REVISIÓN

Dipeptidil peptidasa IV y su implicación en el cáncer

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

RESUMEN

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

Biotecnología Aplicada 2014;31:93-101

ABSTRACT

Dipeptidyl peptidase IV and its implication in cancer. 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 target for the development of new therapeutic assessments in cancer. Current research interests are focused in 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

*I*ntroducción

Las proteasas están involucradas en procesos celulares fisiológicos: crecimiento, diferenciación, nutrición, recambio proteico, migración e invasión, fertilización e implantación del cigoto, muerte celular programada, entre otros. Pero también se relacionan con eventos fisiopatológicos, entre los que resaltan el cáncer, los desórdenes neurodegenerativos, respiratorios y cardiovasculares, así como las infecciones parasitaria, viral y fúngica. El sistema de proteasas involucrado en procesos tan importantes requiere, por tanto, de mecanismos reguladores muy efectivos, entre ellos los inhibidores de proteasas. Estos inhibidores están ampliamente distribuidos en todos los niveles de organización biológicos. Son responsables de impedir la proteólisis en los sitios donde no deba ocurrir, y de su regulación. De modo que, en condiciones normales, garantizan la proteólisis parcial como evento fisiológico. Además, al ser las proteasas cruciales en los mecanismos de replicación e infectividad de muchos organismos patógenos al hombre, plantas y animales, el desarrollo de inhibidores específicos con utilidades terapéuticas potenciales y efectivas se ha convertido en un área emergente del avance científico [1-3]. El síndromde de inmunodeficiencia adquirida (sida), el cáncer, la inflamación, las enfermedades cardiovasculares y respiratorias, la enfermedad de Alzheimer y la diabetes mellitus tipo 2 son algunas de las enfermedades que cuentan con aplicaciones efectivas de los inhibidores de proteasas como herramientas terapéuticas [1-3].

En particular, las proteasas de tipo serino (PS) son la familia de enzimas mejor conocida, como resultado de su estudio exhaustivo, en los últimos 50 años,

1. Turk B. Targeting proteases: successes, failures and future prospects. Nat Rev Drug Discov. 2006;5(9):785-99.

2. Leung D, Abbenante G, Fairlie DP. Protease inhibitors: current status and future prospects. J Med Chem. 2000;43(3):305-41.

3. Abbenante G, Fairlie DP. Protease inhibitors in the clinic. Med Chem. 2005;1(1):71-104.

a partir de técnicas cinéticas, químicas, físicas y genéticas.

Entre ellas destaca la enzima dipeptidil peptidasa IV (DPP-IV, EC 3.4.14.5), conocida también como CD26, es una PS que se expresa en la superficie celular y pertenece a la familia de las prolil-oligopeptidasas. Se caracteriza por una amplia distribución anatómica, y el riñón es el órgano donde ejerce su mayor actividad específica [4]. Además existe una isoforma soluble que se localiza en varios fluidos del organismo [5].

DPP-IV remueve selectivamente el dipéptido del extremo amino de aquellos péptidos que tienen prolina o alanina en la segunda posición. Varias citoquinas, factores de crecimiento y algunos neuropéptidos presentan esta característica estructural, lo que contribuye a sus respectivas actividades biológicas, así como a la estabilidad frente a la degradación proteolítica inespecífica [4]. Entre los sustratos naturales de DPP-IV además hay dos hormonas de naturaleza peptídica, determinantes en el metabolismo de los mamíferos: el péptido similar a glucagón 1 (GLP-1) y el péptido insulinotrófico dependiente de glucosa (GIP), lo que convierte a esta enzima en un nuevo blanco para el estudio de la diabetes mellitus tipo 2. DPP-IV también puede interactuar con numerosas proteínas como la adenosina deaminasa (ADA), la proteína gp120 del virus de la inmunodeficiencia humana tipo 1 (VIH-1), la fibronectina, el colágeno, el receptor de quimoquinas CXCR4 y la tirosinofosfatasa CD45 [6]. Esta última está vinculada con la modulación de varias funciones independientes de su actividad enzimática (AE), algunas de las cuales están relacionadas con enfermedades como el cáncer.

En consecuencia, la DPP-IV ha suscitado gran interés para la comunidad científica: cada año crecen las publicaciones que describen sus múltiples funciones, en campos que incluyen desde la endocrinología y la neuroendocrinología, hasta la inmunología y la oncología [6].

Características generales de la DPP-IV

Localización anatómica, cromosómica y regulación génica

Se han descrito pocas proteasas que puedan escindir el enlace peptídico posprolina, más aún si este residuo se encuentra solamente a dos posiciones del extremo amino en la secuencia polipeptídica. La familia de las dipeptidil aminopeptidasas posprolina está conformada por seis proteínas de la familia genética dipeptidil peptidasa (DP): DPP-IV, proteína de activación de fibroblastos (PAF), DPP-8, DPP-9, la proteína 6 similar a dipeptidil peptidasa (DPL-1, también conocida como DPP-6) y dipeptidasa 10 inactiva (DPL-2; también conocida como DPP 10) [6, 7].

Inicialmente descrita como glicilprolina naftilamidasa en el hígado de rata, durante una preparación comercial de acilasa I por Hopsu-Havu y Glenner [8], DPP-IV (EC 3.4.14.5), posteriormente se denominó dipeptidil aminopeptidasa IV o posprolina dipeptidil peptidasa [9]. Desde entonces se ha aislado a partir de varios tejidos de mamíferos, bacterias y plantas [10-14]. Esta aminopeptidasa es idéntica a la molécula CD26, un marcador de superficie en linfocitos T y B, así como a una proteína de unión a la adenosina deaminasa (ADA). DPP-IV o CD26 es una proteína de superficie celular caracterizada por su ubicuidad. En los seres humanos, se encuentra en las células epiteliales del hígado, del intestino y del riñón. Además, existe una forma soluble en los fluidos biológicos y su expresión es regulada en linfocitos T y B [15]. Las fuentes naturales caracterizadas por la mayor actividad enzimática específica de la DPP-IV son el fluido seminal [6, 16, 17] y el riñón [6, 18].

El gen humano que codifica para DPP-IV o CD26 se localiza en el brazo largo del cromosoma 2 (2q24.3), incluye aproximadamente 70 kb, contiene 26 exones que varían entre 45 pb y 1.5 kb [19], y presenta dominios y sitios de unión a factores de transcripción característicos de genes constitutivos [20]. Aunque solamente se ha detectado un tipo de ARNm para DPP-IV [21], se aprecia una heterogeneidad notable en la proteína expresada, lo que probablemente sea causado por modificaciones postranscripcionales [22].

La DPP-IV se expresa como una proteína integral de membrana (tipo II) altamente glicosilada, [6, 23, 24]. Su forma natural dimérica y soluble existe en fluidos extracelulares como el suero, el líquido seminal, la saliva y la bilis. Esta forma comienza a partir de la S^{39} y se deriva de la molécula CD26 de la superficie celular [25, 26]. El mecanismo de liberación se desconoce, aunque se supone que sea proteolítico [27]. Su nivel sérico en adultos saludables se encuentra alrededor de los 22 nmol/min · mL de *p*-nitroanilina, lo cual se corresponde aproximadamente con 7 µg/mL [18].

Estructura molecular de DPP-IV

DPP-IV se encuentra normalmente en forma de un homodímero con peso molecular de 220-290 kDa [18, 28, 29], aunque también puede formar tetrámeros de alrededor de 900 kDa. Cada subunidad monomérica consiste en dos dominios, un dominio αβ-hidrolasa (residuos del 39-51 y 501-766) y un dominio de propela-β (residuos 59-497) (Figura 1A). DPP-IV presenta nueve sitios de N-glicosilación, ubicados fundamentalmente en el dominio propela-β, cerca de la superficie de dimerización. Se plantea que estas glicosilaciones protegen a la enzima de la proteólisis extracelular [18]. La enzima humana y la porcina tienen la misma longitud (766 aminoácidos), un porcentaje de identidad del 88 % [18], y comparten un conjunto de propiedades funcionales como la estabilidad frente al pH y la temperatura, la susceptibilidad a inhibidores de peptidasas y a iones divalentes, lo que convierte a la DPP-IV porcina en un modelo sustituto adecuado, cuando por razones éticas o económicas, se ha de prescindir de la enzima humana [30]. Varias de las características de la DPP-IV humana y porcina descritas también se identificaron recientemente para la enzima de la rata, lo que indica una elevada conservación en la relación estructura-función para esta enzima en mamíferos [31].

Estructura tridimensional de DPP-IV

La obtención de cristales de DPP-IV ha estado estrechamente ligada al creciente interés en el diseño de inhibidores específicos para esta enzima [28, 30, 31].

El centro activo de DPP-IV

El dominio catalítico de DPP-IV está compuesto por una hoja-β de 8 hebras flanqueada por 12 hélices-α,

4. Itou M, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. World J Gastroenterol. 2013;19(15):2298-306.

5. Gorrell MD, Wang XM, Park J, Ajami K, Yu DM, Knott H, *et al*. Structure and function in dipeptidyl peptidase IV and related proteins. Adv Exp Med Biol. 2006; 575:45-54.

6. Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, *et al*. The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J. 2010;277(5):1126-44.

7. Leiting B, Pryor KD, Wu JK, Marsilio F, Patel RA, Craik CS, *et al*. Catalytic properties and inhibition of proline-specific dipeptidyl peptidases II, IV and VII. Biochem J. 2003;371(Pt 2):525-32.

8. Hopsu-Havu VK, Glenner GG. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide. Histochemie. 1966;7(3):197-201.

9. Palmieri FE, Ward PE. Dipeptidyl(amino) peptidase IV and post proline cleaving enzyme in cultured endothelial and smooth muscle cells. Adv Exp Med Biol. 1989; 247A:305-11.

10. Hu CX, Huang H, Zhang L, Huang Y, Shen ZF, Cheng KD, *et al*. A new screening method based on yeast-expressed human dipeptidyl peptidase IV and discovery of novel inhibitors. Biotechnol Lett. 2009;31(7):979-84.

11. Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, *et al*. Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. Eur J Biochem. 2000;267(17):5608-13.

12. Stano J, Kovacs P, Kakoniova D, Kirilova ND, Komov VP. Activity of dipeptidyl peptidase IV in gingseng callus culture. Biologia. 1994;49:353-7.

13. Koreeda Y, Hayakawa M, Ikemi T, Abiko Y. Isolation and characterisation of dipeptidyl peptidase IV from Prevotella loescheii ATCC 15930. Arch Oral Biol. 2001;46(8):759-66.

14. Davy A, Thomsen KK, Juliano MA, Alves LC, Svendsen I, Simpson DJ. Purification and characterization of barley dipeptidyl peptidase IV. Plant Physiol. 2000;122(2):425-32.

15. Bauvois B, Djavaheri-Mergny M, Rouillard D, Dumont J, Wietzerbin J. Regulation of CD26/DPPIV gene expression by interferons and retinoic acid in tumor B cells. Oncogene. 2000;19(2):265-72.

16. de Meester I, Vanhoof G, Lambeir AM, Scharpe S. Use of immobilized adenosine deaminase (EC 3.5.4.4) for the rapid purification of native human CD26/dipeptidyl peptidase IV (EC 3.4.14.5). J Immunol Methods. 1996;189(1):99-105.

17. Wilson MJ, Ruhland AR, Pryor JL, Ercole C, Sinha AA, Hensleigh H, *et al*. Prostate specific origin of dipeptidylpeptidase IV (CD-26) in human seminal plasma. J Urol. 1998;160(5):1905-9.

18. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, *et al*. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci USA. 2003;100(9):5063-8.

motivo estructural que se conoce como dominio αβ-hidrolasa [32].

El acceso a la cavidad donde se encuentra el centro activo de la DPP-IV humana es a través de una abertura lateral de aproximadamente 15 Å [33]. Por esta razón, solo los péptidos desplegados y los fragmentos de proteína parcialmente desplegados pueden acceder a su interior. Los productos de la hidrólisis por la acción de DPP-IV se liberan a través del túnel formado por el dominio de propela-β (Figura 1B).

La tríada catalítica (S^{630}, D^{708} e H^{740}) se encuentra en la interfase entre los dominios αβ-hidrolasa y propela-β (Figura 1C). El residuo Y^{547}, que no pertenece a esta tríada, es también esencial en la actividad enzimática y parece estabilizar el oxianión tetraédrico intermediario de la reacción [31]. En el bolsillo catalítico existen dos residuos de glutamato (E^{205} y E^{206}), que contribuyen a alinear el sustrato peptídico en el sitio de fijación, mediante puentes salinos que se establecen con el extremo amino del péptido a escindir. Estos residuos solo dejan espacio para dos aminoácidos antes de que el péptido alcance el residuo de serina reactivo en el centro activo, lo cual explica la actividad dipeptidil aminopeptidasa de la enzima. Los datos obtenidos a partir de mutaciones en los residuos E^{205} y E^{206} han ayudado a establecer la importancia de estos en el proceso catalítico de esa enzima [34, 35]. Además, su presencia distingue a los integrantes de la familia de DPP-IV.

