The International Journal of Biochemistry & Cell Biology 44 (2012) 738–747

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Dipeptidyl peptidase-IV inhibits glioma cell growth independent of its enzymatic activity

Petr Busek[a], Jarmila Stremenova[a], Lucie Sromova[a], Marek Hilser[a], Eva Balaziova[a], Dalibor Kosek[a], Jana Trylcova[a], Hynek Strnad[b], Evzen Krepela[a], Aleksi Sedo[a,*]

[a] Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague 12853, Czech Republic
[b] Department of Genomics and Bioinformatics, Institute of Molecular Genetics AS CR, Prague 14220, Czech Republic

ARTICLE INFO

Article history:
Received 9 November 2011
Received in revised form 10 January 2012
Accepted 17 January 2012
Available online 28 January 2012

Keywords:
Protease
Tumor suppression
Primary cell cultures
Astrocytoma

ABSTRACT

Malignant gliomas exhibit abnormal expression of proteolytic enzymes that may participate in the uncontrolled cell proliferation and aberrant interactions with the brain extracellular matrix. The multifunctional membrane bound serine aminopeptidase dipeptidyl peptidase (DPP)-IV has been linked to the development and progression of several malignancies, possibly both through the enzymatic and nonenzymatic mechanisms.

In this report we demonstrate the expression of DPP-IV and homologous proteases fibroblast activation protein, DPP8 and DPP9 in primary cell cultures derived from high-grade gliomas, and show that the DPP-IV-like enzymatic activity is negatively associated with their *in vitro* growth. More importantly, the DPP-IV positive subpopulation isolated from the primary cell cultures using immunomagnetic separation exhibited slower proliferation. Forced expression of the wild as well as the enzymatically inactive mutant DPP-IV in glioma cell lines resulted in their reduced growth, migration and adhesion *in vitro*, as well as suppressed glioma growth in an orthotopic xenotransplantation mouse model.

Microarray analysis of glioma cells with forced DPP-IV expression revealed differential expression of several candidate genes not linked to the tumor suppressive effects of DPP-IV in previous studies. Gene set enrichment analysis of the differentially expressed genes showed overrepresentation of gene ontology terms associated with cell proliferation, cell adhesion and migration.

In conclusion, our data show that DPP-IV may interfere with several aspects of the malignant phenotype of glioma cells in great part independent of its enzymatic activity.

1. Introduction

Gliomas rank among the deadliest human malignancies. The median survival for the most common grade IV tumors (glioblastoma multiforme) is one year despite multimodality treatment with surgery, chemotherapy and radiotherapy (Wen and Kesari, 2008). Disease recurrence is almost a rule due to the uncontrolled proliferation of glioma cells that extensively infiltrate the surrounding brain parenchyma. In addition to genetic alterations, deregulated expression of para- as well as autocrine mediators and their receptors (Hoelzinger et al., 2007), components of the extracellular matrix and proteolytic enzymes jointly contribute to the malignant phenotype of glioma cells (Rao, 2003; Louis et al., 2002; Levicar et al., 2003).

In our previous work, we detected dipeptidyl peptidase (DPP)-IV-like enzymatic activity in permanent glioma cell lines (Sedo et al., 2004) as well as in astrocytic tumors *in situ* (Stremenova et al., 2007). This enzymatic activity is an attribute of a limited number of proteases such as the canonical DPP-IV (CD26, EC 3.4.14.5) and homologous proteases fibroblast activation protein (FAP), DPP8 and DPP9 belonging to the MEROPS (http://merops.sanger.ac.uk) S9B subfamily (Sedo and Malik, 2001). The most thoroughly characterized of these molecules is DPP-IV, a multifunctional plasma membrane-bound serine dipeptidyl aminopeptidase. It is also found as a cleaved ectodomain in body fluids (Durinx et al., 2000) and is thought to proteolytically modify and thus fine tune the bioavailability and receptor binding of a large number of biologically active peptides in the pericellular space (Mentlein, 1999).

Abbreviations: DPP, dipeptidyl peptidase; FAP, fibroblast activation protein; SDF-1, stromal cell derived factor; SP, substance P; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; BSA, bovine serum albumin; MACS, magnetic cell sorting; bFGF, basic fibroblast growth factor.

* Corresponding author at: Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague, Laboratory of Cancer Cell Biology, U Nemocnice 5, 128 53 Prague 2, Czech Republic. Tel.: +420 224965748; fax: +420 224965826.
E-mail address: aleksi@cesnet.cz (A. Sedo).
URL: http://www.lf1.cuni.cz/lbnb (A. Sedo).

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2012.01.011

In addition to the well-studied incretins (McIntosh, 2008), the DPP-IV substrates also include several poly- and oligopeptides strongly associated with the malignant behavior of glioma cells, such as the chemokine stromal cell-derived factor (CXCL12, SDF-1) and the neuropeptide substance P (SP) (Mentlein, 1999; Bajetto et al., 2006; Palma and Maggi, 2000). Furthermore, DPP-IV executes several of its functions by protein–protein interactions that are independent of its intrinsic enzymatic activity. The binding partners of DPP-IV include proteins of the extracellular matrix, CD45, caveolin-1, thrombospondin, adenosine deaminase and plasminogen (Ohnuma et al., 2008; Liu et al., 2009; Gonzalez-Gronow et al., 2008). We speculated that plasma membrane localized DPP-IV-like enzymatic activity as well as nonproteolytic protein–protein interactions of DPP-IV may represent an important mechanism regulating the growth properties of human glioma cells (Busek et al., 2004, 2008).

In the current study we explore the role of DPP-IV in gliomagenesis by using primary cell cultures derived from high-grade gliomas and glioma cell lines inducibly expressing DPP-IV. We show that the DPP-IV-like enzymatic activity in primary cell cultures is negatively associated with their *in vitro* growth and DPP-IV overexpressing glioma cells exhibit decreased proliferation *in vitro.* In addition, forced expression of both enzymatically active and enzymatically inactive DPP-IV suppressed glioma growth in an orthotopic xenotransplantation mouse model.

2. Materials and methods

2.1. Glioma primary cell cultures and cell lines

Primary cell cultures were derived from tumor tissue samples collected from patients undergoing astrocytic tumor resection at the Department of Neurosurgery, Hospital Na Homolce in Prague, Czech Republic. The study was approved by the Institutional ethics committee and was conducted in accordance with the Declaration of Helsinki; all biopsy donors gave full informed consent. Each fresh tissue sample was sectioned into small pieces and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma, Czech Republic) supplemented with 20% fetal bovine serum (FBS, Sigma), 100 μg/mL Streptomycin (Sigma) and 100 U/mL Penicillin G (Sigma). After 5–7 days, the explants were removed and the medium was replaced with DMEM supplemented with 10% FBS and antibiotics. GFAP expression was detected with a variable intensity in the majority of primary cell cultures supporting their glial origin.

Human glioma cell lines U373, T98G and U87 were from ATCC (LGC Standards, Middlesex, UK) and were cultured under standard conditions in DMEM supplemented with 10% FBS.

The CD26 positive cell subpopulation was isolated using MACS (magnetic activated cell sorting) by incubating the cells with a mouse anti-CD26 antibody (clone M A261, Acris, Germany) and then Dynabeads pre-coated with anti-mouse IgG antibodies (Invitrogen, CA, USA). After isolation, the cells were expanded and subcultured before ascertaining their growth properties.

2.2. Construction of vectors with enzymatically inactive mutant DPP-IV

The catalytic Ser^{630} of the full-length human DPP-IV inserted in the pGENE or pTRE-Tight vector was mutated to Ala by site-directed mutagenesis (Quik Change II, Agilent Technologies, Inc., CA, USA) using the following primers: forward 5′-GAATTGCAATTTGGGGCTGGGCATATGGAGGGTACGTAACCTC-3′ and reverse 5′-GAGGTTACGTACCCTCCATATGCCCAGCCCCAAATTCGAATTC-3′ (GeneriBioTech, Czech Republic).

