Dipeptidyl peptidase IV and its implication in cancer

REVIEW

Yarini M Arrebola[1, 2], Hansel Gómez[3], Pedro A Valiente[2], Maria de los A Chávez[2], ✍ Isel Pascual[2]

[1] Instituto Nacional de Gastroenterología
Calle 25 e/ H e I, Vedado, Plaza de la Revolución, CP 10400, La Habana, Cuba
[2] Centro de Estudios de Proteínas (CEP), Facultad de Biología, Universidad de La Habana, UH
Calle 25 No. 455, Vedado, CP 10400, La Habana, Cuba
[3] Departamento de Química, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, España
✍ isel@fbio.uh.cu; iselpascual@yahoo.es

ABSTRACT

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), also known as CD26, is a serine aminopeptidase that preferentially cleaves Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of oligopeptides and processes regulatory peptides *in vivo*, leading to their biological activation or inactivation. The ezyme is a homodimer and each subunit is formed by a αβ-hydrolase domain and a β-propeller domain, involved in the enzymatic activity and its interaction with other proteins. It has an important role in multiple physiological functions, including the regulation of glucose metabolism being one of the current targets for the treatment of type II diabetes mellitus. This enzyme also regulates immune system responses mediated by CD4+ T lymphocytes, and recently has been identified a high/low DPP-IV activity regarding physiological levels, in pathologies like thyroid, ovarian, lung, skin, prostate cancers and central nervous system tumors. For these reasons, this enzyme evolves as a new target of attention for the development of more efficient diagnostics being considering as molecular markers for some pathologies and a target for the development of new therapeutic assessments in cancer. Current research interests are focused on depth in the structure-function relation for this enzyme, as a key point for the development of new therapies in pathologies involving DPP-IV activity or its interaction with other proteins.

Keywords: dipeptidyl peptidase IV, serine peptidases, cancer

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RESUMEN

Dipeptidil peptidasa IV y su implicación en el cáncer. La dipeptidil peptidasa IV (DPP-IV, EC 3.4.14.5), también conocida como CD26, es una aminopeptidasa de tipo serino con preferencia de corte por la secuencia Xaa-Pro o Xaa-Ala, presente en el extremo amino de los oligopéptidos, que procesa péptidos regulatorios *in vivo*, y provoca su activación e inactivación. Es un homodímero y cada subunidad consiste en dos dominios: αβ-hidrolasa y propela-β, implicados en su función enzimática y su interacción con otras proteínas. Esta enzima interviene en varios procesos fisiológicos relacionados con el metabolismo de la glucosa, por lo que es uno de los blancos para el tratamiento de la diabetes mellitus tipo 2. Además regula la respuesta inmune mediada por linfocitos CD4+, y recientemente se identificó una alteración de su actividad (elevada o muy baja), en relación con sus niveles fisiológicos normales, en varios tipos de cáncer: de tiroides, ovario, pulmón, piel, próstata, tumores del sistema nervioso central, entre otros. Por tales razones y por considerarse un potencial marcador molecular de varias enfermedades, constituye un foco de atención para el diagnóstico del cáncer y el desarrollo de terapias para combatirlo. Muchos son los estudios encaminados a una mayor comprensión de su relación estructura-función como base para el diseño de tratamientos a aquellas enfermedades en cuyo mecanismo molecular interviene la DPP-IV o interactúa con otras proteínas.

Palabras clave: dipeptidil peptidasa IV, peptidasas serino, cáncer

*I*ntroduction

Proteases are involved in a myriad of physiological cellular processes, including growth, differentiation, nutrition, protein turnover, migration and diapedesis, fertilization and zygote implantation, programmed cell death, and others. They also mediate physiopathological events such as: cancer, neurodegenerative, respiratory and cardiovascular disorders, parasitic infestations, and viral and fungal infections. Hence, the proteases systems have to be tightly controlled by effective metabolic mechanisms, with proteases inhibitors as one of the key mechanisms. Inhibitors are widely distributed throughout all the biological kingdoms, and they are responsible for halting inadequate proteolysis and its tuning. Under normal conditions, they guarantee partial proteolysis as a physiological event. Moreover, since proteases are crucial mediators in the replication and infectivity of several pathogens in man, plants and animals, the development of specific and efficacious inhibitors for potential therapeutic application has emerged as an active research field [1-3]. They have been found as effective therapeutic tools in cancer, the human immunodeficiency syndrome (AIDS), inflammation, cardiovascular and respiratory diseases, Alzheimer's disease, and type 2 diabetes mellitus [1-3].

Particularly, the serine proteases (SP) comprise the best characterized family of proteases due to exhaustive studies conducted in the last 50 years with kinetic, chemical, physical and genetic techniques. A remarkable example is dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), also known as complement differentiation protein 26 (CD26), a SP belonging to the

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prolyl-oligopeptidases with a cell surface expression pattern. It bears a wide anatomic distribution, with its highest specific activity in the kidney [4]. Besides, a soluble isoform is present in several body fluids [5].

