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Modulation of Substance P Signaling by Dipeptidyl Peptidase-IV Enzymatic Activity in Human Glioma Cell Lines

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Summary

Dipeptidyl peptidase-IV (DPP-IV, CD26) is a serine protease almost ubiquitously expressed on cell surface and present in body fluids. DPP-IV has been suggested to proteolytically modify a number of biologically active peptides including substance P (SP) and the chemokine stromal cell derived factor-1α (SDF-1α, CXCL12). SP and SDF-1α have been implicated in the regulation of multiple biological processes and also induce responses that may be relevant for glioma progression. Both SP and SDF-1α are signaling through cell surface receptors and use intracellular calcium as a second messenger. The effect of DPP-IV on intracellular calcium mobilization mediated by SP and SDF-1α was monitored in suspension of wild type U373 and DPP-IV transfected U373DPPIV glioma cells using indicator FURA-2. Nanomolar concentrations of SP triggered a transient dose dependent increase in intracellular calcium rendering the cells refractory to repeated stimulation, while SDF-1α had no measurable effect. SP signaling in DPP-IV overexpressing U373DPPIV cells was not substantially different from that in wild type cells. However, preincubation of SP with the DPP-IV overexpressing cells lead to the loss of its signaling potential, which could be prevented with DPP-IV inhibitors. Taken together, DPP-IV may proteolytically inactivate local mediators involved in gliomagenesis.

Key words

Dipeptidyl peptidase • Substance P • Glioma • NK1 receptor • Calcium signaling

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Introduction

Dipeptidyl peptidase-IV (DPP-IV, CD26) is a widely expressed 240 kDa serine protease with a multitude of functions under both physiological and pathological conditions (for review see Lambeir *et al.* 2003). Its relatively restricted substrate specifity for proline or alanine in the P1 position directs its action on a number of biologically active peptides such as neuropeptide Y, substance P (SP), glucagon-like peptide-1 and -2 and a number of chemokines including stromal cell-derived factor-1α (SDF-1α, CXCL12) (Mentlein 1999, de Meester *et al.* 2000). Proteolytic removal of the two N-terminal amino acid residues by DPP-IV mostly decreases the biological activity of the corresponding peptide, while in some cases it can activate the peptide substrate or influence its binding to specific receptor subtypes (Mentlein 1999). DPP-IV is mostly expressed on cell surface and a soluble form is also present in the serum (Durinx *et al.* 2000). DPP-IV enzymatic activity can therefore affect auto-, para- as well as endocrine signaling of biologically active substances.

DPP-IV is frequently dysregulated in cancer, being significantly down-regulated or lost in some tumors and upregulated in others (Sulda *et al.* 2006). A number of DPP-IV substrates promote the malignant phenotype of cancer cells that express appropriate receptors. Thus, DPP-IV hydrolytic activity can interfere with some pro-oncogenic signaling pathways (Bušek *et al.* 2004, 2006). Indeed, Masur *et al.* (2006) have recently shown that a DPP-IV inhibitor can promote the growth stimulating and migratory effect of glucagon-like peptide-2 in DPP-IV

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positive colon cancer cell lines. Interestingly, *in vitro* studies have mostly demonstrated that artificial upregulation of DPP-IV has an antioncogenic effect (Wesley *et al.* 1999, 2004, 2005), although the mechanism remains elusive.

In brain tumors, DPP-IV substrates SP and SDF-1α trigger a number of intracellular signaling cascades that affect cell proliferation, survival, migration and invasion (Barbero *et al.* 2003, Palma and Maggi 2000, Sharif 1998). Indeed, antagonists of the corresponding receptors NK1 and CXCR4 exhibit significant antitumor activity in gliomas (Palma *et al.* 2000, Rubin *et al.* 2003). We have previously detected expression and activity of DPP-IV and possibly other molecules bearing similar enzymatic activity, e.g. fibroblast activation protein-α, dipeptidyl peptidases 8 and 9 in human glioma tumors and cell lines (Šedo *et al.* 2004, Stremeňová *et al.* 2006). Here we explore, whether DPP-IV enzymatic activity can influence signaling of selected biologically active DPP-IV substrates in glioma cell lines.

