REVIEW

PAPP-A: a promising therapeutic target for healthy longevity

Cheryl A. Conover[1] and Claus Oxvig[2]

[1]Endocrinology, Mayo Clinic, Rochester, MN, USA
[2]Department of Molecular Biology and Genetics, Aarhus Universitet, Aarhus, Denmark

Summary

Pregnancy-associated plasma protein-A (PAPP-A) is a proteolytic enzyme that was discovered to increase local insulin-like growth factor (IGF) availability for receptor activation through cleavage of inhibitory IGF binding proteins (IGFBPs). Reduced IGF signaling has been associated with increased lifespan and healthspan. Therefore, inhibition of PAPP-A represents a novel approach to indirectly decrease the availability of bioactive IGF. Here, we will review data in support of PAPP-A as a therapeutic target to promote healthy longevity.
Key words: aging; IGF; PAPP-A.

Introduction

PAPP-A was first identified as a placental protein of primates, but no biochemical function was known prior to our discovery in 1999 that PAPP-A is a proteolytic enzyme. We showed that cultured human fibroblasts secrete PAPP-A, and that PAPP-A cleaves IGFBP-4 (Lawrence *et al.*, 1999). Importantly, IGFBP-4 becomes a PAPP-A substrate only following its binding of IGF-I or -II. The two proteolytic fragments resulting from PAPP-A cleavage have very low affinity for the IGFs, causing the IGFBP-4/IGF complex to dissociate.

Further studies found PAPP-A to be expressed in many different cells types besides fibroblasts, including human osteoblasts, vascular smooth muscle cells, pre-adipocytes, ovarian granulosa cells, and kidney mesangial cells (Lawrence *et al.*, 1999; Conover *et al.*, 2001, 2004b, 2006; Resch *et al.*, 2004). It was characterized as a metalloproteinase of the metzincin superfamily and defined a new subfamily, the pappalysins, distinct from previously recognized subfamilies including the matrix metalloproteinases (Boldt *et al.*, 2001). Since the discovery of its function, PAPP-A and its proteolytic activity have been studied in a diverse range of species, including human, baboon, cow, pig, mouse, naked mole rat, marsupial, and zebrafish (Mazerbourg *et al.*, 2001; Conover *et al.*, 2004a; Tchoukalova *et al.*, 2009; Juengel *et al.*, 2010; Phang *et al.*, 2010; Kjaer-Sorensen *et al.*, 2013; Brohus *et al.*, 2015). Based on genomic data, a gene encoding PAPP-A appears to be present in all vertebrates.

The main known function of PAPP-A is to increase local IGF bioavailability through cleavage of inhibitory IGFBPs, in particular IGFBP-4. Indeed, PAPP-A is probably the only physiological IGFBP-4 proteinase (Conover *et al.*, 2004a; Laursen *et al.*, 2007; Ning *et al.*,

2008; Conover, 2012; Oxvig, 2015). Secreted PAPP-A tethers to cells through binding to surface glycosaminoglycans (Fig. 1). IGF bound to IGFBP-4 is not bioactive. However, the liberation of bioactive IGF upon cleavage of IGFBP-4 in the pericellular environment initiates IGF signaling. PAPP-A-induced enhancement of local IGF action through proteolysis of IGFBP-4 has been demonstrated *in vitro* and *in vivo* in several different systems (Conover, 2012; Oxvig, 2015). The only exception to date is in early fetal development of zebrafish (Kjaer-Sorensen *et al.*, 2013). Curiously, a paralog of IGFBP-4 appears to be lacking in zebrafish (Li *et al.*, 2009).

Reduced IGF signaling has been associated with longevity and increased healthspan (Katic & Kahn, 2005). Therefore, a reduction in PAPP-A proteolytic activity represents a novel approach to indirectly decrease the availability of bioactive IGF. For therapeutic intervention, such a strategy is expected to moderately restrain IGF signaling and hence cause fewer adverse effects compared to direct inhibition by targeting the IGF receptor. Here, data in support for the development of PAPP-A as a therapeutic target are reviewed. These are principally based on studies with genetically modified mice and a recently developed monoclonal antibody specific for PAPP-A, which selectively inhibits PAPP-A cleavage of IGFBP-4 (Mikkelsen *et al.*, 2008, 2014). The focus of this review is on curbing aging and age-related diseases.