DPP-IV selectively removes the aminoterminal dipeptide from peptides having proline or alanine in the second position. Various cytokines, growth factors and some neuropeptides bear this structural motif, what contributes to their respective biological activities and their protection against unspecific proteolysis [4]. Additionally, there are two peptide hormones naturally targeted by DPP-IV as substrates which are determinant in mammalian metabolism: the glucagon-like peptide type 1 (GLP-1) and the glucose dependent insulinotropic peptide (GIP). This makes DPP-IV a new target for therapeutic intervention in type 2 diabetes mellitus.

DPP-IV can also interact with several other proteins, such as adenosine deaminase (ADA), the gp120 protein of the human immunodeficiency virus (HIV), fibronectin, collagen, the chemokine receptor CXCR4 and the CD45 tyrosine phosphatase [6]. This last enzyme also bears several functions aside its enzymatic activity (EA), some related diseases like cancer.

Consequently, DPP-IV has raised a considerable interest in the scientific community: there are a climbing number of publications every year describing its multiple functions, in fields so varied as endocrinology and neuroendocrinology, immunology and oncology [6].

General properties of DPP-IV

Anatomical distribution, chromosomic location and gene regulation

Few proteases have been described which may be able to cleave the post-proline peptide bond, particularly if that residue is located in the second aminoterminal position of the polypeptidic sequence. The posproline aminopeptidase family comprises six proteins of the dipeptidyl peptidase (DP) family: DPP-IV, the fibroblasts activation protein (FAP), DPP-8, DPP-9, the dipeptidyl peptidase-like protein 6 (DPL-1; also known as DPP-6) and the inactive dipeptidyl peptidase 10 (DPL-2; also known as DPP-10) [6, 7].

DPP-IV (EC 3.4.14.5) was initially described as glycyl-prolyl naphtylamidase, by Hopsu-Havu and Glenner [8] in a commercial preparation of acylase I from rat liver, and further denominated DPP-IV or posproline dipeptidyl peptidase [9]. It was subsequently isolated from various mammalian tissues, in bacteria and plants [10-14]. This aminopeptidase is identical to the CD26 molecule, a surface marker in b and T lymphocytes, and also a protein binding ADA. Moreover, DPP-IV exists as a cell surface protein and is characterized by its ubiquity, being found in humans in epithelial cells in the liver, intestines and kidneys. A soluble form is also found in body fluids, and its expression is regulated in B and T lymphocytes [15]. The highest specific enzymatic activity of this protease is found in the seminal fluid [6, 16, 17] and the kidney [6, 18].

Its human gene is located in the large arm in chromosome 2 (2q24.3), spanning approximately 70 kb and including 16 exons of 45 bp-1.5 kb in length [19], containing domains and transcription factor binding sites for constitutive genes [20]. In spite of the single mRNA identified for DPP-IV [21], a significant heterogeneity has been found in the protein once expressed, possibly caused by postranscriptional modifications [22].

DPP-IV is expressed as a highly glycosylated, type II integral membrane protein [6, 23, 24]. Its natural dimeric and soluble form is present in the seminal fluid, saliva and bile, and derives from the cell surface CD26 molecule, starting from the S^{39} residue [25, 26]. The release mechanism is unknown, although it has been assumed as being proteolytic [27]. Its serum levels in healthy adults reach approximately 22 nmol/min · mL of *p*-nitroaniline, equivalent to 7 µg/mL [18].

Molecular structure of DPP-IV

This protein is normally found as a homodimer of 220-290 kDa molecular weight [18, 28, 29], also forming tetramers of around 900 kDa. Each monomer consists of two domains, a αβ-hydrolase (residues 39-51 and 501-766) and a β-propeller domain (residues 59-497) (Figure 1A). There are nine N-glycosilation sites, most of them located in the β propeller domain, near to dimerization surface. It has been proposed that glycosylations shield the enzyme from extracellular proteolysis [18]. Human and porcine enzymes are similar in size (766 amino acids) with an 88 % homology and share functional properties such as: stability against pH and temperature changes, and

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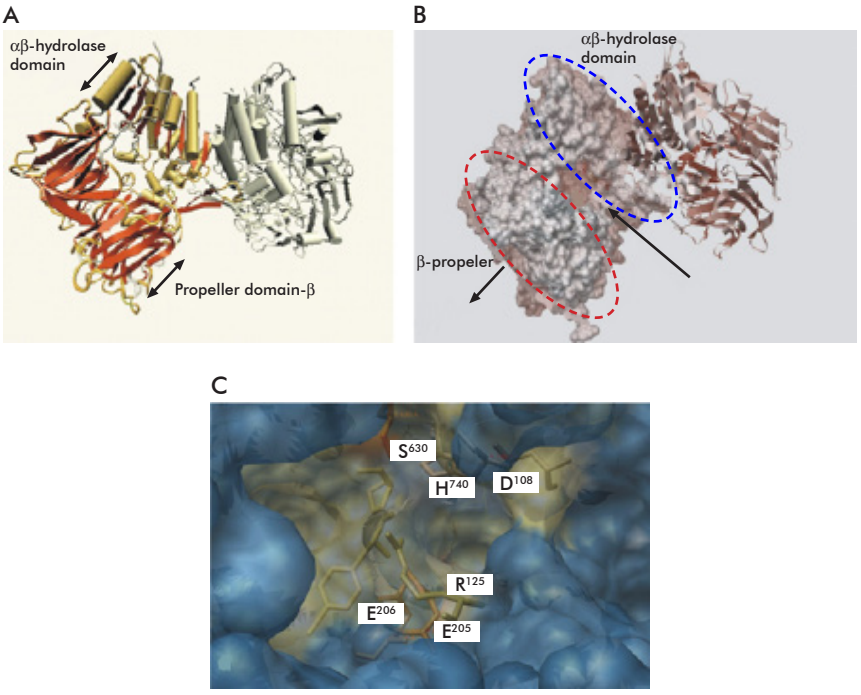