The presence of the anticipated mutation was verified by DNA sequencing using automated DNA sequencer (ABI Prism 3100, Life Technologies Corporation, CA, USA).

2.3. Transfected cells

U373, T98G and U87 cells were transfected with DPP-IV using the mifepristone inducible Gene Switch system (Invitrogen), as described previously (Busek et al., 2006). Mifepristone (Invitrogen) in concentrations of 0.025–5 nmol/L was used to induce DPP-IV expression. In addition, a tetracycline inducible expression system (Clontech, CA, USA) was utilized. U373 cells were transfected with 4 μg of the regulatory pTet-On-Advanced plasmid and selected clones were co-transfected with the pTRE-Tight vector containing either the wild-type full-length human DPP-IV or the enzymatically inactive DPP-IV carrying an active site S630A substitution, and a linear Hygromycin marker (Clontech) using Lipofectamine 2000 (Invitrogen). Stable transfected clones inducibly expressing DPP-IV were subsequently selected with 400 μg/mL G418 (Sigma) and 200 μg/mL Hygromycin B (Invitrogen).

2.4. DPP-IV-like enzymatic activity assay

The cell surface DPP-IV-like enzymatic activity was measured in suspensions of viable cells by a continuous-rate fluorimetric kinetic assay using a plasma membrane impermeable (Bank et al., 2011) H-Gly-Pro-7-amino-4-methylcoumarin (Bachem, Bubendorf, Switzerland) as a substrate at pH7.5 and 37 °C; the total DPP-IV-like enzymatic activity was measured under the same conditions after permeabilization of the cells with 0.1% Triton X-100 (Sedo et al., 1989).

2.5. Characterization of the growth properties of glioma primary cell cultures

Cells were grown in DMEM supplemented with 10% FBS and counted every 2–3 days using a Coulter Counter Z2 (Beckman Coulter, CA, USA). The population doubling time was determined from the least square regression fit of the exponential part of the growth curve. The clonogenic assay was performed by seeding cells at a density of 50 and 150 cells/cm^2 in triplicates and counting the colonies after 2–5 weeks.

2.6. Growth of DPP-IV transfected cells, co-culture experiments

Glioma cells were grown in the presence of various concentrations of the inducing agent mifepristone and in some experiments together with a DPP-IV inhibitor Diprotin A (Bachem; 5 mmol/L).

In co-culture experiments, 4000 cells/well of wild type cells, DPP-IV transfected cells or a mixture of 1:1 wild type:DPP-IV transfected U373 cells were seeded in 96-well plates. After 24 h, the medium was exchanged with or without the addition of mifepristone, and the cells were allowed to grow for additional 72 h.

For quantification, cells were fixed and stained with methylene blue (5 g/l in 50%, v/v, ethanol) at the indicated time points, lysed with 1% sodium dodecyl sulfate and the relative cell number was determined by reading absorbance at 630 nm using a 96-well plate reader (Sunrise; Tecan, Männedorf, Switzerland). In some experiments, the cells were counted using a Coulter Counter Z2 (Beckman Coulter) to verify the results of colorimetric quantification.

2.7. Cell cycle analysis

Nuclear DNA in ethanol fixed cells was stained for 1 h with 50 μg/mL propidium iodide (Sigma) in PBS with 0.1% NaN_3 and 1 mg/mL bovine serum albumin (BSA; Sigma) in the presence of

1 mg/mL RNAse A (Sigma). Samples were analyzed using the flow cytometer FACS Canto II with Diva software (Becton Dickinson) for data acquisition. Histograms were analyzed using the Dean-Jett-Fox model in Flow-Jo (TreeStar Inc., OR, USA).

2.8. Migration assay

6×10^4 cells in DMEM were applied to the cell culture inserts with 8 μm pores (Becton Dickinson) and allowed to migrate for 24 h. Nonmigrated cells were removed using a cotton swab; cells on the lower surface of the inserts were fixed with 5% glutaraldehyde in PBS and stained with methylene blue. Five microscopic fields per insert were counted manually.

2.9. Adhesion assay

Cell adhesion was assessed in BioCoat™ Fibronectin 96-well plates (Becton Dickinson). 5×10^4 cells were added into the wells blocked with 0.1% BSA in DMEM for 15 min, and allowed to attach at 37 °C for the indicated times, after which the nonadherent cells were gently removed with three PBS washes. The adhered cells were fixed with 5% glutaraldehyde and stained with methylene blue. Colorimetric quantification was performed as described above.

2.10. Orthotopic xenograft glioma model

The experimental use of animals was approved by The Commission for Animal Welfare of the First Faculty of Medicine of the Charles University in Prague and The Ministry of Education, Youth and Sports of the Czech Republic according to the animal protection laws.

Male adult NOD.129S7(B6)-Rag1tm1Mom/J mice (The Jackson Laboratory, ME, USA) weighting approximately 25 g were used. All animals were anesthetized prior to surgery. 10^6 DPP-IV transfected U373 cells in 5 μL of DMEM were injected with a Hamilton syringe 1.2 mm anterior from the bregma and 2.5 mm sagital from the midline to a depth of 3 mm using a stereotactic device (Stoelting Co., IL, USA).

The expression of DPP-IV in the experimental group was initiated with the appropriate induction agent 1–3 days after cell implantation and maintained till the sacrification of animals. Mifepristone in sesame oil (Sigma) was administered intraperitoneally at a dose of 270 μg/kg (100 μL total volume) three times a week; the control group received 100 μL of the sesame oil alone at the same intervals. Doxycyclin hyclate (Sigma) was administered in drinking water at a concentration of 2 mg/mL; the doxycyclin solution was changed three times a week.

2.11. Tumor volume assessment

Serial 25 μm thick coronal sections were cut on a cryostat at −20 °C. Every fifth section was stained with hematoxylin and eosin and digitized at 20× magnification. Photographs were used for unbiased tumor volume estimation according to the Cavalieri principle (Mayhew and Olsen, 1991).

2.12. Immunodetection of DPP-IV and GFAP

For flow cytometric detection of DPP-IV, cells were fixed in 2% paraformaldehyde, stained for 30 min at room temperature with a phycoerythrin conjugated monoclonal anti-CD26 antibody (clone 222113, 1:40, RD Systems, MN, USA); samples were analyzed using FACS Canto II as described above. For immunocytochemistry, cells were grown on glass coverslips, air-dried at 4 °C, blocked with 3% heat inactivated FBS and incubated overnight at 4 °C with the primary antibody (anti-CD26 [clone M A261, 1:100, Acris, Herford, Germany]; anti-GFAP [GF-01, 1:200, Exbio, Czech Republic]) and then for 1 h at room temperature with the corresponding Alexa Fluor 488 conjugated secondary antibody (Invitrogen). The primary antibodies were omitted in the staining control. Slides were mounted in Aqua Polymount (Polysciences, PA, USA) and viewed on

