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Review

Prolactin inducible protein in cancer, fertility and immunoregulation: structure, function and its clinical implications

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Abstract. Prolactin inducible protein (PIP) is a 17-kDa single polypeptide chain, known by various names due to its versatile nature and function in human reproductive and immunological systems. It is expressed in several exocrine tissues such as the lacrimal, salivary, and sweat glands. Its expression is up regulated by prolactin and androgens, and estrogens down regulate it. Due to its over-expression in metastatic breast and prostate cancer, presently PIP is considered as a prognostic biomarker. Moreover, its aspartyl-proteinase nature suggests its role in tumor progression. PIP has unique features because it is small in size and plays multiple important functions. Its ability to bind potentially with CD4-T cell receptor, immunoglobulin G (IgG), actin, zinc α2-glycoprotein (ZAG), fibronectin and enamel pellicle, reveals its important biological functions. This is the first comprehensive review on the structure and functional analysis of PIP and its clinical applications.

Keywords. Prolactin inducible protein, antisperm antibody, tumor progression and metastasis, apoptosis, CD4-T cell receptor, fertilization.

Introduction

Prolactin inducible protein (PIP) is a 17-kDa glycoprotein present in human seminal plasma [1, 2]. This protein was independently identified and characterized as "prolactin-inducible protein" (PIP), a prolactin and androgen regulated product of the human breast tumor cell line T47D [1, 3], which is identical to gross cystic disease fluid protein 15 (GCDFP-15) and identified as a major component of breast cyst fluid (BCF), human milk and saliva [4, 5]. A similar 17-kDa actin binding protein in human seminal fluid was discovered and named a secretory actin binding protein (SABP) [6] also termed as "gp17" [6]. In different studies it was named as "extraparotid glycoprotein" (EP-GP) from human submandibular/sublingual saliva [2, 7]. The human gene of PIP has been isolated and mapped to chromosome 7 [8, 9]. The name used for GCDFP-15/PIP/SABP/gp17/EP-GP in the human and mouse genomic nomenclature is PIP; this is the name that will be used in this review.

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PIP is synthesized as a 146-amino acid long polypeptide and shows high sequence similarity with mouse submaxillary gland [1, 9] with a single glycosylation site. The exact biological functions of PIP are still uncertain but various functions have been assigned to PIP due its occurrence at high concentration in biological fluids. PIP binds to many proteins such as fibrinogen, actin, keratin, myosin and tropomyosin [2]. PIP has strong affinity for the Fc fragment of immunoglobulin G (IgG), and it could bind with antisperm antibody (ASA), which meant to protect spermatozoa from the damage by IgG. PIP also has high affinity for CD4-TCR (cluster of differentiation – T cell receptor), which modulates the immune response during the viral infection by interfering with the functions mediated by CD4 [6, 10]. Recently, many studies have documented for PIP expression in breast and prostate carcinomas and its mitogenic activity on breast cancer cell lines [11], which correlates its active role in tumor proliferation [12]. Finally, it was designated as a sensitive and specific marker for monitoring and defining apocrine differentiation in breast cancer [13]. PIP also binds to enamel pellicle of teeth and oral bacteria and it was suggested that PIP may be involved in modulating the colonization of bacteria in many oral and other biological fluids [14]. Lopez-Ottin and Diamandis [15] have reviewed five common proteins present in breast and prostate cancers and found that PIP is among them and it shares every common feature [13, 16]. Interestingly, the expression of PIP is similarly up-regulated by androgens and glucocorticoids and down-regulated by estrogens [17, 18]. Now it becomes apparent that steroid hormones exert their actions by binding and activating transcription factors which, in turn, regulate a large number of other genes. These other gene products mediate additional events by acting as growth factors in an autocrine/paracrine fashion.

Although PIP is a small protein, it plays multiple important functions in biological systems. It actively participates in fertility, immunoregulation, antimicrobial activity, apoptosis and tumor progression. Intense research efforts continue in the biochemical characterization of this protein but its exact function is not well understood. Here our aim is to organize all the biological information regarding this protein in the shade of its structure and function.

