HIV-Tat protein transduction domain specifically attenuates growth of polyamine deprived tumor cells

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Abstract

Polyamines are essential for tumor cell growth, and the polyamine pathway represents an attractive target for cancer treatment. Several polyamine transport proteins have been cloned and characterized in bacteria and yeast cells; however, the mechanism of polyamine entry into mammalian cells remains poorly defined, although a role for proteoglycans has been suggested. Here, we show that the HIV-Tat transduction peptide, which is known to enter cells via a proteoglycan-dependent pathway, efficiently inhibits polyamine uptake. Polyamine uptake–deficient mutant cells with intact proteoglycan biosynthesis (CHO MGBG) displayed unperturbed HIV-Tat uptake activity compared with wild-type cells, supporting the notion that HIV-Tat peptide interferes with polyamine uptake via competition for proteoglycan binding sites rather than a putative downstream transporter. HIV-Tat specifically inhibited growth of human carcinoma cells made dependent on extracellular polyamines by treatment with the polyamine biosynthesis inhibitor α-difluoromethylornithine; accordingly, the Tat peptide prevented intracellular accumulation of exogenous polyamines. Moreover, combined treatment with α-difluoromethylornithine and HIV-Tat efficiently blocked tumor growth in an experimental mouse model. We conclude that HIV-Tat transduction domain and polyamines enter cells through a common pathway, which can be used to target polyamine-dependent tumor growth in the treatment of cancer. [Mol Cancer Ther 2007;6(2):782–8]

Introduction

Polyamines and polybasic peptides have been shown to efficiently traverse the plasma membrane barrier of mammalian cells (1, 2). Polybasic peptides, including the HIV-Tat transduction domain, have gained much attention lately due to their ability to carry large cargo (e.g., nucleic acids and proteins) over the cell membrane into the cytoplasm and nuclear compartment of mammalian cells (3, 4). This capacity has been used for introducing therapeutic macromolecules, such as tumor suppressors and proapoptotic proteins, into tumor cells as a possible strategy to treat cancer (5–7). The uptake mechanism of HIV-Tat and other transduction peptides has been extensively studied, and both clathrin- and caveolae-mediated endocytosis and membrane raft-dependent macropinocytosis have been suggested as the major entry pathway (8–12). Moreover, heparan sulfate proteoglycans seem to have an important role in HIV-Tat uptake (1, 2, 11).

Polyamines are essential for cellular proliferation, and elevated polyamine levels have been associated with cell transformation and cancer disease (13–15). Accordingly, depletion of intracellular polyamines by α-difluoromethylornithine, a specific inhibitor of ornithine decarboxylase that catalyses the first step in the polyamine pathway, results in efficient growth inhibition of tumor cells *in vitro* and, in some cases, reversal of cell transformation and tumorigenesis in animal models and cancer patients (14–16). Although α-difluoromethylornithine is still under investigation in clinical antitumor trials (17), the clinical outcome has been overall disappointing due to compensatory recruitment of extracellular polyamines from the circulation by α-difluoromethylornithine–treated cancer cells *in vivo* (14). Thus, successful antitumor therapy directed at the polyamine system requires efficient inhibition of both *de novo* polyamine synthesis and uptake of polyamines from the extracellular milieu.

Several polyamine transport proteins have been cloned and characterized in bacteria and yeast cells, but attempts to find similar transporters in mammalian cells have, thus far, been unsuccessful (18–22). Previous studies from our group have shown that heparan sulfate proteoglycans facilitate spermine uptake, and that combined inhibition of polyamine and heparan sulfate biosynthesis attenuates tumor growth (23, 24). These and other findings identify cell surface heparan sulfate proteoglycans as an attractive target in the treatment of malignant disease (25).

Here, we have explored the possibility that the structurally diverse HIV-Tat transduction peptide and polyamines share a common transport mechanism in human carcinoma cells

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and that HIV-Tat may be an efficient inhibitor of polyamine uptake and polyamine-dependent tumor growth *in vivo*.

Materials and Methods

Chemicals and Reagents

Polyamines, ornithine, and fine grade chemicals were from Sigma Chemical Co. (St. Louis, MO). [^{14}C]polyamines and [^{3}H]ornithine were from Amersham International (Bucks, United Kingdom). HIV-Tat peptide (GRKKRRQRRRPPQC), either unlabeled or labeled at the C terminus with Alexa-Fluor-647, was synthesized by Innovagen AB (Lund, Sweden). Heparan sulfate preparations were the same as previously described (26). α-Difluoromethylornithine was from Ilex Oncology (San Antonio, TX). Cell media and supplements were from Chemicon Europe, Ltd. (Hampshire, United Kingdom). HiTrap heparin column was from Pharmacia-Amersham.

Cell Culture

Human T24 bladder carcinoma cells were provided by Prof. Inge Ohlsson (Lund University, Lund, Sweden) and cultured in DMEM. Chinese hamster ovary (CHO)-K1 cells were obtained from American Type Culture Collection (Manassas, VA) and cultured in F12K nutrient mixture. Routine culture was done in a humidified 5% CO_2 incubator at 37°C, using the respective media supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (growth medium).

