THEMATIC REVIEW

40 YEARS OF IGF1

PAPP-A and cancer

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Abstract

The zinc metalloproteinase, PAPP-A, enhances local insulin-like growth factor (IGF) action through cleavage of inhibitory IGF-binding proteins, thereby increasing IGF available for IGF receptor-mediated cell proliferation, migration and survival. In many tumors, enhanced IGF receptor signaling is associated with tumor growth, invasion and metastasis. We will first discuss PAPP-A structure and function, and post-translational inhibitors of PAPP-A expression or proteolytic activity. We will then review the evidence supporting an important role for PAPP-A in many cancers, including breast, ovarian and lung cancer, and Ewing sarcoma.

Introduction

The insulin-like growth factor (IGF) system is complex with IGF ligand–IGF receptor (IGF-IR) interaction initiating multiple downstream intracellular signaling pathways and feedback loops (refer the article by Shin-Ichiro Takahashi for a review of IGF-IR-mediated signaling pathways). A family of six high-affinity IGF binding proteins (IGFBPs) that can modulate this interaction and, thus, IGF-IR signaling (refer the article by Leon Bach for a review of IGFBPs) are included. In turn, the IGFBPs and their actions can be modified by specific proteinases and proteinase inhibitors. This review will focus on what is currently known about the zinc metalloproteinase, PAPP-A, and its role in cancer.

PAPP-A was first identified in 1974 as one of the four placental antigens of unknown function found in high concentrations in the plasma of pregnant women, hence the name ‘pregnancy-associated plasma protein-A’ (Lin et al. 1974). In 1999, Lawrence and coworkers discovered that PAPP-A was the enzyme responsible for IGF-dependent IGFBP-4 proteolysis in media conditioned by normal human fibroblasts (Lawrence et al. 1999). Subsequent studies found PAPP-A mRNA to be expressed in a variety of cell types and tissues unrelated to pregnancy (Overgaard et al. 1999, Conover 2012). PAPP-A was shown to enhance IGF action through specific cleavage of inhibitory IGFBPs, primarily IGFBP-4 (Fig. 1). Cleavage of IGFBP-4 results in loss of binding affinity for IGF, causing increased IGF bioavailability for IGF-IR-mediated proliferation, survival and migration. In many cancers, enhanced IGF-IR signaling is associated with tumor growth and...
metastasis (Samani et al. 2007, Pollak 2012). In addition, PAPP-A has the potential to increase local IGF-II available for activation of insulin receptor isoform-A, which is prevalent in tumor tissue and mediates a mitogenic signal (Belfiore & Malaguarnera 2011). The ability of secreted PAPP-A to associate with proteoglycans on the cell surface of the secreting as well as neighboring cells (Laursen et al. 2002) promotes IGF interaction with an activation of IGF-IR signaling in an autocrine/paracrine fashion (Laursen et al. 2007). Newly described inhibitors of PAPP-A activity, stanniocalcin (STC1 and STC2), add another layer of control over IGF-IR signaling (Oxvig 2015).

**PAPP-A primary structure and classification**

The PAPP-A mRNA encodes a secreted glycoprotein of 1547 residues (Kristensen et al. 1994). Based on the presence of a proteolytic module of approximately 300 residues (Boldt et al. 2001), PAPP-A belongs to the superfamily of metzincin metallopeptinases, which share the elongated zinc-binding motif of the catalytic site (Cerda-Costa & Gomis-Ruth 2014). PAPP-A shows no global sequence similarity to other metzincins, which also include the large group of matrix metalloproteinases (MMPs). Its only close relative is PAPP-A2, which shares 45% of its amino acids with PAPP-A and is also an IGFBP proteinase (Overgaard et al. 2001). Together, PAPP-A and PAPP-A2 comprise the ‘pappalysin’ family (Boldt et al. 2001).

Following purification of PAPP-A from pregnancy serum (Oxvig et al. 1994), it was discovered that PAPP-A circulating in pregnant women is in fact a 500-kDa 2:2 heterotetramer composed of two PAPP-A subunits and two subunits of the proform of eosinophil major basic protein (proMBP), denoted as PAPP-A:proMBP (Oxvig et al. 1993). In the eosinophil leukocyte, mature MBP is stored in granules that can be released as a cytotoxic protein, but during human pregnancy, the PAPP-A and proMBP subunits are both synthesized in the placenta and secreted into the maternal circulation in increasing amounts (Bonno et al. 1994). At term, only a minor fraction (less than 1%) of PAPP-A circulates as an uncomplexed dimer of 400kDa (Overgaard et al. 2000), but the uncomplexed fraction is higher in the first trimester (Gyrup et al. 2007). The use of polyclonal antibodies toward PAPP-A has biased earlier reports with polyspecificity resulting from immunization with the PAPP-A:proMBP complex (Oxvig et al. 1993) or with preparations contaminated by other proteins (see below).

