[Frontiers in Bioscience 13, 2319-2326, January 1, 2008]

Dipeptidyl peptidase-IV enzymatic activity bearing molecules in human brain tumors - good or evil?

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1. ABSTRACT

Dipeptidyl peptidase-IV (DPP-IV) represents a unique proteolytic activity cleaving N-terminal X-Pro dipeptides. In addition to canonical DPP-IV/CD26, a number of other molecules have been discovered which exhibit DPP-IV-like enzymatic activity and various degree of structural similarity. These comprise enzymatically active fibroblast activation protein-alpha, DPP-II, DPP8, DPP9 and enzymatically inactive DPP6 and DPP10 that have been grouped as "DPP-IV activity and/or structure homologues" (DASH). Because the enzymatically active DASH can share similar sets of biologically active substrates and are frequently coexpressed within single cell or on tissue level, it is tempting to consider their participation on biological function(s) previously attributed to DPP-IV/CD26. It is speculated that disrupted expression and enzymatic activity of some DASH might corrupt the message carried by their substrates, with consequent promotion of abnormal cell behavior. Thus, modulation of activity of a particular enzyme using e.g. inhibitors, specific antibodies or modifying its expression may be an attractive therapeutic concept in cancer treatment. This review summarizes current knowledge of the expression and possible function of DPP-IV enzymatic activity bearing molecules in human brain tumors.

2. INTRODUCTION

Dipeptidyl peptidase-IV (DPP-IV, EC 3.4.14.5) is a serine protease originally described by Hopsu-Havu and Glenner (1) in liver homogenates as an activity cleaving glycyl-prolyl-beta-naphthylamide. This protease is unique as it is able to remove two N-terminal amino acids from peptides and small proteins with Pro or Ala in the penultimate position that are otherwise rather resistant to proteolytic degradation (2). In the course of time, further molecules exhibiting DPP-IV enzymatic activity and varying degree of structural homology to canonical DPP-IV have been discovered and grouped as Dipeptidyl peptidase-IV activity and/or structure homologues (DASH, (3)). The group comprises enzymatically active DPP-II (also referred to as DPP7 or quiescent cell proline dipeptidase), DPP8, DPP9 and fibroblast activation protein alpha (FAP, also referred to as seprase) and enzymatically inactive DPP6 and DPP10. Attractin, which is structurally unrelated to DPP-IV (4), was formerly supposed to belong to DASH on the basis of its putative enzymatic activity, but a recent report by Friedrich *et al* (5) strongly argues against its intrinsic hydrolytic potential.

DPP-IV and some other DASH exhibit biological functions independent of their hydrolytic activity. For

example, DPP-IV has been shown to be identical to CD26 expressed on the T lymphocyte surface and to act as a signaling coreceptor in the immune system (6), to bind adenosine deaminase, plasminogen (7, 8) and some components of the extracellular matrix (9, 10). DPP8 and 9 are speculated to influence cell migration and adhesion independently of their intrinsic enzymatic activity (11). DPP6 and DPP10, both structurally related to DPP-IV but enzymatically inactive, were shown to be part of the neuronal voltage gated K^+ channels (12, 13). However, the DPP-IV-like hydrolytic activity driven cleavage of a number of biologically active peptides including chemokines and various neuropeptides is considered to be the main mechanism, by which DASH molecules can execute their biological functions. Proteolytic nicking of N-terminal dipeptides of DASH biologically active substrates is considered to be an important regulator of both their half-lives as well as receptor preference, fine-tuning their signaling capacity prior to receptor binding (14, 15). For example, cleavage of the chemokine RANTES 1-68 (regulated on activation normal T-cell expressed and secreted, CCL5) produces RANTES 3-68 that is inactive at receptors CCR1 and CCR3, but retains the ability of full-length molecule to activate CCR5. This conversion abrogates the ability of RANTES to induce migration of monocyte (16-18). Similarly, DPP-IV-like enzymatic activity cleaves neuropeptide Y 1-36 (NP Y) yielding NP Y 3-36 that has decreased affinity for the Y1 receptor subtype, but retains binding capacity for the Y2 and Y5 receptors (19, 20). As a physiological consequence, NP Y loses the vasoconstrictive potential while retaining the ability to promote angiogenesis via Y2 receptors (21). Moreover, expression of NP Y during angiogenesis seems to be orchestrated with expression of its cognate receptors Y1-Y5 and DPP-IV, which increases its proangiogenic potential (22, 23).