En la segunda posición del sustrato, a partir del extremo amino, solo los aminoácidos con pequeñas cadenas laterales, como prolina, alanina o glicina, pueden encajar en el estrecho bolsillo hidrofóbico S1, formado por los residuos V^{711}, V^{656}, Y^{662}, Y^{666}, W^{659} y Y^{631} de DPP-IV [31]. Ello permite explicar la especificidad de sustrato de esta enzima.

La homodimerización es un requisito para la actividad catalítica de DPP-IV. Ese proceso requiere del dominio αβ-hidrolasa [35], así como de una protuberancia de la cuarta hoja de la propela-β. Una mutación puntual cerca del C-terminal de la proteína, como por ejemplo, H^{750} por E, es suficiente para impedir la dimerización de la enzima [36].

El dominio de propela-β de DPP-IV

Las propelas-β están constituidas por agrupaciones de entre cuatro y ocho hojas-β que contienen de 30 a 50 aminoácidos organizados en cuatro hebras antiparalelas [37]. Estas hojas-β se distribuyen radialmente alrededor de un túnel central de ~30-45 Å, y forman una estructura altamente simétrica.

El dominio de propela β se describió por primera vez en la neuraminidasa del virus de la influenza [37], que posee seis hojas-β. Posteriormente, se incluyeron otras enzimas como metilamina deshidrogenasa [38] y galactosa oxidasa [39], ambas con siete hojas-β, así como metanol deshidrogenasa [40], con ocho hojas-β. Desde 1998 se han incrementado las estructuras propela-β identificadas. Las características de estas estructuras supramoleculares fueron resumidas sucesivamente por Murzin [41], Fülöp y Jones [42], Paoli [43], y Jawad y Paoli [44].

Comúnmente, las propelas-β actúan como andamiaje para las interacciones proteína-proteína [42, 45] y también pueden estar involucradas en la actividad catalítica de muchas enzimas [46, 47].

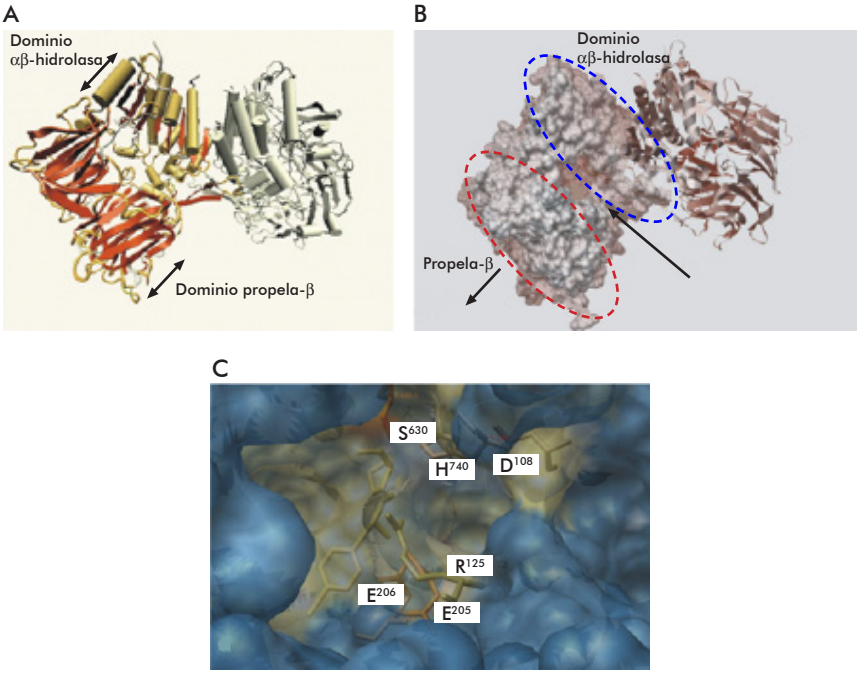

Figura 1. Elementos estructurales de la enzima DPP-IV porcina. A) Estructura de la DPP-IV porcina (PDB: 1 orv). Cada monómero está constituido por los dominios αβ-hidrolasa y propela-β. Para la elaboración de esta imagen se empleó el programa Visual Molecular Dynamics (VMD; http://www.ks.uiuc.edu/Research/vmd/). B) Acceso al centro activo de la DPP-IV porcina (PDB: 1 orv). Las flechas señalan el lugar de entrada de los sustratos de la enzima y de salida de los dipéptidos resultantes de su actividad catalítica. Se destacan los residuos E^{205} y E^{206}, involucrados en la fijación del sustrato. La línea discontinua azul encierra el dominio α/β-hidrolasa. La línea discontinua roja encierra el dominio β-propela. C) Centro activo de la DPP-IV porcina. Se utilizó el PDB: 1orw, con la enzima cristalizada en presencia de un análogo de sustrato no hidrolizable. Se muestran los residuos de la tríada catalítica (S^{630}, H^{740} y D^{708}) y los residuos E^{205}, E^{206} y R^{125}, involucrados en la fijación del sustrato. Esta imagen se confeccionó con el programa CHIMERA (http://www.cgl.ucsf.edu/chimera/).

Algunas de las proteínas que en su estructura contienen un dominio de propela-β se vinculan con la patogénesis de varias afecciones, como cáncer, enfermedad de Alzhéimer, enfermedad de Huntington, artritis, hipercolesterolemia familiar, retinitis pigmentosa, hipertensión arterial e infecciones [48].

La estructura de DPP-IV es única entre las moléculas de superficie leucocitaria que tienen un dominio de propela-β, pues las otras dos, el CD100 [49] y la cadena α de la integrina [50], presentan un dominio propela-β constituido por siete hojas-β. Es más desordenado el dominio propela-β de DPP-IV que la mayoría de los descritos, y está constituido por ocho hojas-β, dispuestas alrededor de una cavidad de 30 a 45 Å de diámetro [5]. Como DPP-IV es una proteína integral de membrana tipo II, este dominio está expuesto al medio extracelular y su estructura influye en la interacción de DPP-IV con varias moléculas como adenosina deaminasa (ADA), la proteína gp120 del VIH, la fibronectina y el colágeno [23].

Entre los elementos estructurales que distinguen al dominio propela-β de DPP-IV se encuentra una hoja-β antiparalela que se inserta entre las hebras 1 y 2 de la segunda hoja-β de la enzima. En ella se localiza el residuo R^{125}, que forma un puente salino con el residuo E^{205}. Este último se sitúa en el giro C-terminal de una hélice-α constituida por los residuos W^{201}-E^{205} e insertada entre las hojas tres y cuatro del dominio propela-β. Otra hoja-β antiparalela, formada por los

19. Abbott CA, Baker E, Sutherland GR, McCaughan GW. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. Immunogenetics. 1994;40(5):331-8.

20. Bohm SK, Gum JR, Jr., Erickson RH, Hicks JW, Kim YS. Human dipeptidyl peptidase IV gene promoter: tissue-specific regulation from a TATA-less GC-rich sequence characteristic of a housekeeping gene promoter. Biochem J. 1995;311(Pt 3): 835-43.

21. Hong WJ, Petell JK, Swank D, Sanford J, Hixson DC, Doyle D. Expression of dipeptidyl peptidase IV in rat tissues is mainly regulated at the mRNA levels. Exp Cell Res. 1989;182(1):256-66.

22. Kahne T, Kroning H, Thiel U, Ulmer AJ, Flad HD, Ansorge S. Alterations in structure and cellular localization of molecular forms of DP IV/CD26 during T cell activation. Cell Immunol. 1996;170(1):63-70.

23. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond). 2005;108(4): 277-92.

24. Yu DM, Ajami K, Gall MG, Park J, Lee CS, Evans KA, *et al*. The *in vivo* expression of dipeptidyl peptidases 8 and 9. J Histochem Cytochem. 2009;57(11):1025-40.

25. Lee KN, Jackson KW, Christiansen VJ, Chung KH, McKee PA. A novel plasma proteinase potentiates alpha2-antiplasmin inhibition of fibrin digestion. Blood. 2004;103(10):3783-8.

residuos D^{230}-N^{263}, se encuentra entre las hebras tres y cuatro pertenecientes a la hoja-β número cuatro, la cual es parte importante de la interfase de dimerización además de estar involucrada en el proceso de fijación del sustrato [31] (Figura 2).

El residuo R^{125} establece contactos con sustratos y con inhibidores, y es de gran interés para el diseño de moléculas que bloqueen la actividad de esta enzima [18, 28, 31, 32, 51-53]. Este residuo está conservado en las enzimas DPP-IV de todas las especies, desde bacterias hasta seres humanos. A su vez, el motivo de secuencia de la hélice-α que contiene al residuo E^{205} (D-W-X-Y-E-E^{205}-E-X) está conservado en las proteínas que conforman la familia génica de DPP-IV [54].

DPP- IV y el cáncer

La pérdida progresiva de los procesos de regulación durante la carcinogénesis provoca alteraciones celulares esenciales que determinan la aparición de fenotipos malignos en las células: crecimiento celular autosuficiente; insensibilidad a señales inhibidoras del crecimiento; evasión de la muerte celular; potencial replicativo ilimitado; angiogénesis sostenida; invasión tisular y metástasis [55]. La mayoría de estas alteraciones está asociada a trastornos en los circuitos de señalización celular, en muchos de los cuales están implicados oncogenes sobreexpresados o expresados constitutivamente, o genes supresores de tumor no expresados o disminuidos. Sin embargo, la iniciación de gran parte de esos circuitos se debe a señales derivadas de moléculas que pueden ser secretadas por el tumor o su microentorno. De esta manera, la regulación autocrina, paracrina y yuxtacrina, determinada por factores de crecimiento, citoquinas, hormonas y señales peptídicas, ejerce una función esencial durante la proliferación, la inhibición de la muerte celular programada, la angiogénesis, la invasión y la migración. La abundancia de los ligandos que dependen del balance proteolítico extracelular, es crucial durante la evolución del tumor [56].

Es conocido que DPP-IV participa en la regulación del crecimiento y la diferenciación mediados por péptidos, así como en la regulación de interacciones con la matriz extracelular [6]. La regulación de la proteólisis mediada por DPP-IV puede tener efectos marcados sobre la disponibilidad de factores de crecimiento o factores inhibidores del crecimiento en un microentorno determinado [6, 56, 57]. Por ello, la pérdida o no de la expresión de esta enzima en varios tipos de cáncer, y su expresión o la de sus ligandos en células cercanas, pueden ser cruciales en los eventos de progresión y metástasis. Estos eventos relacionados con DPP-IV generan evidencias multifactoriales, cuya interpretación dependerá siempre de las características del tejido donde ocurra la carcinogénesis (Tabla).

La expresión de DPP-IV está disminuida en melanoma [58], en algunos cánceres de pulmón [60-62] y de próstata [63, 79, 80], así como en el suero de pacientes con cáncer oral [81] o colorrectal [82, 83]. Se ha observado una disminución progresiva en adenocarcinoma de endometrio [69]. A su vez, la expresión de DPP-IV aumenta en ciertos cánceres de pulmón [59], próstata [63] y ovario [70], carcinoma de tiroides [64], carcinoma de células basales dérmicas [71], adenocarcinoma esofágico [72], leucemia crónica de

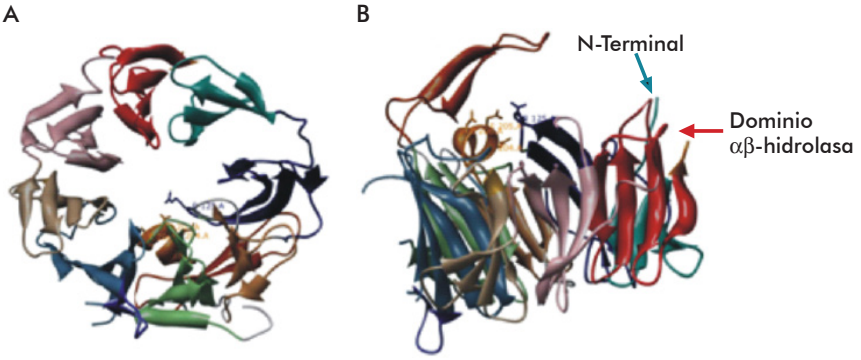

Figura 2. Dominio de propela-β de la DPP-IV porcina (PDB: 1orv). A) Vista anterior del dominio de propela-β, donde se observa la disposición de las hojas-β alrededor de un túnel central. B) Vista lateral. La flecha horizontal señala la hoja-β antiparalela que forma parte de la interfase de dimerización de la DPP-IV. Se representaron las cadenas laterales de los residuos E^{205}, E^{206} y R^{125}. Estas imágenes se confeccionaron con el programa CHIMERA (http://www.cgl.ucsf.edu/chimera/).

células B [73, 74] y en algunos tipos de cánceres de células T: linfoma linfoblástico de células T, linfoma de células largas T-anaplásticas y leucemias T-linfoblásticas agudas [6, 75].

