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Dipeptidyl peptidase IV (DPP IV/CD26) is a cell-surface plasminogen receptor

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1. ABSTRACT

Binding of plasminogen (Pg) to cell-surface receptors colocalized with plasminogen activators promotes Pg activation and enables cells to utilize the proteolytic activity of plasmin (Pm). Proteolysis by Pm is necessary in several physiological and pathological processes requiring extracellular matrix degradation including cell migration, tumor cell invasion and metastasis. The binding of Pg to cell-surface receptors is regulated by two major structural features: L-lysine binding sites (LBS) and negatively charged sialic acid residues located on its carbohydrate chains. Pg uses its LBS to bind to a wide spectrum of cell-surface receptors whereas binding through its sialic acid residues is limited only to receptor proteins containing cationic pockets or lectin-like modules. In this review, we discuss both mechanisms, including the identification of DPP IV as a Pg receptor and the possible physiological role of Pg/Pm in complex with DPP IV and adenosine deaminase (ADA) and /or the Na^+/H^+ exchanger isoform NHE-3 in prostate cancer.

2. INTRODUCTION

In eukaryotic cells, plasminogen (Pg) receptors are represented by a heterogeneous family of proteins and non-protein molecules including $alpha_{IIb}Beta_3$ (1), alpha-enolase (2), actin (3), annexin II (4) gangliosides (5) or NHE-3 (6). Pg binds to these molecules on the cell surface via a mechanism involving L-lysine binding sites (LBS) displayed in double looped disulfide-bonded structures named kringles (7) where kringles 1 and 4 are the primary sites for receptor binding (8). In addition to the above listed receptors, our laboratory identified dipeptidyl peptidase IV (DPP IV) as a Pg receptor in both human rheumatoid synovial fibroblasts and a prostate tumor cell line (9, 10). Unlike sialic acid-binding immunoglobulin-like lectins (siglecs) which recognize a wide spectrum of sialic acid containing glycoproteins (11), DPP IV lectin-like modules interact specifically with Pg Thr^{345}- linked carbohydrate chain sialic acid residues (12). This interaction induces a rise in cytosolic Ca^{2+} which promotes synthesis of matrix metalloproteinase-9 (MMP-9) (10). DPP IV on the cell