Figure 1. Structural elements of the porcine DPP-IV enzyme. A) Porcine DPP-IV structure (PDB: 1 orv). Each monomer is composed of one αβ-hydrolase and one β-propeller domain. The image was elaborated by using the Visual Molecular Dynamics (VMD; http://www.ks.uiuc.edu/Research/vmd/). B) Access to the active site of porcine DPP-IV (PDB: 1 orv). The arrows indicate the entry and exit points for substrates and dipeptide products for the enzyme catalytic activity, respectively. The E^{205} and E^{206} residues, involved in substrate binding, are highlighted. The discontinuous black line encloses the α/β-hydrolase domain and the red one the β-propeller domain. C) Active site of porcine DPP-IV. The data file PDB: 1orw was used, corresponding to the enzyme crystallized together with a non-hydrolizable substrate analogue. Residues of the catalytic triad (S^{630}, H^{740} and D^{708}) and E^{205}, E^{206} and R^{125}, involved in substrate binding, are highlighted. The image was obtained by using the CHIMERA (http://www.cgl.ucsf.edu/chimera/).

susceptibility to peptidases and divalent ions, what makes porcine DPP-IV an adequate surrogate model when the human enzyme is not available due to ethical or economic reasons [30]. Some of the properties of the human and porcine DPP-IV have been recently described for the rat counterpart, indicative of a highly conserved structure-function relationship of this enzyme in mammals [31].

Tridimensional structure

The elucidation of the tridimensional structure of DPP-IV, based on obtaining crystals for structure characterization studies, was fostered by the growing interest in designing inhibitors specific for this enzyme [28, 30, 31].

DPP-IV active site

The catalytic domain of DPP-IV is formed by a β-sheet of 8 strands flanked by 12 α-helixes, a structural motif known as αβ-hydrolase domain [32]. The active site can be accessed through a lateral gap of approximately 15 Å through the cavity where it is located [33]. For this reason, only unfold peptides and partially unfold protein fragment can reach it. Hydrolysis products are released through the tunnel formed by the β-propeller domain (Figure 1B).

The catalytic triad (S^{630}, D^{708} and H^{740}) located in the interphase between the αβ-hydrolase and β-propeller domains (Figure 1 C). Residue Y^{547}, outside this triad, is also essential for the enzyme's activity and seems to stabilize the reaction intermediary tetrahedral oxyanion [31]. There are two glutamate residues in the catalytic pocket (E^{205} and E^{206}) contributing to align the peptidic substrate to the binding site, through salt bridges with the amino terminus of the peptide to be excised. These residues just make room for two amino acids, what determines the dipeptidyl aminopeptidase nature of the enzyme. Data obtained from mutations of E^{205} and E^{206} residues allowed to establish its relevance for the enzyme catalytic activity [34, 35]. Furthermore, its presence is a molecular fingerprint of the DPP-IV family of proteins.

The second aminoterminal residue in the substrate can only be a small sidechain amino acid, such as proline, alanine or glycine, the only ones that could fit in the narrow hydrophobic pocket S1 of DPP-IV formed by residues V^{711}, V^{656}, Y^{662}, Y^{666}, W^{659} and Y^{631} [31]. This further determines the substrate specificity of the enzyme.

Homodimerization is a requisite for the catalytic activity of DPP-IV. That process involves the αβ-hydrolase domain [35] and the bulge of the fourth sheet of the β-propeller. A point mutation near the C-terminus of the protein, for example H^{750} to E, is enough to halt the enzyme dimerization [36].

β-*propeller domain of DPP-IV*

The β-propeller domains are formed by four to eight β-sheets of 30-50 amino acids each, organized in four antiparalel strands [37]. Those β-sheets are radially displaced from a central tunnel of approximately 30-45 Å, forming a highly simmetric structure. This type of domain was firstly described for the influenza virus neuraminidase [37], which bears six β-sheets. Afterwards, other enzymes were described carrying this domain, such as: the methylamine dehydrogenase [38] and the galactose oxidase [39], both with seven β-sheets, and the methanol dehydrogenase [40] with eight. The number of proteins identified as carrying this domain has considerably grown since 1998, the properties of their supramolecular structures been subsequently described by Murzin [41], Fülöp and Jones [42], Paoli [43], and Jawad and Paoli [44].

β-propellers commonly serve as scaffolds for protein-protein interactions [42, 45] and also mediate in the catalytic activity of the enzymes carrying them [46, 47]. Particularly, some of those enzymes are related to the pathogenesis in some diseases, as in cancer, Alzheimer's disease, Huntington disease, arthritis, familial hypercholesterolemia, retinitis pigmentosa, arterial hypertension and also infections [48].