A laminin G-like module of unknown function is present N-terminal to the proteolytic domain of PAPP-A (Boldt et al. 2006), and five short consensus repeats (SCR1-5) are found in the C-terminal end of the protein. Modules SCR3 and SCR4 bind tightly to glycosaminoglycans and allow PAPP-A to bind to the surface of cells and hence be in close proximity to the IGF-IR. Unlike PAPP-A, PAPP-A2 is unable to bind to cell surfaces (Laursen et al. 2002). The PAPP-A subunit has three Lin-12/Notch repeat (LNR) modules. Two of these (LNR1-2) are inserted into the proteolytic module, and one (LNR3) is located close to the C-terminal end of the protein. Each of the modules binds a calcium ion (Boldt et al. 2004), and they are believed to form trimeric units in which LNR1-2 of one PAPP-A subunit combines with
the LNR3 module of the other subunit in an antiparallel arrangement (Weyer et al. 2007). The PAPP-A subunit has a total of 82 cysteine residues, 81 of which have been accounted for when present in the PAPP-A:proMBP complex (Overgaard et al. 2003); only Cys-S63 was not isolated at the peptide level and it is thought to be unpaired. In this complex, the PAPP-A subunits are held together by one disulfide bond, and each PAPP-A subunit is connected to a proMBP subunit by at least two disulfide bonds, which form in a process where proMBP at the same time detaches PAPP-A from the cell surface (Glerup et al. 2005).

**Proteolytic activity of PAPP-A**

Following the finding that PAPP-A is responsible for cleavage of IGFBP-4 (Lawrence et al. 1999), it was shown that IGFBP-5 (Laarsen et al. 2001) and IGFBP-2 (Monget et al. 2003) are also PAPP-A substrates, and that PAPP-A2 cleaves IGFBP-3 and -5 (Overgaard et al. 2001). No other pappalysin substrates are known. PAPP-A cleavage of IGFBP-4 and -5 is highly specific (Laarsen et al. 2002) and highly efficient (Gyrup et al. 2007), but unlike IGFBP-5, IGFBP-4 must bind IGF to become a PAPP-A substrate (Laarsen et al. 2001). In addition to the active site, there are multiple sites of PAPP-A that interact with IGFBP-4 during the process of proteolytic cleavage, including the trimeric unit formed by LNR1-3 (Weyer et al. 2007). Specific and selective inhibition of IGFBP-4 cleavage can be obtained by targeting such a substrate-binding exosite of PAPP-A with monoclonal antibodies (Mikkelsen et al. 2008).

PAPP-A is active while bound to the cell surface and thought to facilitate IGF-IR stimulation because cleavage of IGFBP-IGF complexes causes release of IGF in its proximity (Laarsen et al. 2002). Within tissues, the IGF ‘pressure’ toward the receptor obviously depends on the ratio of IGF to one or more IGFBPs – and also on the proteinases present that might be able to cleave and inactivate IGFBPs. However, the status is difficult to predict in tissue fluids containing complex mixtures of different binding proteins. For example, IGFBP-5 has higher affinity for the IGFs than IGFBP-4, which means that only after the majority of IGFBP-5 molecules are cleaved by PAPP-A or other proteinases, IGFBP-4 gets a chance to bind IGF and become a PAPP-A substrate. PAPP-A may be the only, or at least the most important, proteinase that cleaves IGFBP-4 in vivo. Therefore, together with PAPP-A, IGFBP-4 plays the role of facilitating IGF ‘delivery’ to the IGF-IR rather than antagonizing IGF activity (Laarsen et al. 2007, Ning et al. 2008).

**Post-translational control of PAPP-A activity in vivo**

Although the pappalysins and the MMPs are related, the family of tissue inhibitors of metalloproteinases shows no inhibitory activity toward PAPP-A. Until recently, the only known physiological inhibitor of PAPP-A (but not PAPP-A2) was proMBP. For inhibitory activity, proMBP must bind to PAPP-A, resulting in irreversible formation of the covalent PAPP-A:proMBP complex (Overgaard et al. 2004). The circulating concentration of placenta-derived PAPP-A during pregnancy is elevated more than 10,000-fold compared to the levels in non-pregnant individuals, but the vast majority of PAPP-A is inactive because it is present as the PAPP-A:proMBP complex. It may be speculated that proMBP functions to protect tissues other than the placenta from excessive PAPP-A and IGF activity during human pregnancy. How the degree of complex formation is regulated is unknown (Kloverpris et al. 2013).