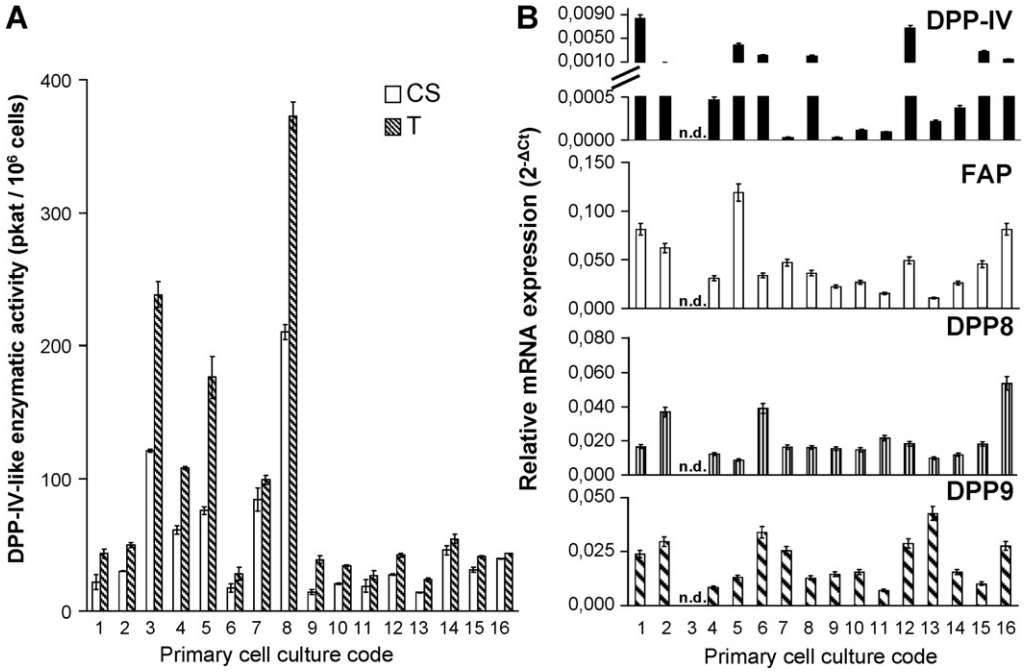

Fig. 1. Dipeptidyl peptidase (DPP)-IV and homologous proteases in glioma primary cultures. (A) DPP-IV-like enzymatic activity and (B) expression of mRNAs encoding proteases known to exhibit the DPP-IV-like activity in primary cell cultures derived from high-grade gliomas. CS: cell surface; T: total DPP-IV-like enzymatic activity. The expression of the investigated mRNAs was normalized to the expression of human β-actin using the ΔCt method. n.d.: not determined; bar graphs depict mean ± SD.

the Olympus IX 70 microscope equipped with the DP30BW camera or the Olympus IX81 confocal microscope (FluoView 300, Olympus, Czech Republic).

2.13. DPP-IV-like catalytic histochemistry

25 μm brain sections were fixed in a 1:1 mixture of acetone and chloroform for 2 min at 4 °C and incubated with 7-(glycyl-L-prolylamido)-4-methoxy-β-naphthylamide hydrochloride (0.83 mmol/L, Sigma) as a substrate and Fast Blue B (Sigma) in PBS (pH 7.4) at room temperature for several minutes (Lojda, 1981).

2.14. Real time RT-PCR and microarray analysis (see supplementary methods)

The expression of DPP-IV, FAP, DPP8 and DPP9 was quantified as described previously (Stremenova et al., 2007; Busek et al., 2008).

For the microarray analysis, 0.75 μg of the amplified RNA from control and induced (72 h, 1 nmol/L mifepristone) DPP-IV transfected U373 cells was hybridized on Illumina HumanRef-8 v3 Expression BeadChip (Illumina, CA, USA) according to the manufacturer's instructions. Wild type, untransfected U373 cells were processed identically to correct for the effects of the inducing agent itself. The data were deposited to the ArrayExpress database under the accession number E-MTAB-583.

Functional annotation and gene set enrichment analysis (GSEA) was performed on transcripts with Storey's *q*-value ≤0.1 using the DAVID database (Huang et al., 2009, http://david.abcc.ncifcrf.gov/summary.jsp).

2.15. Statistical analysis

All statistical analyses were performed using the Statistica software (StatSoft CR s.r.o., Czech Republic) and a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. DPP-IV-like enzymatic activity is associated with slower growth in glioma primary cell cultures

In order to study the function of DPP-IV in a model that closely resembles the heterogeneous cell populations present in gliomas *in vivo*, we examined the DPP-IV-like activity and growth properties of 16 primary cell cultures derived from high-grade gliomas. Primary cell cultures in early passage expressed DPP-IV mRNA as well as mRNAs encoding several proteases known to exhibit the DPP-IV-like enzymatic activity (Fig. 1B). Expression of the canonical DPP-IV was further confirmed using flow immunocytometry and immunocytochemistry (Supplementary Fig. 1). The cell surface DPP-IV-like enzymatic activity (Fig. 1A), probably representing the sum of enzymatic activities of the membrane bound DPP-IV and FAP, was variable and correlated rather poorly with the expression of the corresponding transcripts (DPP-IV mRNA $r = 0.17$, not significant ($p = 0.55$), FAP mRNA $r = 0.51$, $p < 0.05$). mRNA expression of the intracellularly localized DPP8 and DPP9 did not correlate with the DPP-IV-like enzymatic activity.

There was a statistically significant negative correlation of the cell surface DPP-IV-like enzymatic activity and the ability to form colonies ($r = -0.52$, $p < 0.05$). In addition, there was a trend for an increase in the doubling time (median 102.5 *vs.* 143 h, $p = 0.08$, Mann–Whitney test) in primary cell cultures with high DPP-IV-like enzymatic activity (Fig. 2). We further isolated the DPP-IV expressing cells using immunomagnetic separation (MACS) from some of the primary cell cultures and assessed their growth properties. The resulting DPP-IV positive subpopulation exhibited minimal *in vitro* growth or progressively lost the DPP-IV-like enzymatic activity (Supplementary Fig. 2). These results corroborate the association between DPP-IV and decreased growth of the primary cell cultures.

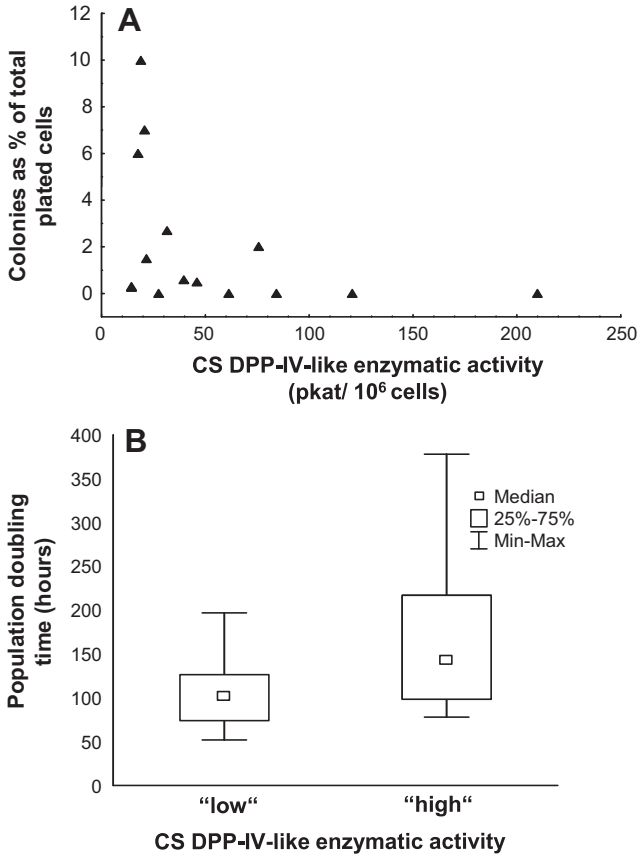

Fig. 2. Growth properties of primary cell cultures derived from high-grade gliomas in relation to their cell surface (CS) dipeptidyl peptidase (DPP)-IV-like enzymatic activity. (A) Negative correlation of the CS DPP-IV-like enzymatic activity and the ability to form colonies ($r = -0.52$, $p < 0.05$, Spearman correlation coefficient). (B) A trend for shorter population doubling time of primary cell cultures with "low" CS DPP-IV-like enzymatic activity. An arbitrary threshold for categorizing the cells as having "low" (median = 19.8 pkat/10^6 cells, $n = 8$) or "high" (median = 68.3 pkat/10^6 cells, $n = 8$) CS DPP-IV-like enzymatic activity is based on the median CS DPP-IV-like enzymatic activity of all investigated primary cell cultures (30.3 pkat/10^6 cells).