The structure of DPP-IV is unique by having a β-propeller domain of eight β-sheets, compared to the other two leucocyte surface molecules carrying a β-propeller domain, of seven β-sheets each: CD100 [49] and the integrin α chain [50]. Its domain is distinctively disorganized among those described and the eight β-sheets are displaced forming a 30-45 Å in diameter cavity [5]. Since DPP-IV is a type II integral membrane protein, this structural domain is exposed to the extracellular milieu, its structure influencing the interaction of the molecule with other molecules such as ADA, HIV gp120, fibronectin (FN) and collagen [23].

One depicting element in the DPP-IV β-propeller domain is an antiparalel β-sheet which inserts between strands 1 and 2 of the second β-sheet of the enzyme. That antiparalel β-sheet contains the R^{125} residue, which forms a salt bridge with E^{205}. This last residue locates at the C-terminal turn of the W^{201}-E^{205} α-helix which intrudes between β-sheets three and four in the β-propeller domain. Another antiparalel β-sheet is located between strands 3 and 4 in the fourth β-sheet, comprising residues D^{230}-N^{263}. This antiparalel β-sheet is essential for the dimerization interphase and is also involved in the substrate binding process [31] (Figure 2).

Residue R^{125} establishes contacts with both substrates and inhibitors, and is a common target for the design of molecules inhibiting the enzyme activity [18, 28, 31, 32, 51-53]. This residue is highly conserved in DPP-IV structure throughout species, from bacteria

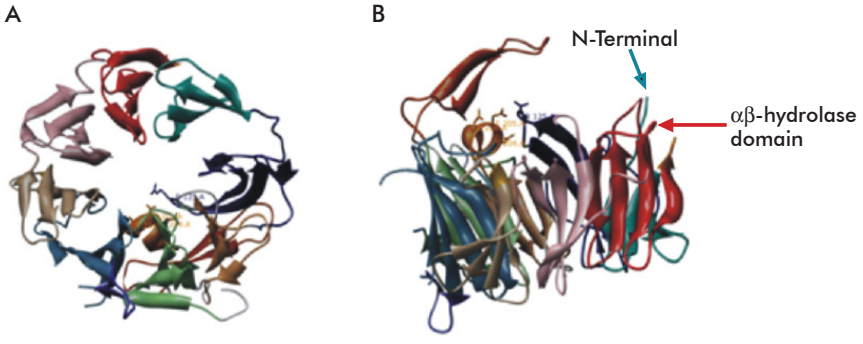

Figure 2. β-propeller domain of porcine DPP-IV (PDB: 1orv). A) Front view of the β-propeller domain, showing the displacement of β-sheets around a central tunnel. B) Side view. The horizontal arrow indicates the antiparalel β-sheet at the dimerization interphase of DPP-IV. Side chains of residues E^{205}, E^{206} and R^{125} are also depicted. Images were elaborated with CHIMERA (http://www.cgl.ucsf.edu/chimera/).

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to humans. At the same time, the sequence motif of the α-helix bearing the E^{205} residue (D-W-X-Y-E-E^{205}-E-X) is conserved in the entire DPP-IV gene family [54].

DPP- IV and cancer

The progressive loss of cellular and molecular regulatory mechanisms that occurs during carcinogenesis promotes alterations on key cellular processes, which ultimately determine the raise of malignant phenotypes displaying: autonomous cell growth, irresponsiveness to growth inhibitory signals, cell death evasion, unlimited replicative potential, sustained angiogenesis, tissue invasion and metastasis [55]. Most of these alterations are related to abnormal cell signaling circuits, with overexpressed or constitutively expressed oncogenes, or tumor suppressor genes with null or decreased expression. Particularly, most of those circuits are triggered by molecules secreted by the tumor or its microenvironment. In this context, the auto, para and yuxtacrine regulations determined by growth factors, cytokines, hormones and peptide signals are determinant for the altered abovementioned processes, with the abundance of these ligands depending on the extracellular proteolytic rate which is essential for tumor evolution [56].

It is known that DPP-IV participates in peptide-mediated growth regulation and differentiation and in the regulation of extracellular matrix interactions [6]. The regulation of the DPP-IV-mediated proteolysis could have marked effects on the availability of growth promoting or inhibitory factors in a given microenvironment [6, 56, 57]. Therefore, the loss or lack of DPP-IV expression, and its expression or that of its ligands in the tumor neighboring cells can be crucial for the progression and metastasis events in several tumor types. The evidences of such events are multifactorial, and their interpretations depend on the properties of the carcinogenesis affected tissues (Table).

DPP-IV expression is decreased in several cancers: melanoma [58], lung [60-62] and prostate [63, 79, 80] cancers, and in serum of oral [81] and colorectal cancers [82, 83]. It has also seen as progressively decreasing during endometrial adenocarcinoma [69]. The opposite effect has been shown in other cancer types, such as: primary lung tumors [59], prostate [63], ovarian carcinoma [70], thyroid carcinoma [64], dermal basal cell carcinoma [71], esophageal adenocarcinoma [72], B-cells chronic leukemia [73, 74] and certain types of T cell cancers (T-cell lymphoblastic lymphoma, anaplastic large cell lymphomas and T-cell acute lymphoblastic leukemia) [6, 75].