También se ha demostrado la implicación de DPP-IV en su interacción con varios componentes de la matriz extracelular en procesos vinculados con el cáncer. Se ha descrito, además, que la unión de DPP-IV con plasminógeno II (Pg 2) sobre la superficie de la línea 1-LN de cáncer de próstata induce un aumento de la concentración intracelular de Ca^{2+}, lo cual activa una cascada que conduce al aumento del pH citosólico. Es probable que esa cascada proceda por la activación de la fosfolipasa C, que promueve la formación de inositol 3,4,5-trifosfato, conocido inductor de la liberación de Ca^{2+} desde el retículo [84]. Además, también es probable que el Pg 2 pueda regular el pH por la asociación con el intercambiador Na+/H+ miembro de la familia NHE (NH3E) unido a la DPP-IV. Tales evidencias sugieren que la unión DPP-IV/Pg 2 tiene el potencial de regular simultáneamente las concentraciones de Ca^{2+}, Na^{+} e H^{+} necesarias para la proliferación y la invasión del tumor [84].

La función de la DPP-IV de unirse a un grupo de proteínas de la matriz extracelular, posiblemente involucre a la región de β propela [85]. Se ha demostrado la afinidad de la DPP-IV por el colágeno tipo I y la fibronectina (FN). Hasta ahora, la unión más importante entre DPP-IV y la FN parece ocurrir en la colonización del pulmón por células cancerosas provenientes de la sangre. Cheng *et al.* [86] demostraron que el arresto vascular de células metastáticas en pulmón era mediado por la adhesión de la DPP-IV a la FN asociada a la superficie cancerosa. El gen de la FN se sobreexpresa en células capaces de colonizar el pulmón provenientes de varios cánceres en seres humanos, rata y ratón. Tal propiedad metastática está relacionada con la facultad de la FN de autopolimerizar de modo disperso y aleatorio sobre las superficies de numerosos tipos de cánceres de pulmón [87], y formar polímeros largos y fibrilares. Este evento ocurre mediante la exposición a la DPP-IV de secuencias consenso de reconocimiento, que facilitan la unión a esta enzima presente en endotelios [86]. Se ha demostrado que esta unión es independiente de la actividad serino proteasa [86]. Por el

26. Ajami K, Abbott CA, McCaughan GW, Gorrell MD. Dipeptidyl peptidase 9 has two forms, a broad tissue distribution, cytoplasmic localization and DPIV-like peptidase activity. Biochim Biophys Acta. 2004;1679(1):18-28.

27. Delacour D, Gouyer V, Leteurtre E, Ait-Slimane T, Drobecq H, Lenoir C, *et al*. 1-benzyl-2-acetamido-2-deoxy-alpha-D-galactopyranoside blocks the apical biosynthetic pathway in polarized HT-29 cells. J Biol Chem. 2003;278(39):37799-809.

28. Rasmussen HB, Branner S, Wiberg FC, Wagtmann N. Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. Nat Struct Biol. 2003;10(1):19-25.

29. Duke-Cohan JS, Morimoto C, Rocker JA, Schlossman SF. Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. J Immunol. 1996;156(5):1714-21.

30. Pascual I, Gomez H, Pons T, Chappe M, Vargas MA, Valdes G, *et al*. Effect of divalent cations on the porcine kidney cortex membrane-bound form of dipeptidyl peptidase IV. Int J Biochem Cell Biol. 2011;43(3):363-71.

31. Gomez H, Chappe M, Valiente PA, Pons T, Chavez Mde L, Charli JL, *et al*. Effect of zinc and calcium ions on the rat kidney membrane-bound form of dipeptidyl peptidase IV. J Biosci. 2013;38(3):461-9.

32. Thoma R, Loffler B, Stihle M, Huber W, Ruf A, Hennig M. Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. Structure. 2003;11(8):947-59.

33. Aertgeerts K, Ye S, Tennant MG, Kraus ML, Rogers J, Sang BC, *et al*. Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. Protein Sci. 2004;13(2):412-21.

34. Abbott CA, McCaughan GW, Gorrell MD. Two highly conserved glutamic acid residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. FEBS Lett. 1999;458(3):278-84.

35. Ajami K, Abbott CA, Obradovic M, Gysbers V, Kahne T, McCaughan GW, *et al*. Structural requirements for catalysis, expression, and dimerization in the CD26/DPIV gene family. Biochemistry. 2003;42(3):694-701.

Tabla. Aspectos relevantes de la expresión de la DPP-IV en varios tipos de cáncer en seres humanos

Tipo de cáncer	Expresión de DPP-IV	Relación con metástasis	Relación con desórdenes inmunológicos	Utilidad como marcador molecular	Referencias
Melanoma	Nula	No identificada	No identificada	No identificada	[58]
Carcinoma de células escamosas de pulmón	Alta	No identificada	No identificada	Nula	[59]
Carcinoma de células pequeñas de pulmón	Baja	No identificada	No identificada	No identificada	[60]
Carcinoma de células largas de pulmón	Baja	No identificada	No identificada	No identificada	[60]
Carcinoma de células no pequeñas de pulmón	Muy baja	Potencial	No identificada	No identificada	[61]
Adenocarcinoma pulmonar	Muy baja	Nula	No identificada	No identificada	[62]
Carcinoma primario de próstata	Muy alta	Nula	No identificada	Discriminar con respecto al tumor secundario	[63]
Carcinoma secundario de próstata	Muy baja	Alta	No identificada	Discriminar con respecto al tumor primario	[63]
Carcinoma papilar de tiroides	Muy alta	No identificada	No identificada	Discriminar entre tumor maligno y neoplasia benigna	[64-68]
Carcinoma folicular de tiroides	Muy alta	No identificada	No identificada	Discriminar entre tumor maligno y neoplasia benigna	[64-66, 68]
Adenocarcinoma de endometrio	Baja	No identificada	No identificada	Discriminar entre tumor maligno y neoplasia benigna	[69]
Carcinoma de ovario	Alta	Alta	No identificada	Potencial	[70]
Carcinoma de células basales dérmicas	Alta	No identificada	No identificada	No identificada	[71]
Adenocarcinoma esofágico	Alta	No identificada	No identificada	Potencial	[72]
Leucemia crónica de células B	Alta	No identificada	Alta	Potencial	[73, 74]
Linfoma T linfoblástico	Alta	No identificada	Alta	Potencial	[75]
Leucemias T linfoblásticas agudas	Alta	No identificada	Alta	Potencial	[75]
Linfoma de células B largas y anaplásticas	Alta	No identificada	Alta	Potencial	[75]
Glioma	Alta	Potencial	No identificada	Propuesta para discriminar el grado de progresión del tumor	[76]
Meningioma	Baja	No identificada	No identificada	Discriminar con respecto a los gliomas	[77]
Neuroblastoma	Baja	Potencial	No identificada	No identificada	[78]

contrario, la DPP-IV posee una débil actividad de unión de la FN soluble del plasma, lo cual sugiere que la FN polimerizada adquiere una conformación distinta a la FN plasmática [86].

Se ha confirmado la participación de la DPP-IV y la poliFN en la metástasis hacia pulmón, mediante varios hallazgos: 1) un polipéptido soluble que mimetiza la región extracelular de la DPP-IV, suprime la adhesión entre la DPP-IV y las células metastáticas provenientes del pecho al pulmón, lo que previene la colonización de este órgano; 2) la abundante presencia de la poliFN en las células de metástasis de pulmón provenientes de varios cánceres en seres humanos, rata y ratón, así como su abundancia en líneas de melanoma humano y de ratón, que colonizan el pulmón; 3) la expresión de poliFN en clones de rhabdomiosarcoma se correlacionan con la metástasis de pulmón [88]. Aún está por dilucidar si esta afección vascular en la metástasis de pulmón ocurre solo por la asociación la DPP-IV con la poliFN o si se debe a la interacción de este complejo con otras moléculas de adhesión, como por ejemplo: proteoglicanos, CD44 y sulfato de heparina [86] .

Experimentos de unión entre un péptido con igual secuencia que la repetición 14 de la FN-III (péptido FN-III14) y la DPP-IV nativa, han demostrado que la unión de ambas en la línea MTF-7, compite con la unión de la DPP-IV y la poliFN, lo que provoca efectos antimetastáticos en el pulmón, pues se ha constatado la inhibición del 50 % de la adhesión, la reducción de las colonias y la disminución de su tamaño. Este comportamiento es similar al obtenido con el bloqueo del dominio extracelular de la DPP-IV [86] por un anticuerpo específico. También se ha descrito que la presencia de otro péptido sintético que contiene la secuencia FN-III14 (péptido 22-mer), induce efectos antimetastáticos en la colonización del bazo y el hígado por linfoma de células T [89].

DPP-IV y melanoma

La DPP-IV se expresa *in vitro* e *in vivo* por los melanocitos, pero no por el melanoma. La pérdida de la expresión de la enzima, probablemente ocurre en una etapa temprana de la transformación del melanocito en melanoma. Wesley *et al.* [58] demostraron que la transfección de células del melanoma con DPP-IV impedía la tumorigenicidad y el crecimiento independiente de anclaje, para lo cual era necesaria la preservación de la actividad enzimática. Adicionalmente, la reexpresión conduce a la reaparición de la dependencia a factores exógenos de crecimiento [6, 58].

DPP- IV y cáncer de pulmón

En el desarrollo del tumor pulmonar intervienen factores de crecimiento como el neuropéptido Y (NPY) y

36. Chien CH, Huang LH, Chou CY, Chen YS, Han YS, Chang GG, *et al*. One site mutation disrupts dimer formation in human DPP-IV proteins. J Biol Chem. 2004; 279(50):52338-45.

37. Varghese JN, Laver WG, Colman PM. Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 A resolution. Nature. 1983;303(5912):35-40.

38. Vellieux FM, Huitema F, Groendijk H, Kalk KH, Jzn JF, Jongejan JA, *et al*. Structure of quinoprotein methylamine dehydrogenase at 2.25 A resolution. EMBO J. 1989;8(8):2171-8.

39. Ito N, Phillips SE, Stevens C, Ogel ZB, McPherson MJ, Keen JN, *et al*. Novel thioether bond revealed by a 1.7 A crystal structure of galactose oxidase. Nature. 1991;350(6313):87-90.

40. Xia ZX, Dai WW, Xiong JP, Hao ZP, Davidson VL, White S, *et al*. The three-dimensional structures of methanol dehydrogenase from two methylotrophic bacteria at 2.6-A resolution. J Biol Chem. 1992; 267(31):22289-97.

41. Murzin AG. Structural principles for the propeller assembly of beta-sheets: the preference for seven-fold symmetry. Proteins. 1992;14(2):191-201.

42. Fülöp V, Jones DT. Beta propellers: structural rigidity and functional diversity. Curr Opin Struct Biol. 1999;9(6):715-21.

la sustancia P: dos de los sustratos de DPP-IV. La escisión del NPY y su consecuente inactivación conducen a la inactivación de los efectos promotores del crecimiento [6, 90, 91]. Ello sugiere que la pérdida de la actividad proteolítica podría provocar el crecimiento de ciertas células tumorales de pulmón, pese a que se desconoce si la DPP-IV actúa mediante la regulación de otros procesos o vías de señalización independientes de su actividad, o si ocurre por mediación de otra molécula de superficie como PAF, cuyo incremento estromal se asocia con una mayor supervivencia [61].

Algunos carcinomas de pulmón normalmente expresan DPP-IV, aunque los de células largas, pequeñas-largas y pequeñas no lo hacen o lo hacen con una marcada reducción [60]. En el carcinoma de células no pequeñas, esta carencia ocurre a nivel de expresión (lo cual disminuye su actividad a menos de 40 pM/min/µg de proteína) como de niveles de ARNm [61], debido a que en esta enfermedad hay frecuentes pérdidas del cromosoma 2q, donde se encuentra el *loci* de la DPP-IV [92-94].

