Published in final edited form as:

Trends Endocrinol Metab. 2012 May ; 23(5): 242–249. doi:10.1016/j.tem.2012.02.008.

Key Questions and Answers about Pregnancy-Associated Plasma Protein-A

Cheryl A. Conover

Division of Endocrinology and Metabolism, Endocrine Research Unit, Mayo Clinic, Rochester, MN.

Abstract

Twenty-five years after it was identified as a circulating protein derived from the placenta but of unknown function, pregnancy-associated plasma protein-A (PAPP-A) was discovered to be a novel zinc metalloproteinase expressed by a variety of cell types. Great progress has been made in understanding the biology of PAPP-A and its regulation during recent years, especially in regard to physiological and pathophysiological inflammatory injury responses. But much remains to be learned about this complex protein and its potential clinical implications outside of pregnancy. In this article we address some of the outstanding questions about PAPP-A, in particular about its newly emerging role in the insulin-like growth factor (IGF) system.

What is PAPP-A?

In 1974, PAPP-A was one of four proteins identified in the plasma of pregnant women, and, accordingly, given the name ‘pregnancy-associated plasma protein-A’ [1]. During pregnancy, PAPP-A is produced by placental syncytiotrophoblasts and secreted into the maternal circulation where its concentration increases until term [2]. Maternal concentrations of PAPP-A are reduced in the first trimester of pregnancies affected by fetal Down's syndrome (trisomy 21 and trisomy 18) [3,4]. Placental PAPP-A mRNA and protein expression is not different between normal and trisomy pregnancies, suggesting that the decrease in maternal serum PAPP-A in trisomic pregnancies is due to altered post-translational events such as mechanisms that affect protein secretion or transportation across the placenta [4]. Low maternal circulating PAPP-A levels during first trimester are also associated with pre-eclampsia, premature birth, and low birth weight of full-term babies [5-7]. Although a valuable clinical marker, there was no known biological function attributed to this pregnancy-associated protein.

Twenty years later, several laboratories described a novel proteolytic activity against IGF binding protein-4 (IGFBP-4), in ovarian follicular fluid and in conditioned medium from fibroblasts, osteoblasts, granulosa cells, lung cells, and smooth muscle cells [reviewed in 8]. The novelty of this enzymatic reaction derived from the apparent obligatory need for IGF to be bound to the substrate, IGFBP-4, for proteolysis [9,10]. In 1999, this IGF-dependent proteolytic activity was identified by Lawrence *et al.* [11] as PAPP-A. Subsequently, PAPP-

© 2012 Elsevier Ltd. All rights reserved.

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

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.

A was found to be ubiquitously expressed and with particularly high expression in kidney and bone, clearly indicating a role for PAPP-A outside of pregnancy [12].

Biochemical characterization of the PAPP-A molecule showed that it possesses the two structural motifs that define members of the metzincin superfamily (glossary), i.e., the characteristic three histidine residue motif -- **HEXXHXXGXXH** -- involved in coordinating the catalytically essential zinc ion, as well as a strictly conserved methionine-containing 1,4- β -turn called the Met-turn [13,14]. However, the metzincin superfamily is divided into subfamilies based on specific residues and on the variable length between the zinc-binding consensus sequence and the Met-turn. Structural analysis of PAPP-A indicated that it did not conform to any of the four existing subfamilies (matrix metalloproteases, astacins, adamalysins/reprolysins, and serralsins), and is the founding member of a new metzincin subfamily, pappalysins [15]. There is also a homolog of PAPP-A, PAPP-A2 (pappalysin2), that shares 46% sequence identity with PAPP-A and can cleave IGFBP-5 but not IGFBP-4 [15]. The physiological role of PAPP-A2 is unclear, although it has been implicated in human pregnancy and in the regulation of body size in mice [16-18]. Since the published data on PAPP-A2 are limited, this review will feature PAPP-A.

PAPP-A exists in two major forms. In pregnancy PAPP-A circulates as two molecules of PAPP-A covalently linked to two molecules of eosinophil major basic protein (proMPB). In this 2:2 heterotetrameric complex, PAPP-A is proteolytically inactive [19]. On the other hand, PAPP-A secreted by cells in culture and PAPP-A produced by vascular cells *in vivo* is a proteolytically active PAPP-A dimer that is not covalently-linked with proMBP [11,20]. This distinction is important when evaluating assays for PAPP-A [20].

As discussed later in this article, *in vitro* studies with several different cell types and *in vivo* studies using PAPP-A-deficient and PAPP-A-overexpressing transgenic mice suggest a general function of this IGFBP protease as an important regulator of local IGF bioavailability. This is significant since IGFs have been shown to play a central role in normal and abnormal growth, maintenance, and regeneration of essentially all tissues and throughout life. This review consists of questions and answers and concludes with more questions about the regulation and biological function of PAPP-A focusing on its role outside of pregnancy.

How is PAPP-A regulated?

The most potent stimulators of PAPP-A expression in cultured human dermal fibroblasts and human coronary artery endothelial and smooth muscle cells are the pro-inflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-1 β [21-23]. Another pro-inflammatory cytokine, IL-6, also increases PAPP-A gene and protein expression in coronary artery smooth muscle cells, but not in endothelial cells [8,23]. TNF- α , IL-1 β , IL-4, and transforming growth factor- β (TGF- β) stimulate PAPP-A expression in human osteoblasts [24]. On the other hand, interferon- γ (INF- γ), which can have pro- and anti-inflammatory properties [25], decreases PAPP-A expression in human fibroblasts [21]. A potent inhibitor of cytokine-induced PAPP-A expression in coronary artery smooth muscle cells was found to be resveratrol, an anti-inflammatory agent found in grape products such as red wine that has been purported to have beneficial effects on the cardiovascular system [22].

