REVIEW

Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders

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ABSTRACT

DP (dipeptidyl peptidase) IV is the archetypal member of its six-member gene family. Four members of this family, DPIV, FAP (fibroblast activation protein), DP8 and DP9, have a rare substrate specificity, hydrolysis of a prolyl bond two residues from the N-terminus. The ubiquitous DPIV glycoprotein has proved interesting in the fields of immunology, endocrinology, haematology and endothelial cell and cancer biology and DPIV has become a novel target for Type II diabetes therapy. The crystal structure shows that the soluble form of DPIV comprises two domains, an α/β-hydrolase domain and an eight-blade β-propeller domain. The propeller domain contains the ADA (adenosine deaminase) binding site, a dimerization site, antibody epitopes and two openings for substrate access to the internal active site. FAP is structurally very similar to DPIV, but FAP protein expression is largely confined to diseased and damaged tissue, notably the tissue remodelling interface in chronically injured liver. DPIV has a variety of peptide substrates, the best studied being GLP-1 (glucagon-like peptide-1), NPY (neuropeptide Y) and CXCL12. The DPIV family has roles in bone marrow mobilization. The functional interactions of DPIV and FAP with extracellular matrix confer roles for these proteins in cancer biology. DP8 and DP9 are widely distributed and indirectly implicated in immune function. The DPL (DP-like) glycoproteins that lack peptidase activity, DPL1 and DPL2, are brain-expressed potassium channel modulators. Thus the six members of the DPIV gene family exhibit diverse biological roles.

INTRODUCTION

Few proteinases are capable of cleaving the post-proline bond and very few can cleave a prolyl bond two positions from the N-terminus. The latter small subset of serine proteinases, the post-proline dipeptidyl aminopeptidases, consists of the four enzymes of the DP (dipeptidyl peptidase) IV gene family, DPIV, FAP (fibroblast activation protein), DP8 and DP9 [1], and DP-II (E.C. 3.4.14.2) [2]. DPIV (E.C. 3.4.14.5) is a ubiquitous, multifunctional homodimeric glycoprotein with roles in nutrition, metabolism, the immune and endocrine systems, bone marrow mobilization, cancer growth and cell adhesion. DPIV ligands include ADA (adenosine deaminase) [3], kidney Na^+/H^+ ion exchanger 3 [4] and fibronectin [5]. Important DPIV substrates include at

Key words: adenosine deaminase, CD26, diabetes, dipeptidyl peptidase IV, fibroblast activation protein, post-proline aminopeptidase.

Abbreviations: ADA, adenosine deaminase; DP, dipeptidyl peptidase; DPL, DP-like; ECM, extracellular matrix; FAP, fibroblast activation protein; G-CSF, granulocyte colony-stimulating factor; GIP, glucose-dependent insulinotropic peptide; GKO, gene knockout; GLP, glucagon-like peptide; GRP, gastrin-releasing peptide; HSC, hepatic stellate cell; IFN, interferon; IL, interleukin; MAb, monoclonal antibody; MMP, matrix metalloproteinase; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating peptide; POP, prolyl oligopeptidase; PPAR, peroxisome proliferator-activated receptor; sCD26, soluble CD26; SREBP, sterol regulatory element-binding protein; srhCD26, soluble recombinant human CD26; uPA, urokinase plasminogen activator; VIP, vasoactive intestinal peptide.

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Table 1 Some natural substrates of DPIV

MDC, macrophage-derived chemokine; Mig, monokine induced by IFN γ; IP10, IFN γ-induced protein; I-TAC, IFN-inducible T-cell α chemoattractant; IGF-1, insulin-like growth factor-1; hCGα, human chorionic gonadotrophin α chain; GHRF, growth hormone releasing factor; LHα leutinizing hormone α chain.