Site of expression

The PIP gene is expressed in most organs that contribute to human body fluids. The relative expression level of a PIP gene is found to be maximum in salivary gland (56.42 %) followed by lacrimal gland (16.2 %), prostate (8.63 %), muscle (2.87 %), trachea (2.85 %), mammary gland (1.84 %), lung (0.51 %) and other organs (10.67 %). It was first identified as a major component of breast cyst fluid that is also present in human milk [4], saliva [4, 19], human breast carcinomas, and particularly those with apocrine [11, 19] and exocrine organs [8]. PIP is a product of normal apocrine eyelid, ear canal, axillar, perineum, bronchial submucosal, seminal vesicle, lacrimal, and salivary glands [11, 20]. Moreover, PIP is expressed in pathological conditions of the mammary gland and in several exocrine tissues, such as the lacrimal, salivary, and sweat glands. Because of its association with secretory cell differentiation, PIP has been used in diagnostic evaluation of tumors of breast, salivary gland, and skin [21–23]. Mirels et al. [24] observed the presence of PIP by using immunohistochemical analysis of salivary, lacrimal, bronchial, submucosal and apocrine glands of the skin, and finally they determined the possible antimicrobial role of PIP in these regions. Haagensen et al. [5] reported the presence of PIP in amniotic fluid, maternal plasma, and cord blood of pregnant women. The presence of PIP in prostatic secretion such as seminal fluid has been extensively studied [6, 25]. Human seminal fluid is a rich source of PIP from where it can easily be purified and characterized [26, 27]. Moreover, its role in human seminal fluid has been fully established [27–31]. PIP is present at high concentration in BCF [32, 33] and considered as a secretory marker of apocrine differentiation in breast carcinoma [34, 35]. A large number of studies have been documented in the favor of aberrantly high expression of PIP in breast cancer specimens both at gene and protein levels [3, 36–39]. Osawa et al. [40] determined the expression profile of PIP gene; a variety of tissues was examined by RT-PCR employing the sequence-specific primers. They found that PIP gene was negligibly expressed in brain, lung, liver, spleen, heart, skeletal muscle, kidney, stomach, uterus, and ovary. On the other hand, it is abundantly expressed in lacrimal, parotid, sublingual, submandibular, mammary gland and seminal vesicle.

Gene structure and regulation

Murphy et al. [1] for the first time have isolated and determined the cDNA sequence and gene regulation of and expression of PIP in the human breast cancer cell line, T47D. The gene of PIP is located on chromosome 7 q32±36 region, and the same protein homologues have been found in the mouse and rat [9]. The mRNA of PIP is about 900 bases in length with a poly(A) tail of approximately 200 nucleotides [41]. However, the cDNA of PIP is of 577 nucleotides,

which reveals that 120 nucleotides are present in the 5' untranslated region. In other experiments, Myal et al. [9] determined that the PIP gene is 7 kb long that contains 4 exons ranging from 106 bp to 223 bp in length. The human and rodent genes are expressed in exocrine organs and through secretory vesicles PIP is transferred in the fluid secretions from these organs. PIP is synthesized as a similar protein in all the organs. However, a posttranslational modification yields two isoforms in seminal and breast cyst fluid [32].

Analysis of the mechanisms controlling PIP expression has revealed an interesting parallelism with those regulating the other major seminal plasma proteins [15]. Various studies have been documented which favor the role of prolactin in up-regulation of PIP gene expression in T47D cell line [1, 42, 43] and breast tumors [3, 8, 9, 13, 17, 42, 44]. Myal et al. [43] demonstrated that the mechanism for hormonal control of PIP expression is a complex process that may involve numerous parameters such as gene structure differences and species- and tissue-specific transacting factors. Interestingly, the expression of the PIP trans-gene was regulated differently by androgen in two different tissues (salivary and lacrimal glands), which reveals the role of tissue-specific transacting factors. Furthermore, they observed that 13.7 kb human genomic DNA containing the PIP gene and its flanking sequences (6.3 kb) must contain functional tissue- and hormone-specific regulatory elements. Recently, Carsol et al. [42] used luciferase constructs containing the 5'-flanking region of the PIP gene, and showed that prolactin-activated Stat5 and DHT-activated AR stimulate PIP gene transcription in a synergistic way. Furthermore, they demonstrated that the synergistic action of prolactin and DHT on transcriptional activity of the PIP gene promoter was also observed both with T47D human breast cancer cells and HeLa human cervical cancer cells. Finally, they concluded that both the Stat5 trans-activation domain and the AR trans-activation domain participate in the functional synergy involving Stat5 and AR signaling pathways. Apart from androgens, it has been demonstrated that interleukin-1 alpha and interleukin-6 also play stimulatory role on the gene expression of PIP and apolipoprotein D [45, 46]. Various studies are available in support of PIP gene regulation by glucocorticoids and androgen in different tissues [47–53]. The inhibitory action of estrogen on the PIP gene has been extensively studied, and it provided a better understanding of the antagonism between progestins in breast cancer cells and estrogens, androgens, and glucocorticoids [17, 54, 55].