3.2. DPP-IV overexpression in glioma cells leads to decreased cell growth and a cell cycle block

The inherent heterogeneity of primary cell cultures together with the possible contribution of DPP-IV activity by multiple molecular species precluded a precise analysis in these cells of the effects of DPP-IV on glioma growth. We therefore utilized model glioma cell lines, which frequently exhibited low to undetectable endogenous DPP-IV, and transfected them with DPP-IV using a mifepristone inducible expression system. Transfected cells exhibited a concentration dependent increase of the DPP-IV enzymatic activity (Fig. 3A) that appeared within hours after the addition of the inducing agent mifepristone; DPP-IV expression was further confirmed by flow immunocytometry and immunocytochemistry (Supplementary Fig. 4A).

DPP-IV overexpression in glioma cells led to a decreased *in vitro* cell growth in different clones of T98G, U373 (Fig. 3B) and U87 cells (not shown).

In order to analyze the mechanisms of the growth inhibitory effect of DPP-IV, we performed the cell cycle analysis in DPP-IV

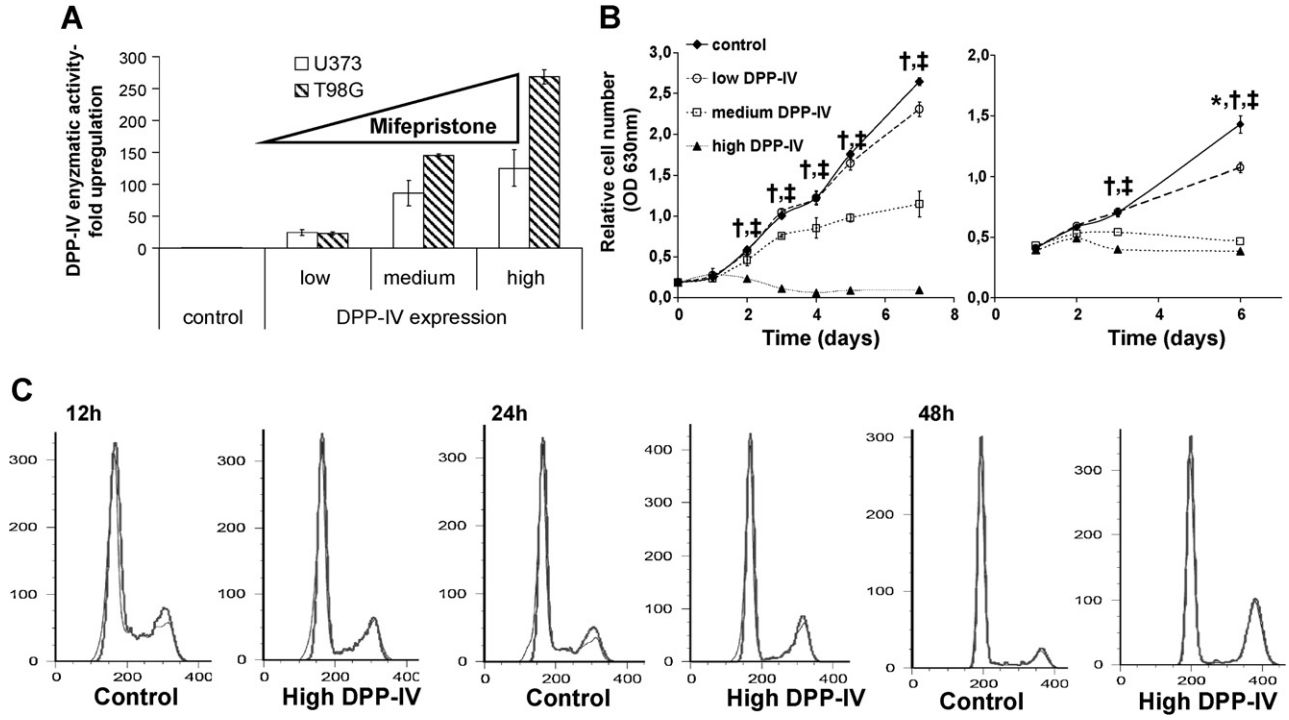

Fig. 3. Effect of dipeptidyl peptidase (DPP)-IV on glioma cell growth and cell cycle. (A) Concentration dependent increase in DPP-IV enzymatic activity in transfected glioma cell lines. DPP-IV enzymatic activity was measured 48 h after the addition of increasing concentrations of the inducing agent mifepristone. Bar graphs depict mean ± SD. (B) Growth curves of DPP-IV transfected U373 (left panel) and T98G (right panel) cells. DPP-IV expression was induced with various concentrations of mifepristone, cells were fixed and stained with methylene blue followed by colorimetric quantification. $^{*,\dagger,\ddagger}p < 0.05$ for control *vs.* low, medium and high DPP-IV expression respectively (repeated measurement ANOVA, Tukey *post hoc* test). (C) Flow cytometric analysis of the cell cycle in DPP-IV transfected U373 cells at various time points after the induction with mifepristone. Representative histograms (vertical axis – number of cells, horizontal axis – relative fluorescence intensity) of control cells and cells with induced DPP-IV expression are shown. Control: transfected cells not induced with mifepristone.

overexpressing cells. The proportion of the cells in the S phase declined early after DPP-IV induction and a G2/M block developed within 24–48 h (Fig. 3C). Apoptotic cell death did not significantly contribute to the growth inhibition as we did not observe a significant G0/G1 subpeak or an increase in annexin V staining (not shown). The changes were not due to the expression-inducing agent itself since mifepristone in concentrations up to 10 nmol/L had no effect on the growth or cell cycle in untransfected glioma cells (Fig. 4B and data not shown) and the concentrations used were much lower than those described to have pharmacological effects on glioma cells (Pinski et al., 1993).

We further aimed at elucidating, whether the effects of DPP-IV on glioma cells might be linked to an increased breakdown of putative soluble growth factor(s) and thus depend on its intrinsic enzymatic activity. Culturing the DPP-IV expressing cells in the presence of Diprotin A, an inhibitor that completely abrogated their DPP-IV enzymatic activity at the concentrations used, only mildly affected the growth decrease (Fig. 4A). We also observed no changes in the growth or the morphology of the wild type, untransfected cells when these were co-cultured with DPP-IV overexpressing cells using cell culture inserts (Supplementary Fig. 3). In addition, when wild type and transfected cells were seeded as a mixture at various ratios, which allowed direct interaction of the cells, the observed decline in cell numbers upon the addition of mifepristone corresponded to the decreased growth of transfected cells (Fig. 4B). Finally, expression of an enzymatically inactive mutant DPP-IV with catalytic site S630A substitution hampered the growth and induced a G2/M cell cycle arrest in U373 cells similarly to the wild type DPP-IV (data not shown). Collectively, these data suggest that the effect of DPP-IV on the growth of glioma cells *in vitro* is in large part independent of its enzymatic activity.

DPP-IV was described to interact with proteins of extracellular matrix such as collagen and fibronectin (Loster et al., 1995; Cheng et al., 2003) with possible effects on cell adhesion and migration. However, using cell adhesion and migration assays we observed decreased adhesion and spreading on fibronectin (Fig. 5A) as well as decreased migration (Fig. 5B) and collagen I induced haptotaxis (not shown) in glioma cells with high DPP-IV expression.