Wesley *et al.* [61] han probado que las líneas H28, H226, H441, SK-LUC-8, SK-LUC-17, SK-LUC-13, SK-LUC-9 y SW-900 en seres humanos, todas de cáncer de células no pequeñas, muestran una marcada disminución en la expresión de la DPP-IV. La restitución de la DPP-IV en la línea SK-LUC-8, particularmente atractiva por la indetectable expresión de la enzima, llevó a profundas reversiones del fenotipo maligno, independientes de la actividad enzimática: cambios morfológicos *in vitro* (células alargadas, ligeramente dendríticas, que adoptaron forma de epitelio cilíndrico o plano), inhibición del crecimiento en cultivo (con retraso de entrada a la fase logarítmica), inhibición del crecimiento independiente de anclaje (facilidad de formar colonias en agar suave, disminuida entre 50 y 70 %), disminución de la migración *in vitro*, y disminución de la confluencia lateral (probablemente por aparición de inhibición por densidad cuando la confluencia superó el 50 %). Ello también estuvo relacionado con el aumento en la expresión del gen p21, un drástico estancamiento del ciclo celular en G1 e inducción de apoptosis (posiblemente por inactivación de péptidos desconocidos). Al mismo tiempo se observó la expresión aumentada de CD44 y PAF, proteínas de superficie asociadas con la supresión del crecimiento y de la metástasis [95, 96]. La posterior implantación de estas células en ratones atímicos permitió corroborar la esperada inhibición del crecimiento tumoral en comparación con lo observado en el control, implantado con células de la misma línea que no habían sido transfectadas con la DPP-IV [61].

Recientemente se ha probado la escasa expresión de la DPP-IV en las líneas A549, de adenocarcinoma pulmonar, y SK-MES-1, de carcinoma de células escamosas; sin embargo, aparecen matices determinados por la historia del proceso de carcinogénesis. A549 exhibe una actividad total de la DPP-IV disminuida entre 8 y 10 veces, con una actividad superficial relativa del 93 %. Ello indica una rápida externalización de la proteína recién sintetizada (probablemente por la preservación de características secretoras inherentes al tipo celular que da origen a esta línea: los neumocitos alveolares tipo II). La actividad total de DPP-IV disminuye 7 veces frente a SK-MES-1, con un patrón granular de deposición intracelular y una actividad superficial relativa del 35 %, consistente con el escaso potencial secretor de la línea. Tales observaciones sugieren que la distribución de la enzima durante la carcinogénesis se correlaciona con alteraciones que puedan originarse en el sistema de tráfico membranoso intracelular [62].

DPP-IV y carcinoma de ovario

La presencia de la DPP-IV en carcinoma de ovario y su relación con la adhesión al mesotelio fue probada *in vitro* por Kikkawa *et al.* [70], al demostrar que células de la línea SKOV-3 con restitución de la DPP-IV se unían mucho más eficientemente a las células mesoteliales que aquellas en las que no hubo restitución de la proteína. También observó una marcada superioridad en la unión a fibronectina y a colágeno inmovilizados.

La unión *in vitro* de las células restituidas con la DPP-IV al mesotelio tiene un efecto dependiente de la dosis, con respecto a la fibronectina soluble. Ello sugiere que la DPP-IV es una proteína clave para la adhesión de la célula tumoral al mesotelio, la proliferación y la invasión. También el carcinoma de ovario y el mesotelio del peritoneo, por el que generalmente se expande, presentan expresión de la DPP-IV, y la ascitis y el suero maligno contienen elevadas cantidades de fibroconectina soluble y de fibroconectina de la matriz extracelular. Esto pudiera llevar a suponer que la DPP-IV capta grandes cantidades de fibroconectina procedente de estos líquidos. Como resultado, las células del carcinoma tendrían la propiedad de adherirse fácilmente al endotelio o al mesotelio, una vez que porten suficiente cantidad de fibroconectina para unirse a la DPP-IV expresada en la superficie de estos [70].

Se ha demostrado que ratones desnudos inoculados con células de la línea tumoral SKOV-3, donde la DPP-IV se ha restituido, presentan menos diseminación peritoneal y sobreviven más que aquellos cuyo tumor no presentaba la proteína [97]. Aunque la causas de ese fenómeno no se comprende del todo aún, pudiera especularse que elevados niveles de la DPP-IV provocan una fuerte adhesión célula-célula, de manera que las células del carcinoma tengan dificultad para desprenderse del tumor. Sin embargo, cuando estas logran separarse, la DPP-IV del tumor y del mesotelio facilita el ataque al peritoneo [70]. A pesar de que la actividad de la enzima no influye aparentemente en la adhesión celular, se puede predecir o por lo menos es probable, que la continua y elevada actividad podría influenciar indirectamente en tal evento.

DPP- IV y cáncer de próstata

La progresión del cáncer de próstata benigno a un estado fatal refractario hormonal se asocia a la sobrexpresión de péptidos factores de crecimiento que median señales alternativas mitogénicas [98-102]. En una situación normal, la DPP-IV interviene en la regulación del crecimiento y diferenciación mediante la regulación de la actividad de estos péptidos [103, 104] (por ejemplo: quimiosina estromal derivada del factor 1) [63].

Otras evidencias sugieren que la metástasis del cáncer de próstata está asociada con la pérdida de DPP-IV (más del 50 % de los casos) [63, 79] y con un incremento

43. Paoli M. Protein folds propelled by diversity. Prog Biophys Mol Biol. 2001;76(1-2): 103-30.

44. Jawad Z, Paoli M. Novel sequences propel familiar folds. Structure. 2002; 10(4):447-54.

45. Adams J, Kelso R, Cooley L. The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol. 2000; 10(1):17-24.

46. Russell RB, Sasieni PD, Sternberg MJ. Supersites within superfolds. Binding site similarity in the absence of homology. J Mol Biol. 1998;282(4):903-18.

47. Todd AE, Orengo CA, Thornton JM. Evolution of function in protein superfamilies, from a structural perspective. J Mol Biol. 2001;307(4):1113-43.

48. Pons T, Gomez R, Chinea G, Valencia A. Beta-propellers: associated functions and their role in human diseases. Curr Med Chem. 2003;10(6):505-24.

49. Love CA, Harlos K, Mavaddat N, Davis SJ, Stuart DI, Jones EY, *et al*. The ligand-binding face of the semaphorins revealed by the high-resolution crystal structure of SEMA4D. Nat Struct Biol. 2003;10(10):843-8.

50. Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL, *et al*. Crystal structure of the extracellular segment of integrin alpha Vbeta3. Science. 2001; 294(5541):339-45.

51. Hiramatsu H, Yamamoto A, Kyono K, Higashiyama Y, Fukushima C, Shima H, *et al*. The crystal structure of human dipeptidyl peptidase IV (DPPIV) complex with diprotin A. Biol Chem. 2004;385(6):561-4.

52. Oefner C, D'Arcy A, Mac Sweeney A, Pierau S, Gardiner R, Dale GE. High-resolution structure of human apo dipeptidyl peptidase IV/CD26 and its complex with 1-[([2-[(5-iodopyridin-2-yl)amino]-ethyl]amino)-acetyl]-2-cyano-(S)-pyrrolidine. Acta Crystallogr D Biol Crystallogr. 2003;59(Pt 7):1206-12.

53. Weihofen WA, Liu J, Reutter W, Saenger W, Fan H. Crystal structure of CD26/dipeptidyl-peptidase IV in complex with adenosine deaminase reveals a highly amphiphilic interface. J Biol Chem. 2004; 279(41):43330-5.

54. Abbott CA, Gorrell MD. The family of CD26/DPP-IV and related ectopeptidases. In: Langner J, Ansorge S, editors. Ectopeptidases. CD13/aminopeptidase N and CD26/dipeptidylpeptidase IV in medicine and biology. New York: Kluwer Academic / Plenum Publishers; 2002. p. 171-95.

55. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.

56. Carl-McGrath S, Lendeckel U, Ebert M, Rocken C. Ectopeptidases in tumour biology: a review. Histol Histopathol. 2006; 21(12):1339-53.

57. Iwata S, Morimoto C. CD26/dipeptidyl peptidase IV in context. The different roles of a multifunctional ectoenzyme in malignant transformation. J Exp Med. 1999; 190(3):301-6.

58. Wesley UV, Albino AP, Tiwari S, Houghton AN. A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. J Exp Med. 1999; 190(3):311-22.

en la actividad del factor básico de crecimiento fibroblástico (bFGF), un poderoso agente mitógeno y angiogénico [98-102, 105], que se expresa en una isoforma citoplasmática de bajo peso molecular y en otra nuclear de peso elevado. La acción del bFGF transcurre mediante la activación de la cascada proteína quinasa activada por mitógeno (MAPK)-quinasa regulada por señal extracelular (ERK1/2), vía que promueve la progresión y migración del cáncer [106, 107]. Además, durante la migración celular y la angiogénesis, la activación de ERK1/2 por bFGF incrementa la producción del activador de plasminógeno tipo uroquinasa (uPA): proteasa de tipo serino que cataliza la conversión del plasminógeno en plasmina, y que a su vez promueve la metástasis por medio de la destrucción de la matriz extracelular [108, 109].

Se ha probado que la restitución de la DPP-IV en células de la línea DU-145 bloquea la localización nuclear de bFGF y los niveles de expresión de la isoforma ligera y de la pesada, y elimina, por tanto, el estímulo para los mecanismos MAP-ERK1/2. Esto acarrea una disminución de los niveles de ARNm de uPA, así como la adopción de formas cúbicas o planas, la recuperación de la inhibición por contacto, la pérdida del crecimiento independiente de anclaje y la inhibición de la proliferación y la migración del tumor [80].

Aunque el mecanismo por el que la DPP-IV afecta la producción de bFGF no se conoce, se especula que en una célula normal, la DPP-IV escinde la región NH_2 terminal, y provoca el confinamiento del bFGF en el núcleo. Esta escisión pudiera ser el primer paso para su degradación [80]. De igual manera podría ocurrir una asociación directa con bFGF que interfiriera con modificaciones postranscripcionales como la metilación del NH_2 terminal, necesaria para el confinamiento en el núcleo [110-112].

La reexpresión de la DPP-IV en DU-145 también estimula la transcripción del gen P27, inhibidor de quinasas dependientes de ciclina, que detiene el ciclo celular en la transición G2-M y aumenta la apoptosis del 24 al 34 % de las células [61].

DPP- IV y la glándula tiroidea: neoplasias, carcinomas papilares y carcinomas foliculares

La DPP-IV es fuertemente positiva en los carcinomas folicular y papilar del tiroides, en contraste con las neoplasias benignas, que son marcadamente negativas [64, 67]. Incluso, en la medida que un adenoma benigno comienza a malignizar, se observa un aumento de la expresión de la DPP-IV, como lo demuestra la mayor expresión del adenoma folicular con la invasión capsular incompleta, con respecto al adenoma folicular sin invasión capsular [65]. La diferencia de expresión de la enzima entre ambos estadios es tal, que actualmente se considera que los niveles de la DPP-IV son el medio más eficaz para discriminar entre el carcinoma folicular y el adenoma folicular. Incluso, con prioridad por encima de variables canónicas como el tamaño de la lesión, la edad del paciente, la apariencia de la lesión al ultrasonido y los niveles séricos de tiroglobulina [68].

DPP- IV y tumoraciones de queratinocitos

Experimentos en ratones transgénicos InvEE, caracterizados por la presencia de queratinocitos con activación constitutiva de MAP quinasa 1 (MEK-1), evidenciaron la sobrerregulación de la DPP-IV en queratinocitos de epitelios tumorales, fenómeno particularmente notorio hacia los bordes de contacto intercelular. Aunque los fibroblastos dérmicos asociados con el daño también mostraron sobrerregulación de la DPP-IV, en el estroma tumoral se observó una infrarregulación. Se constató que la actividad de la DPP-IV estuvo estimulada por la adhesión intercelular inducida por Ca^{2+}, y que la administración de interleuquina 1α (IL-1α) a fibroblastos dérmicos contribuyó a la sobrerregulación de la DPP-IV. La inhibición de la DPP-IV redujo el crecimiento tumoral; y además, el bloqueo de la actividad de la IL-1α condujo a la disminución de la incidencia tumoral o al retraso en la aparición del tumor en individuos sanos [113].

DPP- IV y el tejido neural: gliomas, meningiomas y neuroblastomas

Los tejidos cerebrales humanos normales contienen una actividad tipo DPP-IV que es atribuida fundamentalmente a la acción de DPP8 y DPP9. En cambio, el grado del glioma, tipo tumoral al que pertenecen más del 50 % de los tumores del sistema nervioso central, se ha correlacionado con la marcada disminución de las actividades de DP8 y DP9 y con el simultáneo y drástico aumento de las expresiones de DPP-IV y PAF, localizadas hacia el parénquima y las zonas vascularizadas. Paralelamente, se ha observado un aumento en la expresión de CXCR4, receptor del factor derivado del estroma (SDF-1α) [76]. SDF-1α, uno de los sustratos endógenos de la DPP-IV, en su forma activa constituye la principal quimiosina que media la supervivencia del glioma [114]. Después de ser truncado por la acción de la DPP-IV, pierde sus propiedades quimotácticas e, incluso, puede actuar como antagonista del CXCR4 [115] . La marcada sobrerregulación de CXCR4 observada en el glioma, pareciera actuar como compensación a la sobrexpresión de la DPP-IV, lo cual sugiere la posibilidad de una regulación simultánea de ambas moléculas [76].