Primary Structure of PIP

Human PIP is synthesized as a single chain immature pre-protein that consists of 146 residues [1, 4]. Schaller et al. [27] demonstrated for the first time the full amino acid sequence of mature PIP by using peptide cleavage and amino acid sequencing. After the cleavage of signal peptide (28 amino acid residues) the mature polypeptide is 118 residues long with theoretical molecular mass of 13 kDa and pI of 5.47. Interestingly, the N-terminus of PIP is glutamine that is cyclized to form pyroglutamine which in turn, is not accessible for N-terminal amino acid sequencing [56]. Experimentally, PIP showed a band on SDS-PAGE corresponds to 20 000 Da, which is relatively high as compared to the calculated mass of 13 506 Da revealing the high carbohydrate content (approximately 22 %) as determined by Schaller et al. [27]. Furthermore, due to posttranslational modification and different glycan content PIP exists in many isoforms with different molecular mass and isoelectric points. Due to carbohydrate micro heterogeneity there exists multiple PIP forms in human secretions and T47D breast carcinoma cells [57, 58]. The 12-kDa isoform is presumably a proteolytic fragment of PIP since it is smaller than the theoretical molecular mass. PIP may exist as a dimer or tetramer in different fluids. The protein forms dimers in saliva and tetramers in breast cyst fluid and human seminal fluid [6]. PIP consists of four cysteine residue where Cys37 and Cys61 form disulfide linkages with Cys63 and Cys95, respectively. Recently, we have determined the first crystal structure of PIP [M. I. Hassan, S. Bilgrami, V. Kumar, N. Singh, S. Yadav, P. Kaur and T.P. Singh, unpublished data]. However, the secondary structure predictions of PIP by the method of Garnier et al. [59] indicates 26 % helical and 49 % extended conformation, 19 % β-turns and only 6 % random coil that is totally different from our crystal structure. Furthermore, PIP is classified as a peripheral protein with no membrane-spanning segments, according to calculations by the method of Klein et al. [60].

Multiple sequence analysis of human PIP with that of other mammalian PIP whose sequences are available in SWISS PROT sequence data bank reveals close resemblance among one another (Fig. 1). Human PIP [1, 9] shows sequence identity of 97 % with chimpanzee [61], 94 % with gorilla [61], 93 % with orangutan [61], 90 % with gibbon [61], 89 % syndactylus [61], 71 % with Japanese monkey [40], 61 % with guinea pig [40], 55 % with rabbit [40], 52 % with bovine [40], 44 % with mouse [62] and 38 % with rat [24]. Interestingly, all the PIPs contain conserved cysteine residues. Moreover, the single potential glycosylation site was observed at position 77 where all PIPs have N-

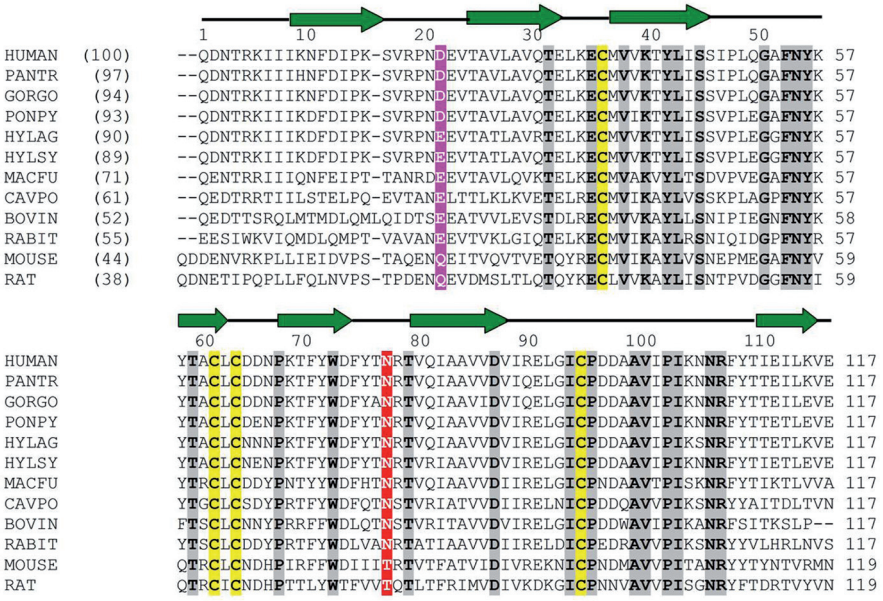

Figure 1. Multiple sequence alignment of PIP from mammalian sources. The conserved residues are highlighted in grey and the conserved cysteine residues, which form disulfide linkages, are shaded in yellow. The potential glycosylation site is shaded in red and Asp22 a proposed active site residue is shown in pink. The sequence identity with human PIP is written in parentheses. Secondary structure elements are shown on the top of amino acid sequence where loop residues are shown in black line, however, β-strands are in filled arrows (green). The amino acid sequences were taken from Swiss-Prot-TrEMBL protein sequence database (www.expasy.org). The primary accession numbers are: HUMAN, P12273; BOVIN, P60986; CAVPO, P60987; GORGO, A0A885; HYLAG, A0A890; HYLSY, A0A889; MACFU, P60988; MOUSE, P02816; PANTR, P60989; PONPY, A0A888; RABIT, P60990; and RAT, O70417.