In vivo, PAPP-A expression has been shown to be upregulated in response to acute injury, in several systems. In healing human skin wounds, PAPP-A expression is induced over time in dermal granulation tissue [26]. Upregulation of PAPP-A expression also occurs after acute vascular injury in pig and mouse models, and precedes neointimal formation [27,28], and in

a skeletal muscle crush injury in mice, preceding regeneration [29]. Furthermore, PAPP-A may play a role in chronic injury responses, since PAPP-A expression is significantly increased in atherosclerotic lesions in humans [30,31] and in mice [32], and intense immunostaining for PAPP-A is found in human atherosclerotic plaques in association with activated macrophages and smooth muscle cells [30,31]. Gruber et al. [33] recently reported increased PAPP-A expression in degenerated human intervertebral discs.

Additional reports show that PAPP-A gene expression is induced by forskolin and prostaglandin E₂ (PG E₂) in human osteoblasts [24], by bone morphogenetic protein-2 (BMP-2) in human mesenchymal stem cells [34], and by follicle-stimulating hormone (FSH) in bovine granulosa cells in culture [35]. However, in another study, FSH had no direct effect on PAPP-A mRNA abundance in bovine granulosa cells [36]. In general, peptide growth factors (IGF, insulin, platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor) and steroid hormones (dexamethasone, β -estradiol, progesterone, testosterone) have not been found to regulate PAPP-A expression [21,23,24,37,38]. However, these studies have been limited to one or two cell lines. Table 1 presents a summary of the regulators of PAPP-A expression identified to date. These *in vitro* and *in vivo* data on PAPP-A regulation strongly implicate PAPP-A in inflammatory injury responses and tissue remodeling. However, there is clear evidence of cell-specific regulation. Therefore, these studies need to be extended and include other cell types and tissues.

Although extensive work has been done to identify factors regulating PAPP-A expression *in vitro* and *in vivo*, very little is known about the mechanism of regulation. Resch et al. [39] indicated that NF κ B activation is the primary mediator of cytokine-stimulated PAPP-A expression in human fibroblasts. Searching non-transcribed genomic sequences ~1000 base pairs upstream of the coding sequence of amino-terminal PAPP-A, has revealed three potential NF κ B binding sites. However, it has not been determined if any of these candidate transcription factor binding sites are active. Chandler *et al.* [40] recently identified a p53 binding site in intron 1 of the human PAPP-A gene. Interestingly, binding of wild-type p53 results in transcriptional repression of PAPP-A, while binding of denatured p53 results in transcriptional activation of PAPP-A. Expanding the concept of specific transcription factors in PAPP-A gene expression awaits cloning of the promoter region. Moreover, studies of microRNAs that can exert post-translation effects on PAPP-A gene expression are currently lacking. Knowledge of this regulation is particularly important, since microRNAs are known to regulate protein translation in response to oxidative stress and inflammation in vascular and adipose tissues [41] -- tissues, that involve PAPP-A.

What is the evidence for a PAPP-A – IGF system connection?

The first recognized connection was the discovery of PAPP-A as the IGF-dependent IGFBP-4 protease, secreted by human fibroblasts, in culture [11]. Pericellular IGFBP-4 binds IGF with high affinity, and prevents IGF interaction with cell surface receptors. PAPP-A cleaves IGFBP-4 (when IGF is bound to IGFBP-4), markedly reducing its affinity for IGF and thereby freeing IGF for receptor binding and activation (Fig. 1). There have been studies from several laboratories and in multiple cell types demonstrating the PAPP-A mediated enhancement of IGF bioactivity *in vitro* by its degradation of IGFBP-4 [21,28,29,37,42]. Data are accumulating to support a similar function *in vivo*.

Studies using genetically-engineered mice have been particularly enlightening about the PAPP-A - IGF connection. Rehage *et al.* [29] showed that transgenic mice over-expressing PAPP-A in skeletal muscle have enhanced skeletal muscle mass. Transgenic mice over-expressing PAPP-A in osteoblasts had increased bone formation [43]. However, reduction of

free IGFs in bone by dual overexpression of a protease-resistant IGFBP-4 with PAPP-A, in this mouse model, completely blocked the anabolic effect of PAPP-A on bone [44]. Thus, an increase in local IGF bioavailability through IGFBP proteolysis appeared to be the primary, if not the only, basis for PAPP-A's anabolic effects in bone *in vivo*. Using a similar approach, Ryan *et al.* [45] reported that protease-resistant IGFBP-4 blocked IGF activity, tumor growth and angiogenesis in a mouse model of breast cancer, where PAPP-A is expressed by the surrounding host tissue. Apolipoprotein E knock-out (ApoE KO) mice (an established mouse model of atherosclerosis) that express the human PAPP-A transgene in arterial smooth muscle have three-fold enhanced plaque development when challenged with a high fat diet, compared with ApoE KO mice on the same diet [46]. Expression of an IGF-responsive gene was markedly elevated in lesions of these PAPP-A transgenic mice, supporting the association of increased PAPP-A expression with increased IGF bioactivity *in vivo* [46]. Similar conclusions were drawn from studies using PAPP-A KO mice, as discussed below.

The IGFs fit the concept of antagonistic pleiotropy whereby certain genes/gene products are important early in life but detrimental to the organism later in life [47]. Thus, the IGFs are necessary for normal fetal growth and optimal reproduction. On the other hand, the IGFs are associated with aging and age-related diseases. Therefore, if PAPP-A serves to enhance IGF activity, then the lack of PAPP-A in the PAPP-A KO mouse should have negative effects on fetal growth and reproduction and positive effects in adult mice promoting a healthy lifespan. A series of studies indicated this to be the case. PAPP-A KO mice are born as proportional dwarfs and this growth phenotype could be rescued through upregulation of IGF-II during embryogenesis [12,48]. During their reproductive period, PAPP-A KO mice had compromised ovarian steroidogenesis and reduced litter size [49,50]. Furthermore, Ning *et al.* [51] employed extensive crossbreeding of PAPP-A KO and IGFBP-4 KO mice and presented compelling evidence that the growth defect in the PAPP-A KO mouse is completely dependent on the presence of IGFBP-4. Conversely, adult PAPP-A KO mice have reduced/delayed atherosclerotic plaque, thymic involution and incidence of cancer, and they live ~ 30% longer than their wild-type littermates [32,52,53].