It was recently discovered that the stanniocalcins, STC1 (Kloverpris et al. 2015) and STC2 (Jepsen et al. 2015), are potent inhibitors of both pappalysin members. STC2 inhibition of PAPP-A requires covalent binding, similar to proMBP, but STC1 inhibits by high-affinity (KD = 75 pM) reversible interaction. Similar to the phenotype of PAPP-A knockout mice (Conover et al. 2004), transgenic STC2 mice are growth-retarded (Gagliardi et al. 2005). However, transgenic expression of a mutated STC2 variant, STC2 (Cys-120-Ala), which is unable to bind to PAPP-A, has no effect on growth (Jepsen et al. 2015), suggesting that the growth-suppressive effect of STC2 is based on its ability to inhibit PAPP-A.

It has been hypothesized that PAPP-A and the stanniocalcins are part of the same regulatory system that functions to control proteolytic cleavage of IGFBPs and, thus, IGF bioactivity (Oxvig 2015). But what is the experimental evidence that these highly specific proteinases and proteinase inhibitors are involved in human IGF physiology? First, humans that lack PAPP-A2 show a growth-retardation phenotype (Dauber et al. 2016) that can be rescued by administration of IGF-I (Munoz-Calvo et al. 2016). Second, a large genetic study showed that individuals carrying a single-residue variant of STC2 (Arg-44-Leu) are taller by 2.1 cm. This variant shows slightly compromised inhibition of PAPP-A (Marouli et al. 2017). There are currently no reports linking PAPP-A2 and cancer. Third, the existence of complexes between STCs and PAPP-A has been demonstrated in follicular fluid from normal human ovaries (Jepsen et al. 2016) and in the wall of human atherosclerotic arteries (Steffensen et al. 2016).
PAPP-A and the STCs have been studied separately for several years in different kinds of cancer. However, because proteinase–inhibitor interactions between these molecules are likely to occur if they are present in the extracellular environment of the same tissue, we will also include reports of STC and cancer below.

**PAPP-A and breast cancer**

The earliest study proposing a link between PAPP-A and breast cancer was published in 1985, i.e. before the discovery of its proteolytic activity and connection with the IGF system. Kuhajda and Eggleston reported that strong PAPP-A immunostaining was an independent predictor of early recurrence of stage I breast cancer (Kuhajda & Eggleston 1985). However, four years later, the same group reported that the polyclonal antibody used in this study for immunostaining also detected the 16.5-kDa α-chain and 40-kDa β-chain of haptoglobin-related protein (Hrp), which is expressed in breast cancer (Kuhajda et al. 1989). Thus, not only Hrp but also proMBP may be recognized by preparations of polyclonal antibodies toward PAPP-A. Nevertheless, using a specific monoclonal antibody for immunohistochemistry, PAPP-A antigen was recently shown to be expressed in human breast cancer associated with an aggressive phenotype (Mansfield et al. 2014).

Most rodent models of breast cancer suggest that PAPP-A acts as an oncogene. A study by Chander and coworkers provided insight into possible regulatory factors in PAPP-A expression. They studied MMTV-Skp2B transgenic mice, which develop mammary tumors that are associated with increased IGFBP-4 proteolysis and increased PAPP-A expression (Chander et al. 2011), and found that binding of wild-type p53 to a site in intron 1 of the PAPP-A gene transcriptionally repressed PAPP-A. On the other hand, binding of a mutated p53 to this site transcriptionally activated PAPP-A. An elegant regulatory system was put forth whereby Skp2B degrades prohibitin, a chaperone of p53, which causes p53 to denature and results in increased PAPP-A expression, increased proteolysis of IGFBP-4 and increased IGF signaling, thus contributing to mammary tumor development. The involvement of p53 in PAPP-A transcriptional regulation was later confirmed by others (Melo et al. 2013).