linked glycosylation except the mouse and rat which have O-linked due to the presence of Thr77 instead of Asn. The motif search program reveals the presence of actin binding motifs in the sequence of PIP [63]. Furthermore, PIP also contains two probable fibronectin binding motifs in the protein segments of the 42–57 and 109–118 [58]. Both these motifs occur at the opposite sides of the β-fold of PIP. They contain characteristic patches of Ser, Asn, Lys, Tyr and Thr residues as required by fibronectin binding. PIP is also considered as an aspartyl proteinase due to its fibronectin degrading ability and the presence of Asp22 (proposed active site) [64]. Interestingly, in most of the species Asp22 is conserved or replaced by homologous residue Glu22 (Fig. 1). The presence of immunoglobulin fold in the structure of PIP clearly indicates its immunoregulation capabilities and strong binding with IgG and CD4 molecules.

Three Dimensional Structure of PIP

Due its diverse functions, PIP received considerable attention by immunologists as well as cancer biologists. Its structural information was not available until last year. Caputo et al. [64] generated a three dimensional model structure of PIP and explained many important functions. Although their model is not as accurate as the recently determined crystal structure [M. I. Hassan, S. Bilgrami, V. Kumar, N. Singh, S. Yadav, P. Kaur and T.P. Singh, unpublished data], they have nonetheless described several structural features of PIP with special consideration of its aspartyl proteinase nature and its role in metastasis. They reported that PIP has a proper β-fold, which is a typical feature of several aspartic proteinases [65]. Furthermore, fibronectin is one of the major protein constituents of the seminal coagulum [66] and PIP constitutes at least 1% of seminal plasma proteins [67], suggesting that it may contribute to fibronectin cleavage during liquefaction.

In the current year, we have successfully determined the crystal structure of PIP from human seminal fluid in the complex form with other protein i.e., zinc α2-glycoprotein (ZAG) [M. I. Hassan, S. Bilgrami, V. Kumar, N. Singh, S. Yadav, P. Kaur and T.P. Singh, unpublished data]. Our crystal structure is a landmark revolution for both immunologists and structure biologists and opens a new promising channel. The crystal structure indicates that PIP is basically a β-rich protein without any α-helical structure. The structure of PIP comprised seven antiparallel β-strands and seven loops (Fig. 2). The β-strands are organized in the form of two β-sheets that are arranged in a sandwiched β-sheet structure with a large number of hydrophobic residues, filling the space between the two sheets. The

Table 1. List of structure of proteins which are closely related to PIP.

Name of Protein	PDB code	Number of residues		RMSD	Identity (%)	
		Total	Super-imposed		sequence	2° structure
Prolactin Inducible Protein	2icn:b	118	118	0.00	100	100
M1 Domain of Titin	2bk8:a	97	78	2.24	8	75
Ig-Like Domain of Myosin-Binding Protein C	2dav:a	126	80	2.40	11	75
Second Ca^{2+} Binding Domain of the Na/Ca Exchanger (NCX1)	2fwu:a	157	83	2.46	10	75
23rd Filamin Domain From Human Filamin-B	2eec:a	125	76	2.79	5	86
Estrogen Receptor alpha Ligand-Binding Domain	2ocf:d	93	76	2.90	7	75
Fn3 Domain of Human Contactin 1	2ee2:a	119	78	2.95	5	75
Human C1q	2jg8:e	132	62	2.98	5	75
The Sixth Fibronectin Type Iii Domain of Human Netrin Receptor Dcc	2ede:a	114	77	3.06	6	86
Tig Domain of Human Calmodulin-Binding Transcription Activator 1 (Camta1)	2cxk:b	85	77	3.71	8	75