Methods

Chemicals and cell lines

Human SDF-1α was purchased from PeproTech (UK), SP, Diprotin A and Lys[Z(NO2)]-pyrrolidide were from Bachem (Switzerland). Glioma cell lines U373 and T98G (ATCC, USA) and their transfectants (U373DPPIV, T98GDPPIV) were cultured in Dulbecco's Modified Eagle's Medium with 10 % fetal bovine serum (Sigma, Czech Republic) under standard conditions. DPP-IV was transfected into U373 and T98G cells using the mifepristone inducible Gene Switch System (Invitrogen, USA). The transfectants inducibly expressed DPP-IV upon treatment with 5 nM Mifepristone (mife). Maximum DPP-IV upregulation was achieved after 24 hours in U373DPPIV and after 48 hours in T98GDPPIV cells.

Real time RT-PCR

Total RNA was isolated using the TriZol Reagent (Invitrogen, UK) according to the manufacturer's instructions. Spectrophotometric analysis, carried out in 10 mmol/l Tris/HCl buffer, pH 7.5, revealed that the samples of total RNA had an A260 nm/A280 nm ratio greater than 1.8. The concentration of total RNA was determined using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, USA).

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, USA) and were synthesized at Proligo (France) and Applied Biosystems (UK), respectively (Table 1). The expression of DPP-IV, NK1 receptor, CXCR4 and β-actin mRNA (an internal reference transcript) was quantified by coupled real time RT-PCR assays. The RT-PCR reaction mixtures of a total volume of 50 μl contained 25 μl of ThermoScript Reaction Mix (a buffer with 3 mmol/l $MgSO_4$ and 0.2 mmol/l of each dGTP, dCTP, dATP and dTTP) and 1 μl of ThermoScript Plus Reverse Transcriptase/ Platinum Taq DNA polymerase Mix (both mixes were from Platinum Quantitative RT-PCR ThermoScript One-Step System, Invitrogen), the respective gene-specific primers and TaqMan probe, 40 units of RNase inhibitor RNaseOUT (Invitrogen), and an input of 200 ng of total RNA. 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 operated by the 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 and 45 cycles of denaturation at 95 °C for 15 s and of annealing/extension at 58 °C for 1 min. The threshold cycle (Ct) values of the amplification reactions, represented by the plots of background-subtracted fluorescence intensity (ΔFI) of the reporter dye (6-FAM or VIC) against PCR cycle number, were determined with the Sequence Detection System software. Target transcript expression was normalized to β-actin mRNA expression using the ΔCt method and the linearized ΔCt (i.e. $2^{-\Delta Ct}$) was used for comparative purposes (Livak and Schmittgen 2001).

DPP-IV enzymatic activity assay

Cell surface DPP-IV enzymatic activity was assessed in suspensions of viable cells by a continuous rate fluorimetric assay using 7-(glycyl-L-prolylamido)-4-methylcoumarin (Gly-Pro-AMC; Bachem, Switzerland) as substrate at pH 7.5 and 37 °C (Šedo *et al.* 1989).

Measurement of intracellular calcium

Mobilization of intracellular Ca^{2+} was monitored in cell suspensions at 37 °C after loading cells with the ratiometric indicator FURA-2 (final concentration 4 μmol/l) for 30 min and scraping the cells gently into a Krebs buffer (120 mmol/l NaCl, 4.75 mmol/l KCl,

Table 1. 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 nmol/l Reverse primer: 5'-GATAGAATGTCCAAACTCATCAAATGT-3', 200 nmol/l TaqMan probe: 5'-(6-FAM)CACCGTGCCCGTGGTTCTGCT(TAMRA)-3', 200 nmol/l
NK1	NM_001058	Forward primer: 5'-CAGTGGTGAACTTCACCTATGCT-3', 400 nmol/l **Reverse primer: 5'-GATGTATGATGGCCATGTACCTATC-3', 400 nmol/l** TaqMan probe: 5'-(6-FAM)TCCACAACTTCTTTCCCATCGCCG(TAMRA)-3', 200 nmol/l
CXCR4	NM_001008540	Forward primer: 5'-CATGGGTTACCAGAAGAAACTGA-3', 400 nmol/l Reverse primer: 5'-GACTGCCTTGCATAGGAAGTTC-3', 400 nmol/l TaqMan probe: 5'-(6-FAM)CACCTGTCAGTGGCCGACCTCCT(TAMRA)-3', 200 nmol/l
β-Actin	NM_001101	Forward primer: 5'-CTGGCACCCAGCACAATG-3', 200 nmol/l Reverse primer: 5'-GGGCCGGACTCGTCATAC-3', 200 nmol/l TaqMan probe: 5'-(VIC)AGCCGCCGATCCACACGGAGT(TAMRA)-3', 200 nmol/l

Table 2. Expression of DPP-IV and receptors of its biologically active substrates in wild type glioma cell lines (U373 and T98G) and transfected cells (U373DPPIV and T98GDPPIV) stimulated (mife +) or not stimulated (mife -) to express DPP-IV.

