

# Expression and enzymatic activity of dipeptidyl peptidase-IV in human astrocytic tumours are associated with tumour grade

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**Abstract.** Alterations in dipeptidyl peptidase-IV (DPP-IV) enzymatic activity are characteristic of malignant transformation. Through its well-characterized functionality in regulating the activity of bioactive peptides by removal of the N-terminal dipeptide, DPP-IV activity may have profound effects upon metastatic potential and cell growth. Although DPP-IV/CD26 (EC 3.4.14.5) is the canonical representative of the group, a number of other proteins including DPP-7, 8, 9, and seprase/fibroblast activation protein- $\alpha$  (FAP- $\alpha$ ) have been shown to have similar enzymatic activity. This study was set up to address the relative representation and enzymatic activity of plasma membrane localized DPP-IV/CD26 and FAP- $\alpha$  in human brain and astrocytic tumours. In parallel, expression of CXCR4, receptor for glioma cell growth stimulator chemokine SDF-1 $\alpha$  known to be a DPP-IV substrate, was investigated. This is the first report showing that non-malignant brain tissue contains a DPP-IV-like enzymatic activity attributable mostly to DPP-8/9, while the substantial part of the activity in glioma is due to increased DPP-IV/CD26, localized in both the vascular and parenchymal compartments. DPP-IV enzymatic activity increased dramatically with tumour grade severity. A grade-related increase in CXCR4 receptor paralleled the rise in DPP-IV expression and activity. These data might support a role for DPP-IV regulation of the CXCR4-SDF-1 $\alpha$  axis in glioma development.

## Introduction

Malignant gliomas, characterized as highly proliferative and locally invasive neoplasias, account for more than 50%

of central nervous system tumours. Despite intensive basic research and efforts in the fields of therapeutic regimens and modalities, prognosis of patients suffering from these tumours remains dismal. A major area of current investigation is identification of alterations in mRNA and protein expression in glioma cells that might contribute to gliomagenesis and also be amenable as therapeutic targets.

Dipeptidyl peptidase-IV (DPP-IV/CD26) is a multifunctional, typically plasma membrane localized protein, widely distributed in various cell systems. It has been for years believed to be a unique protease cleaving peptides and proteins with a proline residue on the penultimate position from their N-terminal. Subsequently, other 'DPP-IV activity and/or structure-homologues' (DASH), were discovered (1). Together with CD26, the canonical DPP-IV, enzymatically active DASH include plasma membrane fibroblast activation protein- $\alpha$  (FAP- $\alpha$ ) and the intracellular DPPs-7, 8, 9. Because of their similar enzymatic activity, it is tempting to speculate on their participation in or interference with biological function(s) previously attributed solely to DPP-IV/CD26 although relative activity will be a function of local DASH involvement. Among the multiple physiological and pathological functions so far described, DPP-IV may play a critical role in immunoregulation as well as in carcinogenesis (2-5). In contrast to the proteases involved in structural protein degradation during malignant progression, plasma membrane associated DPP-IV/CD26 probably function dominantly as the regulators of humoral signaling. As such, they participate in postsecretory processing of biologically active peptides implicated in control of cell growth, migration and invasion. Thus, although some biological functions of DPP-IV seem to be independent of its intrinsic hydrolytic activity (6), limited proteolysis of DASH substrates such as substance P, neuropeptide Y, and RANTES and SDF-1 $\alpha$  chemokines appears to be critical for quantitative and also qualitative changes of their signaling potential (5).

Chemokine SDF-1 $\alpha$  together with its cognate receptor CXCR4 is functionally involved in several cancer progression-related events including chemotaxis, invasion, adhesion and vascularization (7,8) in a variety of malignant tumours (9-11). In glioma cells, CXCR4 represents the major chemokine receptor mediating their survival (12,13). DPP-IV enzymatic

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activity is responsible for cleavage of SDF-1 $\alpha$  at its amino-terminal consensus sequence. Truncation of SDF-1 $\alpha$  by DPP-IV-like activity inhibits chemotactic activity, while the resulting fragment may even act as an antagonist of CXCR4 (14). Co-distribution of DPP-IV with CXCR4 and their SDF-1 $\alpha$  induced internalization has been observed in human lymphocytes and in several cell lines (15). Such orchestrated regulation of DPP-IV hydrolytic potential within the CXCR4-SDF-1 $\alpha$  regulatory axis might serve as an example of the putative regulatory 'DASH system' (5), consisting of plasma membrane localized DPP-IV enzymatic activity-bearing molecules, their biologically active autocrine/paracrine substrates and relevant receptors.