amino acid sequence of PIP contains one Asn-X-Ser/Thr motif at Asn77-Arg78-Thr79, indicating a potential glycosylation site. The crystallographic analysis clearly reveals a glycan attachment at Asn77 as N-acetyl glucosamine (NAG) residue. Interestingly, the structure of PIP is quite similar to that of domain 7 of fibronectin type III (FN-7) [68]. Both the PIP and FN-7 contains seven β-strands in the form of β-sheet and devoid of any α-helical content. Despite the low sequence identity (13%) the r.m.s. shift between PIP and FN-7 was found to be 1.4 when C^{α} atoms were superimposed. The overall structure of PIP reveals the presence of immunoglobulin fold, where the β-sheet is frequently observed and to be functionally more important. Until now, structural coordinates were not available in the protein data bank (PDB) showing a high sequence similarity with PIP. We have listed some proteins from the PDB, which have maximum sequence identity and minimum r.m.s. deviations in Table 1. There is only a single structural complex of PIP known which reveals that a loss of 892.8 $Å^2$ of solvent accessible surface area upon complex formation and the gain in solvation free energies of folding from isolated chains to the complex of -14.50 kcal/mol. The coordinates of PIP was analyzed in order to determine various parameters and it was observed that the accessible surface area corresponds to 6697.6 $Å^2$, whereas the total and contact areas are 7605 $Å^2$ and 2015.8 $Å^2$, respectively. The average temperature factor of PIP was found to be 35.14 $Å^2$ due to its C^{α} traces. Interestingly, three regions in the protein have higher value of their B-factor (>40); these regions are Lys16-Asp22, Ile48-Gly52 and Phe74-Val80. Residues in these regions fall in loop regions of the protein and it appears that they are involved in the function and binding.

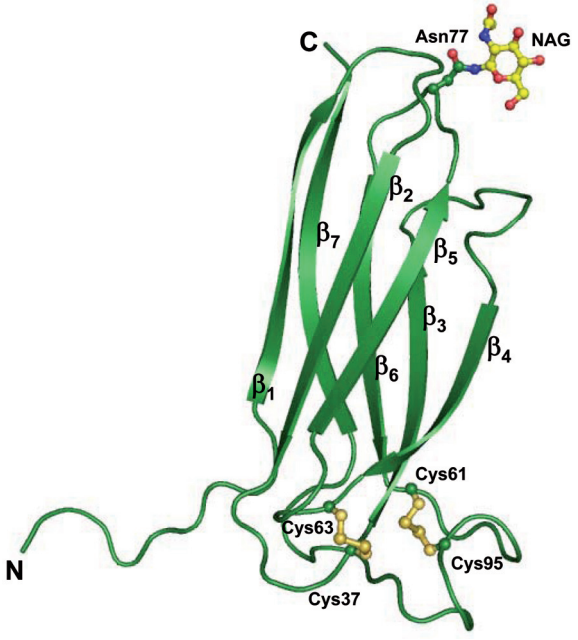

Figure 2. Three-dimensional structural fold of PIP represents seven β-stranded structure. A single potential glycosylation site (Asn77) which is accommodated by N-acetyl glucosamine (NAG) shown in ball and stick (yellow). Two disulfide bonds formed by four cysteine residues are indicated in ball and stick (dark yellow).

Functions of PIP

A large number of studies have been documented on structure and function of PIP [2, 27, 32, 47, 58, 69, 70]. Although exact function(s) of PIP is (are) still not known, due to its presence in different body fluids and similarity with proteins of known functions, various possible functions have been proposed.

Inhibition of bacterial growth

The salivary form of PIP binds to the surface of bacterial strains (*Streptococcus* and *Gemella*, and *Staphylococcus hominis*) which are colonizing in oral

tract, ear canal and skin [71], resulting in the inhibition of bacterial growth [24]. Its predominance in mucosal-type tissues, as well as its presence in saliva, tears, submucosal glands of the bronchi and apocrine glands of the skin, suggests that PIP may play an important role in mucosal immunity [24]. Schenkels et al. [14] used the replica-plate assay to determine the binding of PIP with several bacterial strains in saliva, specifically, *Gemella haemolysans, Gemella morbillorium, Streptococcus acidominimus, Streptococcus oralis, Streptococcus salivarius* and *Streptococcus parasanguis*. Similarly, bacteria from the ear canal and skin were identified as *Staphylococcus hominis*. They concluded that PIP selectively binds to several oral and non-oral bacterial species and may inhibit their growth.

Fertilization

The binding ability of PIP to the Fc fragment of IgG has improved our understanding of the function of PIP in the human seminal fluid [72]. Furthermore, the reduced level of PIP might be associated with infertility, particularly in men with anti-sperm antibody (ASA) [73]. Moreover, PIP secreted in the seminal fluid is precisely localized on the postacrosomal region of ejaculated spermatozoa. This reveals the possibility of PIP for playing a significant role in fertilization [74]. Human seminal fluid contains a large array of proteins which causes immunosuppressive properties [75]. ASA is an IgG and nature has provided PIP in seminal fluid to counter the detrimental effects of ASA by binding and neutralization. This mechanism of action of PIP has been extensively studied [29, 30, 72, 76, 77]. Among the major constituents of human seminal fluid, fibronectin is one [66] and it acts as a substrate for PIP [64]. This may suggest that PIP contributes to fibronectin cleavage during liquefaction, which in turn leads to fertilization.