To identify potential functional partners and molecular mechanisms underlying the observed effects of DPP-IV in our model, we compared the whole genome expression profile in induced, DPP-IV overexpressing U373 cells and uninduced control cells. Gene set enrichment analysis performed on differentially expressed genes identified overrepresentation of genes linked to cell proliferation, cell adhesion, migration and regulation of cell development and neuron differentiation (Supplementary Tables 1–4). Transcripts for several growth factor receptors (*e.g.* PDGFRA, CALCRL, GRPR), proteins promoting cell cycle progression (*e.g.* CCND1, CDK6, PTP4A3) and involved in cell adhesion (CD97, COL8A1, COL13A1, NLGN1, NLGN4X, PCDH20, SCARF2, NrCAM) were downregulated (Supplementary Table 1). DPP-IV overexpression also led to the elevation of several putative or proven glioma associated tumor suppressors such as BEX2 (Foltz et al., 2006), RAP1GAP (Zheng et al., 2009), DUSP26 (Patterson et al., 2010), SYT13 (Jahn et al., 2010), TSPYL2 (Tu et al., 2007). On the contrary, several genes typically overexpressed in gliomas (*e.g.* CALCRL, COL8A1, HAS2, NES, RRM2; Cancer Genome Atlas Research Network, http://cancergenome.nih.gov/) were downregulated. Expression of DPP-IV in glioma cells *in vitro* thus reverses several changes in the expression profile typical of glioblastoma multiforme.

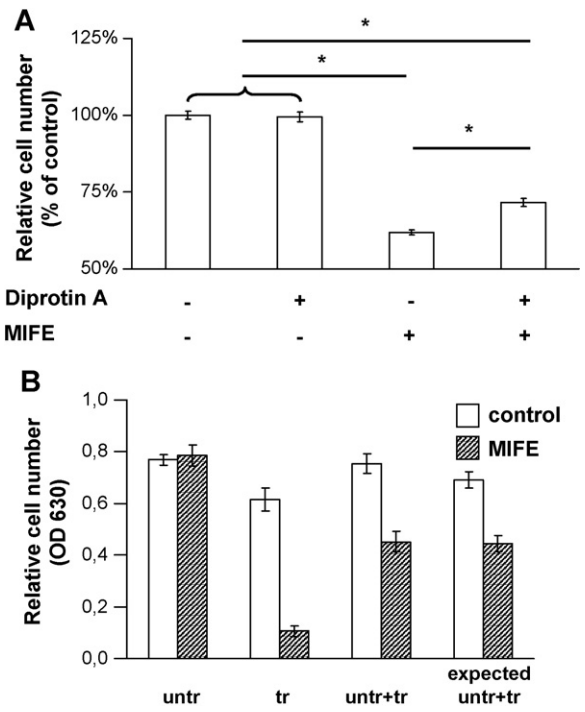

Fig. 4. Effect of dipeptidyl peptidase (DPP)-IV-like enzymatic activity inhibition on the growth of DPP-IV overexpressing glioma cells. (A) Control uninduced U373 cells and cells induced with 1 nmol/L mifepristone (MIFE) to achieve medium overexpression of DPP-IV were cultured for 72 h in the presence or absence of 5 mmol/L DPP-IV inhibitor Diprotin A. Mean ± SEM of 3 experiments performed in hexaplicates, $^{*}p < 0.05$, ANOVA, Tukey *post hoc* test. (B) Co-culture of untransfected (untr) and DPP-IV transfected (tr) U373 cells. "untr + tr" – cell suspensions of "untr" and "tr" mixed 1:1. "expected untr + tr" – calculated based on the values in "untr" and "tr" without co-culture. 2.5 nmol/L mifepristone was added as indicated to induce DPP-IV expression in transfected cells; cells were grown for 72 h. Bar graphs depict mean ± SD.

3.3. Forced DPP-IV expression reduces glioma growth in an orthotopic xenograft model

To test the effect of DPP-IV on tumor growth *in vivo*, transfected U373 cells inducibly expressing DPP-IV were orthotopically implanted into immunodeficient mice and DPP-IV expression was induced with mifepristone. DPP-IV induction *in situ* was confirmed by enzyme catalytic histochemistry (Fig. 6B) and immunohistochemistry (Supplementary Fig. 4C). Tumors developed in control mice as well as in animals with DPP-IV expressing cells and both exhibited features typical of high-grade gliomas, *i.e.* high cellularity and infiltrative growth with occasional necrotic areas (Supplementary Fig. 4D). The volume of tumors overexpressing DPP-IV was decreased by 42 ± 18% (mean ± SD; $p < 0.05$; $N = 16$ per experimental group) (Fig. 6A) compared to controls 5 weeks after implantation, accompanied by a statistically significant decrease of the Ki67 labeling index (median 23.1% *vs.* 18.5%, $p < 0.05$; data not shown).

To verify these results we used the tetracycline inducible expression system for transgenic DPP-IV expression. Although this expression system achieved much lower DPP-IV expression compared to the mifepristone system (Supplementary Fig. 4A and B), thus reducing the putative risk of non-specific effects of protein overexpression, we observed similar decrease in the size of the implanted tumors. Importantly, identical results were also obtained with U373 cells transfected with an enzymatically inactive mutant DPP-IV (Fig. 6A).

Neither mifepristone nor doxycycline alone affected the size of tumors originating from untransfected U373 at the doses used for the induction of DPP-IV expression (not shown).

These data demonstrate that DPP-IV functions as an inhibitor of glioma cell growth *in vivo* and that these growth inhibitory effects are independent of its intrinsic enzymatic activity.

4. Discussion

Several proteases were shown to participate on the promotion of malignancies due to their involvement in the regulation of cell proliferation, invasion into surrounding tissue or support of neovascularization (Kessenbrock et al., 2010). This predominant view of tumor associated proteases as molecules that support tumor progression may however be oversimplified. Protease inhibitors have so far failed in halting tumor progression in clinical trials and several reports demonstrate that proteases may act as tumor suppressors (reviewed in Lopez-Otin and Matrisian, 2007). Abnormal expression of DPP-IV and its association with malignant transformation was demonstrated in a number of malignancies including brain tumors (Stremenova et al., 2007). DPP-IV-like enzymatic activity and DPP-IV expression are often increased in tumor tissue (Sedo et al., 2008), but the clinical implications and the biological effects of DPP-IV on transformed cells are diverse. On the one hand, DPP-IV expression is associated with a more malignant behavior in some T cell malignancies (Sato et al., 2005), thyroid cancer (Hirai et al., 1999), gastrointestinal stromal tumors (Yamaguchi et al., 2008), and has recently been described as a marker of a subpopulation of colorectal cancer stem cells responsible for the metastatic spread of the disease (Pang et al., 2010). On the other hand, DPP-IV was reported to act as a tumor suppressor in ovarian (Kajiyama et al., 2002), prostate (Wesley et al., 2005) as well as non-small cell lung cancer cells (Wesley et al., 2004) and also in the tumor cells derived from neuroectoderm such as melanoma (Wesley et al., 1999) and neuroblastoma (Arscott et al., 2009). The underlying mechanisms of these disparate effects on cancer cells are only scarcely understood and are likely dependent on the cell type and the molecular context within the tumor microenvironment. In neuroblastoma and prostate cancer cells, DPP-IV may proteolytically inactivate the growth promoting and prometastatic chemokine CXCL12 (SDF-1) (Arscott et al., 2009; Sun et al., 2008). However, in several experimental models (Wesley et al., 1999, 2004; Pethiyagoda et al., 2000) overexpression of a mutant, enzymatically inactive form of DPP-IV was shown to produce similar results to the enzymatically active DPP-IV suggesting that in addition to the inactivation of biologically active peptides, nonproteolytic mechanisms must contribute to its tumor suppressive effects. DPP-IV overexpression in cell lines frequently triggers profound changes in cell morphology, growth, migration or invasion (Yu et al., 2010), which may reflect the changed expression of adhesion molecules (*e.g.* E cadherin, Kajiyama et al., 2003), CD44 (Wesley et al., 2004), protease inhibitors (Kajiyama et al., 2003), altered expression and subcelullar localization of bFGF (Wesley et al., 2005) or upregulation of the related protease FAP (Wesley et al., 1999, 2004).

