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PAPP-A: A New Anti-Aging Target?

Cheryl A. Conover
Division of Endocrinology, Metabolism, and Nutrition, Endocrine Research Unit, Mayo Clinic, Rochester, MN

Abstract

This article focuses on the role of PAPP-A in mammalian aging. It introduces PAPP-A and a little of its history, briefly discusses the function of PAPP-A in the insulin-like growth factor (IGF) system and the regulators of PAPP-A expression, and then reviews data concerning PAPP-A in aging and age-related diseases especially in regard to the PAPP-A knock-out (KO) mouse. The PAPP-A KO mouse is a valuable new model to test hypotheses concerning the control of the tissue availability of IGF, independent from systemic levels, on healthspan as well as lifespan.

What is PAPP-A?

PAPP-A is the acronym for pregnancy-associated plasma protein-A because the protein was first identified as one of four proteins found at high concentrations in the plasma of pregnant women (Lin *et al.* 1974). Its function was unknown for 25 years but had, and continues to have, clinical utility in screens for Down Syndrome (van Heesch *et al* 2010). In the early 1990s, several laboratories noted novel insulin-like growth factor (IGF)-dependent proteolytic activity against IGF binding protein (IGFBP)-4 in culture media from a variety of cells, including fibroblasts, osteoblasts, and vascular smooth muscle cells (Fowlkes & Freemark 1992, Conover *et al.*1993, Durham *et al.*1994, Parker *et al.*1995). The protease was characterized as a highly glycosylated, zinc-binding metalloproteinase with a native molecular weight greater than 200 kD (Lawrence *et al.* 1999a). However, the protein responsible for this proteolytic activity remained elusive until Lawrence *et al.* (1999b) successfully isolated it from human fibroblast conditioned media and identified it, using mass spectrometry and molecular probes, as PAPP-A. Subsequent studies demonstrated that PAPP-A clearly had purpose outside of pregnancy.

What does PAPP-A do?

PAPP-A is as an important regulator of local IGF action through proteolysis of IGFBPs (reviewed in Boldt & Conover 2007). Its primary physiological substrate is IGFBP-4, although proteolytic activity against IGFBP-2 and -5 has also been described (Laursen *et al.* 2001, Monget *et al.* 2003, Rivera *et al.* 2003, Kumar *et al.* 2005). IGFBP-4 is considered an inhibitory IGFBP in that it binds IGF-I and IGF-II with high affinity thereby restricting their interaction with IGF receptors on cells. IGFBP-4 is a substrate for PAPP-A only when it is complexed with IGF, hence the observed IGF-dependent proteolytic activity in cell-free assay (Byun *et al.* 2000, Laursen *et al.* 2001)). Moreover, this IGFBP-4/IGF complex can serve as a pericellular reservoir (Ning *et al.* 2008). PAPP-A cleaves the IGFBP-4 into low binding-affinity fragments, releasing the IGF for receptor activation. In this way, IGF action can be augmented without changes in IGF ligand or receptor expression. PAPP-A regulation of local IGF receptor activation has been assessed by phosphorylation of IGF receptor and

Address all correspondence to: Cheryl A. Conover, Ph.D, Mayo Clinic, 200 First Street, 5-194 Joseph, Rochester, MN 55905, Phone: 507-255-6415, Fax: 507-255-4828, Conover.Cheryl@Mayo.edu.

downstream signaling intermediate, IGF-stimulated ^{3}H-thymidine incorporation and IGF-responsive gene expression *in vitro* and *in vivo* in various systems (Ortiz *et al.* 2003, Conover *et al.* 2004, Resch *et al.* 2006, Harrington *et al.* 2007, Miller *et al.* 2007, Conover *et al.* 2010). Importantly, PAPP-A is secreted and then binds to cell surfaces in an autocrine/paracrine manner (Laursen *et al.* 2002, Conover *et al.* 2007). The preserved protease activity of cell-associated PAPP-A would serve to amplify local IGF action. Although IGF-independent actions of PAPP-A are possible and even plausible (Jadlowiec *et al.* 1995), several studies using different approaches support regulated proteolysis of IGFBP-4 as the major function of PAPP-A *in vivo* (Bale & Conover 2005, Ning *et al.* 2008, Phang *et al.* 2010).

What regulates PAPP-A expression *in vitro*?

The most potent stimulators of PAPP-A expression in human fibroblasts and in human vascular smooth muscle and endothelial cells are the proinflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Resch *et al.* 2004, Conover *et al.* 2006, Conover *et al.* 2008a,). IL-6 is also stimulatory in human coronary artery smooth muscle cells (Boldt & Conover 2007) and transforming growth factor-β is stimulatory in human osteoblasts (Ortiz *et al.* 2003). Interestingly, PAPP-A expression was found to be markedly increased with human osteoblast senescence (Kveiborg *et al.* 2000). Direct inhibitors of cytokine-stimulated PAPP-A expression include the polyphenol, resveratrol, and the anti-oxidant, *N*-acetyl cysteine (Conover *et al.* 2006, Boldt & Conover 2007). These *in vitro* findings may be relevant to the regulation and biological function of PAPP-A *in vivo*, as will be touched upon in a later section.