Immune regulation

The fact that PIP potentially binds to CD4 with high affinity led to proposals of many possible functions of PIP in immune regulation [10, 67]. The ability of PIP to bind to CD4+T cell receptor and to block CD4-mediated T cell programmed death, reveals that PIP may modulate the immune response during insemination [10, 28, 78]. The CD4 molecule plays a key regulatory role in the immune system by acting as a co-receptor for Ag recognition of peptides associated with MHC class II proteins [79]. Furthermore, the proper binding of CD4 with nonpolymorphic regions of MHC class II molecules [2, 7] and subsequent formation of a ternary complex with the TCR [80] is the basic requirement for antigen processing and presentation. It has been reported that human CD4 also serves as the primary cellular receptor for HIV-1 retroviruses [18, 81]. In fact, it was demonstrated that the binding to CD4 of multimeric HIV gp120 or of gp120/anti-gp120 antibody complexes blocked the TCR-mediated activation of CD41-T cells *in vitro* [82] through induction of apoptosis [83, 84]. As PIP was shown to interfere with gp120 binding to CD4, interaction of PIP with certain regions of CD4 and the envelope protein gp-120 of HIV-1, it is speculated that PIP may interfere with HIV envelope protein/CD4 binding by inhibiting syncytium formation between transfected cells [10]. The binding of PIP to CD4 also inhibits T-lymphocyte apoptosis, a mechanism that is mediated via CD4 cross-linking and TCR activation [32]. In another study, Mirels et al. [24] demonstrated that abundance of PIP in mucosal-type tissues, as well as in saliva, tears, submucosal glands of the bronchi and apocrine glands of the skin, indicates that PIP may play an important role in mucosal immunity. The potent binding ability of PIP for IgG further suggests its role as an immunomodulatory protein [29, 30].

Formation of enamel pellicle

Recently, PIP was discovered as a major protein of enamel pellicle by using a proteomics-based approach and its functions proposed in the salivary system [85–89]. Rathman et al. [7, 90, 91] have identified and extensively characterized the proteins from the enamel by using hydroxyapatite column; they found that PIP was among the enamel and suggested PIP has a protective role in the salivary system. Moreover, it was postulated that since PIP is capable to bind with hydroxyapatite (a major component of tooth enamel), it may participate in formation of the enamel pellicle [88]. It appears that PIP modulates bacterial colonization because it binds to oral bacteria such as *Streptococcus* and *Gemella* [14]. Apart from human, PIP has also been identified and purified in rat saliva [24].

Tumor progression and biomarker

Over-expression of PIP gene in apocrine differentiation of breast carcinoma has revealed its proposed function in prognostic relevance [13]. A large number of studies reveals the role of PIP in tumor proliferation and metastasis, particularly in breast and prostate cancer [15, 16, 39, 48, 52, 92–97]. Shiu et al. [3], for the first time demonstrated the mechanism of action of PIP in breast cancer development, which is mediated by over-expression of prolactin. The peptide based nucleotide method (highly sensitive) has clearly established a marked relation between PIP expression and breast cancer development [98]. In earlier days, PIP was considered a biomarker only for breast cancer because in immunohistochemical

analysis and *in-situ* mRNA hybridization experiments, it had been observed that the mRNA of PIP was not detected in a small number of human tumors such as prostate, bladder, colon, uterus, thyroid, and kidney cancer, however, it was detected only in some benign breast tissue [1, 99]. In two other independent studies it was demonstrated that the androgen regulated over-expression of two important proteins in T47D human breast cancer cells were ZAG and PIP [100, 101]. It is worthwhile to mention that ZAG and PIP are among the very few proteins which are induced by androgens in human breast cells and may be an important component of tumor progression [15, 50, 102].

Although the PIP is considered as a valuable tumor marker, the exact mechanism is still not clearly understood. Cassoni et al. [12] has demonstrated the effect of PIP in the proliferation of 4 human breast-cancer cell lines (MCF7, BT474, MDA-MB231 and T47D) and in a "normal" human immortal breast-cell line (MCF10A). Interestingly, these breast-cell lines showed a mitogenic response to PIP (10 μg/mL) with an enhanced cell growth. These mitogenic effects of PIP in both normal and malignant breast epithelial cells suggest a possible relationship between the PIP and development of breast epithelial hyperplasia. PIP has an aspartyl proteinase like activity with specific fibronectin-degrading ability, which clearly indicates its significant role in mammary tumor progression [64]. PIP from human seminal plasma and breast tumors binds with CD4+T cells strongly, suggesting a possible mechanism by which it may affect the activity of tumor-infiltrated CD4+T cells [10]. In other reports, it has been recommended that PIP may constitute a breast tumor-specific antigen [32, 78, 103, 104].