Due to the frequently observed coexpression of multiple DASH at the cellular as well as tissue level, their participation on the overall DPP-IV- like hydrolytic activity is evident and mutual functional overlap possible. However, plasma membrane localization, presence in body fluids and the ability to cleave larger substrates make DPP-IV and FAP most feasible as regulators of biologically active peptides. Thus, the cleavage of some of the "classical" DPP-IV substrates demonstrated for intracellular DPP8, 9 (24, 25) is likely of limited physiological impact.

Changes in expression of DPP-IV enzymatically active molecules have been described in a number of pathological states, including cancer. However, the functional consequences of such alterations are not straightforward (26) and may depend on the tissue specific presence of relevant biologically active substrates and respective receptors.

Here, we review data on the expression and possible function of DPP-IV enzymatic activity bearing molecules in the central nervous system focusing on human brain tumors.

3. DPP-IV ENZYMATIC ACTIVITY IN GLIOMA CELL LINES – POSSIBLE ASSOCIATION WITH CELL DIFFERENTIATION AND GROWTH

DPP-IV was detected in D384 astrocytoma cells by Medeiros *et al* (27) and subsequently by Sedo in human neuroblastoma SK-N-SH, rat C6 as well as human U373 and U87 glioma cell lines (28, 29). Moreover, in the C6 cell line DPP-IV enzymatic activity was upregulated during chemically induced differentiation (30). In subsequent studies with rat C6 (31) as well as human glioma cell lines U373, T98G, Hs 683, U138 and U87, substantial heterogeneity of molecules bearing DPP-IV-like enzymatic activity was demonstrated by gel chromatography, enzyme inhibition studies and native electrophoresis (32). These observations implied either the presence of multiple molecular forms of DPP-IV or expression of other enzymatically active DASH. Indeed, RT PCR confirmed expression of DPP-IV, FAP, DPP8 and DPP-II transcripts (32). Furthermore, subcellular localization and inhibition studies suggest that the majority of DPP-IV like enzymatic activity in all glioma cell lines studied may in fact be attributed to intracellular soluble DPP8/9 (unpublished data). However, cell differentiation and growth arrest induced by starvation were accompanied by rise of DPP-IV-like enzymatic activity localized predominantly in the plasma membrane. Indeed, we observed upregulation of both DPP-IV and FAP mRNA upon starvation induced growth arrest in U87 cells ((33) and unpublished data). Our results also revealed positive correlation ($r = 0.9$, $p < 0.05$) between the expression of DPP-IV and FAP mRNA in different glioma cell lines (Figure 1). This is analogous to the results demonstrating that DPP-IV upregulation is accompanied by growth arrest and restoration of non-malignant phenotype in melanoma and lung cancer cells (34, 35). Moreover, reexpression of DPP-IV in these cells was associated with upregulation of FAP. In our experimental setting, overexpression of DPP-IV in T98G human glioma cell line lead to growth inhibition and accumulation of the cells in G2/M phase of the cell cycle (Figure 2). The mechanisms of growth inhibition and antioncogenic activity, which has been attributed to DPP-IV by several authors, may involve proteolytic processing of its biologically active substrates (for review cf. (36)). In gliomas, substance P (SP), RANTES and stromal cell derived factor-1alpha (SDF-1alpha or CXCL12), can promote malignant behavior of the tumor cells (37-39). We have demonstrated the ability of glioma cells highly expressing DPP-IV to prevent signaling of SP by its proteolytic degradation (40). This cleavage however could not explain the growth arrest observed in T98G cells, which are devoid of functional SP receptor (40). In this case, other substrate(s) or non-enzymatic functions of DPP-IV are likely involved.

Taken together, so far available data suggests possible coregulation of both plasma membrane localized DASH, DPP-IV and FAP, and their link to cell growth and differentiation, although the underlying molecular mechanisms remain to be elucidated.