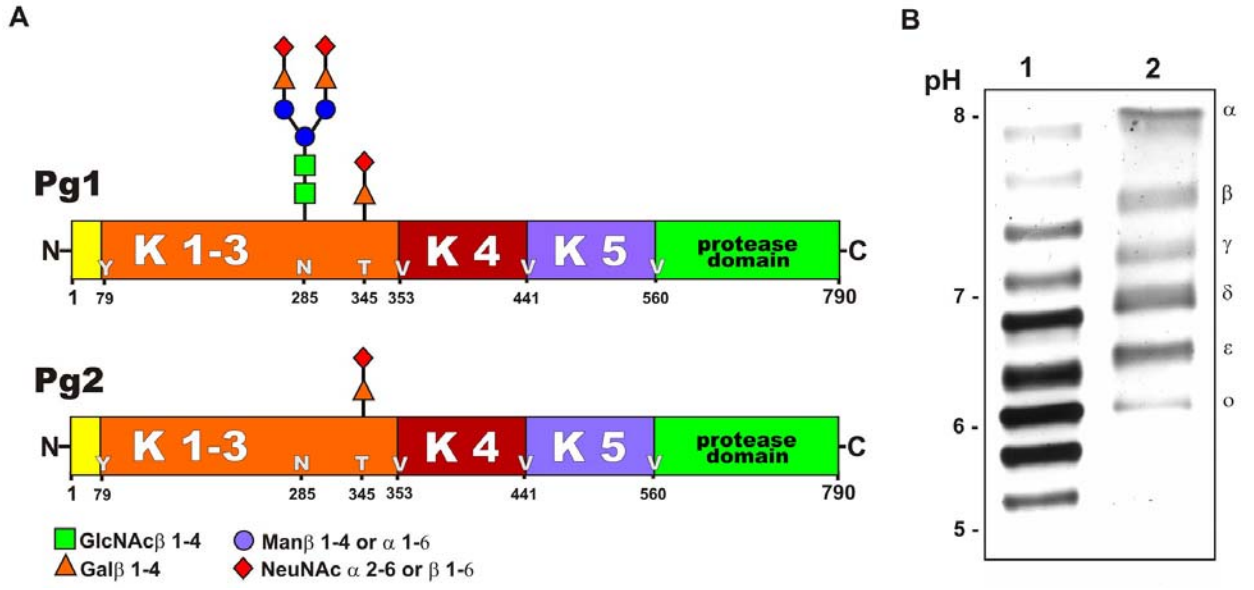

Figure 1. (A) Structural domains of Pg 1 and Pg 2 showing the segments containing kringles 1-3, kringle 4, kringle 5 and the protease domain. Both Pg glycoforms contain the same number of amino acids, but differ in their carbohydrate chains. Pg 1 contains two carbohydrate chains, one bi-antennary N-linked to Asn^{285}, and one single O-linked to Thr^{345}. Pg 2 contains only the O-linked to Thr^{345} (B) Lane 1: Isoelectric focusing analysis of Pg 1 (10 µg). Lane 2: Isoelectric focusing analysis of Pg 2 (10 µg). They were designated 2 alpha-2 phi, according to their decreasing pIs.

surface also associates with ADA (13) and the Na^+/H^+exchanger isoform NHE-3 (14). In this review, we summarize current knowledge of the mechanisms underlying the interaction between DPP IV when associated with ADA or NHE-3 and Pg.

3. PLASMINOGEN STRUCTURAL FEATURES

Pg in the circulation exhibits two major glycosylated variants, Pg 1 and Pg 2, which differ in their LBS affinity (15). Pg 1 and Pg 2 have identical amino acid composition (Figure 1A), but differ in the number of sites of glycosylation. Pg 1 contains two carbohydrate linkages, an N-linked glycosylation at Asn288 and an O-linked glycosylation at Thr345. Pg 2 has just a single oligosaccharide linkage at Thr345 (16,17). Pg 1 and 2 not only differ in their carbohydrate contents, but also differ in many of their physicochemical properties. The activation of Pg 1 is enhanced more than that of Pg 2 in the presence of fibrin by either urokinase or streptokinase (18). Although the concentration of Pg 2 in the circulation is twice that of Pg 1, the latter is a more efficient fibrinolytic enzyme when activated (19). Additionally, Pg 2 is degraded by elastase faster than Pg 1 (18). Pg 1 appears in plasma at half the rate of Pg 2 (20), and secondary glycosylation is essential for the secretion of Pg 1 (21). All these differences result from additional glycosylation of Asn288 in Pg 1 that normally does not occur in Pg 2 (22). Multiple isoforms of Pg 1 and Pg 2 have been identified in plasma (Figure 1B). In our laboratory, we have purified and analyzed the sialic acid contents of Pg 2 glycoforms (Table 1) which explain dramatically different isoelectric points (Figure 1B).

3.1. Plasminogen binding through L-lysine binding sites

Many types of cells bind Pg in a kringle-dependent reaction to membrane-associated receptors displaying L-lysine residues (23). Pg 2 binds to these receptor(s) considerably better than Pg 1 (24). The latter is completely unable to displace Pg 2 from the receptor (24). The association of Pg with cell surface receptors changes the K_m between Pg and its activators, promoting plasmin (Pm) formation even in the absence of fibrin. These processes are the result of a much more open conformation of the Pg molecule, induced by occupation of the LBS, which affects the position of entire domains, thereby promoting conversion of Pg to Pm by its physiologic activators (25). The formation of Pm from Pg on the cell surface has important pathophysiological ramifications leading to enhanced cell migration and proteolytic activation of metalloproteinases (26), thereby facilitating metastatic activity by tumor cells (26). Interestingly, since cell-bound Pm is protected from inhibition by the primary Pm inhibitor $alpha_2$-antiplasmin, these functions are greatly stimulated (26).