	Relative cell surface DPP-IV enzymatic activity/10^6 cells	**Relative mRNA expression ($2^{-\Delta Ct}$) x10^{-3}**		
		DPP-IV	**NK1**	**CXCR4**
Wild cells				
U373	1.0 ± 0.1	3.148 ± 1.138	1.129 ± 0.338	0.627 ± 0.055
T98G	0.7 ± 0.1	0.004 ± 0.001	0.011 ± 0.003	0.286± 0.067
Transfected cells				
U373DPPIVmife -	2.4 ± 0.1	89.630 ± 17.872	2.314 ± 0.628	0.195 ± 0.067
U373DPPIV mife +	27.4 ± 0.5	663.675 ± 63.162	5.097 ± 1.062	0.665 ± 0.095
T98GDPPIV mife -	1.3 ± 0.1	36.818 ± 10.271	0.143 ± 0.083	0.078 ± 0.042
T98GDPPIV mife +	543.7 ± 90.0	2444.720 ± 326.319	0.005 ± 0.001	0.074 ± 0.026

The expression of DPP-IV, NK1 receptor and CXCR4 mRNAs was normalized to the expression of human β-actin mRNA. Data are presented as means ± SEM of at least three measurements.

1 mmol/l KH_2PO_4, 5 mmol/l $NaHCO_3$, 1.44 mmol/l $MgSO_4$, 1.1 mmol/l $CaCl_2$, 0.1 mmol/l EGTA, 11 mmol/l glucose, 25 mmol/l NaHEPES, 0.1 % bovine serum albumin fraction V, pH 7.4). Fluorescence was measured at 340 nm/380 nm (excitation) and 510 nm (emission) on a Perkin Elmer spectrofluorometer. Stock solution of SP was added to the cuvette with tested cells either directly or after 10-30 min of preincubation at 37 °C with T98GDPPIV cells induced or not induced to upregulate DPP-IV. Concentration of intracellular calcium was calculated using the equation $[Ca^{2+}]_i = K_d \times (R-R_{min})/(R_{max}-R) \times SFB$, where R is the emission ratio value (340 nm/380 nm). R_{max}, the maximum 340 nm/380 nm ratio, was determined by lysing the cells with 0.1 % Triton X-100 in the presence of 1 mmol/l $CaCl_2$. The R_{min} 340 nm/380 nm ratio was obtained by adding 40 mmol/l EGTA. K_d is the dissociation constant of the Fura-2/Ca^{2+} complex (225 nmol/l) and SFB is the ratio of 380 nm fluorescences under Ca^{2+}-free/Ca^{2+} saturated conditions. Trypsin, which is known to induce calcium oscillations in glioma cells (Ubl *et al.* 1998), was used at a final concentration of 10^{-4} g/l as a positive control for the above described $[Ca^{2+}]_i$ rise measuring method.

Results

To quantify the potential of our model cell lines to proteolytically process biologically active DPP-IV substrates, cell surface DPP-IV enzymatic activity was determined in wild type U373 and T98G cells and in their transfected counterparts U373DPPIV and T98GDPPIV. Cell surface DPP-IV enzymatic activity was upregulated