Using a rat model, Henning and coworkers reported that the non-coding mammary carcinoma susceptibility locus, Mcs5c, regulates PAPP-A gene expression during a critical time period in mammary development that can affect breast cancer risk (Henning et al. 2016). A novel mechanism of PAPP-A regulation during this ‘Window of Susceptibility’ involves mammary-gland-specific chromatin looping, where a temporal control element within Mcs5c physically interacts with the Pappa locus. The authors reported genotype-dependent DNA methylation of Pappa, as well, during this specific time period. These data support a potential protective benefit of decreased PAPP-A levels during adolescent development.

Pregnancy is another condition associated with a temporal increased risk of breast cancer. A mouse model of pregnancy-associated breast cancer was created by transgenic expression of PAPP-A in the mammary gland (Takabatake et al. 2016). Transgenic mice showed an increased deposition of collagen and increased proliferative signaling. However, the effect of prolonged lactation was protective causing an increased presence of inhibitory STC1 and STC2 in the tissue, while a short lactation period was associated with delayed mammary gland involution and tumor formation. Curiously, the tumors of the latter group were characterized by a reorientation of collagen fibers into the tumor-associated collagen signature (TACS-3).

In contrast to the aforementioned studies that point at tumor-promoting properties of PAPP-A, Loddo and coworkers suggest that PAPP-A acts as a tumor suppressor gene. They found that PAPP-A was epigenetically silenced in breast cancer precursor lesions, and that tumors became more invasive after downregulation of PAPP-A (Loddo et al. 2014). Dysfunctional IGF-IR signaling through loss of PAPP-A function delayed transit through mitosis. A detailed understanding of the molecular function of PAPP-A during tumor development and progression may cause apparent conflicts like these to be resolved.

Several studies have suggested a role of STCs in breast cancer. STC1 expression is stimulated by the tumor suppressor gene, BRCA1, and mutant BRCA1 was reported to cause a loss of STC1 expression in breast tumor cells (Welsh et al. 2002). Reduced STC2 promotes breast cancer cell proliferation, migration and invasion (Raulic et al. 2008, Hou et al. 2015), and STC2 expression has been found to be an independent prognostic factor for overall survival in breast cancer (Todd et al. 2016). Furthermore, elevated expression of STC1 and STC2 has been reported to correlate with tumor dormancy and, hence, late relapse in breast cancer patients (Joensuu et al. 2008). These studies were published prior to the identification of STC1 and STC2 as inhibitors of PAPP-A proteolytic activity, and although consistent with the inhibition of PAPP-A activity and decreased IGF signaling, this mechanism of action remains to be supported experimentally. Again, several other studies are conflicting, reporting increased...
expression of STC1 or STC2 in tumors (Bouras et al. 2002, Parssinen et al. 2008). As the only documented biochemical activity of the STCs in mammals is the inhibitory activity toward the pappalysins (Oxvig 2015), a detailed mechanistic explanation independent of PAPP-A for the involvement of the STCs in any type of cancer is yet to be proposed.

**PAPP-A and ovarian cancer**

Tanaka and coworkers reported that downregulation of PAPP-A mRNA by using an antisense strategy decreased IGF signaling and ovarian cancer cell growth and migration *in vitro*, and high PAPP-A protein expression promoted metastatic ovarian cancer *in vivo*. Bikunin, a Kunitz-type protease inhibitor, was found to negatively regulate PAPP-A mRNA expression, and thus suggested to represent a link between the PAPP-A and the IGF system on the one side and the urokinase-type plasminogen activator (uPA) and its receptor, uPAR, on the other side (Tanaka et al. 2004).

In another study, a human ovarian cancer cell line (SKOV3) with little PAPP-A expression and low tumorigenic potential (long tumor latency and low frequency of tumor formation *in vivo*) was used to generate stable clones overexpressing wild-type PAPP-A or mutated PAPP-A with markedly reduced proteolytic activity (Boldt & Conover 2011). Wild-type, but not mutated, PAPP-A accelerated anchorage-independent growth in soft agar, and the clone with the highest PAPP-A expression and proteolytic activity against IGFBP-4 stimulated cell invasion through Matrigel. Addition of recombinant wild-type PAPP-A to vector control SKOV3 cells also increased invasiveness. *In vivo* wild-type PAPP-A, but not mutated or control SKOV3 clones, showed significantly accelerated tumor growth and neovascularization. This study suggests an oncogenic role for PAPP-A in ovarian cancer, which depends on its proteolytic activity. Interestingly, the level of PAPP-A expression may affect the transition between expansive and invasive growths of cancer cells. Thus, xenografts based on cells with moderate PAPP-A expression induced earlier onset and increased solid tumor growth than xenografts based on cells with high PAPP-A expression, which were more invasive. Furthermore, mRNA expression of IGF system components in this study highlights the different contributions of the tumor and the host to tumor growth. PAPP-A was expressed by the SKOV3 cells, IGFBP-4 was expressed by the mouse and IGF-I, IGF-II and IGF-IR were expressed by both tumor and mouse cells.