4. DPP IV AS A PLASMINOGEN RECEPTOR

4.1. Physiologic role of DPP IV glycosylation

DPP IV is a ubiquitous serine protease known to function in immune and endocrine system regulation, glucose homeostasis, cell adhesion and tumor growth (27, 28). DPP IV has a large number of known substrates including chemokines, neuropeptides, and glucagon-like peptides (GLP-1 and GLP-2) (28). As mentioned above, in addition to its numerous substrates, DPP IV also serves as a ligand for ADA, NHE-3 and fibronectin (14, 29, 30).

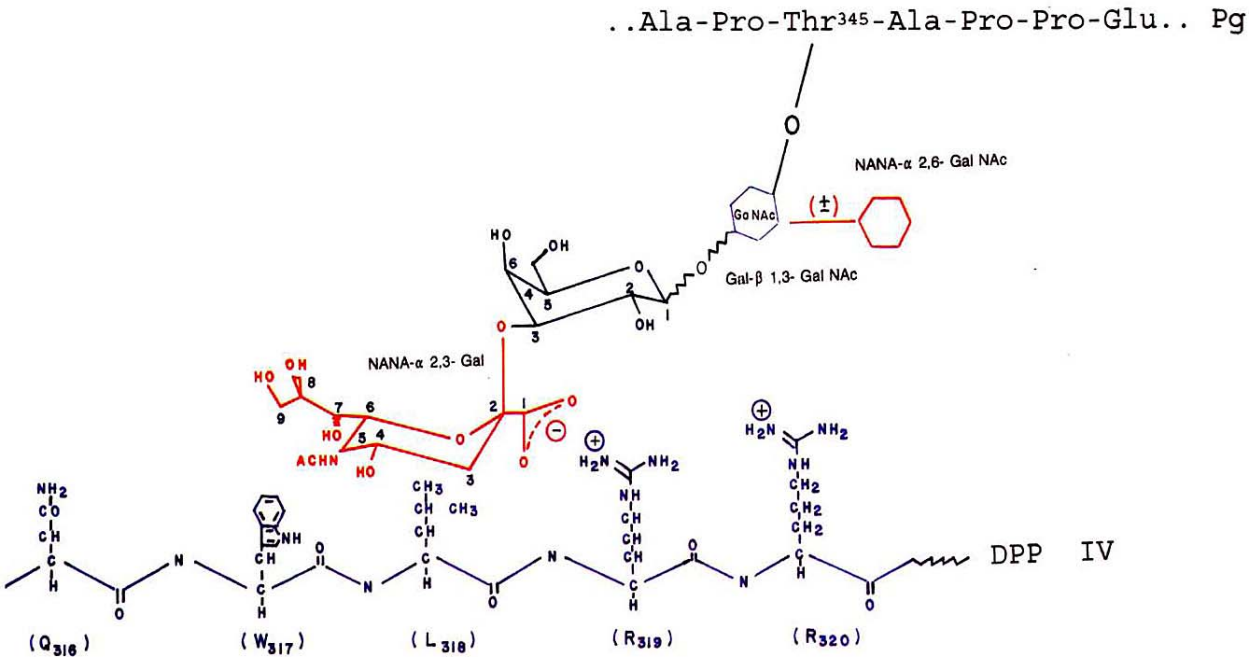

Figure 2. Model of interaction between the alpha 2, 3-linked sialic acid in the carbohydrate chain bound to Pg-Thr^{345} and the DPP IV sequence L^{313}QWLRRI^{319}.

DPP IV is a N-glycosylated type II plasma membrane homodimer protein with non-covalently linked subunits (31). DPP IV is anchored to the plasma membrane via a polypeptidic chain containing the first 39 NH_2-terminal amino acid residues, and contains eight potential N-glycosylation sites (32). DPP IV purified from plasma membranes of kidney and liver consists of several glycoforms of different isoelectric points due to differences in the degree of sialylation (33). DPP IV follows two secretory pathways in kidney cells, apical and basolateral, which are finely regulated through sorting mechanisms involving N- and O-linked glycosylation of the molecule (34). Both glycosylation types are critical for the apical kidney cell surface expression of DPP IV, whereas the basolateral secretion is not affected by them (34). Sialylation is also involved in the control of the apical targeting of secreted DPP IV (35). Although the molecule is glycosylated at several Asn residues, only N-glycosylation at Asn^{319} is critical for DPP IV correct protein folding and secretion (36). In addition to its role in apical sorting of DPP IV, sialylation enables the cell to control the enzymatic activity of the secreted circulating enzyme, as observed in DPP IV purified from plasmas of human patients with rheumatoid arthritis and other autoimmune diseases (37, 38). Hypersialylation blocks access of substrates to the active site of DPP IV, thereby inhibiting its enzymatic activity (37), a phenomenon also observed in the hypersialylated neonatal Pg 1 or Pg 2 glycoforms (39).