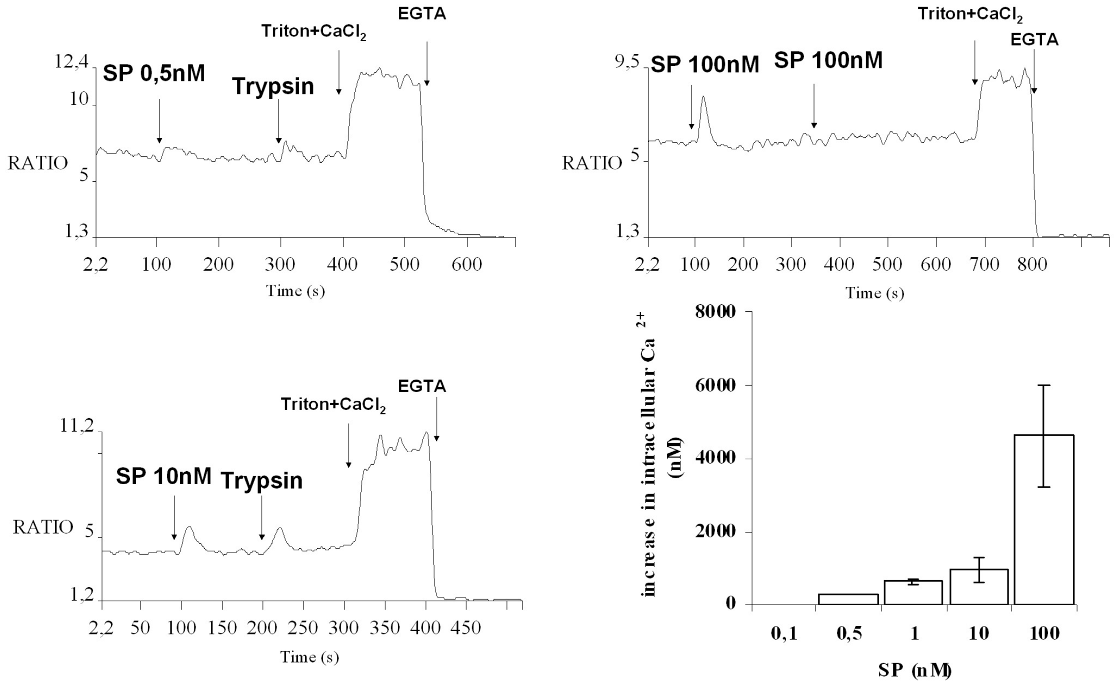

Fig. 1. Substance P triggers concentration-dependent mobilization of intracellular calcium in U373 cell line. Intracellular Ca^{2+} release was measured using the fluorescent probe Fura-2 AM. The fluorescence ratio at the two excitation wavelengths (vertical axis) is presented as an indicator of intracellular Ca^{2+} mobilization. Intracellular Ca^{2+} concentration was calculated as described in Materials and Methods; bars represent means ± SEM. SP: substance P, concentration in nmol/l [nM]

upon mifepristone stimulation 10 times in U373DPPIV and several hundred times in T98GDPPIV in comparison with the corresponding mifepristone unstimulated transfectants and wild type cells. Upregulation of DPP-IV was also confirmed at the transcriptional level (Table 2). We also determined the expression of transcripts of NK1 receptor and CXCR4, the receptors of SP and SDF-1α, respectively (Table 2).

Thereafter we tested the ability of DPP-IV substrates SP and SDF-1α to trigger calcium signaling in our model cell lines. SP caused a concentration dependent transient rise of $[Ca^{2+}]_i$ in U373 rendering the cells refractory to repeated stimulation (Fig. 1). This rise of $[Ca^{2+}]_i$ induced by SP was, however, not significantly affected by upregulation of DPP-IV in U373DPPIV cells (data not shown). To assess the possible effect of prolonged exposure of SP to high DPP-IV activity, U373 cells were exposed to SP preincubated with DPP-IV upregulating T98GDPPIV glioma cells. Such pre-treatment of SP abrogated its signaling potential (Fig 2b). SP exposed to DPP-IV upregulating T98GDPPIV cells also lost its capacity to induce the abovementioned resistance of U373 to repeated stimulation by SP not exposed to DPP-IV enzymatic cleavage (Fig. 2). On the contrary, preincubation of SP with T98GDPPIV cells not upregulating DPP-IV did not affect its potential to trigger calcium signaling (Fig. 2a). DPP-IV inhibitors Diprotin A and Lys[Z(NO2)]-pyrrolidide preserved the signaling potential of SP, which confirms that SP was inactivated specifically by DPP-IV enzymatic activity (Fig. 2d-f).

SDF-1α did not cause measurable changes of $[Ca^{2+}]_i$ in any cell line tested (data not shown).