PAPP-A and lifespan studies

Both male and female PAPP-A knockout (KO) mice on chow diet live 30–40% longer than wild-type (WT) littermates, with no secondary endocrine abnormalities (Conover *et al.*, 2010b). Circulating levels of growth hormone (GH), IGF-I, glucose, and insulin were not significantly different between PAPP-A KO and WT mice in this study. PAPP-A KO mice also live longer when fed a high fat diet starting as adults (Conover *et al.*, 2015). Thus, PAPP-A deficiency can promote longevity without dietary restriction. Furthermore, this extended lifespan is not a secondary consequence of a small body size because PAPP-A KO mice rescued from the dwarf phenotype by enhanced IGF-II expression during fetal development retain their longevity advantage (Conover *et al.*, 2010a). Finally, conditional knockout of the PAPP-A gene in adult mice using tamoxifen-inducible Cre recombinase methodology (Conover *et al.*, 2013a) also resulted in a 20% extension of lifespan ($P < 0.0001$; manuscript in preparation). End-of-life pathology showed delayed occurrence of fatal neoplasias and indicated decreased incidence and severity of conditions with age-related degenerative changes, such as cardiomyopathy, nephropathy, and thymic atrophy in PAPP-A KO mice compared to WT littermates (Conover *et al.*, 2010b).

Several mouse models with reduced GH-stimulated IGF-I expression by liver and low levels of circulating IGF-I (Snell, Ames dwarf, GH receptor KO) have also been found to have extended longevity (Junnila *et al.*, 2013). On the other hand, transgenic mice over-expressing GH exhibit a shortened lifespan (Bartke, 2003). It is important to note that PAPP-A KO mice have normal levels of circulating IGF-I (and GH) and their phenotype reflects reduction in local IGF action. Unlike the GH mutant mice that have postnatal growth retardation, deletion of the PAPP-A gene manifests itself early in fetal development as proportional

Correspondence

Cheryl A. Conover, Endocrinology, Mayo Clinic, 200 First Street SW5-194 Joseph, Rochester, MN 55905, USA. Tel: 507-255-6415; fax: 507-255-4828; e-mail: conover.cheryl@mayo.edu
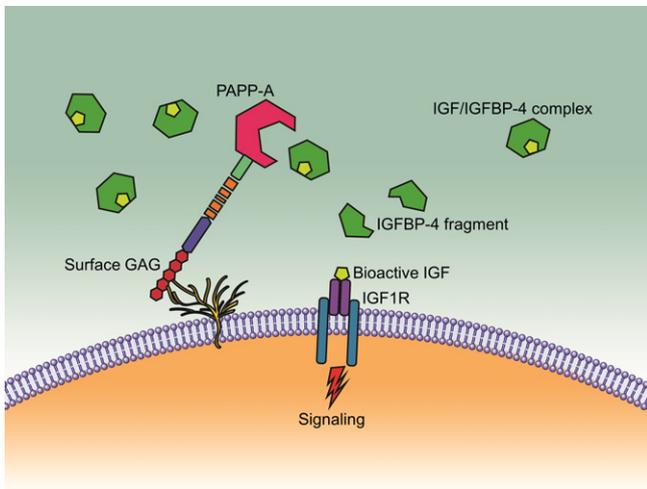
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Fig. 1 Role of PAPP-A and IGFBP-4 in the local control of IGF signaling.

dwarfism (Conover et al., 2004a). The lifespan extension in the Snell, Ames dwarf, and GH receptor KO models reflects GH tone rather than IGF-I bioavailability.

PAPP-A and age-related diseases

Thymic involution

Thymic involution, the shrinking and morphological changes of the thymus in aging vertebrates, results in immune senescence that has the potential to impact aging and age-related diseases. As reported previously, the thymi of PAPP-A KO mice are relatively resistant to normal age-dependent involution (Vallejo et al., 2009). At 18 months of age, thymi of WT mice are markedly reduced in size and cellularity with extensive adipose infiltration. In contrast, the similarly aged PAPP-A KO mice maintain the lobular thymic structure and cellularity and the ability to produce diverse immune-competent T cells.

We recently completed a study of young PAPP-A KO and WT mice that were fed a high fat diet for 20 weeks. Compared to WT littermates, the thymi of male and female PAPP-A KO mice were 122% ($P = 0.007$) and 158% ($P < 0.0001$) larger, respectively. Even with the larger size, the thymi of female PAPP-A KO mice contained 40% less lipid ($P = 0.033$) than WT mice. In a preliminary study, once a week intraperitoneal treatment of WT mice on high fat diet with an inhibitory monoclonal antibody against PAPP-A (mAb-PA) resulted in thymic size that was 120% of treatment with isotype control antibody. Targeting PAPP-A to reduce/delay thymic involution could, therefore, play a central role in regulating immune competence and healthy aging. However, further studies are needed to better characterize and understand the role of PAPP-A in thymic biology.