While DPP-IV has been studied in numerous cancer types, there are no reports of DPP-IV activity in human brain and astrocytic tumours, despite the importance of SDF-1 $\alpha$  CXCR4 in glioma development and DPP-IV regulation of SDF-1 $\alpha$  activity. In this study, we ascertain the relative representation and enzymatic activities of DPP-IV-like proteases in normal brain and astrocytomas of varying grades, and relate this to the presence of SDF-1 $\alpha$  receptor CXCR4.

## Materials and methods

**Patients, sample preparation and characterization.** Tumour specimens were collected from 31 patients undergoing astrocytic tumour resection. Tumours were graded in compliance with the current WHO Classification Criteria. Non-tumorous brain specimens were obtained from eleven patients in whom brain surgery was performed for drug-resistant temporal lobe epilepsy (Table I). Tissue samples, clear of macroscopic vessels and necrosis, were frozen on solid CO<sub>2</sub> and then stored at -80°C. Vascularization and necrotic areas in paraffin sections were scored semi-quantitatively on a 5-titered scale (0-4 crosses) and expressed as the average calculated from the individual values of each optical field determined. Written informed consent was obtained from the patients before their entry into the study, according to the guidelines of institutional Ethics Committee.

**Isolation and quantification of total RNA.** Total RNA was isolated using the TRIzol Reagent (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Spectrophotometric analysis, carried out in 10 mM Tris-HCl buffer, pH 7.5, confirmed that the samples of total RNA had an A<sub>260nm</sub>/A<sub>280nm</sub> ratio >1.8. The concentration of total RNA was determined using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR, USA).

**Real-time RT-PCR.** Gene coding region-specific oligonucleotide primers and fluorogenic TaqMan probes for the real-time RT-PCR assays of expression of the investigated transcripts were designed with the program Primer Express (Applied Biosystems, Foster City, USA) and were synthesized at Proligo (Paris, France) and Applied Biosystems (Warrington, UK), respectively. The sequences and final concentrations of the primers and probes that were used in the real-time RT-PCR assays are indicated in Table II. The expression of DPP-IV, FAP- $\alpha$ , and CXCR4 mRNAs and  $\beta$ -actin mRNA (internal reference transcript) was quantified by coupled

Table I. Characterization of the experimental cohort.

Diagnosis	Patient code	Age	Sex	Vascularisation	Necrosis
Control	26	46	F	1	0
	27	29	F	1	0
	43	44	M	0	0
	45	43	M	0	0
	46	54	F	0	0
	48	34	F	0	0
	49	30	M	1	0
	50	50	M	0	0
	53	40	F	0	0
	7	72	M	ND	ND
Grade II	5A	34	M	0	0
	5	34	M	1	0
	11	34	F	1	0
Grade III	19	34	F	1	0
	2	25	M	1	0
	15	68	M	1	1
	16	52	M	2	1
Grade IV	55	33	M	2	0
	1	70	M	2	2
	3	53	F	2	0
	4	52	F	2	1
	6	54	F	2	1
	9	59	F	1	0
	10	48	F	3	2
	12	57	F	3	2
	13	32	M	3	1
	14	70	M	3	1
	22	49	M	3	3
	25	67	F	2	2
	28	72	F	2	1
	29	67	F	2	1
	31	46	M	3	3
	32	65	F	3	1
	33	62	M	2	4
	35	73	F	2	1
	36	32	F	2	4
	37	32	F	ND	ND
	39	33	F	2	0
	40	55	M	1	3
	42	57	F	2	0
	44	61	M	2	1
	47	66	F	2	2

ND, not determined.

Table II. Primers and TaqMan probes used for real-time RT-PCR quantitation of expression of the investigated transcripts.