Primary ovarian cancer patient tumorgrafts in immunocompromised mice (referred to as Avatars) recapitulate the patient experience in terms of metastases, ascites-related complications and chemo-resistance. These intraperitoneal engrafted tumors allow for the development and interaction of the tumor cells with stroma in an environment similar to the source patient (Siolas & Hannon 2013, Weroha et al. 2014). As a result, experiments in Avatars are more likely to produce clinically relevant outcome parameters and significantly influence individualized treatment decisions. Becker and coworkers found that administration of a novel PAPP-A neutralizing antibody (mAb-PA) could not only limit ovarian tumor growth, but also prevent ascites accumulation, induce ascites regression and reverse platinum resistance in different ovarian cancer Avatars (Becker et al. 2015). Importantly, effectiveness of PAPP-A inhibition depended upon the levels of PAPP-A in the tumor/ascites. Avatars expressing relatively high PAPP-A were responsive to mAb-PA treatment, whereas those expressing low PAPP-A did not. Thus, PAPP-A expression may serve as a biomarker to identify patients who might benefit from therapy that involves inhibition of PAPP-A proteolytic activity.

Patient ovarian tumors were found to express PAPP-A, IGFBP-4 and IGF-I and -II, and these proteins were present in the ascites of women with ovarian cancer (Thomsen et al. 2015). PAPP-A was particularly high in ascites, with levels approximately 50-fold greater than that in serum. There was no difference in serum PAPP-A levels between patients and controls, emphasizing the importance of PAPP-A activity locally rather than in the circulation.

Reports on the involvement of the STCs in ovarian cancer appear to be somewhat conflicting. Ismail and coworkers found that STC1 gene expression is downregulated seven-fold in cultured epithelial cancer cells compared to normal human ovarian surface epithelium (Ismail et al. 2000), whereas Liu and coworkers showed increased immunostaining for STC1 in ovarian cancer compared to normal human ovarian surface epithelium (Ismail et al. 2000). However, although a simplistic view would predict reduced expression of STC, causing less inhibition of PAPP-A, the opposite is generally observed for both STC1 (Yeung et al. 2005, Zhang et al. 2013) and STC2 (Law & Wong 2010, Wu et al. 2015).

**PAPP-A and lung cancer**

Bulut and coworkers first reported that serum PAPP-A levels were slightly increased in patients with lung cancer (Bulut et al. 2009), illustrating that in some cases,
increased local synthesis of PAPP-A is in fact reflected by an increased level in the circulation. In a recent study, PAPP-A and interleukin-6 levels were found to be elevated in malignant pleural fluid approximately 50-fold and more than 100-fold, respectively, compared to serum levels (Espelund US 2017 (in press)). The level of PAPP-A activity-derived IGFBP-4 fragments and bioactive IGF were also elevated in pleural fluid, and correlated inversely with the level of STC2.

Several studies using lung cancer cell lines are beginning to shed light on mechanism. Salim and coworkers found miRNA-214 to be a biomarker predicting metastasis in non-small cell lung cancer (NSCLC), i.e., reduced miRNA-214 increased the invasive potential of NSCLC cells, whereas overexpression of miRNA-214 decreased it (Salim et al. 2013). Gene expression analysis of miRNA-241 knocked down in NSCLC cells identified PAPP-A as a metastasis-related target gene of miRNA-214. In addition, membrane-associated PAPP-A was strongly expressed in NSCLC tumors. A lung cancer cell line that overexpressed PAPP-A, which was secreted, increased tumor growth in vivo in a xenograft model. However, if the overexpressed PAPP-A is not secreted, it inhibited tumor growth (Pan et al. 2012). Downregulation of PAPP-A expression and secretion by RNAi in A549 lung cancer cells, which normally show high PAPP-A expression, decreased tumor growth in vivo. The tumor-promoting activity appeared to be mediated, in large part, through enhancement of IGF signaling. Similarly, inhibition of PAPP-A activity with weekly intraperitoneal administration of inhibitory monoclonal antibody against PAPP-A (mAb-PA) in a murine xenograft model of A549 cells inhibited tumor growth, IGFBP-4 proteolysis and IGF signaling (Mikkelsen et al. 2014).