Se ha encontrado que los meningiomas WHO tipo I y los meningiomas WHO atípicos tipo II expresan la DPP-IV a muy bajos niveles, en detrimento de las actividades aumentadas de DPP8 y DPP9. La diferencia de expresión de DPP-IV entre meningiomas y gliomas pudiera deberse al origen embriológico y, paradójicamente, pudiera ser una de las causas de la menor agresividad de los meningiomas en comparación con los gliomas. En consonancia con la expresión disminuida de DPP-IV, el meningioma expresa cantidades normales de CXCR4 [77]; de modo que al no existir suficiente actividad de la DPP-IV, no parece activarse el "efecto de compensación" , que sí ocurre en la vía de recepción del SDF-1α en el glioma.

Varias líneas derivadas de neuroblastomas humanos muestran una expresión de la DPP-IV notoriamente deprimida. *In vitro*, la reexpresión de la enzima conduce a la pérdida del fenotipo maligno: adquisición de morfología neuronal o de epitelio plano; inhibición de la proliferación; inducción de apoptosis por activación de caspasas; disminución de la fosforilación de Akt y de la actividad de la metaloproteinasa de matriz MMP9, conocidos efectores de la vía activada por la interacción de SDF-1α con CXCR4 y la disminución de la migración celular. La inhibición de la proliferación

59. Sedo A, Krepela E, Kasafirek E. Dipeptidyl peptidase IV, prolyl endopeptidase and cathepsin B activities in primary human lung tumors and lung parenchyma. J Cancer Res Clin Oncol. 1991;117(3):249-53.

60. Asada Y, Aratake Y, Kotani T, Marutsuka K, Araki Y, Ohtaki S, *et al*. Expression of dipeptidyl aminopeptidase IV activity in human lung carcinoma. Histopathology. 1993;23(3):265-70.

61. Wesley UV, Tiwari S, Houghton AN. Role for dipeptidyl peptidase IV in tumor suppression of human non small cell lung carcinoma cells. Int J Cancer. 2004; 109(6):855-66.

62. Dimitrova M, Ivanov I, Todorova R, Stefanova N, Moskova-Doumanova V, Topouzova-Hristova T, *et al*. Comparison of the activity levels and localization of dipeptidyl peptidase IV in normal and tumor human lung cells. Tissue Cell. 2012;44(2):74-9.

63. Bogenrieder T, Finstad CL, Freeman RH, Papandreou CN, Scher HI, Albino AP, *et al*. Expression and localization of aminopeptidase A, aminopeptidase N, and dipeptidyl peptidase IV in benign and malignant human prostate tissue. Prostate. 1997;33(4):225-32.

64. Frohlich E, Maier E, Wahl R. Interspecies differences in membrane-associated protease activities of thyrocytes and their relevance for thyroid cancer studies. J Exp Clin Cancer Res. 2012;31:45.

65. Kotani T, Asada Y, Aratake Y, Umeki K, Yamamoto I, Tokudome R, *et al*. Diagnostic usefulness of dipeptidyl aminopeptidase IV monoclonal antibody in paraffin-embedded thyroid follicular tumours. J Pathol. 1992;168(1):41-5.

66. Tanaka T, Umeki K, Yamamoto I, Sakamoto F, Noguchi S, Ohtaki S. CD26 (dipeptidyl peptidase IV/DPP IV) as a novel molecular marker for differentiated thyroid carcinoma. Int J Cancer. 1995; 64(5):326-31.

67. Tang AC, Raphael SJ, Lampe HB, Matthews TW, Becks GP. Expression of dipeptidyl aminopeptidase IV activity in thyroid tumours: a possible marker of thyroid malignancy. J Otolaryngol. 1996; 25(1):14-9.

68. Maruta J, Hashimoto H, Yamashita H, Yamashita H, Noguchi S. Diagnostic applicability of dipeptidyl aminopeptidase IV activity in cytological samples for differentiating follicular thyroid carcinoma from follicular adenoma. Arch Surg. 2004; 139(1):83-8.

69. Khin EE, Kikkawa F, Ino K, Kajiyama H, Suzuki T, Shibata K, *et al*. Dipeptidyl peptidase IV expression in endometrial endometrioid adenocarcinoma and its inverse correlation with tumor grade. Am J Obstet Gynecol. 2003;188(3):670-6.

70. Kikkawa F, Kajiyama H, Ino K, Shibata K, Mizutani S. Increased adhesion potency of ovarian carcinoma cells to mesothelial cells by overexpression of dipeptidyl peptidase IV. Int J Cancer. 2003;105(6):779-83.

71. Pro B, Dang NH. CD26/dipeptidyl peptidase IV and its role in cancer. Histol Histopathol. 2004;19(4):1345-51.

72. Goscinski MA, Suo ZH, Nesland JM, Florenes VA, Gierckskiy KE. Dipeptidyl peptidase IV expression in cancer and stromal cells of human esophageal squamous cell carcinomas, adenocarcinomas and squamous cell carcinoma cell lines. APMIS. 2008;116(9):823-31.

parece deberse a la inducción de diferenciación, evidenciada en los cambios morfológicos. La pérdida de la actividad migratoria parece deberse a la contribución simultánea de la restitución morfológica y la infrarregulación de MMP9, factor proangiogénico con actividad gelatinasa sobre la matriz extracelular [78].

DPP- IV, GLP-1 y cáncer

La presencia del receptor de GLP-1 se ha demostrado en la línea Hs-7766T de carcinoma pancreático humano y en las líneas CAPAN-1, CFPAC-1 y PL45, todas de adenocarcinoma ductal pancreático humano. Sin embargo, existen diferencias en cuanto a si la estimulación del receptor provoca la activación de ERK1/2 o la inducción de AMPc [114]. Dado que las vías asociadas a ERK1/2, y en menor medida, las asociadas al AMPc, cobran relevancia en eventos como la mitosis, la meiosis, y la carcionogénesis, los péptidos miméticos del GLP-1 o los inhibidores de DPP-IV podrían tener efectos oncogénicos [116]. Basados en la no detección de receptores del GLP-1 en 21 adenocarcinomas pancreáticos humanos, Korner *et al*. [117] sugieren que la expresión de GLP-1 está restringida a determinadas líneas celulares, por lo que en seres humanos podría no ser relevante *in vivo*. Adicionalmente, la exantina, un análogo del GLP-1, no moduló el crecimiento de líneas cancerígenas pancreáticas que expresaban el receptor, ni las rescató de la muerte inducida por drogas. La activación sostenida del receptor por medio de la exantina tampoco estimuló el crecimiento ni la progresión tumoral en ratas [118]. Estas observaciones sugieren que la hipotética aparición de cáncer pancreático a causa de la administración de inhibidores de DPP-IV o de miméticos del GLP-1, puede deberse a la aparición colateral de pancreatitis [116], la cual es favorecida por factores como la obesidad y la diabetes tipo II [119]. En efecto, la incidencia de pancreatitis en pacientes tratados con agonistas del receptor GLP-1 no difiere con la población aquejada de diabetes tipo II [120, 121]. Igualmente, estudios en ratas y monos indicaron que la inducción de pancreatitis por estimulación del receptor de GLP-1 parece muy poco probable [122].

Estudios preclínicos han mostrado un incremento de tumores de células C del tiroides en roedores tratados con análogos del GLP-1. Sin embargo, la expresión del receptor al GLP-1 en células C del tiroides depende mucho de la especie, por lo cual las observaciones en roedores no presentan la misma relevancia en seres humanos [117], al ser su expresión en esos modelos 22 veces superior [123, 124]. Además, se ha demostrado que las ratas son más propensas a padecer neoplasias de células C que son muy raras en seres humanos [125].

Tanto en páncreas como en tiroides, por el corto tiempo de los estudios y las evidencias, sería más probable que el aumento de las concentraciones de GLP-1 se deba más al estímulo de lesiones premalignas que a la inducción de nuevas lesiones [117]. En contraste, se cree que la activación del receptor del GLP-1 puede inhibir el crecimiento tumoral en dos tipos de cáncer muy comunes: colon y mama [117].

La línea murina de cáncer de colon CT26 expresa un receptor de GLP-1 funcional. Su exposición *in vitro* a la exenatida induce cambios morfológicos, inhibe la proliferación, y la formación de colonias en agar sólido, e induce apoptosis. Estos efectos se han confirmado *in vivo* mediante la inducción de apoptosis en CT26 implantadas a ratones, si bien no afectó el peso del tumor [126].

Ligumsky *et al.,* [127] probaron *in vitro* que la exenatida redujo significativamente el número de colonias formadas por las líneas de cáncer de mama MCF-7 (positiva para receptores de estrógeno) y MDA-MB-231 (negativa para receptores de estrógeno). La línea HB-2 no cancerosa permaneció sin afectación. Después de injertar células MDA-MB-231 en ratones, se observó una reducción tumoral dosis dependiente de la exenantida inoculado por vía intraperitoneal [128].

Conclusiones

Actualmente la enzima DPP-IV atrae la atención de la comunidad científica internacional, por las peculiaridades de su estructura tridimensional compleja. Esta determina las propiedades moleculares y funcionales de la DPP-IV, así como su rol en procesos fisiológicos y patológicos, por su actividad enzimática o su interacción con otras proteínas. La DPP-IV está implicada no solo en el mantenimiento de la homeostasis de los mamíferos, sino que el desbalance de su actividad o su expresión se encuentran asociados a los mecanismos moleculares de múltiples enfermedades, en especial el cáncer, y desórdenes inmunológicos. Ello la convierte en un blanco muy atractivo y centro de análisis para una mejor comprensión de la enfermedad y para el diseño de terapias novedosas y efectivas.

Agradecimientos

Al Proyecto 3276/3 de la Fundación Internacional para la Ciencia (IFS), junto con la Organización para la Prohibición de las Armas Químicas , por el apoyo financiero.

73. Bauvois B, De Meester I, Dumont J, Rouillard D, Zhao HX, Bosmans E. Constitutive expression of CD26/dipeptidylpeptidase IV on peripheral blood B lymphocytes of patients with B chronic lymphocytic leukaemia. Br J Cancer. 1999;79(7-8):1042-8.

74. Cro L, Morabito F, Zucal N, Fabris S, Lionetti M, Cutrona G, *et al*. CD26 expression in mature B-cell neoplasia: its possible role as a new prognostic marker in B-CLL. Hematol Oncol. 2009;27(3):140-7.

75. Havre PA, Dang LH, Ohnuma K, Iwata S, Morimoto C, Dang NH. CD26 expression on T-anaplastic large cell lymphoma (ALCL) line Karpas 299 is associated with increased expression of versican and MT1-MMP and enhanced adhesion. BMC cancer. 2013;13:517.

76. Stremenova J, Krepela E, Mares V, Trim J, Dbaly V, Marek J, *et al*. Expression and enzymatic activity of dipeptidyl peptidase-IV in human astrocytic tumours are associated with tumour grade. Int J Oncol. 2007;31(4):785-92.

77. Stremenova J, Mares V, Lisa V, Hilser M, Krepela E, Vanickova Z, *et al*. Expression of dipeptidyl peptidase-IV activity and/or structure homologs in human meningiomas. Int J Oncol. 2010;36(2):351-8.

78. Arscott WT, LaBauve AE, May V, Wesley UV. Suppression of neuroblastoma growth by dipeptidyl peptidase IV: relevance of chemokine regulation and caspase activation. Oncogene. 2009;28(4):479-91.

79. Dinjens WN, Ten Kate J, Kirch JA, Tanke HJ, Van der Linden EP, Van den Ingh HF, *et al*. Adenosine deaminase complexing protein (ADCP) expression and metastatic potential in prostatic adenocarcinomas. J Pathol. 1990; 160(3):195-201.

80. Wesley UV, McGroarty M, Homoyouni A. Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. Cancer Res. 2005;65(4):1325-34.

81. Urade M, Komatsu M, Yamaoka M, Fukasawa K, Harada M, Mima T, *et al*. Serum dipeptidyl peptidase activities as a possible marker of oral cancer. Cancer. 1989;64(6):1274-80.

82. de la Haba-Rodriguez J, Macho A, Calzado MA, Blazquez MV, Gomez MA, Munoz EE, *et al*. Soluble dipeptidyl peptidase IV (CD-26) in serum of patients with colorectal carcinoma. Neoplasma. 2002;49(5):307-11.

83. Cordero OJ, Imbernon M, Chiara LD, Martinez-Zorzano VS, Ayude D, de la Cadena MP, et al. Potential of soluble CD26 as a serum marker for colorectal cancer detection. World J Clin Oncol. 2011;2(6):245-61.

84. Gonzalez-Gronow M, Misra UK, Gawdi G, Pizzo SV. Association of plasminogen with dipeptidyl peptidase IV and Na+/H+ exchanger isoform NHE3 regulates invasion of human 1-LN prostate tumor cells. J Biol Chem. 2005;280(29):27173-8.