It has been further demonstrated the involvement of DPP-IV in the interaction with extracellular matrix components in cancer cells. Its binding to type II plasminogen (Pg 2) on the surface of the 1-LN prostate cancer cell line lead to increased intracellular Ca^{2+} concentrations, with downstream activation of a transduction pathway ultimately resulting in increased cytosolic pH. That pathway may be triggered by phospholipase C activation which promotes the synthesis of inositol 3,4,5-triphosphate, a well-known inducer of endoplasmic reticulum Ca^{2+} release [84]. Moreover, it is possible that Pg 2 may regulate pH though its association to the NHE family Na+/H+ exchanger (NH3E) previously bound to DPP-IV. These evidences suggest that the DPP-IV-Pg2 may regulate simultaneously Ca^{2+}, Na^{+} and H^{+} concentrations required for tumor proliferation and invasion [84].

The binding of DPP-IV to a subset of extracellular matrix proteins is probably mediated by the β-propeller domain [85]. It was shown the affinity of DPP-IV for type I collagen and FN. So far, the most significant interaction between DPP-IV and FN seems to be that reported during the colonization of the lung by blood-derived cancer cells. Cheng *et al.* [86] demonstrated that the vascular arrest of metastatic cells in the lung was mediated by the adhesion of DPP-IV to the FN in the surface of cancerous cells. The FN gene is overexpressed in cells able to colonize the lung derived from several cancers in humans, rats and mice. Such metastatic behavior relies on the ability of FN to randomly and dispersedly self-polymerize on the surface of numerous lung cancer cell types [87], and to assemble into long, fibrillar strands. This event occurs by the exposure of FN consensus recognition sequences to the DPP-IV molecules present in the endothelia [86]. It has been demonstrated that interaction depends on SP activity [86]. By the contrary, DPP-IV displays a weak binding activity to plasma soluble FN, suggesting that polymerized FN acquires a conformation different from that of plasma FN [86].

Several findings have confirmed the involvement of DPP-IV and polyFN in lung metastasis: 1) a soluble peptide mimicking the extracellular region of DPP-IV was able to suppress the adhesion of DPP-IV to breast metastatic cells in lung, preventing colonization; 2) the abundance of polyFN in lung metastatic cells, demonstrated in human rat and mice cancers, and also in mice and human melanoma cell lines able to colonize this organ; 3) the polyFN expression in rhabdomyosarcoma clones correlates with lung metastasis [88]. Still remains to be elucidated if the vascular compromise in lung metastasis is solely mediated by the DPP-IV to polyFN interaction or their complexation with other adhesion molecules, such as: proteoglycans, CD44 or heparin sulphate [86].

Binding experiments with a peptide bearing the FN-III repeat 14 sequence (peptide FN-III14) and the native DPP-IV showed that it was able to compete for the binding of polyFN to DPP-IV in the MTF-7 cell line, deriving in profound antimetastatic effects in lung due to 50 % decrease in adhesion and a reduced number of colonies and their size. Such a behavior was similar to that obtained through blocking the DPP-IV extracellular domain by a specific antibody [86]. Another peptide bearing the FN-III14 sequence (22-mer peptide) was described as inducing antimetastatic effects in spleen and liver colonization in T cell lymphoma [89].

DPP-IV in melanoma

In melanoma, DPP-IV is expressed in melanocytes both *in vitro* and *in vivo*, but not by the melanoma itself. Its loss of expression seems to occur at a very early stage during melanocyte transformation into melanoma. Wesley *et al.* [58] demonstrated that DPP-IV-transfected melanoma cells displayed no tumorigenicity or anchorage-independent growth, this last relying on DPP-IV enzyme activity. Additionally, the protein re-expression led to the reacquisition of

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Table. Relevant aspects for the expression of DPP-IV in several human cancer types

Cancer type	DPP-IV expression	Relevance for metastasis	Relevance to immunological disorders	Use as molecular biomarker	References
Melanoma	Null	Unidentified	Unidentified	Unidentified	[58]
Lung squamous cell carcinoma	High	Unidentified	Unidentified	Null	[59]
Small-cell lung carcinoma	Low	Unidentified	Unidentified	Unidentified	[60]
Large-cell lung carcinoma	Low	Unidentified	Unidentified	Unidentified	[60]
Non-small-cell lung carcinoma	Very low	Potential	Unidentified	Unidentified	[61]
Pulmonary adenocarcinoma	Very low	Null	Unidentified	Unidentified	[62]
Primary prostate carcinoma	Very high	Null	Unidentified	Discriminate against secondary tumor	[63]
Secondary prostate carcinoma	Very low	High	Unidentified	Discriminate against primary tumor	[63]
Papillary thyroid carcinoma	Very high	Unidentified	Unidentified	Discriminate malignant tumor from benign neoplasia	[64-68]
Follicular thyroid carcinoma	Very high	Unidentified	Unidentified	Discriminate malignant tumor from benign neoplasia	[64-66, 68]
Endometrial adenocarcinoma	Low	Unidentified	Unidentified	Discriminate malignant tumor from benign neoplasia	[69]
Ovarian carcinoma	High	High	Unidentified	Potential	[70]
Dermal basal cell carcinoma	High	Unidentified	Unidentified	Unidentified	[71]
Esophageal adenocarcinoma	High	Unidentified	Unidentified	Potential	[72]
B-cells chronic leukemia	High	Unidentified	High	Potential	[73, 74]
T-cell lymphoblastic lymphoma	High	Unidentified	High	Potential	[75]
T-cell acute lymphoblastic leukemia	High	Unidentified	High	Potential	[75]
Anaplastic large cell lymphomas	High	Unidentified	High	Potential	[75]
Glioma	High	Unidentified	Unidentified	Proposed to discrimínate the tumor progression grade	[76]
Meningioma	Low	Unidentified	Unidentified	Discriminate against glioma	[77]
Neuroblastoma	Low	Potential	Unidentified	Unidentified	[78]