We previously reported a grade dependent increase of the DPP-IV-like enzymatic activity in the human glioma tissue (Stremenova et al., 2007). In the present study we therefore aimed at determining the role of DPP-IV and its intrinsic enzymatic activity in the malignant behavior of glioma cells.

We observed varying cell surface DPP-IV-like enzymatic activity and DPP-IV expression on the mRNA as well as protein level in primary cell cultures derived from high-grade gliomas. Somewhat surprisingly, higher DPP-IV-like enzymatic activity was associated with slower proliferation of primary cell cultures at early passage. Although DPP-IV mRNA expression did not correlate with the DPP-IV-like enzymatic activity in the set of primary cell cultures at early passage that were used in this study, we later observed a modest

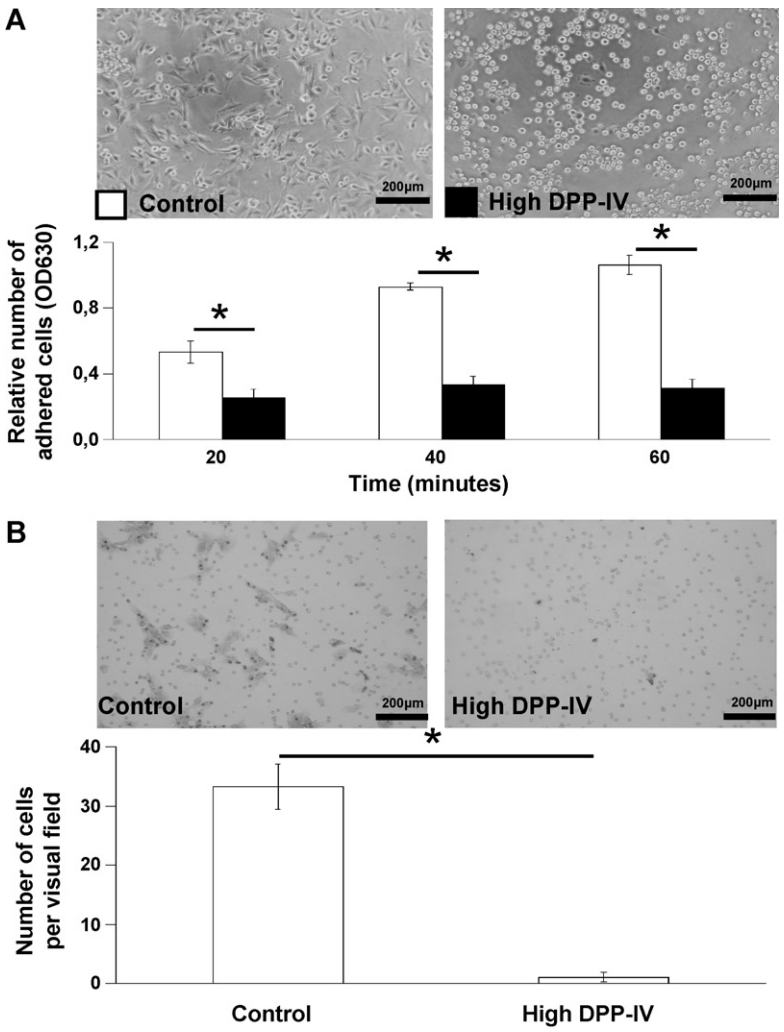

Fig. 5. Effect of dipeptidyl peptidase (DPP)-IV on glioma cell adhesion and migration. (A) Cell adhesion was evaluated 20, 40 and 60 min after seeding the DPP-IV transfected U373 cells into fibronectin-coated wells. Insets – phase contrast microphotographs of cells that were allowed to attach for 180 min without washing. $^{*}p < 0.05$, repeated measurement ANOVA. (B) Cell migration was evaluated by a modified Boyden chamber assay using tissue culture inserts with 8 μm pores. The assay was performed in quadruplicates. Insets – representative microphotographs of the transmigrated cells. $^{*}p < 0.05$, Mann–Whitney test; bar graphs depict mean ± SD.

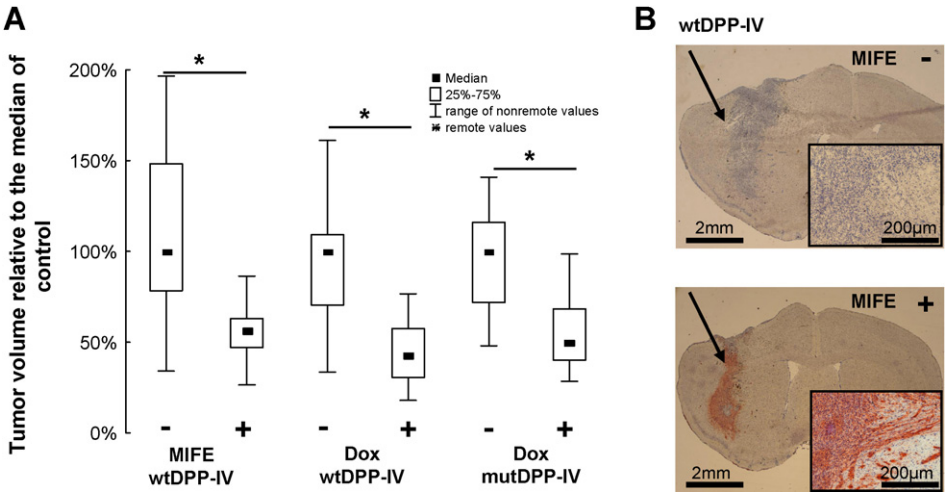

Fig. 6. Effect of dipeptidyl peptidase (DPP)-IV on glioma growth *in vivo*. (A) Tumor volume in mice orthotopically implanted with U373 cells inducibly expressing enzymatically active (wtDPP-IV) or enzymatically inactive (mutDPP-IV) DPP-IV. DPP-IV expression was induced by mifepristone (MIFE) or doxycycline (Dox) as indicated. A total of 14–19 animals per experimental group were analyzed. (B) Representative microphotographs of control and DPP-IV expressing tumors (arrows) with the detection of the DPP-IV-like enzymatic activity by enzyme histochemistry (red); nuclei were counterstained with hematoxylin. $^{*}p < 0.05$, Mann–Whitney test.

correlation ($r = 0.56$, $p \leq 0.05$, $n = 47$) in a substantially extended panel of primary cultures (Balaziova et al., 2011). In addition to the canonical DPP-IV (CD26), the cell surface DPP-IV-like enzymatic activity may in part reflect the presence of FAP. FAP was detected on the surface of glioma cells by Mentlein et al. (2011) and is increased in 40–60% of patients with glioblastoma (Stremenova et al., 2007; Mentlein et al., 2011; Dolznig et al., 2005; The Cancer Genome Atlas Research Network, 2008). So far, there are no data on the possible effects of FAP on glioma cell proliferation and a recent report by Mentlein et al. (2011) suggests that FAP may rather be involved in glioma cell invasion. There is one report suggesting that a minor fraction of the typically intracellularly localized DPP8 and DPP9 might also be loosely bound on the cell surface of immune cells under certain circumstances (Bank et al., 2011). However, the washing steps preceding the enzymatic assay in our studies and no correlation of the DPP8 and DPP9 mRNA expression with the DPP-IV-like enzymatic activity make their putative contribution less probable.

Importantly, the DPP-IV positive subpopulation isolated from our primary cell cultures using immunomagnetic separation showed decreased growth. The antiproliferative effect of CD26 binding, which was previously demonstrated for certain anti-CD26 antibodies in renal cell carcinoma and mesothelioma cells (Inamoto et al., 2006, 2007), cannot be completely ruled out. However, the higher proliferation of the CD26 negative subpopulation compared to the parental population before MACS indirectly argues that it is rather CD26 itself than the antibody binding that is responsible for the slower cell growth. In addition, we routinely use the respective anti CD26 antibody for the enrichment of CD26 transfected cells without significant effects on cell proliferation.