85. Gorrell MD, Gysbers V, McCaughan GW. CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. Scand J Immunol. 2001;54(3):249-64.

86. Cheng HC, Abdel-Ghany M, Pauli BU. A novel consensus motif in fibronectin mediates dipeptidyl peptidase IV adhesion and metastasis. J Biol Chem. 2003;278(27):24600-7.

87. Cheng HC, Abdel-Ghany M, Elble RC, Pauli BU. Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin. J Biol Chem. 1998;273(37):24207-15.

88. Korach S, Poupon MF, Du Villard JA, Becker M. Differential adhesiveness of rhabdomyosarcoma-derived cloned metastatic cell lines to vascular endothelial monolayers. Cancer Res. 1986;46(7):3624-9.

89. Kato Y, Saijo N. Developed new agents for lung cancer. Nihon Geka Gakkai zasshi. 2002;103(2):218-23.

90. Mentlein R, Dahms P, Grandt D, Kruger R. Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul Pept. 1993;49(2):133-44.

91. Ghersi G, Chen W, Lee EW, Zukowska Z. Critical role of dipeptidyl peptidase IV in neuropeptide Y-mediated endothelial cell migration in response to wounding. Peptides. 2001;22(3):453-8.

92. Mathew S, Morrison ME, Murty VV, Houghton AN, Chaganti RS. Assignment of the DPP4 gene encoding adenosine deaminase binding protein (CD26/dipeptidylpeptidase IV) to 2q23. Genomics. 1994;22(1):211-2.

93. Otsuka T, Kohno T, Mori M, Noguchi M, Hirohashi S, Yokota J. Deletion mapping of chromosome 2 in human lung carcinoma. Genes Chromosomes Cancer. 1996;16(2):113-9.

94. Shiseki M, Kohno T, Nishikawa R, Sameshima Y, Mizoguchi H, Yokota J. Frequent allelic losses on chromosomes 2q, 18q, and 22q in advanced non-small cell lung carcinoma. Cancer Res. 1994;54(21):5643-8.

95. Morrison H, Sherman LS, Legg J, Banine F, Isacke C, Haipek CA, *et al*. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. Genes Dev. 2001;15(8):968-80.

96. Yan P, Muhlethaler A, Bourloud KB, Beck MN, Gross N. Hypermethylation-mediated regulation of CD44 gene expression in human neuroblastoma. Genes Chromosomes Cancer. 2003;36(2):129-38.

97. Kajiyama H, Kikkawa F, Maeda O, Suzuki T, Ino K, Mizutani S. Increased expression of dipeptidyl peptidase IV in human mesothelial cells by malignant ascites from ovarian carcinoma patients. Oncology. 2002;63(2):158-65.

98. Ware JL. Growth factor network disruption in prostate cancer progression. Cancer Metastasis Rev. 1998;17(4):443-7.

99. Giri D, Ropiquet F, Ittmann M. Alterations in expression of basic fibroblast growth factor (FGF) 2 and its receptor FGFR-1 in human prostate cancer. Clin Cancer Res. 1999;5(5): 1063-71.

100. Dow JK, deVere White RW. Fibroblast growth factor 2: its structure and property, paracrine function, tumor angiogenesis, and prostate-related mitogenic and oncogenic functions. Urology. 2000;55(6):800-6.

101. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1(1):34-45.

102. Isaacs JT, Isaacs WB. Androgen receptor outwits prostate cancer drugs. Nat Med. 2004; 10(1):26-7.

103. Boonacker E, Van Noorden CJ. The multifunctional or moonlighting protein CD26/DPPIV. Eur J Cell Biol. 2003;82(2):53-73.

104. Proost P, Struyf S, Schols D, Opdenakker G, Sozzani S, Allavena P, *et al*. Truncation of macrophage-derived chemokine by CD26/dipeptidyl-peptidase IV beyond its predicted cleavage site affects chemotactic activity and CC chemokine receptor 4 interaction. J Biol Chem. 1999;274(7):3988-93.

105. Nakamoto T, Chang CS, Li AK, Chodak GW. Basic fibroblast growth factor in human prostate cancer cells. Cancer Res. 1992; 52(3):571-7.

106. Gioeli D, Mandell JW, Petroni GR, Frierson HF, Jr., Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res. 1999;59(2):279-84.

107. Pintucci G, Moscatelli D, Saponara F, Biernacki PR, Baumann FG, Bizekis C, *et al*. Lack of ERK activation and cell migration in FGF-2-deficient endothelial cells. FASEB J. 2002;16(6):598-600.

108. Giuliani R, Bastaki M, Coltrini D, Presta M. Role of endothelial cell extracellular signal-regulated kinase1/2 in urokinase-type plasminogen activator upregulation and in vitro angiogenesis by fibroblast growth factor-2. J Cell Sci. 1999;112 (Pt 15):2597-606.

109. Rabbani SA, Mazar AP. The role of the plasminogen activation system in angiogenesis and metastasis. Surg Oncol Clin N Am. 2001;10(2):393-415.

110. Bugler B, Amalric F, Prats H. Alternative initiation of translation determines cytoplasmic or nuclear localization of basic fibroblast growth factor. Mol Cell Biol. 1991;11(1):573-7.

111. Bikfalvi A, Klein S, Pintucci G, Rifkin DB. Biological roles of fibroblast growth factor-2. Endocr Rev. 1997;18(1):26-45.

112. Delrieu I. The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism. FEBS Lett. 2000;468(1):6-10.

113. Arwert EN, Mentink RA, Driskell RR, Hoste E, Goldie SJ, Quist S, et al. Upregulation of CD26 expression in epithelial cells and stromal cells during wound-induced skin tumour formation. Oncogene. 2012;31(8):992-1000.

114. Ehtesham M, Winston JA, Kabos P, Thompson RC. CXCR4 expression mediates glioma cell invasiveness. Oncogene. 2006;25(19): 2801-6.

115. Christopherson KW, 2nd, Hangoc G, Broxmeyer HE. Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. J Immunol. 2002;169(12):7000-8.

116. Vangoitsenhoven R, Mathieu C, Van der Schueren B. GLP1 and cancer: friend or foe? Endocr Relat Cancer. 2012;19(5):F77-88.

117. Korner M, Stockli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. J Nucl Med. 2007;48(5):736-43.

118. Koehler JA, Drucker DJ. Activation of glucagon-like peptide-1 receptor signaling does not modify the growth or apoptosis of human pancreatic cancer cells. Diabetes. 2006; 55(5):1369-79.

119. Girman CJ, Kou TD, Cai B, Alexander CM, O'Neill EA, Williams-Herman DE, *et al*. Patients with type 2 diabetes mellitus have higher risk for acute pancreatitis compared with those without diabetes. Diabetes Obes Metab. 2010;12(9):766-71.

120. Garg R, Chen W, Pendergrass M. Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. Diabetes Care. 2010;33(11):2349-54.

121. Dore DD, Bloomgren GL, Wenten M, Hoffman C, Clifford CR, Quinn SG, *et al*. A cohort study of acute pancreatitis in relation to exenatide use. Diabetes Obes Metab. 2011; 13(6):559-66.

122. Nyborg NC, Molck AM, Madsen LW, Knudsen LB. The human GLP-1 analog liraglutide and the pancreas: evidence for the absence of structural pancreatic changes in three species. Diabetes. 2012;61(5):1243-9.

123. Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, *et al*. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology. 2010;151(4):1473-86.

124. Waser B, Beetschen K, Pellegata NS, Reubi JC. Incretin receptors in non-neoplastic and neoplastic thyroid C cells in rodents and humans: relevance for incretin-based diabetes therapy. Neuroendocrinology. 2011; 94(4):291-301.

125. Roman S, Lin R, Sosa JA. Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases. Cancer. 2006;107(9):2134-42.

126. Koehler JA, Kain T, Drucker DJ. Glucagon-like peptide-1 receptor activation inhibits growth and augments apoptosis in murine CT26 colon cancer cells. Endocrinology. 2011; 152(9):3362-72.

127. Ligumsky H, Wolf I, Israeli S, Haimsohn M, Ferber S, Karasik A, *et al*. The peptide-hormone glucagon-like peptide-1 activates cAMP and inhibits growth of breast cancer cells. Breast Cancer Res Treat. 2012;132(2):449-61.

128. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science. 1995;270(5240):1326-31.

Recibido en septiembre de 2013.
Aprobado en abril de 2014.

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

Biotecnología Aplicada 2014;31:102-110

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

1. Turk B. Targeting proteases: successes, failures and future prospects. Nat Rev Drug Discov. 2006;5(9):785-99.

2. Leung D, Abbenante G, Fairlie DP. Protease inhibitors: current status and future prospects. J Med Chem. 2000;43(3):305-41.

3. Abbenante G, Fairlie DP. Protease inhibitors in the clinic. Med Chem. 2005;1(1):71-104.

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

4. Itou M, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. World J Gastroenterol. 2013;19(15):2298-306.

5. Gorrell MD, Wang XM, Park J, Ajami K, Yu DM, Knott H, *et al*. Structure and function in dipeptidyl peptidase IV and related proteins. Adv Exp Med Biol. 2006; 575:45-54.

6. Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, *et al*. The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J. 2010;277(5):1126-44.

7. Leiting B, Pryor KD, Wu JK, Marsilio F, Patel RA, Craik CS, *et al*. Catalytic properties and inhibition of proline-specific dipeptidyl peptidases II, IV and VII. Biochem J. 2003;371(Pt 2):525-32.

8. Hopsu-Havu VK, Glenner GG. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide. Histochemie. 1966;7(3):197-201.

9. Palmieri FE, Ward PE. Dipeptidyl(amino) peptidase IV and post proline cleaving enzyme in cultured endothelial and smooth muscle cells. Adv Exp Med Biol. 1989; 247A:305-11.

10. Hu CX, Huang H, Zhang L, Huang Y, Shen ZF, Cheng KD, *et al*. A new screening method based on yeast-expressed human dipeptidyl peptidase IV and discovery of novel inhibitors. Biotechnol Lett. 2009;31(7):979-84.

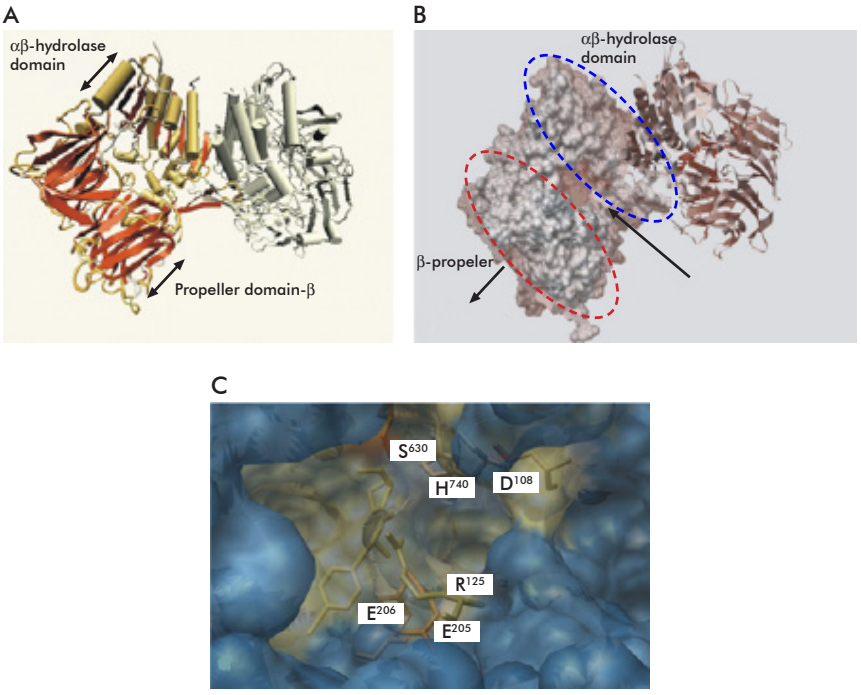

Figure 1. Structural elements of the porcine DPP-IV enzyme. A) Porcine DPP-IV structure (PDB: 1 orv). Each monomer is composed of one αβ-hydrolase and one β-propeller domain. The image was elaborated by using the Visual Molecular Dynamics (VMD; http://www.ks.uiuc.edu/Research/vmd/). B) Access to the 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

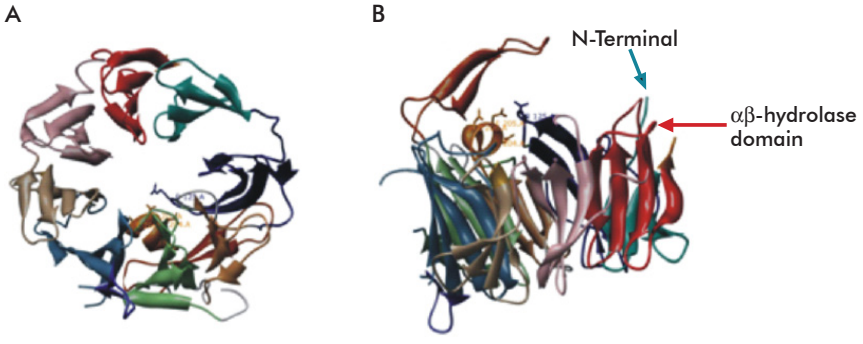

Figure 2. β-propeller domain of porcine DPP-IV (PDB: 1orv). A) Front view of the β-propeller domain, showing the displacement of β-sheets around a central tunnel. B) Side view. The horizontal arrow indicates the antiparalel β-sheet at the dimerization interphase of DPP-IV. Side chains of residues E^{205},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

11. Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, *et al*. Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. Eur J Biochem. 2000;267(17):5608-13.