Transcript	GeneBank accession no.	Sequences and final concentration of primers and TaqMan probes
DPP-IV	NM_001935	Forward primer: 5'-TGGAAGGTTCTTCTGGGACTG-3', 200 nM Reverse primer: 5'-GATAGAATGTCCAACTCATCAAATGT-3', 200 nM TaqMan probe: 5'-(6-FAM)CACCGTGCCCGTGGTTCTGCT(TAMRA)-3', 200 nM
FAP- $\alpha$	NM_004460	Forward primer: 5'-TGCCACCTCTGCTGTGC-3', 200 nM Reverse primer: 5'-GAAGCATTCACACTTTTCATGGT-3', 200 nM TaqMan probe: 5'-(6-FAM)TGCAATTGTCTTACGCCCTTCAAGAGTTC(TAMRA)-3', 200 nM
CXCR4	NM_001008540	Forward primer: 5'-CATGGGTTACCAGAAGAACTGA-3', 400 nM Reverse primer: 5'-GACTGCCTTGCATAGGAAGTTC-3', 400 nM TaqMan probe: 5'-(6-FAM)CACCTGTCACTGGCCGACCTCCT(TAMRA)-3', 200 nM
$\beta$ -actin	NM_001101	Forward primer: 5'-CTGGCACCCAGCACAATG-3', 200 nM Reverse primer: 5'-GGGCCGGACTCGTCATAC-3', 200 nM TaqMan probe: 5'-(VIC)AGCCGCCGATCCACACGGAGT(TAMRA)-3', 200 nM

real-time RT-PCR assay. The RT-PCR reaction mixtures of a total volume of 50  $\mu$ l contained 25  $\mu$ l of ThermoScript Reaction Mix (a buffer with 3 mM MgSO<sub>4</sub> and 0.2 mM of each dGTP, dCTP, dATP and dTTP) and 1  $\mu$ l of ThermoScript Plus Reverse Transcriptase/Platinum *Taq* DNA polymerase Mix (Platinum Quantitative RT-PCR ThermoScript One-Step System, Invitrogen), the respective gene-specific primers and TaqMan probe at the indicated final concentrations (Table II), 40 U of RNase inhibitor RNaseOUT (Invitrogen), and 200 ng of total RNA. The final concentrations of the primers and TaqMan probe adopted for real-time RT-PCR quantification of each indicated transcript were determined in optimization experiments. The real-time RT-PCR assays were run in duplicate in MicroAmp Optical 96-well Reaction Plates on the ABI PRISM 7700 Sequence Detection System using Sequence Detection System software (Applied Biosystems). The reverse transcription was carried out at 58°C for 30 min and the subsequent PCR amplification included a hot start at 95°C for 5 min, 45 cycles of denaturation at 95°C for 15 sec and of annealing/extension at 58°C for 1 min. The threshold cycle ( $C_T$ ) values of the amplification reactions, represented by the plots of background-subtracted fluorescence intensity ( $\Delta FI$ ) of the reporter dye (6-FAM or VIC) against PCR cycle number, were determined with the Sequence Detection System software. The statistical difference of the  $\beta$ -actin mRNA-normalized target transcript expression in tumour and normal tissues was calculated from the linearized  $\Delta C_T$  data (i.e.  $2^{-\Delta C_T}$ ) and the tumour/normal ratio of the  $\beta$ -actin mRNA-normalized target transcript expression was calculated by means of the  $2^{-\Delta \Delta C_T}$  method (16).

**DPP-IV enzymatic activity biochemical assay.** Tissue samples were homogenized in ice-cold phosphate buffered saline (PBS), pH 6.0, with an Ultra-Turrax homogenizer fitted with an S8N-5G probe (IKA, Staufen, Germany) and used

for assay immediately. DPP-IV-like enzymatic activities were determined by continuous rate fluorimetric assay (17) with 7-(Glycyl-L-Prolylamido)-4-methylcoumarin (Gly-Pro-NHMeC) and with H-7-(L-Lysyl-L-alanylamido)-4-methylcoumarin (Lys-Ala-NHMeC) (Bachem AG, Switzerland) as substrates respectively at a final concentration of 50  $\mu$ M. The assays were performed in PBS of pH 7.4 and in citrate/Na<sub>2</sub>HPO<sub>4</sub> buffer of pH 5.5. The release of 4-Amino-7-methylcoumarin was monitored at 380-nm excitation and 460-nm emission wavelengths (spectrofluorimeter Perkin-Elmer LS50B, USA). Selective DPP-IV, DPP-II and DPP8/9 inhibitors (Ferring Pharmaceuticals, UK) were used at the final concentration of 250 nM. Total protein concentration in samples was assayed according to Lowry (18).

**DPP-IV enzymatic activity histochemistry.** DPP-IV-like catalytic histochemistry was performed according to Lojda (19) in 10- $\mu$ m cryostat sections cut at -20°C (Bright Instrument Company Ltd., Huntingdon, UK). The sections were fixed in a 1:1 mixture of acetone and chloroform for 2 min at 4°C and were incubated with Gly-Pro-4-methoxy- $\beta$ -naphthylamide ( $0.83 \times 10^{-3}$  M) as a substrate (Bachem, Bubendorf, Switzerland) and Fast Blue B in PBS of pH 7.4 at 4°C. In controls, the DPP-IV substrate was omitted from the incubation medium.