4.2. DPP IV binding sites

Pg 2 binds non-covalently to DPP IV through a mechanism involving interaction between their negatively charged Thr^{345} O-linked alpha (2-3)-polysialylated carbohydrate chains and exposed cationic sites on the DPP IV primary structure. DPP IV contains several of these cationic pockets including the primary amino acid sequences L^{69}YKQENNIL^{77}, L^{137}NKRQL^{142} and L^{312}QWLRRI^{317} which are easily accessible for secondary interaction (40). However, our observations indicate that only the peptide containing the amino acid sequence L^{312}QWLRRI^{317} is able to bind to Pg (12). Based on a model depicting the interaction between a cationic pocket of HIV-1 Tat protein and hypersialylated DPP IV (41), we constructed a working model (Figure 2) showing the molecular interaction between the cationic pocket including amino acids L^{312}QWLRRI^{317} in DPP IV and a negatively charged region provided by alpha 2, 3-sialic acid in the primary structure of the Pg/Pm molecule (12). DPP IV N-glycosylation at Asn^{319} is involved in DPP IV trafficking and correct protein folding (36), and may also regulate Pg 2 binding. The Pg 2 variant is composed of six glycoforms designated -2 alpha to -2 phi, according to increasing sialic acid contents (42). Only Pg 2 gamma, Pg 2 delta or Pg 2 epsilon, containing the highest contents of sialic acid, are able to bind to DPP IV, whereas Pg 2 alpha, Pg 2 beta or Pg 2 phi do not bind to this protein (Figure 3A). Pg 2 phi, which constitutes less than 1% of the total Pg 2 variant glycoforms,is believed to be a neonatal remnant of hypersialylated and its sialic acid content is similar to that of neonatal Pg 2 whose binding to a receptor in U937 monocytoid cells is greatly decreased (43). In addition to its role in DPP IV trafficking and correct folding (36), N-glycosylation at Asn^{319}, located next to the amino acid sequence L^{312}QWLRRI^{317}, may serve also as a sorting mechanism to regulate Pg 2 binding , as evidenced by the