There also appears to be a strong PAPP-A - IGF connection in ovarian follicular growth and selection, in multiple species. There are documented decreases in IGFBPs in human, bovine, equine, porcine, rodent, and possum follicles due to decreased IGFBP gene expression, as well as to increased PAPP-A [54-57]. IGF bioavailability through active intrafollicular IGFBP proteolysis involving PAPP-A has been shown to be an important determinant of follicular fate, i.e., development of healthy dominant versus atretic follicles [56,58-60].

Thus, there are compelling data for PAPP-A regulation of IGF action during fetal development, folliculogenesis and aging, as well as in normal and abnormal growth of bone, skeletal muscle and vascular tissue. Further study other IGF-responsive tissues and conditions will undoubtedly reveal additional roles for PAPP-A.

Are the biological effects of PAPP-A local or systemic?

The biological effects of PAPP-A are primarily local. PAPP-A is a secreted enzyme that associates with cell surface heparan sulfate proteoglycans through its carboxy-terminal short consensus repeats [61,62]. PAPP-A remains an active protease when is cell-bound and can thereby control IGF signaling, spatially [63]. Cell-associated PAPP-A is well situated to proteolyze IGFBP-4 at the cell surface, and to facilitate IGF action (Fig. 1). The presence of more than one IGFBP in most tissues suggests cooperative function. Proteolysis of IGFBP-4 by cell-associated PAPP-A has been proposed to be the final regulatory step of efficient IGF delivery to the receptor [64]. This cell-association may also explain the intense PAPP-A

immunostaining that is found in activated macrophages, in human atherosclerotic plaque and healing wounds [26,30,31]. Macrophages themselves do not express PAPP-A, but PAPP-A secreted by regional stromal cells (in response to macrophage-derived cytokines) preferentially binds and is internalized by macrophages [63]. Thus, PAPP-A can act as an autocrine/paracrine regulator of IGF action (Fig. 2). Furthermore, models such as the PAPP-A KO mouse, which have reduced autocrine/paracrine IGF bioavailability but normal circulating levels of IGF [12], can be used to distinguish systemic versus local IGF effects.

It is important to emphasize that in this integrated IGF system, PAPP-A functions as a modifier, not a direct inhibitor of IGF action, through proteolysis of IGFBPs, in particular, IGFBP-4. Low expression of PAPP-A, IGF, or IGFBP-4 would alter the degree to which PAPP-A deficiency influenced the pericellular IGFBP-4 reservoir, IGF availability and local IGF signaling. Thus, the influence of PAPP-A KO is likely to be heterogeneous in regard to IGF-I signaling, in different tissues and under different circumstances [65]. In the global PAPP-A KO mouse model, there is evidence for an impact of PAPP-A deficiency under basal conditions, i.e., uninjured, in bone [66]. What about the other tissues that express PAPP-A at moderate- to-high levels, such as kidney, heart, brain, testes? And are there other situations, besides the injury repair response, where PAPP-A, IGF-I and IGFBP-4 are coordinately upregulated [26,28,32]? In regard to antagonistic pleiotropy, does inhibition of PAPP-A early in life affect responses in the adult? The availability of tissue-specific and temporally-regulated PAPP-A KO mice would add valuable tools to the current arsenal for deciphering the PAPP-A - IGF connection in physiology and pathophysiology.

Are there other substrates for PAPP-A besides IGFBPs?

This remains the great unanswered question. Besides IGFBPs, no other protein, or low molecular weight substrate has been identified for PAPP-A. PAPP-A appears to be the primary physiological IGFBP-4 protease, but it has also been reported to cleave IGFBP-2 and IGFBP-5 [67,68]. The ability of PAPP-A to degrade other unidentified proteins is unlikely to be discovered by a simple high throughput screen. Indeed, IGFBP-4 would not have been identified as a substrate if not for the serendipitous addition of IGF to conditioned medium from cells expressing what was later identified as PAPP-A [69]. IGFBP-4 was found to have a complex cleavage recognition site, more dependent on conformation than a linear amino acid sequence [70,71]. Thus, the need for a 'co-factor' and/or a specific conformation will complicate identification of potential substrates for PAPP-A.

Does PAPP-A have IGF-independent effects?

This is another important question looking for an answer. Based on the multi-domain structure of PAPP-A [8,10,15], it is conceivable that there are other potential mechanisms. The need for the proteolytic activity of PAPP-A has been addressed *in vitro* [29], and in a recent *in vivo* study [72], where ovarian cancer cells that expressed wild-type PAPP-A, but not a mutant PAPP-A with reduced proteolytic activity, had increased tumorigenicity in a xenograft model. However, this latter study only provided evidence that the proteolytic activity was necessary for PAPP-A's effect, and not the direct involvement of IGFBPs as substrate or regulation of IGF signaling. Although IGFBP-4 is generally recognized as an inhibitor of IGF action, IGFBP-4 was recently reported to induce cardiomyocyte differentiation, in an IGF-independent fashion [73]. Indirectly, PAPP-A proteolysis of IGFBP-4 could have IGF-independent effects. Clearly, further studies directly assessing the effect of these mutations on IGF signaling *in vivo* and addressing the biology of other domains in PAPP-A are needed to identify potential IGF-independent effects of PAPP-A.

Is PAPP-A functionally detrimental or beneficial?