**Immunohistochemistry.** Detection of DPP-IV/CD26, FAP- $\alpha$  and CXCR4 was performed in cryostat sections preincubated in 3% of heat-inactivated bovine foetal serum for 20 min, followed by overnight incubation at 4°C with the respective primary antibodies: mouse monoclonal anti-human CD26 (1:100, clone M-A261), rabbit polyclonal anti-human CXCR4 (1:200) from Acris, Germany and mouse monoclonal anti-human FAP- $\alpha$  (1:200, clone F11-24, Alexis Biochemical, USA). This was followed by incubation with anti-mouse- (1:200, Sigma, USA) or anti-rabbit (1:200, Sigma, USA)

Table III. Correlations of clinical, morphological and biochemical characteristics of human astrocytic tumours and non-tumorous brain tissues.

	mRNA FAP- $\alpha$	mRNA CXCR4	Total DPP-IV-like activity	Activity after DPP-IV inhibition	Activity after DPP8/9 inhibition	Vascularization	Necroses
mRNA DPP-IV	<b>0.471</b>	<b>0.617</b>	<b>0.590</b>	<b>-0.621</b>	<b>0.458</b>	<b>0.531</b>	<b>0.648</b>
mRNA FAP- $\alpha$		<b>0.522</b>	0.319	-0.327	ns	0.403	0.357
mRNA CXCR4			<b>0.639</b>	-0.387	ns	<b>0.647</b>	<b>0.657</b>
Total DPP-IV-like activity				<b>-0.452</b>	ns	<b>0.769</b>	<b>0.758</b>
Activity after DPP-IV inhibition					<b>-0.565</b>	<b>-0.452</b>	-0.356
Activity after DPP8/9 inhibition						ns	ns
Vascularization							<b>0.712</b>

Spearman's correlation coefficients; probability of correlations: ns,  $p > 0.05$ ; normal font,  $p < 0.05$ ; bold font,  $p < 0.01$ .

IgG-FITC conjugates respectively. In controls, the primary antibodies were omitted from the medium.

The enzyme histochemistry and the immunohistochemistry sections were mounted in antifading Gel/Mount (Biomedica Corp., USA) and examined by transmission or fluorescence microscopy (Axiophot, Zeiss-Opton, Germany). Staining intensity was scored semi-quantitatively on a subjectively determined 5-titered scale (0-4 crosses) relative to the negative staining controls, averaged from 10 segments of 2-3 non-sequential histological sections.

**Statistical analysis.** Statistica 6.0 software (StatSoft, Inc. Tulsa, OK, USA) was used to perform statistical calculations. Differences between groups were evaluated with Mann-Whitney test; correlations were analyzed by means of Spearman's correlation coefficient (Table III).

## Results

Co-expression of both plasma membrane-bound DPP-IV and FAP- $\alpha$ , together with the robust expression of chemokine receptor CXCR4 mRNA was observed in all tissue samples analyzed. Although there were significant relative variations between tumours a significant upregulation of both DPP-IV and FAP- $\alpha$  mRNA expression was found in WHO grade IV glioma (Fig. 1). Furthermore, expression of DPP-IV and FAP- $\alpha$  transcripts was significantly correlated (Table III). Expression level of CXCR4 mRNA tightly correlated with the WHO glioma grade as well and with both DPP-IV and FAP- $\alpha$  mRNA expression (Table III).