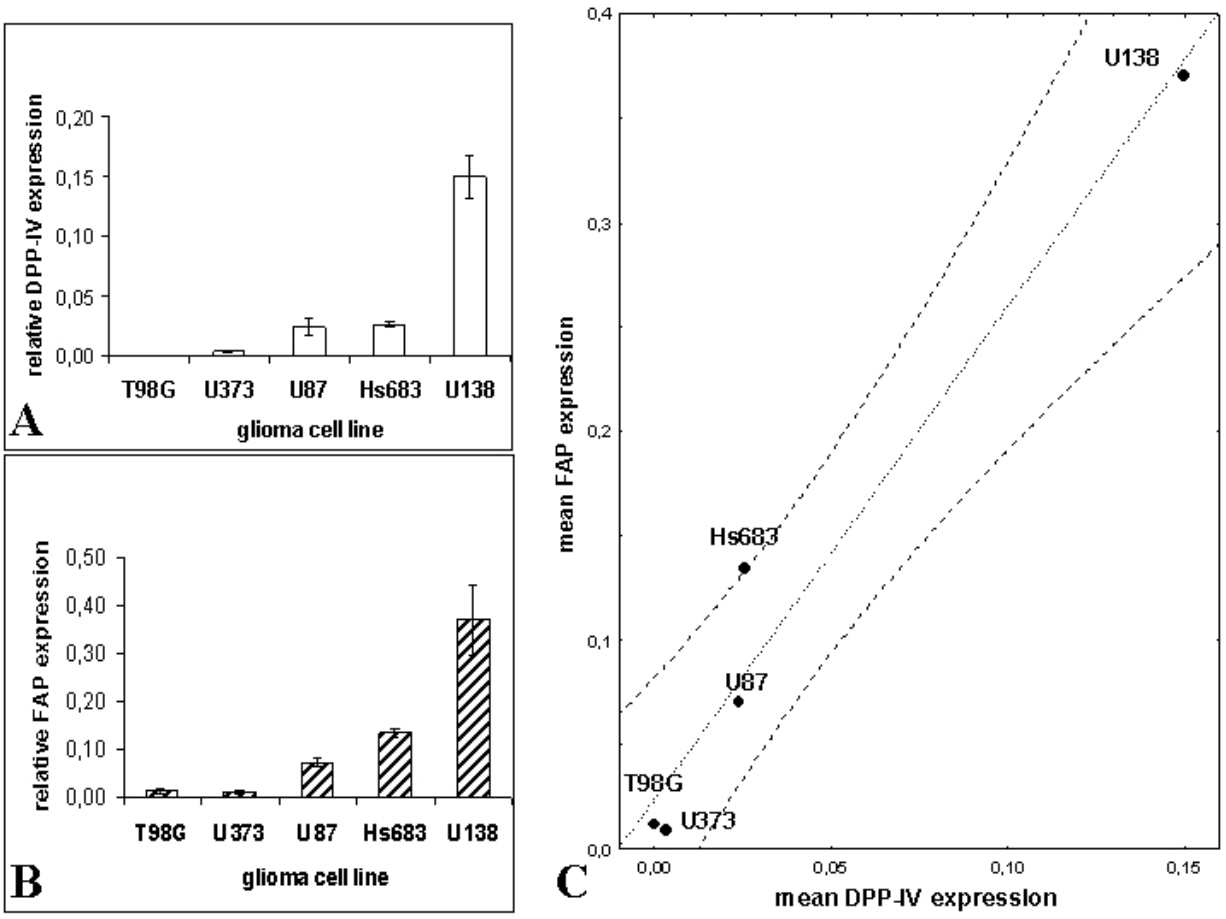

Figure 1. Expression of DPP-IV and FAP transcripts positively correlates in human glioma cell lines. Expression of DPP-IV (panel A) and FAP (panel B) mRNA was assessed using real time RT-PCR with normalization of the values to human beta-actin mRNA. Data are presented as means ± SEM of four measurements. Correlation between the expressions in different glioma cell lines ($r = 0.9$, $p < 0.05$) was assessed by Spearman coefficient using Statistica 7.0 software, regression curve and 95% confidence intervals are shown (panel C).