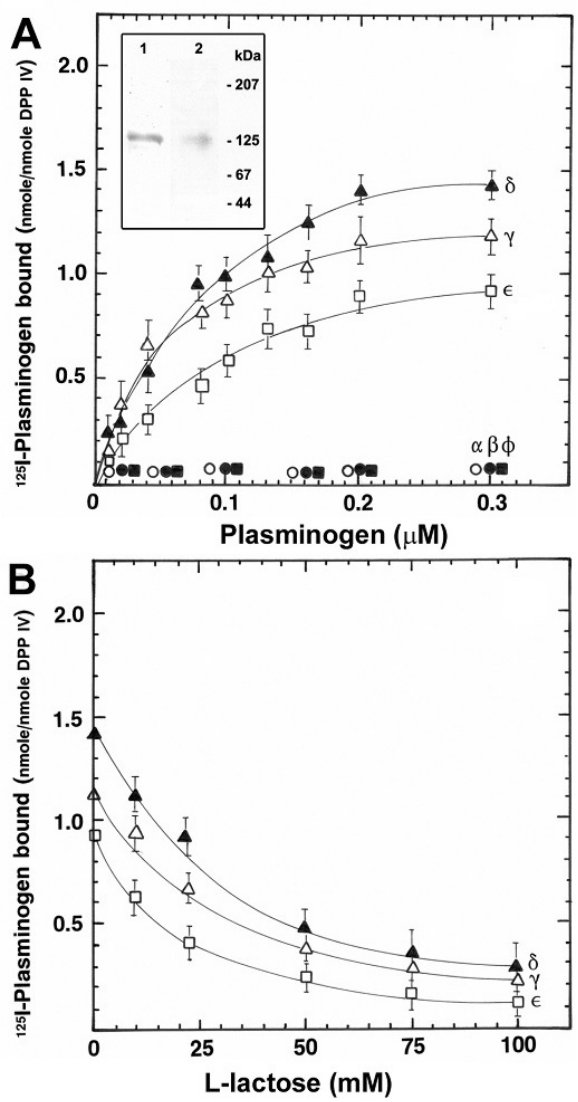

Figure 3. Binding of individual Pg 2 glycoforms to immobilized DPP IV purified from 1-LN cell membranes**.** (A) DPP IV (0.2 μg/well) was used to coat 96-well plates. Increasing concentrations of ^{125}I-labelled Pg 2 alpha (○), Pg 2 beta (●), Pg 2 gamma (Δ), Pg 2 delta (▲), Pg 2 epsilon (□) or Pg 2 phi (■) were added to triplicate wells and incubated at 22 ^{o}C for 1 h. *Inset:* SDS/10% PAGE of purified DPP IV (5 μg) under reducing conditions. Lane 1, Coomassie Brilliant Blue R-250 stained gel; lane 2, blot incubated with a mAb anti-DPP IV IgG followed by reaction with an alkaline phosphatase-conjugated secondary IgG. (B) Inhibition of binding of ^{125}I-labelled Pg 2 gamma (Δ), Pg 2 delta (▲) or Pg 2 epsilon (□) (0.1 μM) to immobilized DPP IV by increasing concentrations of L-lactose.

binding only of the highly sialylated Pg 2 gamma, Pg 2 delta or Pg 2 epsilon to DPP IV (10). Binding of Pg 2 glycoforms to DPP IV was inhibited by L-lactose (Figure 3B), a sugar that interferes with sialic acid binding to lectins, thereby suggesting the alpha 2,3-linked sialic acid of the Pg 2 Thr^{345} O-linked carbohydrate chain as the point of attachment of Pg 2 to DPP IV. Although both Pg 2 delta and Pg 2 epsilon contain almost identical amounts of sialic acid (Table 1), Pg 2 epsilon contains one alpha (2,6)-sialic acid residue which permits its separation from Pg 2 delta by chromatofocusing techniques (42).

5. DPP IV FORMS A COMPLEX WITH ADA AND PLASMINOGEN

5.1. Interaction between ADA and plasminogen

Although mainly cytosolic, an "ecto" form of ADA also exists on activated human T lymphocytes and epithelial cells of many organs because of the association of ADA with the multifunctional cell membrane glycoprotein DPP IV (44). It has been suggested that DPP IV-associated ecto-ADA regulates the levels of extracellular adenosine and thereby controls signal transduction via a family of adenosine receptors (45, 46). We investigated the binding of ADA to 1-LN prostate cancer cells and to isolated, immobilized DPP IV purified from 1-LN cell membranes, in the absence or presence of Pg 2, and found that these three proteins form a ternary complex demonstrating a connection between the Pg activation system and the protein complex DPP IV-ADA (47). A close examination of the human and murine DPP IV segment containing amino acids 310-350 (48, 49) reveals a conserved Pg 2 binding region, as well as an ADA binding region (Figure 4).

5.2. Effect of ADA on plasminogen activation on the cell surface

The interaction between ADA and Pg 2 is mediated by kringle 4 attachment to Lys^{206} in the ADA segment containing amino acids H^{197}VQAYQEAVKSGIH^{210} (47). The three-dimensional structure of ADA shows that both the Pg and the DPP IV binding segment, containing amino acids E^{139}GERD^{143}, do not obstruct each other at the protein surface (Figure 5). Pg 2 associates to ADA in a kringle-dependent mechanism via its LBS promoting a conformational change that stimulates Pg 2 activation by its physiologic activators urokinase (u-PA) or tissue-type plasminogen activator (t-PA) (33). ADA also stimulates Pg 2 activation by t-PA in the presence of poly-D-lysine, which mimics a fibrin surface, thereby suggesting that extracellular ADA may act as a pro-fibrinolytic factor (47). The interaction between ADA and Pg 2 is mediated by Pg kringle 4 (47), thereby mimicking the effect of tetranectin, a specific kringle 4-binding protein occurring in plasma which enhances Pg activation by t-PA in the presence of poly-D-lysine (50).