PIP is also considered a prognostic biomarker for prostate carcinoma [15, 51, 105, 106], although its role in prostate tumor progression is not yet fully established. The comparison between PIP expression in normal prostate tissues and adenocarcinoma of the prostate showed that benign prostate epithelium expresses PIP at low levels, whereas, PIP is over-expressed in carcinomas of the prostate, indicating its role in tumor progression [103, 104].

Apart from breast and prostate cancers the over-expression of PIP has also been observed in other carcinomas. PIP has also been used to discriminate between primary tumors of ovary, breast, and colon [107]. In another study, [108] PIP showed metastasis in renal cell carcinoma, pancreatic carcinoma and the liver tumor along with breast cancer. Immunohistochemical analysis showed the over-expression of PIP in adenocarcinoma of the brain [109] and lung adenocarcinoma [92, 94, 110–112]. Furthermore, the PIP can also be used to differentiate metastatic lung cancer from the breast cancer [94].

The role of PIP in tumors and the prognostic value of its expression was not yet clearly established [16]. The studies have been extended to facilitate characterization of the PIP, which may help in understanding the molecular mechanism of PIP in the tumor progression. A remarkable understanding has been perceived after the discovery of its proteinase nature due to the presence of Asp22, which is homologous to Asp32 of other known aspartyl proteases [64]. Furthermore, the fibronectin is a biological substrate for PIP and considered as a multifunctional extra-cellular matrix protein that plays a central role in cell adhesion. Fibronectin interacts in multiple ways with the cell surface as well as with other extra-cellular matrix components and is sensitive to digestion by various proteases [113, 114]. It has been proposed that the fibronectin-degrading proteases (PIP) could facilitate cell invasion by cleaving the extra-cellular matrix scaffold between cells, which causes cell detachment from adhesion sites. A large number of studies favor a well established relationship between fibronectin-degrading proteases and cell invasion [115–118]. On the other hand, PIP causes a potent inhibition of CD4 T lymphocyte apoptosis concomitantly with a moderate up-regulation of Bcl-2 expression, which emphasizes that PIP may have a functional relevance in tumor pathology [28]. In another study, Caputo et al. [58] demonstrated that the different N-glycosylations may be among the important factors involved in the stabilization of dimeric structure of the protein and, thus, may enhance the proteolytic action of PIP for fibronectin [64] and the metastatic potential of tumor cells [119].

PIP binding proteins

Although PIP is a tiny protein, it has a potential to bind with an enormous number of proteins under physiological conditions and to perform various functions. The potent binding of PIP to CD4 has been extensively studied [6, 10, 28, 67, 78, 79, 120]. In order to define the functional parts, Basmaciogullari et al. [120] have demonstrated the regions of PIP which are involved in CD4 binding by using mutational analysis. It has been observed in their experiments that amino acids Asp87, Arg90, and Glu91 most likely represent contact residues with CD4, while others located both in the N and C-terminal regions presumably play a significant role in the conformational integrity of the CD4-binding site. Moreover, these regions are N-terminal end (residues 1–24, domain I) and the C-terminal part (residues 78–104, domain IV) of PIP, suggesting that CD4 may bind to two distinct sites or that these two regions are spatially

Table 2. List of proteins which have close interactions with PIP.