The answer to this question appears to be “both”. PAPP-A is important in the normal healing response, exemplified by the delayed fracture repair observed in PAPP-A KO mice [74], as well as in healthy ovarian follicular development [56,58-60]. Transgenic models suggest that overexpression of PAPP-A can promote tissue regeneration [29]. However, PAPP-A may exaggerate a pathological injury response, such as in atherosclerotic plaque progression [75]. This potentially deleterious promotion of atherosclerosis is supported by studies in PAPP-A KO and transgenic mice [32,46]. However, the role of PAPP-A in atherosclerosis is controversial, and it has been alternatively proposed that PAPP-A may play a role in reparative processes after plaque rupture, rather than promoting plaque formation [76,77]. It would be of interest to determine whether the degree and/or extent of PAPP-A expression can make the difference between a remedial versus a pathological response.

A negative impact of PAPP-A in aging and age-related diseases is supported by the finding that the PAPP-A KO mice have extended healthy lifespan, with reduced age-related degenerative disease, and reduced incidence and delayed occurrence of spontaneous cancers [53]. There is also recent evidence in humans and in animal models that PAPP-A may promote tumor growth. PAPP-A has been reported to be highly expressed in human lung cancer, granulose cell tumors and some ovarian cancers [78,79]. In a xenograft model, PAPP-A overexpression markedly increased growth of an ovarian cancer cell line with normally low tumorigenic potential [72]. Denatured p53 increases transcription of PAPP-A, which contributes to the oncogenic function of the Skp2 β ubiquitin ligase subunit, in mammary gland tumors [40]. Interestingly, IGF-I receptor inhibitors are currently in clinical trials as mono- and combination therapies for certain cancers, such as lung cancer. Theoretically, PAPP-A inhibitors might be more selective. Further studies are necessary to distinguish between tissue types and conditions for which loss of PAPP-A, and presumably reduced IGF signaling, have favorable versus deleterious effects.

Can what we learn about PAPP-A in mice apply to humans?

Most likely yes. The amino acid sequence identity between human and murine PAPP-A is approximately 91% [80], and there is current support for the mouse as a model for the study of mammalian PAPP-A in cardiovascular disease, follicular development, and depot-specific adipogenesis. However, a major caveat to this translation of findings from mice is that, unlike for human PAPP-A, there are no effective antibodies currently available that recognize mouse PAPP-A protein, so conclusions from the mouse studies so far have been based on PAPP-A mRNA expression or the global PAPP-A KO and transgenic mouse models.

The role of PAPP-A in promoting atherosclerosis in mice has already been discussed [32,45]. In humans, PAPP-A is highly expressed in vulnerable plaque, as shown in some [30,31] but not all [81] studies; and elevated circulating PAPP-A appears to be a marker of acute coronary syndrome and is associated with increased risk of adverse cardiovascular events [30,82-86].

A role for PAPP-A in physiological processes of dominant follicle development, ovulation and luteogenesis has been indicated in multiple species [54-60]. In particular, PAPP-A expression in human and mouse ovary is restricted to dominant follicles and the corpus luteum [56,58].

PAPP-A and fat is an emerging area of research. Investigating mechanisms for regional differences in adipogenesis, microarray analyses of RNA from human preadipocytes isolated

from different fat depots has indicated that PAPP-A is one of the most distinctive genes, with expression levels in preadipocytes from visceral fat depots greatly exceeding those from subcutaneous fat [87]. Similar depot-specific results are seen for PAPP-A expression in preadipocytes from non-human primates and mice [88]. In humans, increased accumulation of visceral fat relative to subcutaneous fat is associated with adverse metabolic effects [89,90].

Interestingly, one tissue where data on PAPP-A do not appear to transfer between mouse and human is the placenta. PAPP-A expression is exceptionally high in human placenta, but only moderate in mouse placenta [91]. This may relate to differences in implantation in humans and mice.

Concluding remarks

As presented in this review, great strides have been made in answering questions about PAPP-A, especially in regard to its role in regulating IGF bioavailability in the pericellular environment of diverse tissues and under various physiologic and pathophysiologic conditions. However, many outstanding questions remain [see Box]. To answer these questions, efforts need to be made to clone the PAPP-A promoter, determine the biological significance of the multidomain structure of PAPP-A, identify possible new substrates for PAPP-A, generate new animal models, investigate potential roles for PAPP-A in currently unexplored tissues (e.g., kidney, brain, testes), and develop antibodies that recognize murine PAPP-A. Answers to these and other questions will undoubtedly lead to additional insight into the biology of PAPP-A as well as new therapeutic approaches targeting this novel metalloproteinase.

Acknowledgments

This work was supported by National Institutes of Health Grants HL074871 and AG028141 and a Senior Scholar Award from the Ellison Medical Foundation.

Glossary

Antagonistic pleiotropy	Theory first proposed by G. C. Williams, 1957, proposing that genes can have opposite effects on biological fitness at different ages, such that their effects are beneficial early in life but are detrimental to the organism later in life
Heparan sulfate proteoglycan	The basic proteoglycan unit consists of a core protein with one or more covalently attached glycosaminoglycan chains, which are carbohydrate polymers, that are negatively charged due to heparan sulfate groups. Proteoglycans are a major component of the extracellular matrix
IGF	Insulin-like growth factors I and II are polypeptides with high sequence similarity to insulin, that promote cell growth, differentiated function, and survival in diverse tissues
IGFBP	Insulin-like growth factor binding proteins are a family of six proteins that bind IGFs with high affinity. These proteins modulate IGF action in complex ways that involve both inhibition and enhancement of IGF action. In addition, some IGFBPs appear to have IGF-independent effects