6. DPP IV IN ASSOCIATION WITH PLASMINOGEN AND Na^+/H^+ EXCHANGER NHE-3

In renal brush-border membranes, DPP IV exists in multimeric complexes with NHE-3, a member of the NHE antiporter family (14). In humans there are nine NHE isoforms that are members of the SLC9A gene family (51). The established plasma membrane isoforms include NHE-1-5 (51); however, Pg 2 binds only to NHE-3 (6). The sequence including Val^{275}-Val^{290} in the second outside loop of NHE-3 contains one L-lysine residue (lys^{280}) which

Table 1. Sialic acid content of plasminogen 2 glycoforms

Glycoform	mol sialic acid/mol protein
alpha	1.31±0.31
beta	2.21±0.20
gamma	2.95±0.03
delta	5.77±2.50
epsilon	5.34±0.68
phi	13.65±4.07

Figure 4. Amino acids 310-350 of DPP IV showing regions involved in Pg and ADA binding.

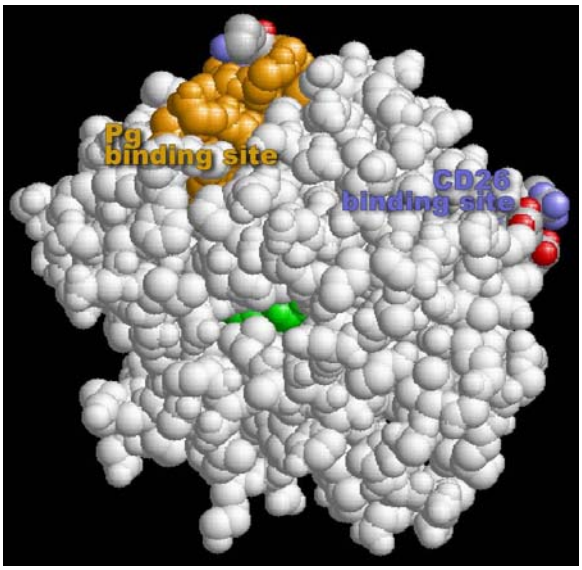

Figure 5. Three-dimensional structure of ADA showing the surface regions containing amino acids His^{197} - His^{210} and Glu^{139}- Asp^{143} involved in Pg and DPP IV binding, respectively.

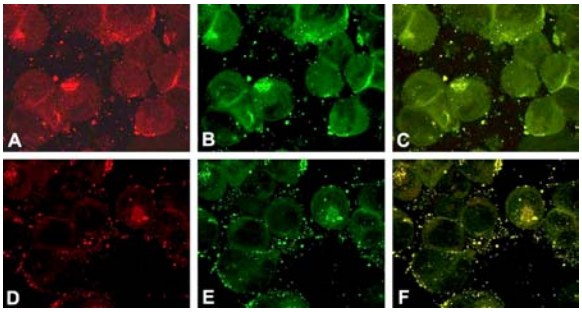

Figure 6. Immunofluorescence microscopy of NHE-3, u-PA or DPP IV on 1-LN prostate cancer cell surface. (A) Immunofluorescent staining with specific antibodies anti-NHE-3 or (B) anti-DPP IV in non-permeabilized cells. (C) confocal fluorescent stain obtained after merging in a single picture the two previous images. (D) immunofluorescent staining with specific antibodies anti-urokinase plasminogen activator (u-PA) or (E) DPP IV . (F) confocal fluorescent stain resulting from merging the two previous images.

serves as a plasma membrane binding site for Pg 2 on 1-LN prostate cancer cells (6). DPP IV and NHE-3 are plasma membrane proteins localized in lipid rafts (52, 53). Immunofluorescence microscopy of 1-LN prostate cancer cells with specific antibodies anti-NHE-3 or anti-DPP IV in non-permeabilized cells (Figures 6A and 6B) demonstrate co-localization of these two proteins (Figure 6C), and show a patchy and punctate staining pattern that is typically observed in raft proteins. Similarly, reactivity with anti-urokinase plasminogen activator (u-PA) or DPP IV (Figures 6D and 6E) also demonstrate co-localization of u-PA with DPP IV (Figure 6F). Since u-PA is also localized in lipid rafts (54), clustering of these three proteins in one membrane compartment would be expected to promote efficient cell surface Pg 2 activation.