The observed negative association of DPP-IV and glioma cell growth is also consistent with our reports of DPP-IV upregulation in serum withdrawal-induced differentiation and growth arrest of glioma cells (Sedo et al., 2004; Balaziova et al., 2011).

To directly address the role of DPP-IV in glioma cells we transfected glioma cell lines with DPP-IV using an inducible expression system. Consistently with the primary cell culture data, we observed a diminished cell growth in cells expressing high levels of transgenic DPP-IV, which was associated with delayed progression of the cell cycle as suggested by the decreased proportion of cells in the S phase and a subsequently developing G2/M block. Similarly to the previous studies in lung cancer cell lines and melanoma (Wesley et al., 1999, 2004; Pethiyagoda et al., 2000), the effects of DPP-IV were largely independent of its intrinsic enzymatic activity as demonstrated using enzymatically inactive DPP-IV with an active site S630A substitution and coculture experiments. These results suggested that similarly to other cancer cell types (Arscott et al., 2009; Wesley et al., 1999, 2004, 2005), DPP-IV may function as a negative growth regulator also in glioma cells. We therefore tested the importance of DPP-IV for *in vivo* growth of glioma induced by orthotopic implantation of glioma cells into immunodeficient mice. Compared to the controls, tumor size was significantly reduced in animals with the overexpression of the transgenic DPP-IV in implanted cells. Similar results were obtained with two different expression systems with differing levels of the transgene expression and irrespective of the DPP-IV enzymatic activity. This further supports specific growth inhibitory effect of DPP-IV on glioma cells and its independence on the enzymatic activity.

The whole genome expression profiling of glioma cells overexpressing DPP-IV revealed alterations of several pathways critical for cell proliferation, as well as cell–cell and cell–extracellular matrix interactions. In addition, DPP-IV overexpression reverted the expression of a number genes typically overexpressed in gliomas. Somewhat surprisingly, DPP-IV overexpression was also accompanied by the upregulation of a number of growth factors (*e.g.* BMP4, BDNF, FGF18, GPI, IL11, PDGFB, TGFB3), invasion promoting genes (MMP15) and genes that may support glioma cell survival (ACSL5; Mashima et al., 2009). These gene expression changes may indicate activation of compensatory mechanisms counteracting the observed growth inhibitory effects of DPP-IV. Functional validation and determining the biological relevance of these changes require further studies. To the best of our knowledge, this is the first study on the changes in the whole genome expression profile induced by DPP-IV in cancer cells.

The growth inhibitory effects of DPP-IV in glioma cells might seemingly be in contradiction to the observation of higher DPP-IV expression and activity in glioma tissue homogenates (Stremenova et al., 2007). The "net" pro- or anti-oncogenic effects of proteases seems to represent an outcome of several factors including their differing functions in individual cell populations of both the tumor parenchyma and stroma, and varying (in)dependence of these functions on the intrinsic enzymatic activity. For example, forced expression of MT1-MMP was described to cause glioma cell death, although its presence in the tumor microenvironment promoted tumor expansion (Markovic et al., 2009). Thus, DPP-IV may – independent of its enzymatic activity – negatively influence the proliferation of glioma cells, slowing their growth possibly as a part of an adaptive response to the limited nutrition supply or hypoxia (Dang et al., 2008), yet support angiogenesis or promote intratumoral deregulation of immune response through the proteolytic processing of neuropeptides and chemokines (Ghersi et al., 2001; Mentlein, 1999). By degrading the chemokines such as CXCL12 (SDF-1), DPP-IV might also impair the recruitment of tumor suppressive neural precursor cells (Chirasani et al., 2010; Charles et al., 2011) and as a result promote glioma progression. Understanding of the complex role and the molecular mechanisms by which DPP-IV participates on gliomagenesis would allow its rational therapeutic targeting with the advantage of the availability of several specific, clinically tested DPP-IV inhibitors that are used in patients with type 2 diabetes (Neumiller et al., 2010).

Taken together, in the present work we demonstrate a growth inhibitory effect of DPP-IV on glioma cells. We show for the first time that primary cell cultures derived from high-grade gliomas express several molecules with DPP-IV-like enzymatic activity, which is negatively associated with their proliferation. DPP-IV overexpression in glioma cell lines does not promote their malignant behavior but rather slows their growth and may decrease their migration and adhesion. Our data also indicate that nonproteolytic mechanisms are important for these effects of DPP-IV in glioma cells. The whole genome expression profiling of DPP-IV overexpressing glioma cells revealed several candidate genes that shed light on the molecular pathways potentially affected by DPP-IV and will serve as a lead for further functional studies.

Acknowledgments

The cooperation of the clinical staff of the Departments of Neurosurgery and Pathology, Hospital Na Homolce in Prague is acknowledged, as well as the excellent technical assistance of Kvetoslava Vlasicova and Karin Roubickova. We are indebted to patients who contributed tissue samples for the derivation of the glioma primary cell cultures. We thank Jonathan Duke-Cohan (Harvard School of Medicine) for critical reading of the manuscript and useful suggestions. The work was supported by the grants of the Ministry of Education, Youth and Sports (MSMT 0021620808) and the Grant Agency of the Charles University in Prague (projects 257896, 251029, SVV-2011-262513).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocel.2012.01.011.

References

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

Bajetto A, Barbieri F, Dorcaratto A, Barbero S, Daga A, Porcile C, et al. Expression of CXC chemokine receptors 1–5 and their ligands in human glioma tissues: role of CXCR4 and SDF1 in glioma cell proliferation and migration. Neurochem Int 2006;49:423–32.

Balaziova E, Busek P, Stremenova J, Sromova L, Krepela E, Lizcova L, et al. Coupled expression of dipeptidyl peptidase-IV and fibroblast activation protein-α in transformed astrocytic cells. Mol Cell Biochem 2011;354:283–9.

Bank U, Heimburg A, Wohlfarth A, Koch G, Nordhoff K, Julius H, et al. Outside or inside: role of the subcellular localization of DP4-like enzymes for substrate conversion and inhibitor effects. Biol Chem 2011;392:169–87.

Busek P, Krepela E, Mares V, Vlasicova K, Sevcik J, Sedo A. Expression and function of dipeptidyl peptidase IV and related enzymes in cancer. Adv Exp Med Biol 2006;575:55–62.

Busek P, Malik R, Sedo A. Dipeptidyl peptidase IV activity and/or structure homologues (DASH) and their substrates in cancer. Int J Biochem Cell Biol 2004;36:408–21.

Busek P, Stremenova J, Krepela E, Sedo A. Modulation of substance P signaling by dipeptidyl peptidase-IV enzymatic activity in human glioma cell lines. Physiol Res 2008;57:443–9.

Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. Glia 2011;59:1169–80.

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

Chirasani SR, Sternjak A, Wend P, Momma S, Campos B, Herrmann IM, et al. Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 2010;133:1961–72.

Dang DT, Chun SY, Burkitt K, Abe M, Chen S, Havre P, et al. Hypoxia-inducible factor-1 target genes as indicators of tumor vessel response to vascular endothelial growth factor inhibition. Cancer Res 2008;68:1872–80.

Dolznig H, Schweifer N, Puri C, Kraut N, Rettig WJ, Kerjaschki D, et al. Characterization of cancer stroma markers: in silico analysis of an mRNA expression database for fibroblast activation protein and endosialin. Cancer Immun 2005;5:10.

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

Foltz G, Ryu GY, Yoon JG, Nelson T, Fahey J, Frakes A, et al. Genome-wide analysis of epigenetic silencing identifies BEX1 and BEX2 as candidate tumor suppressor genes in malignant glioma. Cancer Res 2006;66:6665–74.

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

Gonzalez-Gronow M, Kaczowka S, Gawdi G, Pizzo SV. Dipeptidyl peptidase IV (DPP IV/CD26) is a cell-surface plasminogen receptor. Front Biosci 2008;13:1610–8.