Discussion

DPP-IV is a widely expressed serine protease that can proteolytically modify a number of biologically active peptides. It is considered to be an important regulator of SP plasma half-life (Ahmad *et al.* 1992, Wang *et al.* 1991) and has been shown to affect some SP regulated physiological processes (Grouzmann *et al.* 2002, Guieu *et al.* 2006). Functional SP receptors NK1 are widely expressed in astrocytic brain tumors and have been implicated in the regulation of their growth (Palma *et al.* 2000, Sharif 1998). NK1 receptor triggers a number of signaling cascades including elevation of $[Ca^{2+}]_i$ and activation of mitogen-activated protein kinases that can mediate the growth promoting effect of SP (Luo *et al.* 1996, Palma *et al.* 1999).

We previously detected DPP-IV enzymatic

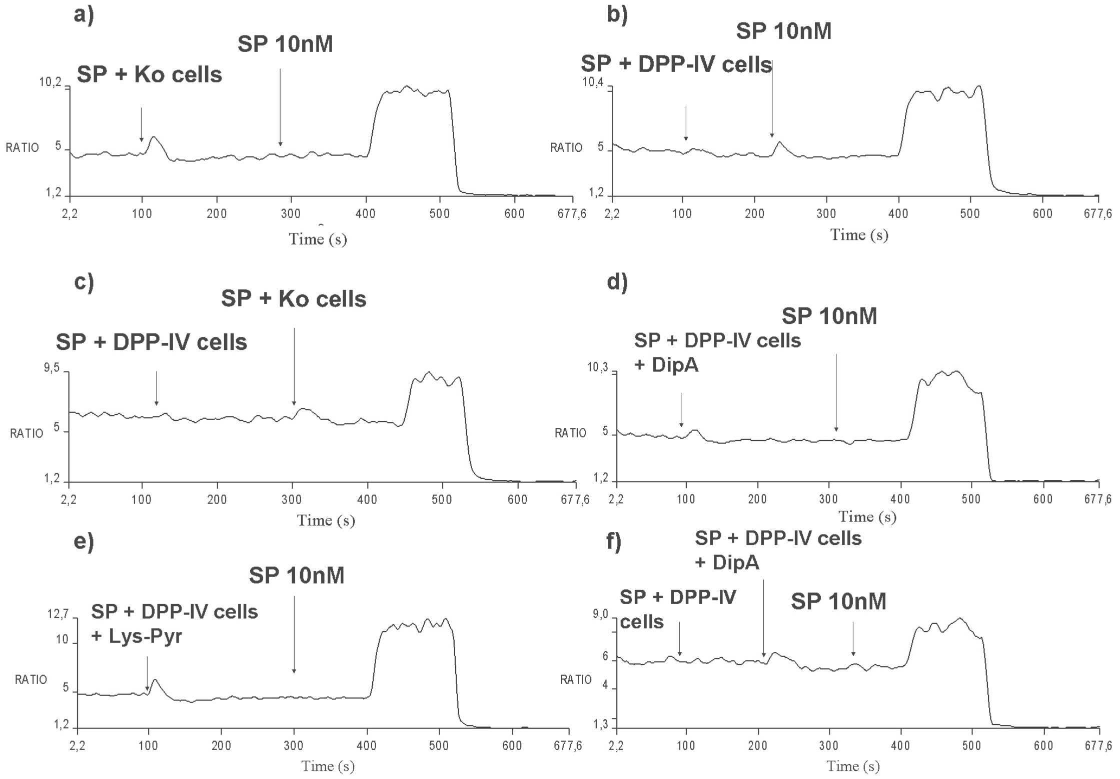

Fig. 2. Substance P loses its ability to trigger calcium signaling in U373 cells after preincubation with DPP-IV overexpressing cells. Intracellular Ca^{2+} release was monitored as described in Fig.1. Substance P (SP, 10 nmol/l [nM]) was preincubated in the suspension of T98GDPPIV cells that (a) had not been (Ko cells) or (b) had been (DPP-IV cells) induced to overexpress DPP-IV. Plots (d), (e), (f): indicated DPP-IV inhibitors DipA (200 µmol/l) and Lys-Pyr (200 µmol/l) were present in the preincubation mixture. Neither T98GDPPIV cells nor inhibitors alone induced calcium signaling in U373 cell line (data not shown). DipA: Diprotin A; Lys-Pyr: Lys[Z(NO2)]-pyrrolidide.