6.1. Changes in cytosolic pH induced by plasminogen

Pg 2 induces a rise in cytosolic pH, mediated by binding to DPP IV and NHE-3 in 1-LN prostate cancer cells, which promotes cell invasiveness (6). A similar cytoplasmic alkalinization is produced by stimulation of NHE-1, a ubiquitously expressed transporter in the plasma membrane with a main function to extrude H^+ from the cytoplasm (55). This is a key mechanism in oncogenic transformation and is necessary for the development and maintenance of the transformed phenotype (56) and appears to be a universal response of quiescent cells to growth-promoting factors (55). Therefore, association of these three proteins on the cell surface affects signaling pathways critical for cell transformation and motility.

7. FUNCTIONS OF DPP IV AS A PLASMINOGEN RECEPTOR

Interaction of Pg 2 with DPP IV on the surface of 1-LN prostate cancer cells produces a rapid increase in cytosolic Ca^{2+} which up-regulates expression of MMP-9 and promotes the invasiveness of these cells in an *in vitro* model (10). Only the highly sialylated Pg 2 gamma, Pg 2 delta or Pg 2 epsilon are able to induce a rise in cytosolic Ca^{2+}; however the expression of MMP-9 is stimulated only by Pg 2 epsilon glycoform (10). As mentioned above, the presence of alpha (2,6)-sialic acid residue confers this Pg glycoform the unique capacity to induce expression of MMP-9 when bound to DPP IV (10). In this context, DPP IV plays a dual role: it locks Pg 2 epsilon in a configuration which facilitates its activation by u-PA and it stimulates release of Ca^{2+} from internal stores. The increase in cytosolic Ca^{2+} is a signal for increased expression of MMP-9 and also stimulates intracellular alkalinization via acid extrusion through NHE-3. These three processes appear to be coordinated by DPP IV. First there is a very specific stimulation in the production of plasmin from the highly sialylated Pg 2 epsilon glycoform, followed by a burst in the expression of proMMP-9 which is rapidly converted by this plasmin into active MMP-9. Plasmin has been implicated in muscle and fibroblast cell proliferation via mechanisms distinct from those involving its activators (57, 58). Therefore, it is reasonable to assume that its association with DPP IV may be critical for a stimulation of cell motility and prostate tumor cell growth and invasiveness.