Hirai K, Kotani T, Aratake Y, Ohtaki S, Kuma K. Dipeptidyl peptidase IV (DPP IV/CD26) staining predicts distant metastasis of 'benign' thyroid tumor. Pathol Int 1999;49:264–5.

Hoelzinger DB, Demuth T, Berens ME. Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J Natl Cancer Inst 2007;99:1583–93.

Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.

Inamoto T, Yamada T, Ohnuma K, Kina S, Takahashi N, Yamochi T, et al. Humanized anti-CD26 monoclonal antibody as a treatment for malignant mesothelioma tumors. Clin Cancer Res 2007;13:4191–200.

Inamoto T, Yamochi T, Ohnuma K, Iwata S, Kina S, Inamoto S, et al. Anti-CD26 monoclonal antibody-mediated G1-S arrest of human renal clear cell carcinoma Caki-2 is associated with retinoblastoma substrate dephosphorylation, cyclin-dependent kinase 2 reduction, p27(kip1) enhancement, and disruption of binding to the extracellular matrix. Clin Cancer Res 2006;12:3470–7.

Jahn JE, Best DH, Coleman WB. Exogenous expression of synaptotagmin XIII suppresses the neoplastic phenotype of a rat liver tumor cell line through molecular pathways related to mesenchymal to epithelial transition. Exp Mol Pathol 2010;89:209–16.

Kajiyama H, Kikkawa F, Khin E, Shibata K, Ino K, Mizutani S. Dipeptidyl peptidase IV overexpression induces up-regulation of E-cadherin and tissue inhibitors of matrix metalloproteinases, resulting in decreased invasive potential in ovarian carcinoma cells. Cancer Res 2003;63:2278–83.

Kajiyama H, Kikkawa F, Suzuki T, Shibata K, Ino K, Mizutani S. Prolonged survival and decreased invasive activity attributable to dipeptidyl peptidase IV overexpression in ovarian carcinoma. Cancer Res 2002;62:2753–7.

Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 2010;141:52–67.

Levicar N, Nuttall RK, Lah TT. Proteases in brain tumour progression. Acta Neurochir 2003;145:825–38.

Liu Z, Christensson M, Forslow A, De Meester I, Sundqvist KG. A CD26-controlled cell surface cascade for regulation of T cell motility and chemokine signals. J Immunol 2009;183:3616–24.

Lojda Z. Proteinases in pathology. Usefulness of histochemical methods. J Histochem Cytochem 1981;29:481–93.

Lopez-Otin C, Matrisian LM. Emerging roles of proteases in tumour suppression. Nat Rev Cancer 2007;7:800–8.

Loster K, Zeilinger K, Schuppan D, Reutter W. The cysteine-rich region of dipeptidyl peptidase IV (CD 26) is the collagen-binding site. Biochem Biophys Res Commun 1995;217:341–8.

Louis DN, Pomeroy SL, Cairncross JG. Focus on central nervous system neoplasia. Cancer Cell 2002;1:125–8.

Markovic DS, Vinnakota K, Chirasani S, Synowitz M, Raguet H, Stock K, et al. Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. Proc Natl Acad Sci USA 2009;106:12530–5.

Mashima T, Sato S, Sugimoto Y, Tsuruo T, Seimiya H. Promotion of glioma cell survival by acyl-CoA synthetase 5 under extracellular acidosis conditions. Oncogene 2009;28:9–19.

Mayhew TM, Olsen DR. Magnetic resonance imaging (MRI) and model-free estimates of brain volume determined using the Cavalieri principle. J Anat 1991;178:133–44.

McIntosh CH. Dipeptidyl peptidase IV inhibitors and diabetes therapy. Front Biosci 2008;13:1753–73.

Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul Peptides 1999;85:9–24.

Mentlein R, Hattermann K, Hemion C, Jungbluth A, Held-Feindt J. Expression and role of the cell surface protease seprase/fibroblast activation protein-alpha (FAP-alpha) in astroglial tumors. Biol Chem 2011;392:199–207.

Neumiller JJ, Wood L, Campbell RK. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes mellitus. Pharmacotherapy 2010;30:463–84.

Ohnuma K, Takahashi N, Yamochi T, Hosono O, Dang NH, Morimoto C. Role of CD26/dipeptidyl peptidase IV in human T cell activation and function. Front Biosci 2008;13:2299–310.

Palma C, Maggi CA. The role of tachykinins via NK1 receptors in progression of human gliomas. Life Sci 2000;67:985–1001.

Pang R, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 2010;6:603–15.

Patterson KI, Brummer T, Daly RJ, O'Brien PM. DUSP26 negatively affects the proliferation of epithelial cells, an effect not mediated by dephosphorylation of MAPKs. Biochim Biophys Acta 2010;1803:1003–12.

Pethiyagoda CL, Welch DR, Fleming TP. Dipeptidyl peptidase IV (DPPIV) inhibits cellular invasion of melanoma cells. Clin Exp Metastasis 2000;18:391–400.

Pinski J, Halmos G, Shirahige Y, Wittliff JL, Schally AV. Inhibition of growth of the human malignant glioma cell line (U87MG) by the steroid hormone antagonist RU486. J Clin Endocrinol Metab 1993;77:1388–92.

Rao JS. Molecular mechanisms of glioma invasiveness: the role of proteases. Nat Rev Cancer 2003;3:489–501.

Sato T, Yamochi T, Yamochi T, Aytac U, Ohnuma K, McKee KS, et al. CD26 regulates p38 mitogen-activated protein kinase-dependent phosphorylation of integrin beta1, adhesion to extracellular matrix, and tumorigenicity of T-anaplastic large cell lymphoma Karpas 299. Cancer Res 2005;65:6950–6.

Sedo A, Busek P, Scholzova E, Malik R, Vlasicova K, Janackova S, et al. 'Dipeptidyl peptidase-IV activity and/or structure homologs' (DASH) in growth-modulated glioma cell lines. Biol Chem 2004;385:557–9.

Sedo A, Krepela E, Kasafirek E. A kinetic fluorometric assay of dipeptidyl peptidase IV in viable human blood mononuclear cells. Biochimie 1989;71:757–61.

Sedo A, Malik R. Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? Biochim Biophys Acta 2001;1550:107–16.

Sedo A, Stremenova J, Busek P, Duke-Cohan JS. Dipeptidyl peptidase-IV and related molecules: markers of malignancy? Exp Opin Med Diagn 2008;2:677–89.

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

Sun YX, Pedersen EA, Shiozawa Y, Havens AM, Jung Y, Wang J, et al. CD26/dipeptidyl peptidase IV regulates prostate cancer metastasis by degrading SDF-1/CXCL12. Clin Exp Metastasis 2008;25:765–76.

The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8.

Tu Y, Wu W, Wu T, Cao Z, Wilkins R, Toh BH, et al. Antiproliferative autoantigen CDA1 transcriptionally up-regulates p21(Waf1/Cip1) by activating p53 and MEK/ERK1/2 MAPK pathways. J Biol Chem 2007;282:11722–31.

Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med 2008;359:492–507.

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

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

Wesley UV, Tiwari S, Houghton AN. Role for dipeptidyl peptidase IV in tumor suppression of human non small cell lung carcinoma cells. Int J Cancer 2004;109:855–66.
Yamaguchi U, Nakayama R, Honda K, Ichikawa H, Hasegawa T, Shitashige M, et al. Distinct gene expression-defined classes of gastrointestinal stromal tumor. J Clin Oncol 2008;26:4100–8.
Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, et al. The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J 2010;277:1126–44.
Zheng H, Gao L, Feng Y, Yuan L, Zhao H, Cornelius LA. Down-regulation of Rap1GAP via promoter hypermethylation promotes melanoma cell proliferation, survival, and migration. Cancer Res 2009;69:449–57.