S. N	Name	Synonyms	Peptide Length	Score#	Ref
1	CD4 *	T-cell surface glycoprotein CD4 precursor (T-cell surface antigen T4/Leu-3)	458	0.983	[78]
2	PGR	Progesterone receptor (PR)	933	0.720	[96]
3	CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5 precursor (Carcinoembryonic antigen) (CEA) (Meconium antigen 100) (CD66e antigen)	702	0.710	[127]
4	ERBB2	Receptor tyrosine-protein kinase erbB-2 precursor (EC 2.7.1.112) (p185erbB2) (C-erbB-2) (NEU proto-oncogene) (Tyrosine kinase-type cell surface receptor HER2) (MLN 19)	1255	0.710	[128]
5	KRT20	Keratin, type I cytoskeletal 20 (Cytokeratin 20) (K20) (CK 20) (Protein IT)	424	0.707	[129]
6	ETFA	Electron transfer flavoprotein alpha-subunit, mitochondrial precursor (Alpha-ETF)	333	0.707	[130]
7	KRT7	Keratin, type II cytoskeletal 7 (Cytokeratin 7) (K7) (CK 7) (Sarcolectin)	469	0.682	[131]
8	SPI1 *	Transcription factor PU.1 (31 kDa transforming protein)	264	0.672	[132]
9	DHTR	Androgen receptor (Dihydrotestosterone receptor)	920	0.608	[133]
10	APOD	Apolipoprotein D precursor (Apo-D) (ApoD)	189	0.581	[46]
11	SDC1	Syndecan-1 precursor (SYND1) (CD138 antigen)	310	0.558	[134]
12	CCL27	Small inducible cytokine A27 precursor (CCL27) (CC chemokine ILC) (IL- 11 Ralpha-locus chemokine) (Skinkine) (ESkine) (Cuteaneous T-cell attracting chemokine) (CTACK)	112	0.558	[135]
13	PRL	Prolactin precursor (PRL)	227	0.539	[10]
14	VIM	Vimentin	466	0.513	[136]
15	UPK3A	Uroplakin-3A precursor (Uroplakin III) (UPIII)	287	0.504	[137]
16	GPR22	Probable G-protein coupled receptor 22	433	0.499	[138]
17	KRT19	Keratin, type I cytoskeletal 19 (Cytokeratin 19) (K19) (CK 19)	417	0.475	[139]
19	LYZ	Lysozyme C precursor (EC 3.2.1.17) (1,4-beta-N-acetylmuramidase C)	148	0.461	[140]
20	TITF1	Thyroid transcription factor 1 (Thyroid nuclear factor 1) (TTF-1) (Homeobox protein Nkx-2.1) (Homeobox protein NK-2 homolog A)	401	0.433	[129]
21	ZAG *	Zinc α2 glycoprotein	278	ND	

* Determined experimentally; # Calculated by the online tool STRING for Proteins and their Interactions developed by EMBL-EBI (http://string.embl.de) [141, 142].

close in the folded protein. They extended their experiment to determine the effect of IgG (another PIP binding molecule) on CD4 binding by PIP. They compared the reactivity of mutant PIP peptides and PIP-Ig molecules toward CD4 binding. Interestingly, five mutations were found to decrease (K10A) or abolish (D87A, R90A, E91A, and D98A) the binding of PIP-Ig molecules to CD4. In another study, Gaubin et al. [28] have demonstrated that the inhibition of CD4-TCR interaction leads to the inhibition of apoptosis by PIP. PIP is unable to modify the Fas-mediated apoptosis. However, it has been experimentally determined that programmed cell death induced by stimulation of the TCR/CD3 complex in mature activated T cells is mediated by the interaction of Fas with Fas ligand [121–123]. The absence of inhibition by PIP in Fas-mediated apoptosis in monocyte-depleted PBMCs consequently supports the conclusion that gp17 exerts its inhibitory effect upstream to the death signals generated specifically by Fas-FasL interaction.

Binding affinity of PIP with IgG and its associated functions have been well documented [29, 30, 34, 72, 76]. It was found that PIP binds to IgG via its Fc fragment. Recently, we have isolated a naturally occurring complex of PIP with ZAG [56] and determined its crystal structure. Our crystallographic analysis suggests that PIP is present in the cleft formed by domain α1-α2 and α3 platform of ZAG and provide a structural organization which is quite similar to an MHC-like arrangement where PIP is placed at the β2M site. The ZAG [124] and PIP [125] have common immunoglobulin like folds and the complex formation occurs similar to two immunoglobulin like domains of PapD-PapK chaperone-subunit complex [126]. This complex formation is accompanied by induction of β-strand to complete the immunoglobulin-like fold of the subunit via a mechanism termed as donor strand complementation. PIP also has close interaction with fibronectin and led to a proper function [64]. It has been determined that the regions of PIP involved in the interaction with fibronectin are residues 109–118 and the 42–57. This specific ability to interact with its binding partners may be relevant toward its function [58]. Apart from the above mentioned proteins, the interactions of PIP with other proteins available in literature are listed in Table 2, which further reveals its multidimensional function in human physiology.

Conclusions

Being a clinically important molecule, all the available information about PIP has been compiled here. The PIP is a small protein but plays a number of significant functions in human body such as immune regulation, fertilization and inhibition of apoptosis. All these

functions of PIP are attributed to its aspartyl protease like nature and its strong binding affinity with CD4 and IgG. Interestingly, PIP in the presence of androgen and estrogen shows similar pattern of its expression as observed for other tumor markers such as PSA, ZAG and progastriscin. In fact, these are highly expressed in several carcinomas. Due to its overexpression, PIP is considered as a prognostic biomarker of various carcinomas, which is helpful to clinicians for diagnosis. Its structure has been recently determined, which shows the presence of predominately β-pleated structure and a meaningful binding with immunoglobulin like domains present in ZAG, IgG and CD4. This review deals with the distribution, localization, biochemical characteristics, structure, function and usefulness of PIP in clinical management.

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