Metzincin superfamily	Metalloproteinases in the metzincin superfamily encode a highly conserved zinc-binding motif that contains three histidine residues, which bind zinc, and a conserved methionine-turn (met-turn) in the active-site helix. The metzincin superfamily includes serralysins, astacins, adamalysins, metalloproteinases, and pappalysins
microRNA	Small highly conserved noncoding RNA molecules that exert post-transcriptional effects on gene expression
NFκB	Protein complex that controls the transcription of DNA in response to stressors
p53	A tumor suppressor protein that plays a key in conserving genome stability by preventing DNA mutations and cancer
PAPP-A	Pregnancy-associated plasma protein A, pappalysin 1, is a secreted metalloproteinase which cleaves insulin-like growth factor binding proteins (IGFBPs). Low plasma PAPP-A concentration is used as a marker in prenatal screening for Down's syndrome. PAPP-A also plays a role outside of pregnancy
PAPP-A KO mice	Targeted deletion of the PAPP-A gene through homologous recombination in embryonic stems cells, resulting in mice with global “knock-out” of PAPP-A
PAPP-A transgenic mice	Mice overexpressing PAPP-A driven by different tissue-specific promoters
proMBP	In pregnancy, PAPP-A circulates as two molecules of PAPP-A, which are covalently linked to two molecules of the precursor form of eosinophil major basic protein (proMBP). In this complex, PAPP-A is proteolytically inactive

REFERENCES

1. Lin TM, et al. Characterization of four human pregnancy-associated plasma proteins. *Am. J. Obstet. Gynecol.* 1974; 118:223–236. 1. [PubMed: 4129188]
2. Folkersen J, et al. Pregnancy-associated plasma protein-A: circulating levels during normal pregnancy. *Am. J. Obstet. Gynecol.* 1981; 139:910–914. [PubMed: 6164292]
3. Wald NJ, et al. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N. Engl. J. Med.* 1999; 341:461–467. [PubMed: 10441601]
4. Brizot ML, et al. Gene expression of human pregnancy-associated plasma protein-A in placenta from trisomic pregnancies. *Placenta.* 1996; 17:33–36. [PubMed: 8710811]
5. Smith GCS, et al. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J. Clin. Endocrinol. Metab.* 2002; 87:1762–1767. [PubMed: 11932314]
6. Poon LCY, et al. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet. Gynecol.* 2009; 33:23–33. [PubMed: 19090499]
7. Smith GCS, et al. Early-pregnancy origins of low birth weight. *Nature.* 2002; 417:916. [PubMed: 12087395]
8. Boldt HB, Conover CA. Pregnancy-associated plasma protein-A (PAPP-A): a local regulator of IGF bioavailability through cleavage of IGFBPs. *Growth Horm. IGF Res.* 2007; 17:10–18. [PubMed: 17218136]
9. Qin X, et al. Evidence that the interaction between insulin-like growth factor (IGF)-II and IGF binding protein (IGFBP)-4 is essential for the action of the IGF-II-dependent IGFBP-4 protease. *Arch. Biochem. Biophys.* 2000; 379:209–216. [PubMed: 10898936]

10. Laursen LS, et al. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett.* 2001; 504:36–40. [PubMed: 11522292]
11. Lawrence JB, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc. Natl. Acad. Sci. USA.* 1999; 96:3149–3153. [PubMed: 10077652]
12. Conover CA, et al. Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. *Development.* 2004; 131:1187–1194. [PubMed: 14973274]
13. Stocker W, et al. The metzincins: topological and sequential relations between the astacins, adamalysins, serravalins, and matrixins (collagenases) define a superfamily of zinc-peptidases. *Protein Sci.* 1995; 4:823–840. [PubMed: 7663339]
14. Boldt HB, et al. Mutational analysis of the proteolytic domain of pregnancy-associated plasma protein-A (PAPP-A): classification as a metzincin. *Biochem. J.* 2001; 358:359–367. [PubMed: 11513734]
15. Overgaard MT, et al. Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase. *J. Biol. Chem.* 2001; 276:21849–21853. [PubMed: 11264294]
16. Yan X, et al. Involvement of pregnancy-associated plasma protein-A2 in insulin-like growth factor (IGF) binding protein-5 proteolysis during pregnancy: a potential mechanism for increasing IGF bioavailability. *J. Clin. Endocrinol. Metab.* 2010; 95:1412–1420. [PubMed: 20103653]
17. Christians JR, et al. PAPP-A2, an enzyme that cleaves an insulin-like growth-factor-binding protein, is a candidate gene for a quantitative trait locus affecting body size in mice. *Genetics.* 2006; 173:1547–1553. [PubMed: 16702428]
18. Conover CA, et al. Pregnancy-associated plasma protein A2 (PAPP-A2): tissue expression and biological consequences of gene knock-out in mice. *Endocrinology.* 2011; 152:2837–2844. [PubMed: 21586553]
19. Oxvig C, et al. Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. *J. Biol. Chem.* 1993; 268:12243–12246. [PubMed: 7685339]
20. Qin Q-P, et al. Molecular distinction of circulating pregnancy-associated plasma protein A in myocardial infarction and pregnancy. *Clin. Chem.* 2005; 51:75–83. [PubMed: 15613709]
21. Resch ZT, et al. Pregnancy-associated plasma protein A gene expression as a target of inflammatory cytokines. *Endocrinology.* 2004; 145:1124–1129. [PubMed: 14657012]
22. Conover CA, et al. Cytokine stimulation of pregnancy-associated plasma protein A expression in human coronary artery smooth muscle cells: inhibition by resveratrol. *Am. J. Physiol. Cell Physiol.* 2006; 290:C183–C188. [PubMed: 16338976]
23. Conover CA, et al. Differential regulation of pregnancy associated plasma protein-A in human coronary artery endothelial cells and smooth muscle cells. *Growth Horm. IGF Res.* 2008; 18:213–220. [PubMed: 17936662]
24. Conover CA, et al. Regulation of pregnancy-associated plasma protein-A expression in cultured human osteoblasts. *Bone.* 2004; 34:297–302. [PubMed: 14962808]
25. Muhl H, Pfeilschifter J. Anti-inflammatory properties of pro-inflammatory interferon-gamma. *Int. Immunopharmacol.* 2003; 3:1247–1255. [PubMed: 12890422]
26. Chen B-K, et al. Localization and regulation of pregnancy-associated plasma protein A expression in healing human skin. *J. Clin. Endocrinol. Metab.* 2003; 88:4465–4471. [PubMed: 12970325]
27. Bayes-Genis A, et al. Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. *Arterioscler. Thromb. Vasc. Biol.* 2001; 21:335–341. [PubMed: 11231911]
28. Resch ZT, et al. Targeted disruption of the pregnancy-associated plasma protein-A gene is associated with diminished smooth muscle cell response to insulin-like growth factor-I and resistance to neointimal hyperplasia after vascular injury. *Endocrinology.* 2006; 147:5634–5640. [PubMed: 16959843]