IGFs and aging

According to the theory of antagonistic pleiotropy, there are gene products that can have opposite effects on biological fitness at different ages such that their effects are beneficial early in life but detrimental to the organism later in life (Williams 1957). This appears relevant to the seeming paradox of the IGF system. IGFs are essential for normal body size during fetal development, peak bone mass during puberty, and optimal fecundity in the reproductive period. On the other hand, IGFs are associated with aging and age-related diseases. These observations are generally true for both mice and humans. Since PAPP-A enhances IGF signaling, then the same might be expected for PAPP-A's effects in early versus late life. And the converse should also hold, i.e., loss of PAPP-A or inhibition of PAPP-A activity should have negative effects early in life and beneficial effects later in life. To test this hypothesis, we developed a mouse with global deletion of PAPP-A gene expression (Conover *et al.* 2004). The prediction was that loss of PAPP-A would have impact during critical periods when heightened IGF activity is important and in specific tissues where PAPP-A expression is upregulated. Moreover, since PAPP-A's effects on IGF activity are moderating rather than absolute, the loss of PAPP-A would not be expected to have the dire consequences seen in IGF-I and IGF-I receptor KO mice (Liu *et al.* 1993).

PAPP-A KO mice

PAPP-A KO mice are born as proportional dwarfs due to the suppression of IGF-II-mediated signaling during early embryogenesis prior to organogenesis (Conover *et al.* 2004). IGF-II is the predominant fetal IGF, and the dwarf phenotype of the PAPP-A KO mouse could be rescued by increasing *Igf2* gene expression during fetal development through disruption of its imprinting (Bale & Conover 2005). The PAPP-A KO mice also have mild deficiencies in bone mass and ovarian steroidogenesis (Tanner *et al.* 2008, Nyegaard *et al.* 2010), processes that are known to be regulated by IGFs during puberty and early adulthood. Otherwise, they appear to grow normally while maintaining their small

body size. Moreover, and in accordance with antagonistic pleiotropy, PAPP-A KO mice have a dramatic 20–40% increase in median and maximum lifespan compared to wild-type littermates (Conover & Bale 2007, Conover *et al.* 2010b). Reduced energy expenditure and altered glucose-insulin homeostasis were excluded as key determinants of the enhanced longevity of PAPP-A KO mice (Conover *et al.* 2008b). Of importance to the model, circulating levels of IGF-I are not different in wild-type and PAPP-A KO mice. Recently, Swindell *et al.* (2010) evaluated gene expression in various tissues of wild-type and PAPP-A KO mice and found both similar and contrasting expression patterns between PAPP-A KO mice and long-lived dwarf mice with reduced circulating IGF-I. It is not surprising that kidney, with one of the highest PAPP-A expression levels in wild-type mice, had the most marked changes in gene expression in PAPP-A KO mice, whereas liver, with very little PAPP-A expression, showed few changes. The recent review by Bartke (2008) presents other comparisons of PAPP-A KO mice to long-lived mouse models with genetic manipulations of the IGF-I axis. Although many of these long-lived mice are dwarfs, PAPP-A KO mice whose body size was normalized during fetal development did not lose their longevity advantage (Conover *et al.* 2010a).

A recent report of end-of-life pathology (Conover *et al.* 2010b) indicated that the incidence of neoplastic disease was not significantly different in wild-type and PAPP-A KO mice. However, it occurred in older-aged PAPP-A KO compared with wild-type mice. Furthermore, PAPP-A KO mice were less likely to show degenerative changes of age even though the average age at death was higher. Co-morbidities were significantly reduced in PAPP-A KO mice, as well. In addition, scheduled sacrifice pathology at 78, 104 and 130 weeks of age indicated that wild-type mice had more degenerative changes earlier than PAPP-A KO mice. Specifically, cardiomyopathy, nephropathy, neurodegenerative lesions, and testicular, ovarian and thymic atrophy were more evident and more severe in wild-type than in PAPP-A KO mice at the time points studied. These results are consistent with a general delay in age-related degenerative disease in PAPP-A KO mice, which likely enables them to maintain a better health status compared with wild-type mice.

As reported previously, the thymi of PAPP-A KO mice are relatively resistant to the normal age-dependent atrophy seen in mice and in humans (Vallejo *et al.* 2009). At 78 weeks of age the thymi of wild-type mice were very small in size with extensive fatty tissue infiltrates and few thymocytes. In contrast, PAPP-A KO mice maintained thymic structure with normal histology and cellularity and a pool of diverse and functionally competent T cells. However, at 104 weeks, obvious thymic tissue was not present in most of the PAPP-A KO mice, suggesting delay rather than absolute resistance to thymic involution later in life (Conover *et al.* 2010b). It is intriguing that this delay in thymic involution paralleled the delayed occurrence of neoplasia, especially lymphomas, in PAPP-A KO mice. However, further study is necessary to determine whether-or-not there is a direct relationship.