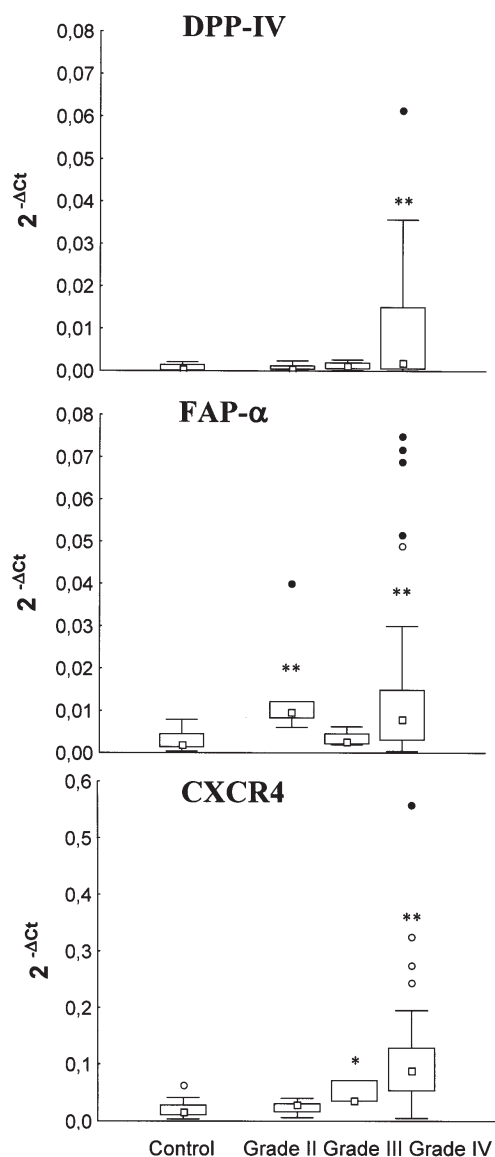


Figure 1. Expression of DPP-IV, FAP- $\alpha$  and CXCR4 mRNA in glioma. Squares, medians; boxes, middle 25-75% of measured values; bars, minimal resp maximal values;  $\circ$ , remote values;  $\bullet$ , extreme values; p, compared to controls; \* $p < 0.05$ , \*\* $p < 0.01$ ; Mann-Whitney U test.

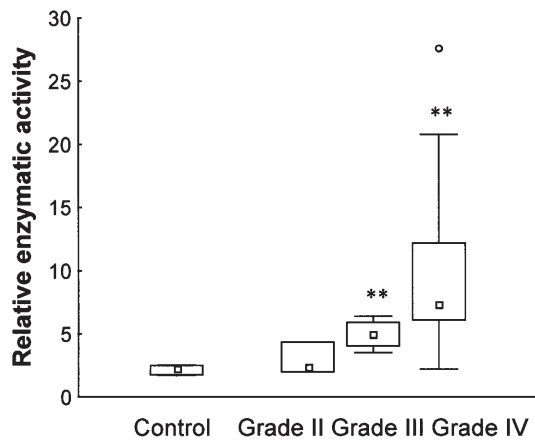


Figure 2. DPP-IV-like enzymatic activity per mg of protein in glioma. Squares, medians; boxes, middle 25-75% of measured values; bars, minimal resp maximal values; ○, remote values; p, compared to controls; \*\*p<0.01; Mann-Whitney U test.

Although DPP-IV-like enzymatic activity varied within the individual tumours, it was significantly higher in high-grade tumours compared to the non-malignant brain tissues (Fig. 2). The entire DPP-IV-like enzymatic activity correlated with FAP- $\alpha$  and even more significantly with DPP-IV/CD26 mRNA expression. In addition, DPP-IV-like enzymatic activity tightly correlated with CXCR4 mRNA expression (Table III).

To assess the relative participation of plasma membrane associated DASH molecules to the whole DPP-IV-like enzymatic activity present in the tissue samples, inhibition studies using selective inhibitors were performed. Using an inhibitor with 60-fold higher potency for DPP-IV/CD26 than for DPP8/9 (20), the DPP-IV-like enzymatic activity was more profoundly inhibited in astrocytic tumours of all WHO grades than that in non-malignant brain (Fig. 3). In contrast, no such differential inhibition was seen using an inhibitor possessing 50-fold higher potency for DPP8/9 than for

DPP-IV (Fig. 3). Residual enzymatic activity after DPP8/9 inhibition correlated positively with mRNA expression of DPP-IV/CD26 (Table III). In line with that, the residual enzymatic activity after DPP-IV inhibition correlated inversely with both total DPP-IV-like enzymatic activity and with DPP-IV/CD26 mRNA expression (Table III). Together, results of expression and inhibition studies suggest the major part of DPP-IV-like hydrolytic activity is carried by intracellular DPP8/9 in non-malignant tissues as well as in gliomas irrespective of their WHO grade. In contrast with that, enzymatic activity of plasma membrane associated DPP-IV and FAP- $\alpha$  seem to grow gradually along with the tumour WHO grade.