29. Rehage M, et al. Transgenic overexpression of pregnancy-associated plasma protein-A increases the somatic growth and skeletal muscle mass in mice. *Endocrinology*. 2007; 148:6176–6185. [PubMed: 17901236]
30. Bayes-Genis A, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *N. Engl. J. Med.* 2001; 345:1022–1029. [PubMed: 11586954]
31. Sangiorgi G, et al. Pregnancy-associated plasma protein-A is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques. *J. Am. Coll. Cardiol.* 2006; 47:2201–2211. [PubMed: 16750685]
32. Harrington SC, et al. Genetic deletion of pregnancy-associated plasma protein-A is associated with resistance to atherosclerotic lesion development in apolipoprotein E-deficient mice challenged with a high-fat diet. *Circ. Res.* 2007; 100:1696–1702. [PubMed: 17510462]
33. Gruber HE, et al. The relationship between pregnancy-associated plasma protein-A (PAPP-A) and human intervertebral disc generation. *Histol. Histopathol.* 2010; 25:1431–1436. [PubMed: 20865665]
34. Jadowiec J, et al. Pregnancy-associated plasma protein-A is involved in matrix mineralization of human adult mesenchymal stem cells and angiogenesis in the chick chorioallantoic membrane. *Endocrinology*. 2005; 146:3765–3772. [PubMed: 15919757]
35. Sudo N, et al. Insulin-like growth factor-I (IGF-I) system during follicle development in the bovine ovary: relationship among IGF-I, type 1 IGF receptor (IGFR-1) and pregnancy-associated plasma protein-A (PAPP-A). *Mol. Cell. Endocrinol.* 2007; 264:197–203. [PubMed: 17116363]
36. Aad PY, et al. Real-time RT-PCR quantification of pregnancy-associated plasma protein-A mRNA abundance in bovine granulosa and theca cells: effects of hormones in vitro. *Domestic Anim. Endocrinol.* 2006; 31:357–372.
37. Ortiz CO, et al. Transforming growth factor- β regulation of the insulin-like growth factor binding protein-4 protease system in cultured human osteoblasts. *J. Bone Miner. Res.* 2003; 18:1066–1072. [PubMed: 12817760]
38. Durham SK, et al. Regulation of insulin-like growth factor binding protein 4 by a specific insulin-like growth factor binding protein 4 proteinase in normal human osteoblast-like cells: implications in bone cell physiology. *J. Bone Miner. Res.* 1994; 9:111–117. [PubMed: 7512304]
39. Resch ZT, et al. Stress-activated signaling pathways mediate the stimulation of pregnancy-associated plasma protein-A expression in cultured human fibroblasts. *Endocrinology*. 2006; 147:885–890. [PubMed: 16269458]
40. Chandler H, et al. Skp2B overexpression alters a prohibitin-53 axis and the transcription of PAPP-A, the protease of insulin-like growth factor binding protein 4. *PLoS ONE*. 2011; 6:1–10.
41. Hulsmans M, et al. MicroRNAs regulating oxidative stress and inflammation in relation to obesity and atherosclerosis. *FASEB J.* 2011; 25:2515–2527. [PubMed: 21507901]
42. Byun D, et al. Pregnancy-associated plasma protein-A accounts for the insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) proteolytic activity in human pregnancy serum and enhances the mitogenic activity of IGF by degrading IGFBP-4 *in vitro*. *J. Clin. Endocrinol. Metab.* 2001; 86:847–854. [PubMed: 11158056]
43. Qin X, et al. Pregnancy-associated plasma protein-A increases osteoblast proliferation in vitro and bone formation in vivo. *Endocrinology*. 2006; 147:5653–5661. [PubMed: 16946002]
44. Phang D, et al. Inactivation of insulin-like-growth factors diminished the anabolic effects of pregnancy-associated plasma protein-A (PAPP-A) on bone in mice. *Growth Horm. IGF Res.* 2010; 20:192–200. [PubMed: 20144555]
45. Ryan AJ, et al. Expression of a protease-resistant insulin-like growth factor-binding protein-4 inhibits tumor growth in a murine model of breast cancer. *Br. J. Cancer.* 2009; 101:278–286. [PubMed: 19536088]
46. Conover CA, et al. Transgenic overexpression of pregnancy-associated plasma protein-A in murine arterial smooth muscle accelerates atherosclerotic lesion development. *Am. J. Physiol. Heart Circ. Physiol.* 2010; 299:H284–H291. [PubMed: 20472761]
47. Williams GC. Pleiotrophy, natural selection, and the evolution of senescence. *Evolution*. 1957; 11:398–411.