4. DPP-IV ENZYMATICALLY ACTIVE MOLECULES IN HUMAN BRAIN AND BRAIN TUMORS

Reports on the expression and possible function of DASH in the central nervous system are scarce and mostly concentrate on DPP-IV and DPP-II. DPP-IV as well as its activity has been detected in the capillaries and meninges in rat (41-44) and on certain neuronal structures in rat and mouse (45). Biochemical studies also suggest its presence in various brain regions in rat and guinea-pig (46, 47). A number of studies with DPP-IV inhibitors and DPP-IV deficient animals implicate DPP-IV in the regulation of nociception and behavior possibly via metabolism of biologically active peptides such as SP, endomorphin-2 and NP Y (48-50). To our knowledge only a report by Bernstein *et al* (51) described the presence of DPP-IV in the human brain with abundant expression in the developing central nervous system and a decrease in adults.

DP-II (for review cf. 52) was detected in brain homogenates (53) and histochemically in specific neuronal populations in rat brain by Gorenstein *et al* (54) with no staining over glia. However, later studies demonstrated presence of DPP-II in glial cells (55), linked it to astrocyte differentiation and described the decrease of its activity during maturation of the rat brain (56). DPP-II was also described in neurons, pericytes and ependymal cells in the spinal cord in rat (57). According to Frerker *et al* (24), DPP-II with a significant contribution of DPP8/9 may in fact constitute the majority of DPP-IV-like enzymatic activity in rat brain.

We detected DPP-IV-like enzymatic activity in homogenates of non-tumorous human brain, astrocytic and non-astrocytic tumors (58). Using real time RT-PCR and immunochemistry we observed very low expression of DPP-IV and FAP on mRNA and protein levels in non-tumorous brain. Selective inhibitors showed that the majority of DPP-IV-like enzymatic activity in non-tumorous brain could be attributed to DPP8/9, which are thought to be localized intracellularly. However, DPP-IV and FAP mRNA and protein were upregulated and the DPP-IV-like enzymatic activity was increased in high-