Inhibitor of DPP-II, which is intracellular and prefers an acidic pH, was devoid of virtually any effect on the enzymatic assays with Gly-Pro-NHMe as a substrate at pH 7.5, suggesting the lack of DPP-II contribution to the overall DPP-IV-like enzymatic activity. In addition, the DPP-II inhibitor negligibly affected enzymatic reactions with Lys-Ala-NHMe run at pH 5.5, i.e. in conditions typically preferred by DPP-II (not shown).

To characterize the tissue distribution of DPP-IV/CD26, FAP- $\alpha$  and CXCR4, enzymatic activity histochemistry and immunohistochemistry analyses were carried out. DPP-IV-like activity as well as DPP-IV/CD26, FAP- $\alpha$  and CXCR4 proteins in non-tumorous brain are either absent or present at trace levels except some solitary segments of the capillary/vascular bed, as best evident for DPP-IV enzymatic activity histochemistry, also in the surrounding pericyte-like cells. In contrast, significant up-regulation of DPP-IV enzymatic activity (Fig. 4) and expression of DPP-IV/CD26, FAP- $\alpha$  and CXCR4 proteins (Table IV) is evident in both vascular and parenchymal tissue compartments of WHO grade IV tumours (Fig. 5). In most tumour samples substantial intra-specimen variation in staining density was observed for all molecules studied.

Extent of neovascularization and necrosis represent morphological indicators used for astrocytoma tumour grading. Expected positive correlation between the tumour

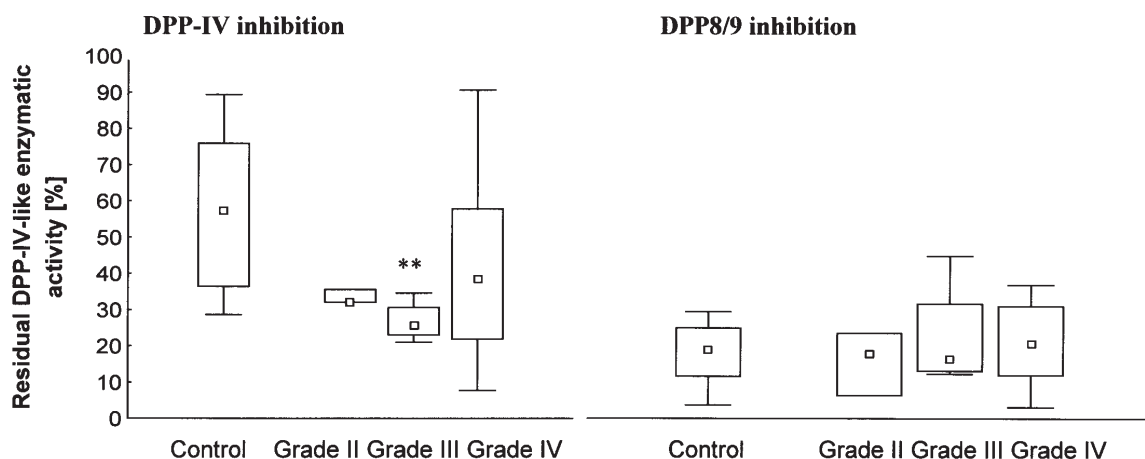


Figure 3. Residual DPP-IV-like enzymatic activity after DPP-IV and DPP8/9 inhibition. Squares, medians; boxes, middle 25-75% of measured values; bars, minimal resp maximal values; p, compared to controls; \*p<0.05; Mann-Whitney U test.

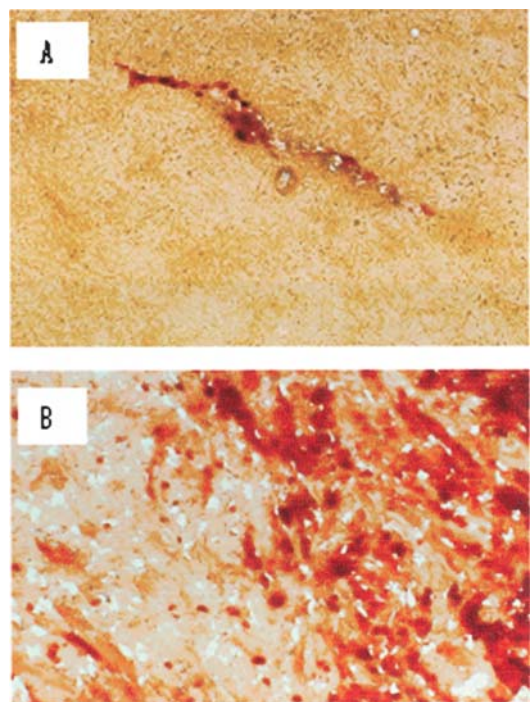


Figure 4. Localization of DPP-IV in non-tumorous (A) and glioblastoma WHO grade IV (B) tissues by enzymatic activity histochemistry. Original magnification x200.