Substrate	N-terminus	Reference
GLP-1	His-Ala-Glu-	[19]
GLP-2	His-Ala-Asp-	[20]
GIP	Tyr-Ala-Asp-	[19]
GRP	Val-Pro-Leu-	[21,22]
Substance P	Arg-Pro-Lys-	[21]
NPY	Tyr-Pro-Ser-	[23]
Peptide YY(1–36)	Tyr-Pro-Ile-	[23]
PACAP38	His-Ser-Asp-	[22,24]
CCL5/RANTES	Ser-Pro-Tyr-	[25]
CCL11/eotaxin	Gly-Pro-Gly-	[25]
CCL22/MDC	Gly-Pro-Tyr-	[25]
CXCL9/Mig	Thr Pro-Val-	[26]
CXCL10/IP10	Val-Pro-Leu-	[25,26]
CXCL11/I-TAC	Phe-Pro Met-	[26]
CXCL12/SDF-1	Lys-Pro-Val-	[27]
IGF-1	Gly-Pro-Glu-	[28]
Prolactin	Thr-Pro-Val-	[21]
hCGα	Ala-Pro-Asp-	[21]
GHRF	Tyr-Ala-Glu-	[19,24]
LHα	Phe-Pro-Asn-	[28]
Thyrotropin α	Phe-Pro-Asp-	[28]
Peptide histidine methionine	His-Ala-Asp-	[19,24]
Enkephalins	Tyr-Pro-Val-	[29]
Vasostatin-1	Leu-Pro-Val-	[30]

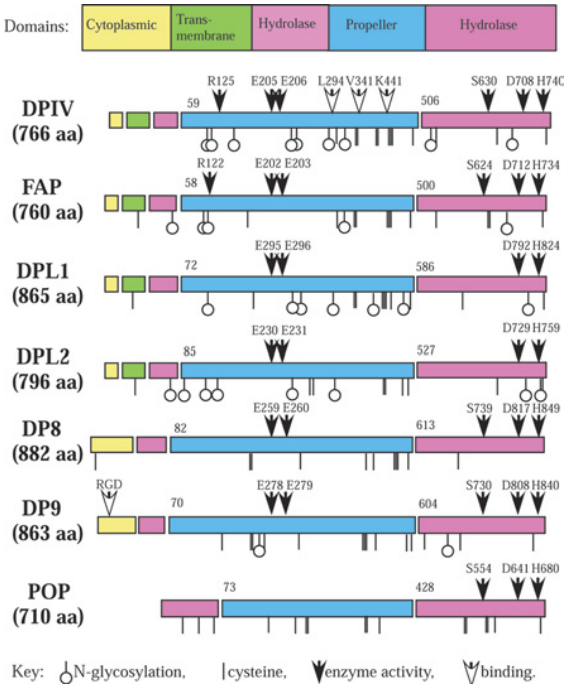

Figure 1 Schematic presentation of the proteins of the DPIV family and of POP

The arrangement of structural domains is depicted. Approximate positions of N-glycosylation sites, cysteine residues and some residues required for enzyme activity and ADA or antibody binding are depicted. Not to scale.

COLOUR

least nine chemokines, NPY (neuropeptide Y), peptide YY, GLP (glucagon-like peptide)-1, GLP-2 and GIP (glucose-dependent insulinotropic peptide; Table 1). DPIV inhibitors are in clinical trials as a new therapy for non-insulin dependent diabetes mellitus (Type II diabetes) [6,7]. Therapeutic benefit is derived from reduced inactivation of GLP-1 and GIP by DPIV-mediated cleavage, thus stimulating greater insulin production. Furthermore, on a high-fat diet, the DPIV GKO (gene knockout) mouse has reduced appetite and increased energy expenditure compared with wild-type animals [8], suggesting that DPIV-selective inhibitors may be useful as anti-obesity agents that might combat liver steatosis. Lymphocytes and endothelial and epithelial cells express DPIV (for review, see [9]). In addition to the integral membrane form, a soluble form of DPIV occurs in serum [10].