12. Stano J, Kovacs P, Kakoniova D, Kirilova ND, Komov VP. Activity of dipeptidyl peptidase IV in gingseng callus culture. Biologia. 1994;49:353-7.

13. Koreeda Y, Hayakawa M, Ikemi T, Abiko Y. Isolation and characterisation of dipeptidyl peptidase IV from Prevotella loescheii ATCC 15930. Arch Oral Biol. 2001;46(8):759-66.

14. Davy A, Thomsen KK, Juliano MA, Alves LC, Svendsen I, Simpson DJ. Purification and characterization of barley dipeptidyl peptidase IV. Plant Physiol. 2000;122(2):425-32.

15. Bauvois B, Djavaheri-Mergny M, Rouillard D, Dumont J, Wietzerbin J. Regulation of CD26/DPPIV gene expression by interferons and retinoic acid in tumor B cells. Oncogene. 2000;19(2):265-72.

16. de Meester I, Vanhoof G, Lambeir AM, Scharpe S. Use of immobilized adenosine deaminase (EC 3.5.4.4) for the rapid purification of native human CD26/dipeptidyl peptidase IV (EC 3.4.14.5). J Immunol Methods. 1996;189(1):99-105.

17. Wilson MJ, Ruhland AR, Pryor JL, Ercole C, Sinha AA, Hensleigh H, *et al*. Prostate specific origin of dipeptidylpeptidase IV (CD-26) in human seminal plasma. J Urol. 1998;160(5):1905-9.

18. Engel M, Hoffmann T, Wagner L, Wermann M, Heiser U, Kiefersauer R, *et al*. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proc Natl Acad Sci USA. 2003;100(9):5063-8.

19. Abbott CA, Baker E, Sutherland GR, McCaughan GW. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. Immunogenetics. 1994;40(5):331-8.

20. Bohm SK, Gum JR, Jr., Erickson RH, Hicks JW, Kim YS. Human dipeptidyl peptidase IV gene promoter: tissue-specific regulation from a TATA-less GC-rich sequence characteristic of a housekeeping gene promoter. Biochem J. 1995;311(Pt 3):835-43.

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

21. Hong WJ, Petell JK, Swank D, Sanford J, Hixson DC, Doyle D. Expression of dipeptidyl peptidase IV in rat tissues is mainly regulated at the mRNA levels. Exp Cell Res. 1989;182(1):256-66.

22. Kahne T, Kroning H, Thiel U, Ulmer AJ, Flad HD, Ansorge S. Alterations in structure and cellular localization of molecular forms of DP IV/CD26 during T cell activation. Cell Immunol. 1996;170(1):63-70.

23. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond). 2005;108(4): 277-92.

24. Yu DM, Ajami K, Gall MG, Park J, Lee CS, Evans KA, *et al*. The *in vivo* expression of dipeptidyl peptidases 8 and 9. J Histochem Cytochem. 2009;57(11):1025-40.

25. Lee KN, Jackson KW, Christiansen VJ, Chung KH, McKee PA. A novel plasma proteinase potentiates alpha2-antiplasmin inhibition of fibrin digestion. Blood. 2004;103(10):3783-8.

26. Ajami K, Abbott CA, McCaughan GW, Gorrell MD. Dipeptidyl peptidase 9 has two forms, a broad tissue distribution, cytoplasmic localization and DPIV-like peptidase activity. Biochim Biophys Acta. 2004;1679(1):18-28.

27. Delacour D, Gouyer V, Leteurtre E, Ait-Slimane T, Drobecq H, Lenoir C, *et al*. 1-benzyl-2-acetamido-2-deoxy-alpha-D-galactopyranoside blocks the apical biosynthetic pathway in polarized HT-29 cells. J Biol Chem. 2003;278(39):37799-809.

28. Rasmussen HB, Branner S, Wiberg FC, Wagtmann N. Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. Nat Struct Biol. 2003;10(1):19-25.

29. Duke-Cohan JS, Morimoto C, Rocker JA, Schlossman SF. Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. J Immunol. 1996;156(5):1714-21.

30. Pascual I, Gomez H, Pons T, Chappe M, Vargas MA, Valdes G, *et al*. Effect of divalent cations on the porcine kidney cortex membrane-bound form of dipeptidyl peptidase IV. Int J Biochem Cell Biol. 2011;43(3):363-71.

31. Gomez H, Chappe M, Valiente PA, Pons T, Chavez Mde L, Charli JL, *et al*. Effect of zinc and calcium ions on the rat kidney membrane-bound form of dipeptidyl peptidase IV. J Biosci. 2013;38(3):461-9.

32. Thoma R, Loffler B, Stihle M, Huber W, Ruf A, Hennig M. Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. Structure. 2003;11(8):947-59.

33. Aertgeerts K, Ye S, Tennant MG, Kraus ML, Rogers J, Sang BC, *et al*. Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. Protein Sci. 2004;13(2):412-21.

34. Abbott CA, McCaughan GW, Gorrell MD. Two highly conserved glutamic acid residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. FEBS Lett. 1999;458(3):278-84.

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

35. Ajami K, Abbott CA, Obradovic M, Gysbers V, Kahne T, McCaughan GW, *et al*. Structural requirements for catalysis, expression, and dimerization in the CD26/DPIV gene family. Biochemistry. 2003; 42(3):694-701.

36. Chien CH, Huang LH, Chou CY, Chen YS, Han YS, Chang GG, *et al*. One site mutation disrupts dimer formation in human DPP-IV proteins. J Biol Chem. 2004; 279(50):52338-45.

37. Varghese JN, Laver WG, Colman PM. Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 A resolution. Nature. 1983;303(5912):35-40.

38. Vellieux FM, Huitema F, Groendijk H, Kalk KH, Jzn JF, Jongejan JA, *et al*. Structure of quinoprotein methylamine dehydrogenase at 2.25 A resolution. EMBO J. 1989;8(8):2171-8.

39. Ito N, Phillips SE, Stevens C, Ogel ZB, McPherson MJ, Keen JN, *et al*. Novel thioether bond revealed by a 1.7 A crystal structure of galactose oxidase. Nature. 1991;350(6313):87-90.

40. Xia ZX, Dai WW, Xiong JP, Hao ZP, Davidson VL, White S, *et al*. The three-dimensional structures of methanol dehydrogenase from two methylotrophic bacteria at 2.6-A resolution. J Biol Chem. 1992; 267(31):22289-97.

41. Murzin AG. Structural principles for the propeller assembly of beta-sheets: the preference for seven-fold symmetry. Proteins. 1992;14(2):191-201.

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

42. Fülöp V, Jones DT. Beta propellers: structural rigidity and functional diversity. Curr Opin Struct Biol. 1999;9(6):715-21.

43. Paoli M. Protein folds propelled by diversity. Prog Biophys Mol Biol. 2001;76(1-2): 103-30.

44. Jawad Z, Paoli M. Novel sequences propel familiar folds. Structure. 2002; 10(4):447-54.

45. Adams J, Kelso R, Cooley L. The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol. 2000; 10(1):17-24.

46. Russell RB, Sasieni PD, Sternberg MJ. Supersites within superfolds. Binding site similarity in the absence of homology. J Mol Biol. 1998;282(4):903-18.

47. Todd AE, Orengo CA, Thornton JM. Evolution of function in protein superfamilies, from a structural perspective. J Mol Biol. 2001;307(4):1113-43.

48. Pons T, Gomez R, Chinea G, Valencia A. Beta-propellers: associated functions and their role in human diseases. Curr Med Chem. 2003;10(6):505-24.

49. Love CA, Harlos K, Mavaddat N, Davis SJ, Stuart DI, Jones EY, *et al*. The ligand-binding face of the semaphorins revealed by the high-resolution crystal structure of SEMA4D. Nat Struct Biol. 2003;10(10):843-8.

50. Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL, *et al*. Crystal structure of the extracellular segment of integrin alpha Vbeta3. Science. 2001; 294(5541):339-45.

51. Hiramatsu H, Yamamoto A, Kyono K, Higashiyama Y, Fukushima C, Shima H, *et al*. The crystal structure of human dipeptidyl peptidase IV (DPPIV) complex with diprotin A. Biol Chem. 2004;385(6):561-4.

52. Oefner C, D'Arcy A, Mac Sweeney A, Pierau S, Gardiner R, Dale GE. High-resolution structure of human apo dipeptidyl peptidase IV/CD26 and its complex with 1-[([2-[(5-iodopyridin-2-yl)amino]-ethyl]amino)-acetyl]-2-cyano-(S)-pyrrolidine. Acta Crystallogr D Biol Crystallogr. 2003;59(Pt 7):1206-12.

53. Weihofen WA, Liu J, Reutter W, Saenger W, Fan H. Crystal structure of CD26/dipeptidyl-peptidase IV in complex with adenosine deaminase reveals a highly amphiphilic interface. J Biol Chem. 2004; 279(41):43330-5.

54. Abbott CA, Gorrell MD. The family of CD26/DPP-IV and related ectopeptidases. In: Langner J, Ansorge S, editors. Ectopeptidases. CD13/aminopeptidase N and CD26/dipeptidylpeptidase IV in medicine and biology. New York: Kluwer Academic / Plenum Publishers; 2002. p. 171-95.

55. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.

56. Carl-McGrath S, Lendeckel U, Ebert M, Rocken C. Ectopeptidases in tumour biology: a review. Histol Histopathol. 2006; 21(12):1339-53.

57. Iwata S, Morimoto C. CD26/dipeptidyl peptidase IV in context. The different roles of a multifunctional ectoenzyme in malignant transformation. J Exp Med. 1999; 190(3):301-6.

58. Wesley UV, Albino AP, Tiwari S, Houghton AN. A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. J Exp Med. 1999; 190(3):311-22.

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

59. Sedo A, Krepela E, Kasafirek E. Dipeptidyl peptidase IV, prolyl endopeptidase and cathepsin B activities in primary human lung tumors and lung parenchyma. J Cancer Res Clin Oncol. 1991;117(3):249-53.

60. Asada Y, Aratake Y, Kotani T, Marutsuka K, Araki Y, Ohtaki S, *et al*. Expression of dipeptidyl aminopeptidase IV activity in human lung carcinoma. Histopathology. 1993;23(3):265-70.

61. Wesley UV, Tiwari S, Houghton AN. Role for dipeptidyl peptidase IV in tumor suppression of human non small cell lung carcinoma cells. Int J Cancer. 2004; 109(6):855-66.

62. Dimitrova M, Ivanov I, Todorova R, Stefanova N, Moskova-Doumanova V, Topouzova-Hristova T, *et al*. Comparison of the activity levels and localization of dipeptidyl peptidase IV in normal and tumor human lung cells. Tissue Cell. 2012;44(2):74-9.

63. Bogenrieder T, Finstad CL, Freeman RH, Papandreou CN, Scher HI, Albino AP, *et al*. Expression and localization of aminopeptidase A, aminopeptidase N, and dipeptidyl peptidase IV in benign and malignant human prostate tissue. Prostate. 1997;33(4):225-32.

64. Frohlich E, Maier E, Wahl R. Interspecies differences in membrane-associated protease activities of thyrocytes and their relevance for thyroid cancer studies. J Exp Clin Cancer Res. 2012;31:45.

65. Kotani T, Asada Y, Aratake Y, Umeki K, Yamamoto I, Tokudome R, *et al*. Diagnostic usefulness of dipeptidyl aminopeptidase IV monoclonal antibody in paraffin-embedded thyroid follicular tumours. J Pathol. 1992;168(1):41-5.

66. Tanaka T, Umeki K, Yamamoto I, Sakamoto F, Noguchi S, Ohtaki S. CD26 (dipeptidyl peptidase IV/DPP IV) as a novel molecular marker for differentiated thyroid carcinoma. Int J Cancer. 1995; 64(5):326-31.

67. Tang AC, Raphael SJ, Lampe HB, Matthews TW, Becks GP. Expression of dipeptidyl aminopeptidase IV activity in thyroid tumours: a possible marker of thyroid malignancy. J Otolaryngol. 1996; 25(1):14-9.