Table IV. Enzymatic activity histochemistry<sup>a</sup> and immuno-histochemistry<sup>b</sup> of DPP-IV, FAP- $\alpha$ , and CXCR4 in human astrocytic tumours and non-tumorous brain tissues.

	DPP-IV-like <sup>a</sup>	DPP-IV/CD26 <sup>b</sup>	FAP- $\alpha$ <sup>b</sup>	CXCR4 <sup>b</sup>
Non-malignant	Traces	Traces	Traces	Traces
Grade II	++	+++	Traces	+++
Grade III	++	++++	+++	++
Grade IV	++++	+++	+++	++++

Values represent modus values of individual samples. Staining intensities were scored as shown in Materials and methods.

grade and both these parameters was indeed observed in our experimental cohort (Table III). There was a substantial degree of correlation of DPP-IV enzymatic activity reached with both vascularization and with relative occurrence of necrosis (Table III). A similar trend was observed for DPP-IV, FAP- $\alpha$  and CXCR4 mRNA expression.

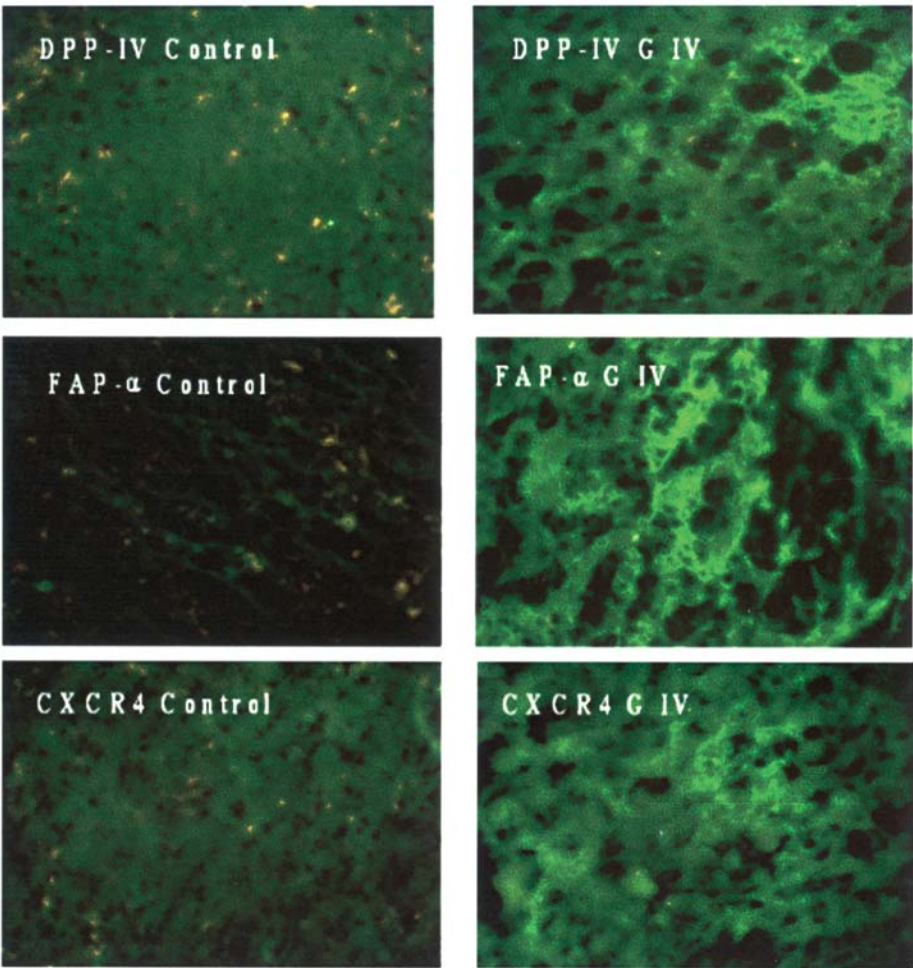


Figure 5. Detection of DPP-IV, FAP- $\alpha$ , and CXCR4 in non-tumorous and glioblastoma Grade IV tissues by immunohistochemistry. Original magnification x400.