DPIV has a post-proline dipeptidyl aminopeptidase activity preferentially cleaving Xaa-Pro or Xaa-Ala dipeptides (where Xaa is any amino acid) from the N-terminus of polypeptides. The POP (prolyl oligopeptidase; EC 3.4.21.26) family, a group of aminopeptidases and endopeptidases able to hydrolyse the post-proline bond, includes the DPIV gene family. The DPIV gene family is distinguished by a pair of glutamate residues that are distant from the catalytic serine in the primary structure (Figure 1) [1], but within the catalytic pocket in the tertiary structure [11]. These glutamate residues, at positions 205 and 206 in DPIV, are essential for DP activity [12,13]. The DPIV gene family has six members, including FAP, DP8, DP9 and the two non-enzymes DPL1 and DPL2 (Table 2).

This review complements recent reviews [14–18] in discussing the structure, activities and roles of the DPIV gene family in T-cell function, chemoattraction of leucocytes, cancer, angiogenesis, fibrinolysis, haematopoiesis and energy metabolism.

IN VIVO EXPRESSION OF DPIV/CD26

DPIV is expressed in all organs, by capillary endothelial cells and activated lymphocytes and on apical surfaces of epithelial, including acinar, cells. In humans, DPIV is present in the gastrointestinal tract, biliary tract, exocrine pancreas, kidney, thymus, lymph node, uterus, placenta, prostate, adrenal, parotid, the sweat, salivary and mammary glands and endothelia of all organs examined, including liver, spleen, lungs and brain (reviewed previously, see [9,14]). DPIV is a 110 kDa glycoprotein

Table 2 **Enzymes of the human DPIV family**

Key: ✓, yes; ×, no; ?, not known.

Characteristic	DP8	DPIV	FAP	DP9
Hydrolysis of H-Gly-Pro	✓	✓	poor	✓
Hydrolysis of H-Ala-Pro	✓	✓	✓	✓
Hydrolysis of H-Arg-Pro	Poor	✓	✓	Poor
Cleavage of chemokines	?	✓	?	?
Gelatinase activity (collagen I)	×	×	✓	×
Dimeric form	×	✓	✓	×
Binding to adenosine deaminase	×	✓	×	×
mRNA expression in normal adult tissues	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous
Protein expression in normal adult	?	Ubiquitous	Serum, pancreas	?
Protein expression by activated fibroblasts	?	✓	✓	?
Expression by fetal mesenchymal cells	?	✓	✓	?
Expression by activated hepatic stellate cells	?	×	✓	?
Expression by lymphocytes	✓	✓	×	✓

that is catalytically active only as a dimer. CD26 cell-surface expression on T-cells increases 5–10- fold following antigenic or mitogenic stimulation.

Human DPIV overexpression in mice produces fewer thymocytes and peripheral blood leucocytes from 2 months of age, more single positive $CD8^+$ thymocytes and more apoptotic $CD8^+$ and $CD4^+$ peripheral blood lymphocytes [31].

Many epithelial tumours and cancer cell lines express DPIV, but DPIV expression is down-regulated or absent in tumour cells [1]. However, solid tumours contain DPIV in the stromal fibroblasts [1,32].

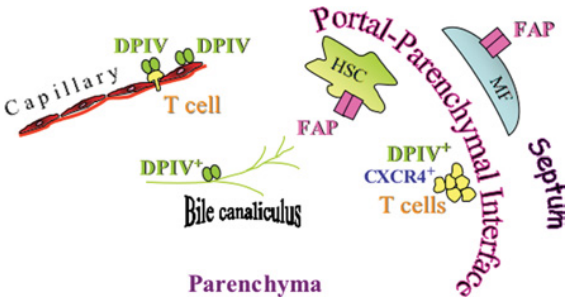

Figure 2 **Schematic representation of FAP and DPIV in cirrhotic liver**

In cirrhotic nodules, FAP is expressed by myofibroblasts (mf) in the septum and by HSCs following activation in the portal–parenchymal interface. DPIV is expressed on activated T-cells, capillary endothelium and the bile canalicular surface of hepatocytes. Not to scale.