Atherosclerosis

As first reported by Bayes-Genis et al. (2001), PAPP-A expression is increased in unstable human atherosclerotic plaques associated with activated macrophages, smooth muscle cells, and endothelium. To assess whether this increased PAPP-A expression was cause or consequence of lesion development, we studied apolipoprotein E (ApoE) KO mice, which are an established murine model of atherosclerosis (Breslow, 1996). Reduction in local IGF bioavailability through PAPP-A gene

deletion in ApoE KO mice inhibited atherosclerotic plaque burden by 70–80% following 20 weeks on a high fat diet. There was no effect on macrophage recruitment or expression of pro-inflammatory cytokines, and no secondary endocrine effects were observed (Harrington et al., 2007). Tang et al. (2012) suggested that PAPP-A inhibits cholesterol efflux in macrophage-derived foam cells through the IGF-I signaling pathway, thus contributing to the pathogenesis of atherosclerosis.

To assess a possible effect of PAPP-A gene knockout during development, a similar experiment was carried out in ApoE KO mice with the PAPP-A gene 'floxed' and crossed with mice with tamoxifen-inducible Cre recombinase (Conover et al., 2013a). Inducible reduction in PAPP-A gene expression in adult ApoE KO mice significantly inhibited established atherosclerotic plaque progression and the development of advanced plaque with necrotic cores (Bale et al., 2014). In this inducible model, reduced PAPP-A expression was not limited to the vasculature, and changes in other tissues could potentially contribute to the observed effects. PAPP-A gene expression was efficiently reduced in thymus and spleen (Conover et al., 2013a), which could dampen the negative effects of the immune response in atherosclerosis (Hansson & Libby, 2006). Moreover, weekly treatment (intraperitoneal injections) of ApoE KO mice with mAb-PA, which specifically inhibits PAPP-A-mediated IGFBP-4 proteolysis, similarly inhibited atherosclerotic plaque progression (Conover et al., 2016b). This latter study was the first to demonstrate proof of principle and provide translational support for the development of novel therapeutic strategies to inhibit human atherosclerosis by targeting the proteolytic activity of PAPP-A.

It is of note that PAPP-A can also cleave IGFBP-5 (Laursen et al., 2007). However, as there are other proteases that have IGFBP-5 as substrate, the impact of PAPP-A-specific IGFBP-5 proteolysis is difficult to ascertain in vivo. The inhibitory mAb-PA was selected to target a specific region of PAPP-A required for proteolytic cleavage of IGFBP-4, but not IGFBP-5 (Boldt et al., 2004; Mikkelsen et al., 2008, 2014), a so-called substrate-binding exosite. Therefore, mAb-PA inhibits proteolysis of IGFBP-4, but not IGFBP-5. This immunoneutralizing antibody was effective in inhibiting atherosclerotic plaque progression (Conover et al., 2016b). In addition, ApoE KO mice expressing a mutated PAPP-A, which selectively lacks proteolytic activity against IGFBP-4 and not IGFBP-5, did not show accelerated atherosclerotic lesion development compared to mice overexpressing native PAPP-A (Boldt et al., 2013). Interestingly, neither did ApoE KO mice expressing proteolytically active PAPP-A, but lacking domains for cell association. Thus, data so far support the model set forth by Laursen et al. (2007) that although cleavage of both IGFBP-4 and IGFBP-5 is required for release of bioactive IGF, cell surface-localized proteolysis of IGFBP-4 represents the final regulatory step of efficient IGF delivery to receptors.