## Discussion

Increased DPP-IV expression and activity has so far been reported in multiple types of cancer, including thyroid, skin and prostate and in some white blood cell-derived malignancies (5). In contrast, decreased DPP-IV expression was observed in melanoma, endometrial adenocarcinoma and non-small cell lung cancer cell lines (21-23). It has been suggested that the balance of inhibitory and stimulatory proteolytic regulation by DPP-IV can have profound effects upon the available soluble growth stimulatory and growth inhibitory factors within the normal microenvironment with drastic consequences if a tumorigenic event occurs (21). Altering the balance of DPP-IV enzymatic activity therapeutically may have significant beneficial effects, as has already been demonstrated in diabetes (24) and suggested for rheumatoid arthritis (4).

In this report, we established that DPP-IV activity represented by several 'Dipeptidyl peptidase-IV activity and/or structure homologues' may be an important therapeutic target for manipulating glioma growth. The observed correlation of DPP-IV and FAP- $\alpha$  mRNA expression fits well with proposed co-regulation of both gene products, where FAP- $\alpha$  upregulation is associated with transfection-induced DPP-IV expression in some cell lines (23). Though in a different cellular context, the identification of DPP-IV-FAP- $\alpha$  heterodimers (25) also indirectly points to the possible functional cooperation of both enzymes. Compared to the non-malignant brain tissue, substantial upregulation of DPP-IV/CD26 and FAP- $\alpha$  expression associated with significantly higher DPP-IV-like enzymatic activity was observed in grade IV gliomas. Moreover, results of the inhibition studies strongly suggest that plasma membrane DPP-IV/CD26 and FAP- $\alpha$  are responsible for the DPP-IV-like enzymatic activity identified in high-grade tumours. Since there is no specific inhibitor, FAP- $\alpha$  participation can not be precisely quantified, but the weaker correlation of FAP- $\alpha$  rather than DPP-IV/CD26 mRNA expression with the whole DPP-IV-like activity argues for a relatively minor contribution of FAP- $\alpha$ . Thus, plasma membrane DPP-IV/CD26 probably represents most of the DASH cleavage activity for susceptible local growth mediators in glioblastoma. Considering the marked effect of the DPP8/9 selective inhibitor, the participation of intracellularly localized DPPs 8 and 9 in astrocytic tumours is apparent. Even in non-malignant tissues the DPP8/9 activity probably represents the dominant component of all cell-associated DASH hydrolytic activity. Nonetheless, as mentioned before, the functional targets of the intracellular and extracellular DASH molecules presumably differ.

The presence of CXCR4 and SDF-1 $\alpha$  were observed across a range of tumours. The pro-malignant potential of the CXCR4-SDF1 axis was noted in the results of Luker and Luker (9), who documented decreased breast cancer growth as a consequence of reduced CXCR4 expression. CXCR4 tumour grade-associated expression has already been reported in gliomas (26,27). The first evidence of a possible functional link of the two so far separately studied molecular groups, chemokine system and DASH molecules, was given by Herrera *et al* (15) who found both structural association as well as a functional relationship of CXCR4 and DPP-IV in

T-cells, where both molecules are internalized together upon the SDF-1 $\alpha$ -mediated signaling. The functional relation of DPP-IV and the CXCR4-SDF-1 $\alpha$  axis in transformed cells was later suggested by several authors (28). In this study, we identified a significant correlation between the chemokine receptor CXCR4 and DPP-IV expression and even tighter correlation between the CXCR4 expression and DPP-IV enzymatic activity in glioblastoma. The marked upregulation of CXCR4 tightly correlating with DPP-IV might be interpreted as a compensation of DPP-IV activity-caused degradation and thus decreased availability of SDF-1 $\alpha$  within the tumour microenvironment. Indeed, inverse correlation of CXCR4 and SDF-1 $\alpha$  expression with the tumour grade previously observed in human endometrial adenocarcinoma was associated with DPP-IV downregulation (22).

Our findings indicate that the DPP-IV-like enzymatic activity in human brain and glioma tissues represents conjunction of hydrolytic action of several DASH members. Moreover, the increase of the hydrolytic activity in glioblastoma compared to non-malignant tissue is probably mostly derived from upregulation of canonical DPP-IV. However, the modification of DPP-IV biologically active substrate turnover at the local level might be counterbalanced by consequent regulation of their receptors. Nevertheless, our observations open up the possibility that pharmacological regulation of DPP-IV-mediated modulation of SDF-1 $\alpha$  function may alter glioma cell migration, providing insight into downstream therapeutic modalities.

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