COLOUR

FAP

FAP has 52 % amino acid identity with DPIV, but FAP and DPIV differ in expression patterns and substrate specificities (Table 2). FAP has a collagen type I-specific gelatinase activity [33,34]. In contrast, we have detected no gelatinase activity from recombinant human DPIV in zymograms of transfected CHO (Chinese-hamster ovary) cells or of purified protein [33] or in gelatinase assays of transfected monkey fibroblastic [14] or human epithelial [35] cell lines. Like DPIV, catalysis depends upon dimerization [33,36]. Interestingly, DPIV and FAP form heterodimers [37]. A soluble form of FAP has been isolated from normal bovine and human serum but, curiously, despite the abundance of serum DPIV, serum FAP is homodimeric [38,39].

Controlling gelatinases is vital for organ structure. Unlike MMPs (matrix metalloproteinases), which have a proenzyme form, the gelatinase activity of FAP is constitutive. FAP is normally restricted to a subset of glucagon: producing α-cells in pancreatic islets [32]. FAP is strongly expressed by activated HSCs (hepatic stellate cells), notably near lipid accumulation, called steatosis, in liver and by mesenchymal cells in other sites of tissue remodelling such as stromal fibroblasts of epithelial tumours and healing wounds and embryonic mesenchymal cells [32–34,40,41]. The FAP GKO mouse has a normal phenotype for body weight, organ weights, histological examination of major organs and haematological analysis [42].

The HSC has an important role in the pathogenesis of cirrhosis. Following liver injury, HSCs undergo activation and transdifferentiation to become myofibroblasts. Significant functional changes accompany this phenotypic change, including alterations in ECM (extracellular matrix) production and expression of various MMPs and their inhibitors. FAP is expressed by myofibroblasts and a subset of activated human HSCs at the tissue–remodelling interface, which is the PPI (portal–parenchymal interface), of cirrhotic liver [33] (Figure 2).

Table 3 Physical attributes of human DPIV-related proteins

✓, yes; ×, no; ?, not known. *The long form is a splice variant.

Attribute	DPIV	FAP	DP8	DP9	DP9 long*	DPL1	DPL2	POP
Synonyms	CD26, ADAbp	Seprase	–	–	–	DPP6, DPPX	DPP10	PEP, PREP
GenBank® accession	M80536	U09278	AF221634	AY374518	AF542510	M96859	AY387785	AB020018
			NM_130434	NM_139159	AF452102	M96860	NM_1004360	
References	[57]	[37]	[45,48]	[13,48,49]	[35]	[52]	[48,50,51]	
Gene location								
Human	2q24.2	2q24.3	15q22.32	19q13.3		7q36.2	2q14.1	6q21
Mouse	2	2	9	17		5	1	10
Number of exons	26	26	20	19	22	26	26	15
Gene size (kb)	81.8	72.8	71	47.3	48.6	911	1402	40.2
Transmembrane domain	✓	✓	×	×	×	✓	✓	×
Monomer mobility	110 kDa	95 kDa	100 kDa	110 kDa	?	97 kDa	97 kDa	80 kDa
Number of amino acids	766	760	882	863	971	865*, 803	796*	712

FAP-positive cells are present in early stages of liver injury, and FAP immunostaining intensity strongly correlates with the histological severity of fibrosis in chronic liver disease [40].

Conferring FAP expression upon a human epithelial cell line increases tumorigenicity in mice, but has the opposite effect on a melanoma cell line [43,44]. The biological importance of these observations is unknown because tumour cells do not naturally express FAP *in vivo*.