growth dependency on exogenously provided growth factors [6, 58].

DPP-IV in lung cancer

Lung cancer development relies on the confluence of different growth factors, such as: neuropeptide Y (NPY) and substance P, DPP-IV substrates both. The excision and subsequent inactivation of NPY abbrogate its growth promoting effects [6, 90, 91]. This suggests that the loss of the DPP-IV proteolytic activity would promote growth in certain lung tumor cells, even without confirmation of DPP-IV acting through the regulation of other processes or signaling pathways independent of its enzyme activity, or mediated by other surface molecules as FAP, which stromal abundance correlates with increased tumor cell survival [61].

Although certain lung carcinomas express DPP-IV, that is not the case in large, small-large and small cell carcinomas, with null or marginal expression [60]. In non-small lung cell carcinoma, such lack occurs both at mRNA and protein expression levels (decreasing its activity to less than 40 pM/min/µg of protein) [61], due to frequent losses of chromosome 2q which bears the DPP-IV *loci* [92-94].

Wesley *et al.* [61] have proven that the human non-small cell lung cancer cell lines H28, H226, H441, SK-LUC-8, SK-LUC-17, SK-LUC-13, SK-LUC-9 and SW-900 show diminished DPP-IV expression. The restitution of DPP-IV in the line SK-LUC-8, particularly attractive by its undetectable expression of the enzyme, significantly reverted the the malign phenotype, independent of the enzyme activity: morphological changes *in vitro* (long and slightly dendritic cells, adopting cylindrical or flat epithelial shape), inhibition of growth in culture (with a lag for the entry in the logarithm phase), inhibition of anchoring-dependent growth (a decreased ability, 50-70 %, to form colonies in soft agar), reduced *in vitro* migration and cell confluence (probably due to the appearance of density inhibition at a confluence higher than 50 %). This was also related to the increased expression of *p21*, a drastic cell cycle arrest on G1 and apoptosis induction (possibly by the inactivation of unknown peptides). Simultaneously, there was a high expression of CD44 and FAP, cell surface proteins associated to suppressed growth and metastasis [95, 96]. The subsequent implantation of these cells on athymic mice allowed to corroborate tumor growth as expected, compared to the control which was grafted with tumor cells from the same cell line but untransfected with DPP-IV [61].

Recently, the marginal expression of DPP-IV in A549 (lung adenocarcinoma) and SK-MES-1 (squamous cells carcinoma) cell lines was corroborated; nevertheless, certain variations arose from the history of the carcinogenic process. A549 exhibited an 8-10 times

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decrease in the overall DPP-IV activity, with a 93 % surface relative activity. This indicates a fast externalization of the recently synthesized protein (probably due to preserved secretory activities inherent to alveolar type II pneumocytes, the original cell type for this cell line).

The whole proteolytic activity of DPP-IV on SK-MES-1 decreased, following a granular intracellular deposition pattern with a 35 % surface relative activity, consistent with the low secretory potential of the cell line. These observations suggest that the distribution of the enzyme during carcinogenesis correlates with alterations that may originate in the intracellular membrane trafficking system [62].

DPP-IV in ovarian cancer

The presence of DPP-IV in ovarian cancer and its involvement in tumor adhesion to the mesothelium was demonstrated by Kikkawa *et al.* [70]. They showed that the SKOV-3 cells attached more efficiently to mesothelial cells when the DPP-IV expression was restituted. A marked increase in the adhesion to immobilized FN and collagen was also detected.

The mesothelium adhesion effect was shown to be dose-dependent *in vitro*, compared to soluble FN. That suggests that DPP-IV is a key protein in the tumor cell adhesion to the mesothelium, proliferation and invasion. Ovarian carcinoma and the peritoneal mesothelium where this tumor spreads out, also express DPP-IV, and high amounts of soluble FN and the fibroconnectin of the extracellular matrix are normally found in ascites and the malignant serum. This leads to assume that DPP-IV captures high amounts of FN from these fluids. As a result, carcinoma cells would develop an easy adhesion capacity to either endothelium or mesothelium, once displaying fibroconectin in amounts enough to bind the DPP-IV molecules on these two layers [70].