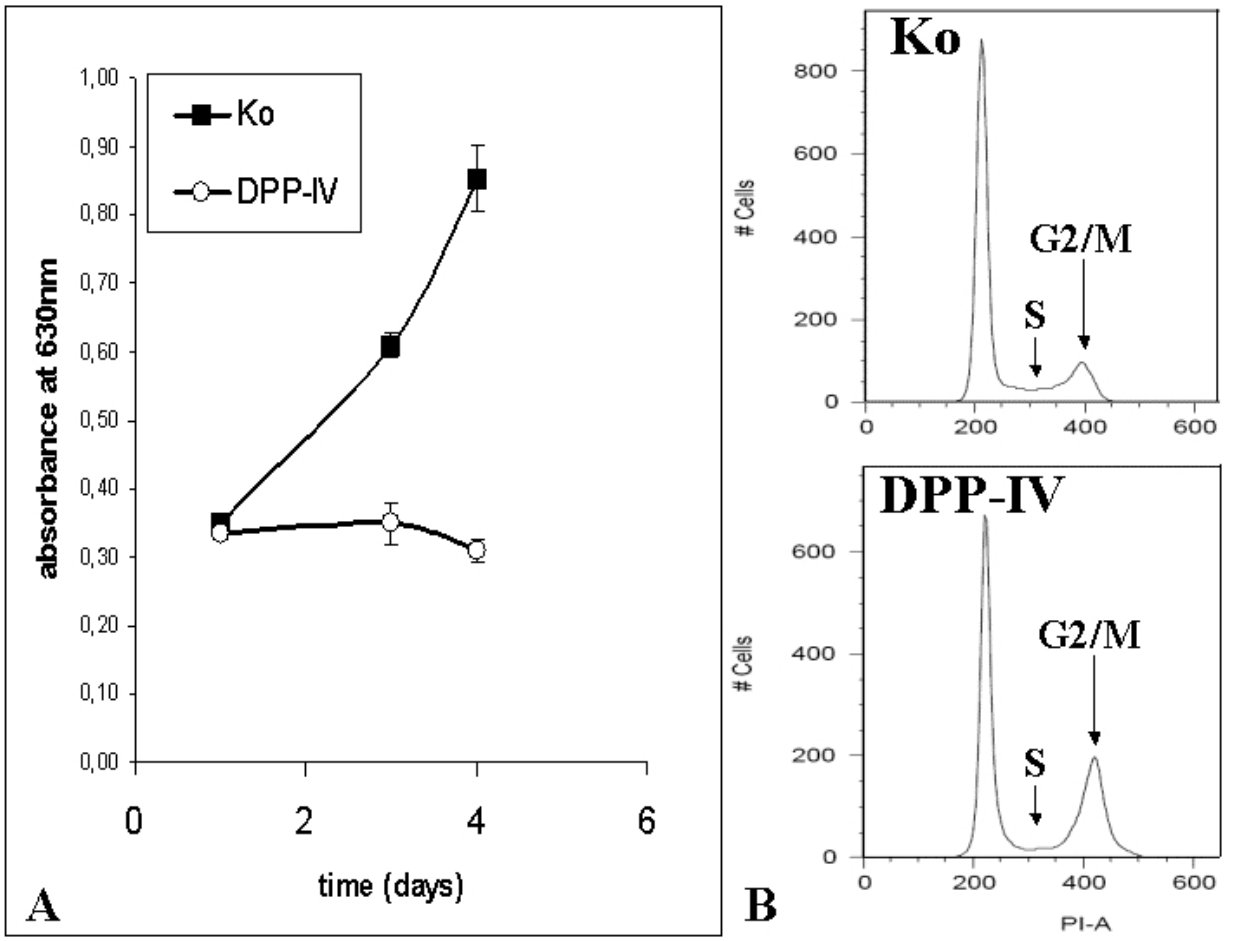

Figure 2. Effect of DPP-IV overexpression on the proliferation of T98G cells. Growth curves (A) and cell cycle analysis (B) of DPP-IV transfected T98G cells without (Ko) and after induction of DPP-IV expression (DPP-IV). T98G cells were transfected with DPP-IV using a mifepristone-inducible expression system (GeneSwitch, Invitrogene). For growth curve construction, cells were fixed and stained with methylene blue followed by colorimetric quantification. Flow cytometric cell cycle analysis was performed 48h after induction of DPP-IV expression. The expression inducing agent mifepristone had no effect on the proliferation of non-transfected cells. Results of a typical experiment are shown. S, G2/M- cells in the S and G2/M phase of the cell cycle, respectively.

grade gliomas compared to non-tumorous brain. Similarly to glioma cell lines (see above), the expression of DPP-IV and FAP positively correlated in tumor tissues. We did not observe a significant difference in contribution of DPP8/9 to the DPP-IV enzymatic activity between non-tumorous and tumorous tissue based on biochemical studies with specific DPP8/9 inhibitors. Our data suggest that a substantial part of the DPP-IV-like enzymatic activity increase in gliomas was due to an upregulation of DPP-IV and possibly FAP (58). This may be seen as contradictory to the antioncogenic properties of DPP-IV observed in several transformed cell lines as mentioned above (section 3.). However, the cellular source of upregulated DPP-IV and FAP in gliomas remains unclear.

Only marginal enzymatic activity in the non-tumorous as well as in the tumorous human brain was detected at the acidic pH 5.5, which suggests that DPP-II does not significantly contribute to the overall DPP-IV-like enzymatic activity in human brain and astrocytic tumors (58).

5. CONCEIVABLE INTERACTION OF DPP-IV WTH THE SDF-1ALPHA- CXCR4 AXIS IN GLIOMAS

Several soluble mediators susceptible to DPP-IV cleavage have been described to promote the malignant behavior of glioma cells (37, 38). SDF-1alpha has been implicated in glioma cell growth, survival, migration and invasion, as well as angiogenesis. Its receptor CXCR4 is abundantly and grade-dependently expressed in gliomas *in vivo* (39, 59-64). DPP-IV is known to effectively cleave SDF-1alpha (65). The functional consequences of SDF-1alpha cleavage by DPP-IV have mostly been documented in hematopoetic system (66-68). In addition, Mizokami *et al* (69) demonstrated that DPP-IV might hamper the growth

of endometrial carcinoma cells probably due to locally decreased availability of SDF-1alpha.