activity in human astrocytic tumor tissues and glioma cell lines (Šedo *et al.* 2004, Stremeňová *et al.* 2006) and observed decreased growth of DPP-IV upregulating transfectants (Bušek *et al.* 2006). Here we demonstrate that DPP-IV enzymatic activity can influence the signaling potential of SP in glioma cell lines. In agreement with the literature (Sharif 1998, Palma *et al.* 1999), SP induced Ca^{2+} signaling in U373 but not in T98G cell line, which well corresponds to the observed substantially lower expression of NK1 receptor mRNA in T98G cells (Table 2). On the contrary, SDF-1α did not cause measurable changes of $[Ca^{2+}]_i$ in any cell line tested, although we detected mRNA expression of its receptor CXCR4. However, Oh *et al.* (2001) also observed SDF-1α induced changes of $[Ca^{2+}]_i$ in glioma cells only after enhancement of CXCR4 expression by cytokines.

Calcium signaling triggered by SP in U373DPPIV cells upregulating DPP-IV was similar in duration and magnitude to that in wild type U373. This is most likely because the second messenger calcium signaling occurs within seconds while degradation of SP by cell surface DPP-IV into the less active SP5-11 fragment may require longer periods of time. The upregulation of DPP-IV was also much lower in U373DPPIV compared to T98GDPPIV, which did not express NK1 receptors (Table 2). However, it should be considered that i) complex cellular programs such as cell proliferation frequently require prolonged exposure to the particular ligand, and ii) the ligand may be subject to functionally relevant proteolytic cleavage by surrounding cells that neither secrete nor respond to it. To simulate functional interaction of two cell types in SP signaling, U373 cells were exposed to SP that had been preincubated with T98GDPPIV glioma cells overexpressing DPP-IV but lacking NK1 receptor. These experiments demonstrated functional inactivation of SP, which was prevented with specific DPP-IV inhibitors.

Cleavage of SP and other susceptible regulatory molecules may contribute to the growth inhibitory effect of DPP-IV that has been observed in several cell lines (Wesley *et al.* 1999, 2004, 2005), including glioma cells (Bušek *et al.* 2006). Our results also suggest that prereceptor modification of signaling peptides by DPP-IV enzymatic activity may be physiologically relevant even for DPP-IV negative cells. Thus, DPP-IV present in the tissue microenvironment may represent an important regulator of local humoral signaling.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

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References

AHMAD S, WANG LH, WARD PE: Dipeptidyl(amino)peptidase-IV and aminopeptidase-M metabolize circulating Substance-P in vivo. *J Pharmacol Exp Ther* **260:** 1257-1261, 1992.

BARBERO S, BONAVIA R, BAJETTO A, PORCILE C, PIRANI P, RAVETTI JL, ZONA GL, SPAZIANTE R, FLORIO T, SCHETTINI G: Stromal cell-derived factor 1 alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. *Cancer Res* **63:** 1969-1974, 2003.

BUŠEK P, KŘEPELA E, MAREŠ V, VLAŠICOVÁ K, ŠEVČÍK J, ŠEDO A: Expression and function of dipeptidyl peptidase IV and related enzymes in cancer. *Adv Exp Med Biol* **575:** 55-62, 2006.

BUŠEK P, MALÍK R, ŠEDO A: Dipeptidyl peptidase IV activity and/or structure homologues (DASH) and their substrates in cancer. *Int J Biochem Cell Biol* **36:** 408-421, 2004.

DE MEESTER I, DURINX C, BAL G, PROOST P, STRUYF S, GOOSSENS F, AUGUSTYNS K, SCHARPE S: Natural substrates of dipeptidyl peptidase IV. *Adv Exp Med Biol* **477:** 67-87, 2000.

DURINX C, LAMBEIR AM, BOSMANS E, FALMAGNE JB, BERGHMANS R, HAEMERS A, SCHARPE S, DE MEESTER I: 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* **267:** 5608-5613, 2000.

GROUZMANN E, MONOD M, LANDIS B, WILK S, BRAKCH N, NICOUCAR K, GIGER R, MALIS D, SZALAY-QUINODOZ I, CAVADAS C, MOREL DR, LACROIX JS: Loss of dipeptidylpeptidase IV activity in chronic rhinosinusitis contributes to the neurogenic inflammation induced by substance P in the nasal mucosa. *FASEB J* **16:** 1132-1134, 2002.

GUIEU R, FENOUILLET E, DEVAUX C, FAJLOUN Z, CARREGA L, SABATIER JM, SAUZE N, MARGUET D: CD26 modulates nociception in mice via its dipeptidyl-peptidase IV activity. *Behav Brain Res* **166:** 230-235, 2006.