Nude mice inoculated with SKOV-3 tumor cells reconstituted with DPP-IV showed lower peritoneal dissemination of the tumor cells and longer survival than those receiving the non-reconstituted cell line [97]. Although the causes for such a phenomenon were not completely understood, it would be speculated that the high levels of DPP-IV would promote a tight cell-to-cell adhesion, which may limit the ability of the carcinoma cells to detach from the tumor and spread away from it. Nevertheless, when they detach, the DPP-IV expressed by the tumor and the mesothelium facilitate the invasion into the peritoneum [70].

It could be predicted at least with low probability that the sustained and increased activity of the enzyme could influence indirectly that event, in spite of its innaparent involvement in cell adhesion [70].

DPP-IV in prostate cancer

The benign prostate cancer progresses to a fatal hormone refractory stage through a process considered to be mediated by the overexpression of peptidic growth factors which trigger alternative mitogenic signals [98-102]. Under normal physiological conditions, DPP-IV participates in cell growth regulation and differentiation by regulating those growth factors [103, 104] (e.g., the factor 1-derived stromal chemokine [63]).

Other evidences suggest that prostate cancer metastasis is associated to the loss of DPP-IV (above 50 % in most of the cases) [63, 79] and the increase in the basic fibroblast growth factor activity (bFGF), this last a potent mitogen and pro-angiogenic factor [98-102, 105] expressed as two isoforms, one cytoplasmic of low molecular weight and another one of high molecular weight in the cell nucleus. bFGF transduces through the extracellular signal-regulated mitogen-activated protein kinase (MAPK)-kinase (ERK1/2) pathway, promoting cell cancer progression and migration [106, 107]. Besides, ERK1/2 activation by bFGF during cell migration and angiogenesis increases the production of urokinase-type collagen activator (uPA), an SP catalyzing the conversion of plasminogen in plasmin, and further promoting metastasis through the destruction of the extracellular matrix [108, 109].

It has been proven that DPP-IV restitution in the DU-145 cell line blocks the nuclear translocation of bFGF and the expression of both isoforms, abrogating the stimulation through the MAP-ERK1/2 pathways. This leads to a decrease in uPA mRNA levels, the acquisition of flat and cube-like cell shapes, the loss of contact and anchoring-dependent growth and tumor migration [80].

Although the mechanism by which DPP-IV affects bFGF production remains to be elucidated, it is speculated that in a normal cell, DPP-IV excises its amino terminal region, causing its confinement within the nucleus. That excision could be the first step for bFGF degradation [80]. Similarly, a direct association may occur with bFGF, that interferes its postranscriptional modification such as methylation of the amino terminal region, which is required for its nuclear confinement [110-112].

The expression of DPP-IV in DU-145 cells also stimulated transcription of the P27 gene, an inhibitor of cyclin-dependent kinases, halting the cell cycle at the G2-M transition and increasing apoptosis from 24 to 34 % [61].

DPP and thyroid gland: neoplasias, papillary and follicular carcinomas

Thyroid and follicle carcinomas are highly positive to DPP-IV screening, in contrast to benign neoplasias which are markedly negative [64, 67]. Furthermore, DPP-IV expression increases during benign adenoma progression to malignancy, demonstrated by its higher expression in follicle adenoma displaying incomplete capsule invasion compared to that without capsular invasion [65]. The expression of the enzyme is so distinctive at both stages that it is currently considered that DPP-IV levels are the most effective fingerprint to discriminate between follicle carcinoma and follicle adenoma, even more precise that canonical variables such as the patient age, lesion size, its ultrasound image and serum thyroglobulin levels [68].

DPP- IV and keratinocyte tumorization

Experiments in InvEE transgenic mice, bearing keratinocytes with constitutively activated MAP kinase 1 (MEK-1), evidenced the upregulation of DPP-IV in epithelial tumor keratinocytes, particularly notorious at the edge of intercellular contact. Noteworthy, although dermal fibroblasts associated to tissue damage also

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shown upregulated DPP-IV expression, it was downregulated in the tumor stroma. It was demonstrated that such activity was stimulated by Ca^{2+}-induced intercellular adhesion, and the upregulation of the enzyme in dermal fibroblasts was promoted by addition of interleukine-1α (IL-1α). DPP-IV inhibition reduced tumor growth, and tumor incidence or its delayed appearance in healthy individuals was decreased by blocking IL-1α activity [113].

DPP- IV and neural tissue: gliomas, meningiomas and neuroblastomas

Healthy human brain tissues display a DPP-IV activity mostly considered as mediated by DPP8 and DPP9. Otherwise, in gliomas, the most significant tumor type in the central nervous system, with more than 50 % of the tumors, this enzyme activity has been correlated with decreased DPP8 and DPP9 activity, and a spike in DPP-IV and FAP expression, in parenchymal and vascularized areas. At the same time, a high expression of CXCR4, receptor for the stroma-derived factor (SDF-1α), was also found [76]. SDF-1α is one of the endogenous substrates of DPP-IV, and its active form, the main chemokine mediating glioma survival [114]. Once excised, SDF-1α loses its chemotactic properties and could even act as CXCR4 antagonist [115]. The marked CXCR4 upregulation seen in glioma would seem to compensate DPP-IV overexpression, suggesting a potential cross-regulation between both molecules [76].