48. Bale LK, Conover CA. Disruption of insulin-like growth factor-II imprinting during embryonic development rescues the dwarf phenotype of mice null for pregnancy-associated plasma protein-A. *J. Endocrinol.* 2005; 186:325–331. [PubMed: 16079258]
49. Nyegaard M, et al. Lack of functional pregnancy-associated plasma protein-A (PAPP-A) compromises mouse ovarian steroidogenesis and female fertility. *Biol. Reprod.* 2010; 82:1129–1138. [PubMed: 20130263]
50. Mason EJ, et al. Insulin-like growth factor (IGF)-I and IGF-II contribute differentially to the phenotype of pregnancy associated plasma protein-A knock-out mice. *Growth Horm. IGF Res.* 2011; 21:243–247. [PubMed: 21802327]
51. Ning Y, et al. Insulin-like growth factor (IGF) binding protein-4 is both a positive and negative regulator of IGF activity *in vivo*. *Mol. Endocrinol.* 2008; 22:1213–1225. [PubMed: 18258685]
52. Vallejo AN, et al. Resistance to age-dependent thymic atrophy in long-lived mice that are deficient in pregnancy-associated plasma protein A. *Proc. Natl. Acad. Sci. USA.* 2009; 106:11252–11257. [PubMed: 19549878]
53. Conover CA, et al. Longevity and age-related pathology of mice deficient in pregnancy-associated plasma protein-A. *J. Gerontol: Biol. Sci.* 2010; 65:590–599.
54. Conover CA, et al. Evidence that the insulin-like growth factor binding protein-4 protease in human ovarian follicular fluid is pregnancy associated plasma protein-A. *J. Clin. Endocrinol. Metab.* 1999; 84:4742–4745. [PubMed: 10599745]
55. Mazerbourg S, et al. Pregnancy-associated plasma protein-A (PAPP-A) in ovine, bovine, porcine, and equine ovarian follicles: involvement in IGF binding protein-4 proteolytic degradation and mRNA expression during follicular development. *Endocrinology.* 2001; 142:5243–4253. [PubMed: 11713222]
56. Hourvitz A, et al. The regulated expression of the pregnancy-associated plasma protein-A in the rodent ovary: a proposed role in the development of dominant follicles and of corpora lutea. *Endocrinology.* 2002; 143:1833–1844. [PubMed: 11956166]
57. Juengel JL, et al. The role of IGFs in the regulation of ovarian follicular growth in the bushtail possum (*Trichosurus vulpecula*). *Reproduction.* 2010; 140:295–303. [PubMed: 20522481]
58. Conover CA, et al. Pregnancy-associated plasma protein-A is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. *Endocrinology.* 2001; 142:2155–2158. [PubMed: 11316785]
59. Mazerbourg S, et al. The insulin-like growth factor system: a key determinant role in the growth and selection of ovarian follicles? A comparative species study. *Reprod. Dom. Anim.* 2003; 38:247–258.
60. Spicer LJ. Proteolytic degradation of insulin-like growth factor binding proteins by ovarian follicles: a control mechanism for selection of dominant follicles. *Biol. Reprod.* 2004; 70:1223–1230. [PubMed: 14668213]
61. Laursen LS, et al. Cell surface targeting of pregnancy-associated plasma protein A proteolytic activity. *J. Biol. Chem.* 2002; 277:47225–47234. [PubMed: 12370176]
62. Weyer K, et al. Cell surface adhesion of pregnancy-associated plasma protein-A is mediated by four clusters of basic residues located in its third and fourth CCP module. *Eur. J. Biochem.* 2004; 271:1525–1535. [PubMed: 15066178]
63. Conover CA, et al. Surface association of pregnancy-associated plasma protein-A accounts for its colocalization with activated macrophages. *Am. J. Physiol. Heart Circ. Physiol.* 2007; 292:H994–H1000. [PubMed: 17040968]
64. Laursen LS, et al. Regulation of insulin-like growth factor (IGF) bioactivity by sequential proteolytic cleavage of IGF binding protein-4 and -5. *Mol. Endocrinol.* 2007; 21:1246–1257. [PubMed: 17312271]
65. Swindell WR, et al. *In vivo* analysis of gene expression in long-lived mice lacking the pregnancy-associated plasma protein A (PappA) gene. *Exp. Gerontol.* 2010; 45:366–374. [PubMed: 20197085]
66. Tanner SJ, et al. Impact of pregnancy-associated plasma protein-A deletion on the adult murine skeleton. *J. Bone Miner. Res.* 2008; 23:655–662. [PubMed: 18086007]