68. Maruta J, Hashimoto H, Yamashita H, Yamashita H, Noguchi S. Diagnostic applicability of dipeptidyl aminopeptidase IV activity in cytological samples for differentiating follicular thyroid carcinoma from follicular adenoma. Arch Surg. 2004; 139(1):83-8.

69. Khin EE, Kikkawa F, Ino K, Kajiyama H, Suzuki T, Shibata K, *et al*. Dipeptidyl peptidase IV expression in endometrial endometrioid adenocarcinoma and its inverse correlation with tumor grade. Am J Obstet Gynecol. 2003;188(3):670-6.

70. Kikkawa F, Kajiyama H, Ino K, Shibata K, Mizutani S. Increased adhesion potency of ovarian carcinoma cells to mesothelial cells by overexpression of dipeptidyl peptidase IV. Int J Cancer. 2003;105(6):779-83.

71. Pro B, Dang NH. CD26/dipeptidyl peptidase IV and its role in cancer. Histol Histopathol. 2004;19(4):1345-51.

72. Goscinski MA, Suo ZH, Nesland JM, Florenes VA, Gierckscky KE. Dipeptidyl peptidase IV expression in cancer and stromal cells of human esophageal squamous cell carcinomas, adenocarcinomas and squamous cell carcinoma cell lines. APMIS. 2008;116(9):823-31.

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.

Acknowledgements

The authors thanks the Project 3276/3 of the International Foundation for Science (IFS), together with the Chemical Weapons Prohibition Organization, for the financial support..

73. Bauvois B, De Meester I, Dumont J, Rouillard D, Zhao HX, Bosmans E. Constitutive expression of CD26/dipeptidylpeptidase IV on peripheral blood B lymphocytes of patients with B chronic lymphocytic leukaemia. Br J Cancer. 1999;79(7-8):1042-8.

74. Cro L, Morabito F, Zucal N, Fabris S, Lionetti M, Cutrona G, *et al*. CD26 expression in mature B-cell neoplasia: its possible role as a new prognostic marker in B-CLL. Hematol Oncol. 2009;27(3):140-7.

75. Havre PA, Dang LH, Ohnuma K, Iwata S, Morimoto C, Dang NH. CD26 expression on T-anaplastic large cell lymphoma (ALCL) line Karpas 299 is associated with increased expression of versican and MT1-MMP and enhanced adhesion. BMC cancer. 2013;13:517.

76. Stremenova J, Krepela E, Mares V, Trim J, Dbaly V, Marek J, *et al*. Expression and enzymatic activity of dipeptidyl peptidase-IV in human astrocytic tumours are associated with tumour grade. Int J Oncol. 2007;31(4):785-92.

77. Stremenova J, Mares V, Lisa V, Hilser M, Krepela E, Vanickova Z, *et al*. Expression of dipeptidyl peptidase-IV activity and/or structure homologs in human meningiomas. Int J Oncol. 2010;36(2):351-8.

78. Arscott WT, LaBauve AE, May V, Wesley UV. Suppression of neuroblastoma growth by dipeptidyl peptidase IV: relevance of chemokine regulation and caspase activation. Oncogene. 2009;28(4):479-91.

79. Dinjens WN, Ten Kate J, Kirch JA, Tanke HJ, Van der Linden EP, Van den Ingh HF, *et al*. Adenosine deaminase complexing protein (ADCP) expression and metastatic potential in prostatic adenocarcinomas. J Pathol. 1990; 160(3):195-201.

80. Wesley UV, McGroarty M, Homoyouni A. Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. Cancer Res. 2005;65(4):1325-34.

81. Urade M, Komatsu M, Yamaoka M, Fukasawa K, Harada M, Mima T, *et al*. Serum dipeptidyl peptidase activities as a possible marker of oral cancer. Cancer. 1989;64(6):1274-80.

82. de la Haba-Rodriguez J, Macho A, Calzado MA, Blazquez MV, Gomez MA, Munoz EE, *et al*. Soluble dipeptidyl peptidase IV (CD-26) in serum of patients with colorectal carcinoma. Neoplasma. 2002;49(5):307-11.

83. Cordero OJ, Imbernon M, Chiara LD, Martinez-Zorzano VS, Ayude D, de la Cadena MP, et al. Potential of soluble CD26 as a serum marker for colorectal cancer detection. World J Clin Oncol. 2011;2(6):245-61.

84. Gonzalez-Gronow M, Misra UK, Gawdi G, Pizzo SV. Association of plasminogen with dipeptidyl peptidase IV and Na+/H+ exchanger isoform NHE3 regulates invasion of human 1-LN prostate tumor cells. J Biol Chem. 2005;280(29):27173-8.

85. Gorrell MD, Gysbers V, McCaughan GW. CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. Scand J Immunol. 2001;54(3):249-64.

86. Cheng HC, Abdel-Ghany M, Pauli BU. A novel consensus motif in fibronectin mediates dipeptidyl peptidase IV adhesion and metastasis. J Biol Chem. 2003;278(27):24600-7.

87. Cheng HC, Abdel-Ghany M, Elble RC, Pauli BU. Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin. J Biol Chem. 1998;273(37):24207-15.

88. Korach S, Poupon MF, Du Villard JA, Becker M. Differential adhesiveness of rhabdomyosarcoma-derived cloned metastatic cell lines to vascular endothelial monolayers. Cancer Res. 1986;46(7):3624-9.

89. Kato Y, Saijo N. Developed new agents for lung cancer. Nihon Geka Gakkai zasshi. 2002;103(2):218-23.

90. Mentlein R, Dahms P, Grandt D, Kruger R. Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul Pept. 1993;49(2):133-44.

91. Ghersi G, Chen W, Lee EW, Zukowska Z. Critical role of dipeptidyl peptidase IV in neuropeptide Y-mediated endothelial cell migration in response to wounding. Peptides. 2001;22(3):453-8.

92. Mathew S, Morrison ME, Murty VV, Houghton AN, Chaganti RS. Assignment of the DPP4 gene encoding adenosine deaminase binding protein (CD26/dipeptidylpeptidase IV) to 2q23. Genomics. 1994;22(1):211-2.

93. Otsuka T, Kohno T, Mori M, Noguchi M, Hirohashi S, Yokota J. Deletion mapping of chromosome 2 in human lung carcinoma. Genes Chromosomes Cancer. 1996;16(2):113-9.

94. Shiseki M, Kohno T, Nishikawa R, Sameshima Y, Mizoguchi H, Yokota J. Frequent allelic losses on chromosomes 2q, 18q, and 22q in advanced non-small cell lung carcinoma. Cancer Res. 1994;54(21):5643-8.

95. Morrison H, Sherman LS, Legg J, Banine F, Isacke C, Haipek CA, *et al*. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. Genes Dev. 2001;15(8):968-80.

96. Yan P, Muhlethaler A, Bourloud KB, Beck MN, Gross N. Hypermethylation-mediated regulation of CD44 gene expression in human neuroblastoma. Genes Chromosomes Cancer. 2003;36(2):129-38.

97. Kajiyama H, Kikkawa F, Maeda O, Suzuki T, Ino K, Mizutani S. Increased expression of dipeptidyl peptidase IV in human mesothelial cells by malignant ascites from ovarian carcinoma patients. Oncology. 2002;63(2):158-65.

98. Ware JL. Growth factor network disruption in prostate cancer progression. Cancer Metastasis Rev. 1998;17(4):443-7.

99. Giri D, Ropiquet F, Ittmann M. Alterations in expression of basic fibroblast growth factor (FGF) 2 and its receptor FGFR-1 in human prostate cancer. Clin Cancer Res. 1999;5(5): 1063-71.

100. Dow JK, deVere White RW. Fibroblast growth factor 2: its structure and property, paracrine function, tumor angiogenesis, and prostate-related mitogenic and oncogenic functions. Urology. 2000;55(6):800-6.

101. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1(1):34-45.

102. Isaacs JT, Isaacs WB. Androgen receptor outwits prostate cancer drugs. Nat Med. 2004; 10(1):26-7.

103. Boonacker E, Van Noorden CJ. The multifunctional or moonlighting protein CD26/DPPIV. Eur J Cell Biol. 2003;82(2):53-73.

104. Proost P, Struyf S, Schols D, Opdenakker G, Sozzani S, Allavena P, *et al*. Truncation of macrophage-derived chemokine by CD26/dipeptidyl-peptidase IV beyond its predicted cleavage site affects chemotactic activity and CC chemokine receptor 4 interaction. J Biol Chem. 1999;274(7):3988-93.

105. Nakamoto T, Chang CS, Li AK, Chodak GW. Basic fibroblast growth factor in human prostate cancer cells. Cancer Res. 1992; 52(3):571-7.

106. Gioeli D, Mandell JW, Petroni GR, Frierson HF, Jr., Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res. 1999;59(2):279-84.

107. Pintucci G, Moscatelli D, Saponara F, Biernacki PR, Baumann FG, Bizekis C, *et al*. Lack of ERK activation and cell migration in FGF-2-deficient endothelial cells. FASEB J. 2002;16(6):598-600.

108. Giuliani R, Bastaki M, Coltrini D, Presta M. Role of endothelial cell extracellular signal-regulated kinase1/2 in urokinase-type plasminogen activator upregulation and in vitro angiogenesis by fibroblast growth factor-2. J Cell Sci. 1999;112 (Pt 15):2597-606.

109. Rabbani SA, Mazar AP. The role of the plasminogen activation system in angiogenesis and metastasis. Surg Oncol Clin N Am. 2001;10(2):393-415.

110. Bugler B, Amalric F, Prats H. Alternative initiation of translation determines cytoplasmic or nuclear localization of basic fibroblast growth factor. Mol Cell Biol. 1991;11(1):573-7.

111. Bikfalvi A, Klein S, Pintucci G, Rifkin DB. Biological roles of fibroblast growth factor-2. Endocr Rev. 1997;18(1):26-45.

112. Delrieu I. The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism. FEBS Lett. 2000;468(1):6-10.

113. Arwert EN, Mentink RA, Driskell RR, Hoste E, Goldie SJ, Quist S, et al. Upregulation of CD26 expression in epithelial cells and stromal cells during wound-induced skin tumour formation. Oncogene. 2012;31(8):992-1000.

114. Ehtesham M, Winston JA, Kabos P, Thompson RC. CXCR4 expression mediates glioma cell invasiveness. Oncogene. 2006;25(19): 2801-6.

115. Christopherson KW, 2nd, Hangoc G, Broxmeyer HE. Cell surface peptidase CD26 dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. J Immunol. 2002;169(12):7000-8.

116. Vangoitsenhoven R, Mathieu C, Van der Schueren B. GLP1 and cancer: friend or foe? Endocr Relat Cancer. 2012;19(5):F77-88.

117. Korner M, Stockli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. J Nucl Med. 2007;48(5):736-43.

118. Koehler JA, Drucker DJ. Activation of glucagon-like peptide-1 receptor signaling does not modify the growth or apoptosis of human pancreatic cancer cells. Diabetes. 2006;55(5):1369-79.

119. Girman CJ, Kou TD, Cai B, Alexander CM, O'Neill EA, Williams-Herman DE, *et al*. Patients with type 2 diabetes mellitus have higher risk for acute pancreatitis compared with those without diabetes. Diabetes Obes Metab. 2010;12(9):766-71.

120. Garg R, Chen W, Pendergrass M. Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. Diabetes Care. 2010;33(11):2349-54.

121. Dore DD, Bloomgren GL, Wenten M, Hoffman C, Clifford CR, Quinn SG, *et al*. A cohort study of acute pancreatitis in relation to exenatide use. Diabetes Obes Metab. 2011;13(6):559-66.

122. Nyborg NC, Molck AM, Madsen LW, Knudsen LB. The human GLP-1 analog liraglutide and the pancreas: evidence for the absence of structural pancreatic changes in three species. Diabetes. 2012;61(5):1243-9.

123. Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, *et al*. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology. 2010;151(4):1473-86.

124. Waser B, Beetschen K, Pellegata NS, Reubi JC. Incretin receptors in non-neoplastic and neoplastic thyroid C cells in rodents and humans: relevance for incretin-based diabetes therapy. Neuroendocrinology. 2011;94(4):291-301.

125. Roman S, Lin R, Sosa JA. Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases. Cancer. 2006;107(9):2134-42.

126. Koehler JA, Kain T, Drucker DJ. Glucagon-like peptide-1 receptor activation inhibits growth and augments apoptosis in murine CT26 colon cancer cells. Endocrinology. 2011;152(9):3362-72.

127. Ligumsky H, Wolf I, Israeli S, Haimsohn M, Ferber S, Karasik A, *et al*. The peptide-hormone glucagon-like peptide-1 activates cAMP and inhibits growth of breast cancer cells. Breast Cancer Res Treat. 2012;132(2):449-61.

128. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science. 1995;270(5240):1326-31.

Received in September, 2013.
Accepted in April, 2014.