It was found that WHO type I and atypical type II meningiomas express DPP-IV at very low levels, in detriment of increased DPP8 and DPP9 activities. The differential DPP-IV expression in meningiomas and gliomas could reside in their embryonic origin and, paradoxically, could be one of the underlying causes for the lowest aggressiveness of mningiomas compared to gliomas. In fact, meningiomas express normal levels of CXCR4, in agreement with the decrease DPP-IV expression [77]. Thus, the putative 'compensatory effect' does not seem to be activated due to insufficient DPP-IV activity, a mechanism that is present in glioma through the SDF-1α activation pathway.

Several human neuroblastoma cell lines show notoriously low DPP-IV expression. The re-expression of the enzyme *in vitro* leads to the loss of the malignant phenotype: neuron-like or flat epithelium morphology, inhibition of proliferation, caspase-activated apoptosis, decreased Akt phosphorylation and MMP9 activity (known effectors of the SDF-1α-CXCR4 activation pathway) and decreased cell migration. The low proliferation seems to be caused by the induction of differentiation as evidenced by morphological changes. The loss of migration activity may be related to the simultaneous contribution of morphology recovery and the underregulation of MMP9, this last a proangiogenic factor displaying gelatinase activity on the extracellular matrix [78].

DPP- IV, GLP-1 and cancer

The presence of the GLP-1 factor was demonstrated in the human pancreatic carcinoma Hs-7766T and human pancreatic duct adenocarcinoma CAPAN-1, CFPAC-1 and PL45 cell lines. Nevertheless, there are differences in the stimulation, mediated either through ERK1/2 activation or AMPc induction [114]. Since transduction pathways triggered by ERK1/2 and, to a lower extent those of AMPc, are relevant in events such as mitosis, meiosis and carcinogenesis, GLP-1 peptide mimetics or DPP-IV inhibitors could exert oncogenic effects [116]. Based on the lack of detection of GLP-1 receptors in 21 human pancreatic adenocarcinomas, Korner *et al*. [117] suggested that GLP-1 expression could be restricted to certain cell lines and, therefore, could be irrelevant in humans. Moreover, exenatide, a GLP-1 analogue, neither modulated the growth of pancreatic cancer cells which expressed the receptor, nor rescued them from drug-induced death. Furthermore, the sustained exantine-mediated activation of the receptor did not stimulate tumor growth or progression in rats [118]. These observations suggested that the hypothetical appearance of pancreatic cancer by administering DPP-IV inhibitors or GLP-1 mimetics could be caused by collateral pancreatitis [116], a condition favored by underlying diseases such as type II diabetes and obesity [119]. In fact, pancreatitis incidence in patients treated with GLP-1 receptor agonists does not differ with that found in populations suffering from type II diabetes [120, 121]. Similarly, studies in rats and monkeys indicated that the induction of pancreatitis through the stimulation of the GLP-1 receptor seems to be quite improbable [122].

Preclinical studies have shown an increased incidence of thyroid C-cell tumors in rodents treated with GLP-1 analogues. Nevertheless, the GLP-1 receptor expression in this cell type is highly dependent on the species, implying that observations in rodents are not necessarily relevant in human [117] due to a differential expression 22-times higher in rodents [123, 124]. Besides, it was shown that rats are more susceptible to develop thyroid C-cell neoplasias, quite rare in humans [125].

The rise in GLP-1 concentrations seems to originate more probably from premalignant lesions stimulation rather than the induction of new lesions [117], both in pancreas and the thyroid, based on the short duration of the studies and the evidences gathered so far. In contrast, it is believed that GLP-1 receptor activation could inhibit tumor growth in two very common cancer types: colon and breast cancers [117].

In the case of colon cancer, the CT26 murine colon cancer cell line expresses a functional GLP-1 receptor. Its exposure to exenatide *in vitro* leads to morphological changes, inhibits proliferation and colony formation in solid agar and induces apoptosis. These effects were confirmed *in vivo* in CT26 cells implanted in mice, even when this did not affect tumor weight [126].

Ligumsky *et al.,* [127] demonstrated in vitro that exentanide significantly reduced the number of colonies formed by the cell lines MCF-1 and MDA-MB-231, positive and negative to strogen receptors, respectively. The non-cancer HB-2 cell line remained unaffected. A significant exentanide dose-dependent tumor reduction was seen in mice implanted with MDA-MB-231 cells, when the drug was admistered by the intraperitoneal route [128].

Conclusions

Currently, DPP-IV gets the attention of the international scientific community, due to its peculiarly complex tridimensional structure. This feature determines

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DPP-IV molecular and functional properties, and its role in both the physiological and pathological processes mediated by its enzyme activity or its interaction with other proteins. It is implicated in mammalian homeostasis maintenance and, significantly, in the molecular mechanisms of multiple diseases, particularly cancer and immunological disorders, where it is found to have altered expression or misbalanced activity. A better comprehension on the role of DPP-IV in those disease-related processes would make it a very attractive target to design and develop more effective therapeutic strategies.

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