67. Kumar A, et al. Pregnancy-associated plasma protein-A regulates myoblast proliferation and differentiation through an insulin-like growth factor-dependent mechanism. *J. Biol. Chem.* 2005; 280:37782–37789. [PubMed: 16115888]
68. Monget P, et al. Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation. *Biol. Reprod.* 2003; 68:77–86. [PubMed: 12493698]
69. Conover CA. A unique receptor-independent mechanism by which insulinlike growth factor I regulates the availability of insulinlike growth factor binding proteins in normal and transformed human fibroblasts. *J. Clin. Invest.* 1991; 88:1354–1361. [PubMed: 1717510]
70. Laursen LS, et al. Substrate specificity of the metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) assessed by mutagenesis and analysis of synthetic peptides: substrate residues distant from the scissile bond are critical for proteolysis. *Biochem. J.* 2002; 367:31–40. [PubMed: 12241545]
71. Weyer K, et al. A substrate specificity-determining unit of three Lin12-Notch repeat modules is formed in trans within the pappalysin-1 dimer and requires a sequence stretch C-terminal to the third module. *J. Biol. Chem.* 2007; 282:10988–10999. [PubMed: 17314100]
72. Boldt HB, Conover CA. Overexpression of pregnancy-associated plasma protein-A in ovarian cancer cells promotes tumor growth *in vivo*. *Endocrinology.* 2011; 152:1470–1478. [PubMed: 21303951]
73. Zhu W, et al. IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. *Nature.* 2008; 454:345–349. [PubMed: 18528331]
74. Miller BS, et al. Pregnancy associated plasma protein-A is necessary for expeditious fracture healing in mice. *J. Endocrinol.* 2007; 192:505–513. [PubMed: 17332520]
75. Li X, et al. PAPP-A: a possible pathogenic link to the instability of atherosclerotic plaque. *Med. Hypotheses.* 2007; 70:597–599. [PubMed: 17714879]
76. Crea F, Andreotti F. Pregnancy associated plasma protein-A and coronary atherosclerosis: marker, friend, or foe? *Eur. Heart J.* 2005; 26:2075–2076. [PubMed: 16141260]
77. Andreotti F, et al. Role of PAPP-A in atherothrombosis: messages to take home. *Atherosclerosis.* 2009; 203:353–354. [PubMed: 18996526]
78. Bulut I, et al. Relationship between pregnancy-associated plasma protein-A and lung cancer. *Am. J. Med. Sci.* 2009; 337:241–244. [PubMed: 19365167]
79. Alexiadis M, et al. Insulin-like growth factor, insulin-like growth factor-binding protein-4, and pregnancy-associated plasma protein-A gene expression in human granulosa cell tumors. *Int. J. Gynecol. Cancer.* 2006; 16:1973–1979. [PubMed: 17177834]
80. See R, et al. Expression of recombinant murine pregnancy-associated plasma protein-A (PAPP-A) and a novel variant (PAPP-Ai) with differential proteolytic activity. *Eur. J. Biochem.* 2002; 269:2247–2256. [PubMed: 11985604]
81. Iversen K, et al. Pregnancy associated plasma protein-A (PAPP-A) is not a marker of the vulnerable atherosclerotic plaque. *Clin. Biochem.* 2011; 44:312–318. [PubMed: 21185276]
82. Lund J, et al. Circulating pregnancy-associated plasma protein A predicts outcome in patients with acute coronary syndrome but no troponin I elevation. *Circulation.* 2003; 108:1924–1926. [PubMed: 14530192]
83. Elseber AA, et al. Prognostic value of circulating pregnancy-associated plasma protein levels in patients with chronic stable angina. *Eur. Heart J.* 2006; 27:1678–1684. [PubMed: 16717071]
84. Qin Q-P, et al. Measurement and clinical significance of circulating PAPP-A in ACS patients. *Clin. Chim. Acta.* 2007; 380:59–67. [PubMed: 17376420]
85. Consuegra-Sanchez L, et al. Pregnancy-associated plasma protein-A (PAPP-A) and cardiovascular risk. *Atherosclerosis.* 2009; 203:346–352.
86. Iversen KK, et al. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. *Atherosclerosis.* 2011; 214:203–208. [PubMed: 21093861]

87. Tchkonina T, et al. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. *Am J. Physiol. Regul. Integr. Comp. Physiol.* 2002; 282:R1286–R1296. [PubMed: 11959668]
88. Tchoukalova YD, et al. Regional variation in adipogenesis and IGF regulatory proteins in the fetal baboon. *Biochem. Biophys. Res. Commun.* 2009; 380:679–683. [PubMed: 19285021]
89. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr. Rev.* 2000; 21:697–738. [PubMed: 11133069]
90. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. *J. Clin. Endocrinol. Metab.* 2008; 93:557–563. [PubMed: 18000086]
91. Qin X, et al. Differential regulation of pregnancy associated plasma protein (PAPP)-A during pregnancy in human and mouse. *Growth Horm. IGF Res.* 2002; 12:359–366. [PubMed: 12213189]

Outstanding Questions about PAPP-A

- What are the molecular mechanisms underlying the transcriptional and translational regulation of PAPP-A?
- What are the relevant tissues for PAPP-A regulation of IGF bioavailability through proteolysis of IGFBP?
- What are the relevant physiological and pathological conditions for PAPP-A regulation of IGF bioavailability through IGFBP proteolysis?
- Are there other substrates for PAPP-A besides IGFBPs?
- Does PAPP-A have IGF-independent effects?
- What determines the favorable versus deleterious effects of altered PAPP-A expression?
- What mouse models reflect the biology of PAPP-A in humans?

Figure 1. Local control of IGF signaling by cell-associated PAPP-A

- 1) PAPP-A is a secreted enzyme that associates with heparin-like proteoglycans on the surface of the secreting and neighboring cells through the third and fourth of five carboxy-terminal short consensus repeats. This tethering localizes PAPP-A's effects.
- 2) IGFBP-4 binds IGF with high affinity and sequesters the ligand from interacting with cell surface receptors.
- 3) PAPP-A cleaves IGFBP-4 in an IGF-dependent manner (i.e., only when IGF is bound to IGFBP-4), markedly reducing affinity of IGFBP-4 for IGF.
- 4) IGF is released in the pericellular environment, facilitating receptor binding.
- 5) IGF binding initiates the specific IGF-I receptor signal transduction, mediating mitogenesis, differentiated function, metabolic effects, and/or survival under tissue- and context-specific conditions.

Figure 2. PAPP-A regulation of autocrine/paracrine IGF action

Activated macrophages do not express PAPP-A but they secrete pro-inflammatory cytokines that stimulate PAPP-A expression in other cells in the tissue environment. This secreted PAPP-A can associate with cells in which it is produced (autocrine), and with macrophages (paracrine), to regulate local IGF action and potentially set up a positive feedback loop.

Table 1**Regulators of PAPP-A Expression**

Agent	Action	Cell Type	References
TNF α	↑	hFB hCASMC hCAEC hOB	21 22 23 24
IL-1 β	↑	hFB hCASMC hCAEC hOB	21 22 23 24
IL-6	↑ -	hCASMC hCAEC	8 23
IL-4	↑	hOB	24
TGF- β	↑ -	hOB hCAEC	24 23
PGE ₂	↑	hOB	24
Forskolin	↑	hOB	24
INF γ	↓	hFB	21
Resveratrol	↓	hCASMC	22
BMP-2	↑	hMSC	34
FSH	↑/-	bGC	35,36

h - human

b - bovine

FB - fibroblasts

OB - osteoblasts

CA - coronary artery

SMC - smooth muscle cells

EC - endothelial cells

MSC - mesenchymal stem cells

GC - granulosa cells

↑ - increase in PAPP-A expression

↓ - decrease in PAPP-A expression

- - no effect on PAPP-A expression