PAPP-A KO mice are also relatively resistant to the development of atherosclerosis (Harrington *et al.* 2007). In these studies, PAPP-A KO mice were crossed with apolipoprotein E (ApoE) KO mice, the latter being an established mouse model of atherosclerosis susceptibility (Meir & Letersdorf 2004). In the absence of PAPP-A, mice on the ApoE-null background (double KO) had a 70–80% reduction in lesion area after 10 weeks on a high fat diet compared to ApoE KO mice with wild-type PAPP-A gene expression. This was in spite of similar elevations in serum cholesterol and triglyceride levels in the two groups of mice. ApoE KO mice had a progressive increase in aortic lesion complexity, whereas double KO mice had lesions resembling more early stage fatty streaks predominantly composed of lipid-enriched macrophages. Interestingly, the absolute amount of macrophage staining in the lesions of ApoE KO and double KO mice did not differ. In addition, there were no significant differences in expression of macrophage-derived

cytokines, TNF-α and IL-1β. Sulchanov et al. (2007) found that systemic infusion of human recombinant IGF-I into ApoE KO mice fed a high fat diet for 12 weeks was associated with a 30% reduction in atherosclerotic lesion size, decreased macrophage accumulation within lesions, and decreased markers of inflammation. Although these findings appear to be contrary to the findings in the PAPP-A KO mice, they likely reflect differences in local versus circulating IGF-I action. In a recent study (Conover *et al.* 2010), overexpression of PAPP-A in arterial smooth muscle accelerated atherosclerosis in ApoE KO mice, further evidence for the importance of PAPP-A in the cardiovascular system.

PAPP-A and inflammatory stress

Both atherosclerosis and aging are characterized by chronic inflammatory stress. Immunostaining of human atherosclerotic plaque for PAPP-A revealed intense staining in the inflammatory regions of unstable plaque associated with activated macrophages and smooth muscle cells (Bayes-Genis *et al.* 2001). Macrophages do not express PAPP-A (Conover *et al.* 2007). However, our working model is that activated macrophages synthesize proinflammatory cytokines that stimulate vascular smooth muscle cells to synthesize and secrete PAPP-A. PAPP-A can function as both an autocrine and paracrine factor by binding to cells in the plaque. Cell-associated PAPP-A thereby enhances local IGF actions on smooth muscle cells and macrophages, representing an important amplification point in atherosclerotic plaque progression (Fig. 1A). With the loss of PAPP-A as a target of these cytokines, the vicious cycle would be blunted in spite of the continued presence of macrophages and similar levels of TNF-α and IL-1β expression (Fig. 1B). If validated, this model of PAPP-A and inflammatory stress could also apply to other situations. Aging is one such situation (Goto 2008, Vasto *et al.* 2009). Adipogenesis is another. PAPP-A is highly expressed in human, baboon, and mouse preadipocytes from visceral fat depots (Tchkonia *et al.* 2007, Tchoukalova *et al.* 2009, our unpublished data) which is known to be an inflammatory environment especially in obese subjects.

PAPP-A and oxidative stress

At this time, little is known about PAPP-A and oxidative stress, a principal cause of aging (Finkel & Holbrook 2000). Activation of NFκB, a transcription factor involved in oxidative stress, mediated cytokine stimulation of PAPP-A expression in human fibroblasts (Resch *et al.* 2004). Preliminary data suggest that skin fibroblasts from PAPP-A KO mice are more resistant to hydrogen peroxide-induced cell death than fibroblasts from wild-type mice (Fig. 2). Also, levels of PAPP-A have been shown to be elevated in renal transplant patients and correlated with levels of F2-isoprostanes, markers of *in vivo* oxidative stress (Coskun *et al.* 2007, Lauzurica *et al.* 2005). Further studies will lead to a better understanding of the relationship between PAPP-A, oxidative stress, and aging.

What's next?

Accumulating data attest to the importance of PAPP-A in mammalian aging. Nevertheless, more research is needed to fully define its role in the aging process. This review suggests some specific areas that warrant further investigation, among them, immune function, inflammatory response, oxidative stress, adipogenesis, and age-related cancer, atherosclerosis and kidney disease. And the impact of PAPP-A on brain function has yet to be studied. The availability of tissue-specific PAPP-A KO and overexpressing mice will be important for identifying the tissues responsible for the longevity phenotype. Conditional PAPP-A KO mice would allow assessment of adult-specific loss of PAPP-A on lifespan without affecting important aspects of early life physiology. Insights gained by such studies will provide for a deeper understanding of the aging process and contribute valuable

information in the further exploration of PAPP-A as a novel drug target for aging and age-related diseases.

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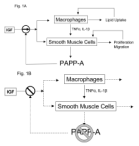

Figure 1.
Working model for (A) PAPP-A enhancement of atherosclerotic lesion development and (B) the consequence of loss of PAPP-A in this context.

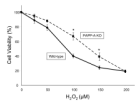

Figure 2.
Skin fibroblasts from wild-type and PAPP-A KO mice were treated with phenol red-free DMEM/10% fetal bovine serum and the indicated concentrations of hydrogen peroxide for 6 h. Cell viability was assessed by trypan blue exclusion. Values are mean ± SEM, N = 4, *P < 0.05.