LAMBEIR AM, DURINX C, SCHARPE S, DE MEESTER I: Dipeptidyl-peptidase IV from bench to bedside: An update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit Rev Clin Lab Sci* **40:** 209-294, 2003.

LIVAK KJ, SCHMITTGEN TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)) method. *Methods* **25:** 402-408, 2001.

LUO WH, SHARIF TR, SHARIF M: Substance P-induced mitogenesis in human astrocytoma cells correlates with activation of the mitogen-activated protein kinase signaling pathway. *Cancer Res* **56:** 4983-4991, 1996.

MASUR K, SCHWARTZ F, ENTSCHLADEN F, NIGGEMANN B, ZAENKER KS: DPPIV inhibitors extend GLP-2 mediated tumour promoting effects on intestinal cancer cells. *Regul Pept* **137:** 147-155, 2006.

MENTLEIN R: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul Pept* **85:** 9-24, 1999.

OH JW, DRABIK K, KUTSCH O, CHOI C, TOUSSON A, BENVENISTE EN: CXC chemokine receptor 4 expression and function in human astroglioma cells. *J Immunol* **166:** 2695-704, 2001.

PALMA C, BIGIONI M, IRRISSUTO C, NARDELLI F, MAGGI CA, MANZINI S: Anti-tumour activity of tachykinin NK1 receptor antagonists on human glioma U373 MG xenograft. *Br J Cancer* **82:** 480-487, 2000.

PALMA C, MAGGI CA: The role of tachykinins via NK1 receptors in progression of human gliomas. *Life Sci* **67:** 985-1001, 2000.

PALMA C, NARDELLI F, MANZINI S, MAGGI CA: Substance P activates responses correlated with tumour growth in human glioma cell lines bearing tachykinin NK1 receptors. *Br J Cancer* **79:** 236-243, 1999.

RUBIN JB, KUNG AL, KLEIN RS, CHAN JA, SUN YP, SCHMIDT K, KIERAN MW, LUSTER AD, SEGAL RA: A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci USA* **100:** 13513-13518, 2003.

ŠEDO A, BUŠEK P, SCHOLZOVÁ E, MALÍK R, VLAŠICOVÁ K, JANÁČKOVÁ S, MAREŠ V: 'Dipeptidyl peptidase-IV activity and/or structure homologs' (DASH) in growth-modulated glioma cell lines. *Biol Chem* **385:** 557-559, 2004.

ŠEDO A, KŘEPELA E, KASAFÍREK E: A kinetic fluorometric assay of dipeptidyl peptidase-IV in viable human-blood mononuclear-cells. *Biochimie* **71:** 757-761, 1989.

SHARIF M: Mitogenic signaling by substance P and bombesin-like neuropeptide receptors in astrocytic/glial brain tumor-derived cell lines. *Int J Oncol* **12:** 273-286, 1998.

STREMEŇOVÁ J, MAREŠ V, DBALÝ V, MAREK J, SYRŮČEK M, KŘEPELA E, VANÍČKOVÁ Z, VLASICOVÁ K, ŠEDO A: Regulation of dipeptidyl peptidase-IV activity and/or structure homologues (DASH) in human brain tumors: an association with WHO grade? *Physiol Res* **55:** 43P, 2006.

SULDA ML, ABBOTT CA, HILDEBRANDT M: DPIV/CD26 and FAP in cancer: A tale of contradictions. *Adv Exp Med Biol* **575:** 197-206, 2006.

UBL JJ, VOHRINGER C, REISER G: Co-existence of two types of $[Ca^{2+}]_i$-inducing protease-activated receptors (PAR-1 and PAR-2) in rat astrocytes and C6 glioma cells. *Neuroscience* **86:** 597-609, 1998.

WANG LH, AHMAD S, BENTER IF, CHOW A, MIZUTANI S, WARD PE: Differential processing of substance-P and neurokinin-A by plasma dipeptidyl(amino)peptidase-IV, aminopeptidase-M and angiotensin converting enzyme. *Peptides* **12:** 1357-1364, 1991.

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* **190:** 311-322, 1999.

WESLEY UV, MCGROARTY A, HOMOYOUNI A: Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. *Cancer Res* **65:** 1325-1334, 2005.

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* **109:** 855-866, 2004.