cell growth are not yet well understood but are likely to involve multiple pathways (Rajasekhar, et al., 2015). Co-localization of A β with the mitotic spindle supports a role of A β in the M phase of the cell cycle (Zitnik, et al., 2007). In a preliminary investigation of biochemical pathways potentially involved in the proliferative effects of A β , we determined the steady state levels of two major candidate genes, cdk4 and p21, as well as S6 kinase activity, a marker of the mTOR pathway, in A β 1-40 treated and untreated cells. We did not observe any significant differences by Western analysis (data not shown).

In summary, we provide some support for a role of A β in the modulation of the proliferation of human somatic cells. The underlying mechanisms and pathophysiological relevance of this observation will require much more research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the technical assistance of Ms. Alexis Jacobson and Ms. Lin Lee. This work was supported by a NIH grant, R24AG42328 (Martin).

References

- Bolos M, Spuch C, Ordonez-Gutierrez L, Wandosell F, Ferrer I, Carro E. Neurogenic effects of beta-amyloid in the choroid plexus epithelial cells in Alzheimer's disease. *Cell Mol Life Sci.* 2013; 70(15):2787–97. [PubMed: 23455075]
- Borysov SI, Granic A, Padmanabhan J, Walczak CE, Potter H. Alzheimer Abeta disrupts the mitotic spindle and directly inhibits mitotic microtubule motors. *Cell Cycle.* 2011; 10(9):1397–410. [PubMed: 21566458]
- Boscolo E, Folini M, Nico B, Grandi C, Mangieri D, Longo V, Scienza R, Zampieri P, Conconi MT, Parnigotto PP, Ribatti D. Beta amyloid angiogenic activity in vitro and in vivo. *Int J Mol Med.* 2007; 19(4):581–7. [PubMed: 17334633]
- Cirrito JR, May PC, O'Dell MA, Taylor JW, Parsadanian M, Cramer JW, Audia JE, Nissen JS, Bales KR, Paul SM, DeMattos RB, Holtzman DM. In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *J Neurosci.* 2003; 23(26):8844–53. [PubMed: 14523085]
- Garcia-Osta A, Alberini CM. Amyloid beta mediates memory formation. *Learn Mem.* 2009; 16(4):267–72. [PubMed: 19318468]

- Huang S, Chen L, Libina N, Janes J, Martin GM, Campisi J, Oshima J. Correction of cellular phenotypes of Hutchinson-Gilford Progeria cells by RNA interference. *Hum Genet.* 2005; 118(3–4):444–50. [PubMed: 16208517]
- Lewczuk P, Beck G, Esselmann H, Bruckmoser R, Zimmermann R, Fiszer M, Bibl M, Maler JM, Kornhuber J, Wiltfang J. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. *Clin Chem.* 2006; 52(2):332–4. [PubMed: 16449222]
- Lopez-Sanchez N, Muller U, Frade JM. Lengthening of G2/mitosis in cortical precursors from mice lacking beta-amyloid precursor protein. *Neuroscience.* 2005; 130(1):51–60. [PubMed: 15561424]
- Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci.* 2008; 28(53):14537–45. [PubMed: 19118188]
- Rajasekhar K, Chakrabarti M, Govindaraju T. Function and toxicity of amyloid beta and recent therapeutic interventions targeting amyloid beta in Alzheimer's disease. *Chem Commun (Camb).* 2015; 51(70):13434–50. [PubMed: 26247608]
- Selkoe DJ. Alzheimer's disease. *Cold Spring Harb Perspect Biol.* 2011; 3(7):1–16.
- Seward ME, Swanson E, Norambuena A, Reimann A, Cochran JN, Li R, Roberson ED, Bloom GS. Amyloid-beta signals through tau to drive ectopic neuronal cell cycle re-entry in Alzheimer's disease. *J Cell Sci.* 2013; 126(Pt 5):1278–86. [PubMed: 23345405]
- Vinters HV. Emerging concepts in Alzheimer's disease. *Annu Rev Pathol.* 2015; 10:291–319. [PubMed: 25387055]
- Zitnik G, Wang L, Martin GM, Hu Q. Localizations of endogenous APP/APP-proteolytic products are consistent with microtubular transport. *J Mol Neurosci.* 2007; 31(1):59–68. [PubMed: 17416970]

- We hypothesize that A β might have evolved for a beneficial effect on normal somatic cells.
- Three different assays showed A β 1-40-mediated enhancement of cell growth.
- Our data support a role of A β in the modulation of the proliferation of human somatic cells.

Figure 1.

Confirmation of the presence of A β 1-40 monomers and morphology of 82-6pBlox fibroblasts: (A) Western analysis of 240 nM A β 1-40 peptide (Std). Note the detectable levels of immunoprecipitated A β in FBS but not in ES. Act: anti-actin. A β : anti-A β antibody. (B) Morphology of 82-6pBlox cells cultured 5 days in FBS media (FBS), ES media (ES), ES media with 100pM of reverse peptide (A β 40-1; REV) or with 100pM of A β 1-40 peptide (A β 1-40).

Figure 2.

Aβ-mediated promotion of cell proliferation: (A) Colony size distributions of 82-6pBlox fibroblasts. Percent of colonies that had undergone the indicated number of doublings over 7 days are shown for each condition. FBS: fetal bovine serum. ES: ES certified serum with supplements (see Materials and Methods); REV: ES media with 10nM Aβ40-1; Aβ: ES media with indicated concentrations of Aβ1-40. * indicates statistical significance. (B) BrdU-labeling index. Percent labeled nuclei are shown for each condition. (C) Cell growth curves. Increase of cell numbers are shown as fold increase compared to the initial counting of day 0.