Visceral obesity

In contrast to subcutaneous fat, the accumulation of visceral fat is strongly associated with severe metabolic complications in humans (Wajchenberg, 2000; Jensen, 2008; Perrini et al., 2008), which shows differential expression of PAPP-A in these two types of adipose tissue (Tchoukalova et al., 2009; Davidge-Pitts et al., 2014). Mouse adipose tissue shows a similar difference with fivefold to 10-fold higher expression of PAPP-A mRNA in visceral (mesenteric) fat compared to subcutaneous (inguinal, subscapular) fat. There was a preferential impact of PAPP-A deficiency in mice on a high fat diet to prevent increases in mesenteric adipocyte size with no significant effect on subcutaneous fat depots, probably due to the high expression of PAPP-A in mesenteric fat (Conover et al., 2013b). The decrease in accumulation

of mesenteric adipose tissue was associated with increased expression of adiponectin, which has anti-inflammatory and cardio-protective effects and enhanced insulin receptor signaling in this depot (Conover et al., 2013b). For these reasons, PAPP-A may be a potential target for treatment and/or prevention strategies for visceral obesity and related morbidities.

Fragility

Aging is associated with loss of skeletal muscle mass and, consequently, compromised muscle function (Marzetti et al., 2009). It has been found that PAPP-A KO mice are resistant to skeletal muscle aging (Conover et al., 2016a). In this study, top genes regulated in muscle from 18-month-old PAPP-A KO compared to WT mice were associated with increased muscle function, increased lipid metabolism, and decreased stress. Fiber cross-sectional area and intrinsic mitochondrial oxidative capacity were significantly increased in skeletal muscle of aged PAPP-A KO compared to WT mice. Moreover, these 18-month-old PAPP-A KO mice exhibited significantly enhanced endurance running on a treadmill. Also, PAPP-A KO mice are resistant to age-related metabolic dysfunction (Hill et al., 2015), largely driven by skeletal muscle.

These studies may seem contrary to other studies where PAPP-A overexpression in skeletal muscle increased muscle mass in response to Injury (Rehage et al., 2007). It is known that enhanced IGF signaling is important for muscle regeneration in response to injury (Clemmons, 2009). However, this response could be different in aging muscle. One possibility is that reduced IGF signaling in pluripotent stem cells residing in skeletal muscle protects against premature depletion. More work is required to delineate the roles of IGFs in this tissue.

Nephropathy

The kidney has been suggested to be an organ of particular interest for targeted PAPP-A inhibition (Swindell et al., 2010). At 18 months of age, there was a higher incidence and severity of chronic nephropathy in WT compared to PAPP-A KO mice (Conover et al., 2010b). It was also found that PAPP-A KO mice are resistant to the development of diabetic nephropathy (Mader et al., 2013). In this model of type 2 diabetes, increased glomerular size and thickened Bowman's capsules were observed in WT mice, but not in PAPP-A KO mice. Glomerular expression of PAPP-A was increased in kidneys of patients with diabetic nephropathy (Mader et al., 2013). Studies in cultured human mesangial cells indicated potent upregulation of PAPP-A mRNA and protein expression by pro-inflammatory cytokines (Donegan et al., 2016), which are prevalent in diabetic nephropathy.

Cancer

The involvement of PAPP-A proteolytic activity in cancer is increasingly suggested. For example, clinical data show that PAPP-A serum levels are elevated in patients with lung cancer (Bulut et al., 2009), and PAPP-A can promote lung cancer growth in vivo (Pan et al. 2012). PAPP-A is highly expressed in aggressive forms of human breast cancer (Mansfield et al., 2014). Interestingly, Chander et al. (2011) found that mutation of the tumor suppressor gene, p53, increases PAPP-A transcription in breast cancer cell lines and mammary gland tissues.

In agreement with such findings, several publications indicate that targeting of PAPP-A proteolytic activity is relevant to the prevention of cancer growth and metastases. Overexpression of proteolytically active, but not proteolytically inactive, PAPP-A in an ovarian cancer cell line was shown to have increased tumor aggressiveness in vivo (Boldt & Conover, 2011). The first report of mAb-PA efficacy was in lung cancer cell xenografts expressing PAPP-A (Mikkelsen et al., 2014). PAPP-A is also overexpressed in a subset of human ovarian tumors, and weekly treatment of mice harboring these intraperitoneal patient tumorgrafts with the inhibitory antibody, mAb-PA, could reduce tumor size and ascites burden. Treatment with mAb-PA also conferred chemo-sensitivity to a high PAPP-A-expressing chemo-resistant patient tumor (Becker et al., 2015). These beneficial effects of inhibiting the proteolytic activity of PAPP-A were only manifested in tumors expressing moderate-to-high levels of PAPP-A. Thus, PAPP-A expression in patient tumors, or ascites (Thomsen et al., 2015), may be a biomarker for patient response to PAPP-A therapies. We also have unpublished data indicating that mAb-PA treatment can prevent ovarian tumor metastases to intraperitoneal lining and perigonadal fat, these being prime sites for metastases via ascites. This fits with the identification of PAPP-A as a migration/invasion-promoting gene in an ovarian cancer cell line (Boldt & Conover, 2011), malignant pleural mesothelioma cells (Huang et al. 2013), nonsmall cell lung cancer (Salim et al., 2013), and melanoma (Prithviraj et al. 2015).