Similarly to Rempel *et al* (60) we observed a tumor grade-related rise in expression of CXCR4 mRNA and protein in human astrocytic tumors (58). We speculate that in high-grade gliomas, upregulated DPP-IV might trim down SDF-1alpha signaling, which may be compensated by the increase in CXCR4. This would favor proliferation of glioma cell populations capable to effectively raise their CXCR4 expression. Indeed, our preliminary data show tight positive correlation between CXCR4 and DPP-IV expression ($r = 0.89$, $p < 0.01$, $N = 8$) in patients with survival under 6 months while no significant correlation ($r = 0.55$, $p > 0.1$, $N = 9$) is present in patients surviving more than one year after surgery (unpublished results). Interestingly, there is not a significant difference in CXCR4 or DPP-IV expression between both groups. This suggests that not the absolute value, but rather a relative ratio of CXCR4 and DPP-IV expression may influence glioblastoma progression.

6. CONCLUSIONS AND PERSPECTIVES

An imbalance of extracellular proteolysis has been demonstrated to be a general hallmark of malignancy (70). Altered proteolytic equilibrium, affecting processing of structural and regulatory proteins within the tumor microenvironment, has multiple downstream projections including regulation of neovascularization, modulation of cancer cell proliferation, migration and invasion.

Altered DPP-IV-like enzymatic activity has been observed in numerous tumors and consequently several roles have been proposed for DPP-IV in cancer pathogenesis. However, the overall DPP-IV-like activity frequently encompasses hydrolytic potential of several coexpressed DASH molecules. It is becoming evident that it is more likely the complex expression pattern of DASH molecules in context with available bioactive substrates and their receptors, which have to be considered to interpret the results of functional studies (36). This broader view may help explain the seemingly contradictory roles of DPP-IV and FAP, which can both act as either tumor suppressors or promoters depending on the tumor type (26).

Plasma membrane localization and slightly alkaline pH optima make the canonical DPP-IV and FAP the most serious candidates among other DASH molecules for proteolytic processing of humoral mediators and for interaction with extracellular matrix. Similarly to several other cancer cell types (34, 35, 71), DPP-IV seems to have a rather antiproliferative effect on glioma cells *in vitro*. This might be due to the locally increased degradation of soluble mediators such as SDF-1alpha or SP. Surprisingly, we observe an increase of DPP-IV like enzymatic activity attributable to DPP-IV or FAP in high-grade gliomas compared to non-tumorous human brain. However, it should be considered that the local proteolytic milieu, endowing tumors with their growth and progression potential, is determined both by transformed and stromal cells. Thus, although DPP-IV might represent “bad guy” for transformed cells themselves, it could still be beneficial to other cell populations within the tumor with a resultant net pro-oncogenic effect.

DPP-IV enzymatic activity is a promising therapeutic target as has been demonstrated by the recent FDA approval of DPP-IV inhibitors for the treatment of diabetes (72) and a number of patents claiming the use of these inhibitors in autoimmune diseases, cancer or stimulation of hematopoiesis. Although few side effects have been reported for the clinically tested DPP-IV inhibitors so far, the severe toxicity of DPP-8 and 9 inhibitors in preclinical studies (73) remains a concern. The objections for DPP-IV targeting in clinical settings might come from two possible mechanisms of adverse effects: (i) Structurally related targets – DASH molecules - might been involved in pathogenesis of different diseases and/or separate metabolic pathways. Since biological functions have not yet been assigned to all DASH, the undesirable consequences of unselective inhibition have to be expected. (ii) Individual DASH molecule may be involved in multiple physiological processes throughout the body. For example, DPP-IV inhibitors used for the treatment of diabetes could increase the risk of promoting an already existing intestinal tumor due to sustained stimulation of tumor cells by glucagon like peptide-2, another DPP-IV substrate (74). „Doublespecific“ inhibitors, targeting specific DASH molecules in an appropriate cell, could address both above-mentioned objections.

7. ACKNOWLEDGEMENTS

The work was supported by grants GAUK 257896 89607 and MSMT 0021620808.

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Abbreviations: DASH: dipeptidyl peptidase-IV activity and/or structure homologues, DPP: dipeptidyl peptidase, FAP: fibroblast activation protein alpha,, NP Y: neuropeptide Y, RANTES: regulated on activation normal T-cell expressed and secreted, RT-PCR: reverse-transcription polymerase chain reaction, SDF-1alpha: stromal cell derived factor-1alpha, SP: substance P

Key Words: Dipeptidyl Peptidase, Glioblastoma, Brain Tumors, Chemokine, Protease, Review

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