DP8 AND DP9

The discovery of DP8 and DP9, which are ubiquitously expressed enzymes with DPIV-like peptidase activity [35,45], means that previous studies using DPIV inhibitors to infer functions of DPIV will require reinterpretation where the inhibitor is found to also inhibit DP8 or DP9 [46]. However, little is known about the expression or functional significance of DP8 or DP9. DP8 and DP9 are both soluble proteins localized in the cytoplasm. Both are DPs that are active as monomers and hydrolyse *H*-Ala-Pro- and *H*-Gly-Pro-derived substrates, although less efficiently than DPIV. Neither DP8 nor DP9 exhibit gelatinase activity and no natural substrates are known.

DP8

DP8 has 26% amino acid identity with the protein sequences of DPIV and FAP and is a dipeptidyl aminopeptidase, hydrolysing the prolyl bond after a penultimate proline [45]. However, some biochemical characteristics of DP8 are similar to the endopeptidase POP (Table 3). Like DP8, POP is a soluble cytoplasmic protein, is active as a monomer and lacks *N*-linked and *O*-linked glycosylation sites. Like DPIV, DP8 mRNA expression is ubiquitous. DP8 mRNA levels are elevated in both activated and transformed lymphocytes. DPIV traverses the TGN (*trans*-Golgi network), which is in a secretion pathway, enters secretory vesicles then moves to the cell surface [47]. Despite finding DP8 in Golgi as well as elsewhere in cytoplasm, we have not found evidence of secretion of DP8 by transfected COS or 293T cells [35].

DP9

DP9 is the closest relative to DP8, having 61% amino acid identity. DP9 has two forms, having open reading frames of 2589 bp and 2913 bp. A ubiquitous predominant DP9 mRNA transcript at 4.4 kb represents the short form and a less abundant 5.0 kb transcript present predominantly in muscle represents the long form (Table 3) [35,48,49]. DP9 has only two potential *N*-linked glycosylation sites. Paradoxically for a cytoplasmic protein, DP9 contains an RGD (Arg-Gly-Asp) potential cell attachment sequence, which is the best characterized integrin-binding motif, near its N-terminus. In contrast, the RGD motif in mouse DPIV has a different location, on propeller blade four where ADA binds to human DPIV. We obtain DP9 sizes on SDS/PAGE of 110 kDa and 95 kDa [35], whereas others report a single band at about 95–100 kDa [48,49]. DP9 has a predicted polypeptide size of 98 263 Da, so we propose that intact fully glycosylated DP9 runs at 110 kDa.

Northern blot analysis on normal tissues shows DP9 mRNA expression predominantly in muscle, liver and leucocytes. However, *in silico* examination of 255 human DP9 ESTs (expressed sequence tags; UniGene Cluster Hs.237617) indicate that DP9 mRNA expression is most abundant in leucocytic cell lines and diseased and tumour-bearing tissues including melanoma [35].

Table 4 ***In vivo*** **immunological and haematopoietic effects of DPIV inhibitors**

*GKO mouse was not inhibitor treated.

		Outcome		
Name of inhibitor	Type of inhibitor	Wild-type mouse	DPIV GKO mouse	Reference
Pro-Pro Diphenyl phosphonate ester	Irreversible, competitive	Prolonged cardiac allograft survival	Not done	[63]
Ala-Pro-nitrobenzoylhydroxylamine	Irreversible, suicide	Suppression of experimental arthritis	Same as wild type	[58]
Lyz-[Z-(NO_2)]-thiazolidide	Reversible, competitive	Suppression of experimental arthritis	Same as wild type	[58]
Isoleucyl-thiazolidide	Reversible, competitive	Exacerbates NPY + Con A-stimulated paw inflammation	Not done	[65]
Val-boro-Pro	Irreversible	Tumour Regression	Same as wild type	[66]
Val-boro-Pro	Irreversible	Accelerates recovery from neutropenia	Same as wild type	[61]
Diprotin A (Ile-Pro-Ile)	Reversible, competitive	Impaired G-CSF-induced haematopoiesis	GKO same as inhibitor*	[67]
Diprotin A or valine-pyrrolidide	Reversible, competitive	Increased bone marrow transplant efficiency	GKO same as inhibitor*	[64]