**PAPP-A and other cancers**

There are more limited data on PAPP-A in other cancers. Huang and coworkers identified PAPP-A as a migration/invasion-promoting gene in malignant pleural mesothelioma. Gene silencing of PAPP-A in mesothelioma cells resulted in decreased migration, invasion and proliferation. Indeed, inoculation of malignant mesothelioma cells expressing PAPP-A with PAPP-A shRNA into the pleural cavity of immune-compromised mice resulted in no tumors or bloody pleural effusion (Huang et al. 2013).

PAPP-A is expressed by metastatic melanoma tumors. Inhibition of PAPP-A decreases migration/invasion of melanoma cells in vitro and in vivo (Prithviraj et al. 2015). Functional data suggest that PAPP-A can enhance melanoma cell migration by inducing IGF-mediated epithelial-to-mesenchymal transition.

Finally, a high-density DNA microarray first identified PAPP-A as an upregulated gene in Ewing Family Tumors (Staeger et al. 2004). Potential targetable cell surface antigens in Ewing sarcoma included PAPP-A as one of the top five membrane-associated proteins overexpressed in Ewing sarcoma (Heitzeneder et al. 2016). Two recent studies support PAPP-A as a potential therapeutic target in Ewing sarcoma, a highly malignant pediatric cancer. Kirschner and coworkers generated T cell receptor (TCR) transgenic T cells directed against PAPP-A. These transgenic T cells killed Ewing sarcoma cell lines in vitro and markedly inhibited tumor growth in vivo (Kirschner et al. 2017) The authors deemed PAPP-A to be a ‘first rate’ target for TCR-based immunotherapy in Ewing sarcoma. Heitzeneder and coworkers showed delayed tumor growth and prolonged survival in Ewing sarcoma-bearing immune-deficient mice with inhibition of PAPP-A activity using mAb-PA, and enhanced efficiency when mAb-PA was used in conjunction with a ligand-blocking antibody targeting IGF-IR. PAPP-A gene knockout in Ewing sarcoma cells showed diminished free IGF-I and decreased cell growth, and downregulation of pathways associated with disrupted IGF signaling. However, there was also an induction of immune-related pathways, suggesting increased immunogenicity in Ewing sarcoma tumors lacking PAPP-A in vitro (Heitzeneder et al. 2016).

A summary of evidence for the regulation and action of PAPP-A in cancer is presented in Fig. 2. In addition
to what was specifically discussed in this review, we included pro-inflammatory cytokines, which are potent stimulators of PAPP-A expression (Conover 2012). Inflammation is prevalent in tumor tissues (Szebeni et al. 2017).

**PAPP-A and cancer: caveats and future directions**

Many studies have considered PAPP-A as a potential target for the treatment of tumor progression. The rationale of inhibiting PAPP-A in such studies is in direct line with the substantiated involvement of IGF signaling in tumor development and progression as discussed above. Although direct inhibition of IGF signaling by targeting of the IGF receptor has largely proven unsuccessful (Pollak 2012), targeting of PAPP-A has the obvious advantage of reducing IGF signaling only in tissues where increased IGF signaling may be secondary to increased PAPP-A activity, and also the advantage of reducing signaling through the insulin receptor isoform-A in such tissues. In turn, measuring levels of PAPP-A antigen may serve as part of the criterion for patient selection, although the possibility that increased PAPP-A activity is a result of decreased levels of STC1 or STC2 should also be considered.

The only known substrates of PAPP-A proteolytic activity are IGFBPs, but it is still possible that (1) PAPP-A has other protein substrates that have not yet been identified, and (2) PAPP-A has biological activities unrelated to its proteolytic activity or the IGF system. The therapeutic benefits of targeting PAPP-A may therefore, possibly in part, be a result of interference with other tumor-promoting mechanisms. As indicated above, several data conflict with a simple model in which PAPP-A promotes and the STCs antagonize tumor development and/or progression by inhibiting PAPP-A. It is possible that the STCs have biochemical activities unrelated to inhibition of PAPP-A, and it is also possible that the STCs may potentially be increased as a response to an increased level of PAPP-A. Future experiments will address these questions and provide a more detailed understanding of the proteolytic network that controls IGFBP proteolytic cleavage in normal tissues and in tumors. Understanding such details may not only increase our understanding of how PAPP-A is involved in tumor biology, but also allow us to refine strategies of targeting PAPP-A and more accurately selecting patients for therapy.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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

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