Inflammation

Aging and many age-related diseases are associated with inflammation. However, the use of anti-inflammatory drugs to alleviate these conditions is problematic because they affect multiple pathways and lack specificity. For example, cyclooxygenase-2 inhibition was used to counteract inflammation, but was associated with serious cardiovascular complications (Sohn & Krotz, 2006). Furthermore, inflammation is important in normal tissue repair and defense against infection. Thus, it is generally recommended that targets should be specific and downstream of inflammatory pathways (Gauldie, 2007). PAPP-A could be such a target.

The pro-inflammatory cytokines, interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) are potent stimulators of PAPP-A expression in human fibroblasts, osteoblasts, arterial smooth muscle cells, endothelial cells, pre-adipocytes, intervertebral disk cells, and mesangial cells (Conover et al., 2004b, 2006, 2008; Resch et al., 2004; Gruber et al., 2013; Davidge-Pitts et al., 2014). In atherosclerotic plaque, macrophage-derived pro-inflammatory cytokines act in a paracrine manner to stimulate vascular smooth muscle and endothelial cells to secrete PAPP-A (Conover et al., 2007). This represents an important amplification point in plaque progression, as PAPP-A can feedback back on activated macrophages and smooth muscle cells to promote IGF-mediated lipid uptake. With the loss of the PAPP-A activity induced by pro-inflammatory cytokines, the vicious cycle would be blunted in spite of the continued presence of macrophages and similar levels of IL-1β and TNF-α expression. We suggest that this model of PAPP-A and inflammatory stress may also apply to other conditions. Aging, associated with chronic low-grade inflammation, is one such condition. With visceral fat representing a highly active inflammatory microenvironment, especially in obese subjects, adipose tissue dysregulation is another.

Contraindications for PAPP-A-directed therapies

Low circulating PAPP-A has been associated with adverse effects on placental function and fetal growth in humans (Smith et al., 2002). Although the role of PAPP-A in human pregnancy is not understood, PAPP-A is believed to be important for placental development. Therefore, targeting PAPP-A during human pregnancy is not likely to be a

viable strategy. Interestingly, placental development in mice does not depend on PAPP-A (Qin *et al.*, 2002; Soe *et al.*, 2002).

The involvement of PAPP-A in normal tissue repair processes also suggests a possible need to suspend PAPP-A targeting temporarily during such conditions. For example, PAPP-A increases bone accretion primarily by increasing IGF bioavailability important for prepubertal bone growth (Mohan *et al.*, 2003; Qin *et al.*, 2006). Fracture repair in PAPP-A KO mice is temporally compromised, but not prevented from normal resolution (Miller *et al.*, 2007). Similarly, controlled increases in PAPP-A expression are seen in healing human skin (Chen *et al.*, 2003), indicating that wound healing may be delayed as a consequence of PAPP-A targeting.

Conclusion

Experimental evidence is accumulating that inhibition of PAPP-A has the potential to promote healthy longevity. It is clearly advantageous that targeting of PAPP-A has the benefit of a single intervention that affects multiple adverse changes with age, not just a single condition (Figueira *et al.*, 2016). PAPP-A is present in the extracellular environment, and its activity is therefore amenable to pharmacologic intervention. Strategies to inhibit PAPP-A have recently been developed and tested in experimental models (Mikkelsen *et al.*, 2008, 2014; Becker *et al.*, 2015; Conover *et al.*, 2016b). Rather than the active site of PAPP-A, a unique substrate-binding exosite, critical for proteolytic cleavage of IGFBP-4, is targeted. This efficiently eliminates activity toward IGFBP-4, but does not interfere with cleavage of other possible substrates of PAPP-A. Inhibition will target discrete conditions with increased PAPP-A activity, resulting in moderate restraint of IGF signaling and minimizing side effects. However, much remains to be learned about stages in life at which mice, and possibly humans, are susceptible to improvements in long-term health by manipulation of PAPP-A.

Author contributions

Drs. Conover and Oxvig contributed equally to the preparation of this review.

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Conflict of interest

None declared.

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