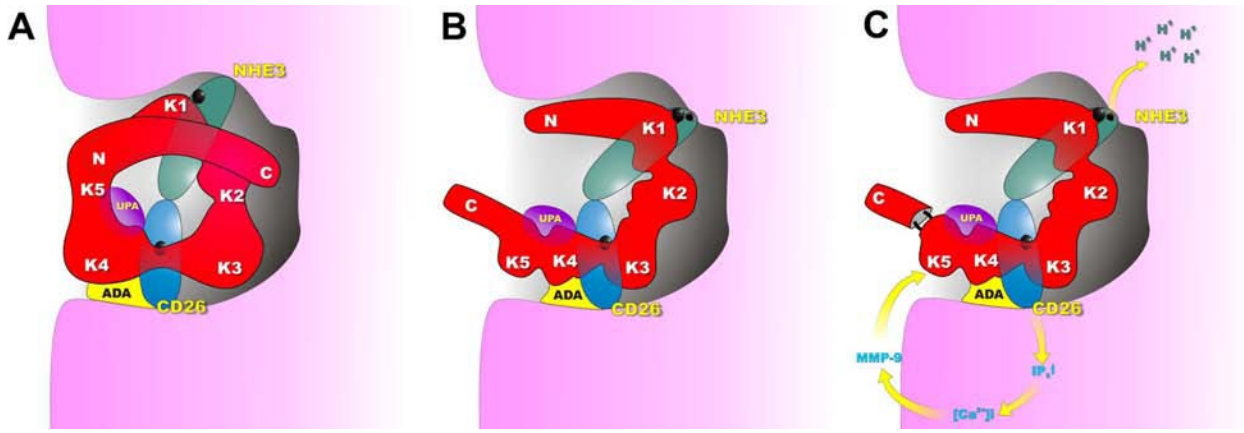

Figure 7. Experimental model of Pg 2 receptor(s) in 1-LN human prostate cancer cells**.** Pg 2 binds to DPP IV via an O-linked carbohydrate chain attached to Thr^{345}. It also binds to NHE-3 via kringle 1 or to ADA via kringle 4. Engagement of the LBS on these kringles induces a conformational change that promotes Pg activation by u-PA localized in the vicinity. Locking Pg 2 in a conformation stabilized by attachment to DPP IV initiates a signaling cascade that promotes expression of MMP-9. Pg 2 may also bind to NHE-3 inducing extrusion of H^+ which induces alkalinization in the cytosolic compartment.

7.1. Regulation of endothelial cell proliferation and tumor cell invasiveness

The Pg molecule is the *in vivo* source for the generation of angiostatin, a potent inhibitor of angiogenesis (59). Angiostatin generation appears to progress in a sequential order beginning with conversion of Pg to Pm, followed by reduction of Pm by disulfide reductases, then serine proteinase-dependent release of kringles 1-4½, and finally matrix metalloproteinase-dependent trimming of kringles 1-4½ to either kringles 1-4 or 1-3 (60). In our laboratory, we isolated angiostatins (kringles 1-3) from all the Pg 2 glycoforms (61). We tested their biological activity and found that only angiostatin derived from Pg 2 epsilon was able to inhibit endothelial cell proliferation and tubule formation. Further, we demonstrated that this angiostatin functions via a mechanism involving inhibition of Pg 2 epsilon binding to DPP IV, thereby blocking the rise in cytosolic Ca^{2+} and expression of MMP-9 induced by this glycoform in these cells (61). Angiostatin 2 epsilon is also able to inhibit 1-LN prostate cancer cell invasiveness stimulated by Pg 2 epsilon via direct binding to DPP IV (61). The mechanism is similar to that observed for endothelial cells. Although these mechanisms were identified under *in vitro* conditions, a similar situation may be operative *in vivo* where expression of MMP-9 resulting from binding of Pg 2 epsilon to DPP IV also serves to generate angiostatin in the peritumoral environment (62). Therefore, tumor growth may be controlled by this mechanism either indirectly via inhibition of angiogenesis or directly by limiting its growth and invasiveness.

8. CONCLUDING REMARKS

Pg receptors are very broadly distributed on both prokaryotic and eukaryotic cells (24); however, mechanisms different to those demonstrating stimulation of Pg activation and protection of the Pm generated on the cell surface from its physiologic inhibitors have not been reported (63). Cell surface proteolysis by Pm is essential in pathological processes requiring degradation of extracellular matrix (26). As discussed above, DPP IV in association with Pg appears to play an essential role in these processes not only because it provides a way to regulate Pg activation, but also because it enables Pg to interact with other DPP IV ligands, such as ADA or NHE-3 (Figure 7). DPP IV is elevated in tissue and prostatic secretions of men with prostate cancer (64). Our work with prostate tumor cells provided us with the knowledge to understand several of the mechanisms involving DPP IV as a receptor for Pg. However, there are still many functional aspects of DPP IV in several diseases that need to be investigated. For instance the consequences of autoimmunity to DPP IV in rheumatoid arthritis patients (37) or in autistic children (65) must be addressed. The answers obtained will provide a key understanding about the physiology of DPP IV in these pathological processes.

9. ACKNOWLEDGEMENT

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Abbreviations: Pg: plasminogen; Pm: plasmin; LBS: L-lysine binding sites; DPP IV: dipeptidyl peptidase IV; ADA: adenosine deaminase; NHE-3: Na^+/H^+ exchanger isoform 3; MMP-9: matrix metalloproteinase-9, u-PA: urokinase plasminogen activator; t-PA: tissue-type plasminogen activator

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