THE NON-ENZYME DPIV GENE FAMILY MEMBERS: DPL1 AND DPL2

Two enzymatically inactive proteins closely related to DPIV lack DPIV catalytic activity due to mutations of the catalytic serine residue and its neighbouring tryptophan residue, giving a surrounding sequence of Gly-Lys-Asp-Tyr-Gly-Gly instead of the motif Gly-Trp-Ser-Tyr-Gly-Gly. Since we cloned the second human DPIV paralogue that lacks the catalytic serine, we use the names DPL (DP-like) 1 and 2 for these proteins to simplify the nomenclature [1,50]. As restoring the enzyme activity of DPL1 or DPL2 would very likely require both the serine and tryptopan residues, their biological activities are probably exerted via binding interactions. It is very unlikely that provision only of a serine residue could rescue an enzyme activity in DPL2, as has been hypothesized [51].

DPL1 was previously called DPPX or DPP6 [52]. Expression of neuronal DPL1 increases in response to kainic acid injection into the hippocampus, suggesting possible involvement in CNS (central nervous system) plasticity [53]. DPL1 has a crucial role in the trafficking, membrane targeting and function of A-type potassium channels in somatodendritic compartments of neurons, which are important in neuronal function and in dysfunction, such as Parkinson's disease [54]. Despite the absence of DP activity, DPL1 exerts an important developmental function. The mouse *rump white* mutation, which lacks expression of the DPL1 gene, is embryonic lethal in homozygotes and causes a pigmentation defect in heterozygotes [55].

DPL2 has been cloned by others and called DPP10 [48,51,56]. Like DPL1, DPL2 is alternatively spliced. The DPL2, DPIV and FAP genes are all on chromosome 2, and DPL2 is more closely related to DPIV and FAP than is DPL1. Intronic portions of the DPL2 gene link to asthma [51]. DPL2 mRNA expression occurs in brain, adrenal gland and pancreas [48,50]. This is similar to the expression pattern of the long form of DPL1 [52,53]. Like DPL1, DPL2 associates with and modulates A-type potassium channels [56].

INHIBITOR DATA INDICATE IMPORTANCE OF DPIV-RELATED DPs

It is likely that many DPIV inhibitors are selective for the DPIV family rather than DPIV itself [48]. Therefore some potential functions of DP8 and DP9 may be inferred from studies in which a function has been attributed to DPIV by observing diminution of that function in cells or animals treated with a DPIV inhibitor. In several paradigms, DPIV inhibitor treatment elicits similar responses in cells and animals irrespective of possession or lack of DPIV expression (Table 4). For example, DPIV inhibitors suppress collagen-induced and alkyldiamine-induced arthritis to similar extents in DPIV-deficient German Fischer344 and wild-type rats [58]. Moreover, T-cell proliferation *in vitro* [59] and immune responses *in vivo* [58] are diminished in the presence of DPIV inhibitors; however, the DPIV-deficient rat has normal immune responses [60]. These data imply that the peptidase activities of DP8 and/or DP9 have important functions in activated lymphocytes. FAP is excluded from consideration because it is not present in leucocytes.

The DPIV/DP-II inhibitor Val-boro-Pro stimulates haematopoiesis to similar extents in DPIV GKO and wild-type mice [61], indicating that bone marrow expresses important DPIV-related enzymes. In animal models, DPIV inhibitors suppress antibody production [62] and prolong the survival of heart transplants [63]. It is possible that these phenomena also relate to functions of DP8 and/or DP9, rather than DPIV. Therapeutic DPIV inhibitors should either avoid inhibition of DP8 and DP9, or the effects of inhibiting DP8 and DP9 should be understood.

Recently, the non-selective DPIV inhibitors valine-pyrrolidide and diprotin A have been shown to greatly

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