Heparin Affinity Chromatography of Tat and Spermine

Samples (100 μg Tat, 0.5 mg spermine supplemented with 0.1 μCi [^{14}C]spermine, or 0.5 mg ornithine supplemented with 0.1 μCi [^{3}H]ornithine) were dissolved in 0.5 mL of buffer A (0.1 mol/L Tris, 0.01 mol/L citrate, pH 7.4) and applied to the heparin affinity column, which was eluted using a gradient ranging from 15% to 100% of buffer B (2 mol/L NaCl in buffer A). The effluent was collected in 0.5-mL fractions, and eluted radioactive [^{14}C] and [^{3}H] species were analyzed by liquid scintillation counting. In the case of Tat peptide, the elution profile was registered by an online UV cell measuring absorbance at 215 nm.

Tat Peptide and Polyamine Uptake Experiments

AlexaFluor-647–labeled Tat peptide (0.1 or 1 μg/mL) in serum-free medium was added to extensively rinsed subconfluent cells, and incubation was allowed to proceed for 20 min at 37°C. To exclude the presence of cell surface–associated peptide, cells were extensively rinsed before flow cytometry measurements: three washes with cold PBS supplemented with 100 μg/mL dextran sulfate (molecular weight, 500,000 kDa); trypsination and blocking with 10% FCS; and two consecutive wash/centrifugation cycles using PBS with 1% bovine serum albumin. Cells were finally suspended in PBS with 1% bovine serum albumin and analyzed for fluorescence in a FACS-Calibur (BD Biosciences, San Jose, CA) instrument integrated with Cell-Quest software.

For polyamine uptake experiments, T24 cells were plated in 24-well culture plates at 4×10^4 per well in growth medium, with or without 5 mmol/L α-difluoromethylornithine. Cells were grown for 3 days to subconfluency, after which varying concentrations of polyamines and [^{14}C]polyamines (specific activity, 31 Ci/mol) in serum-free MEM was added, and then cells were incubated for 20 min at 4°C or 37°C. The incubation medium was removed, and the cells were rinsed with cold MEM containing 1 mmol/L of unlabeled polyamine and then once with MEM and lysed with 0.5 mol/L NaOH for 1 h at 37°C. An aliquot of the cell homogenate was neutralized with 0.5 mol/L HCl and then analyzed for radioactivity by scintillation counting. The difference in cell-associated polyamine levels obtained at 37°C and 4°C was defined as temperature-dependent polyamine internalization.

In vitro Proliferation Assays

The [^{3}H]thymidine incorporation assay was used to assess DNA synthesis. T24 cells were seeded into 24-well culture plates at 70,000 per well in growth medium. After 4 h, the cells were placed in serum-free medium supplemented with insulin (10 ng/mL) and transferrin (25 ng/mL) and allowed to proliferate in the absence or presence of 5 mmol/L α-difluoromethylornithine for 72 h. The growth medium was then supplemented with 0.5 μmol/L spermidine and/or 100 μg/mL HIV-Tat, and cells were incubated for another 16 h in the presence of 3 μCi/mL [^{3}H]thymidine. The cells were then washed with PBS, after which proteins were precipitated with 10% cold trichloroacetic acid for 30 min. DNA from cell extracts was obtained by incubation in 0.5 mol/L NaOH, 0.5% SDS for 5 min at room temperature, and the amount of incorporated [^{3}H]thymidine was determined by liquid scintillation.

Polyamine Analysis

T24 cells were seeded into 75-cm^2 flasks and grown for 72 h in the presence or absence of 5 mmol/L α-difluoromethylornithine, and growth media were supplemented as described in the appropriate figure legend. After another growth period of 16 h, cells were collected either by scraping or trypsinization, counted and pelleted at $900 \times g$ for 10 min at 4°C and the pellets were stored at −80°C until analysis. Polyamine content in cell pellets extracted with 0.2 mol/L perchloric acid was determined by HPLC (Hewlett Packard 1100; Global Medical Instrumentation, Inc., Ramsey, MN) with o-phthaldialdehyde as the reagent (27).

Metabolic Labeling and Isolation of Proteoglycans

Proteoglycans were metabolically labeled in confluent 75-cm^2 cell culture flasks using a sulfate-poor medium supplemented with 50 μCi/mL [^{35}S]sulfate as described previously (28, 29). After 24 h of incubation, the cell layer was extracted with Triton X-100. Proteoglycan material was recovered by passage over DEAE-cellulose. Size separation of proteoglycans and various degradation products thereof was done on a Superose 6 column (Amersham Biosciences, AB, Uppsala, Sweden). Fractions containing heparan sulfate proteoglycan were pooled and further analyzed by ion exchange chromatography on MonoQ (linear gradient elution with 0.3, 1.2, and 2 mol/L NaCl in 7 mol/L urea, pH 8.0; 0.1% Triton X-100). Alternatively, the proteoglycan material obtained from the DEAE-cellulose was analyzed for polyamine affinity on a spermine-substituted HiTrap column (linear gradient elution with 0.15, 1.2, and 2 mol/L

NaCl in 10 mmol/L Tris, pH 7.4). Gradients are depicted in respective figures.

Animal Experiments and Tumor Formation *In vivo*

Female SCIDnodCA mice were kept under pathogen-free conditions in the animal barrier facility at the Biomedical Center, Lund University, in accordance with the Swedish guidelines for humane treatment of laboratory animals. The experimental setup was approved by the ethical committee for animal research in Malmö/Lund, Sweden. For assessment of antitumor activity, human bladder carcinoma T24 cells (1×10^6 in 200 μL PBS) were injected s.c. in the dorsal region of 7- to 8-week-old mice ($N = 40$). The animals were then randomly divided into control and treatment groups and received (*a*) no additives, (*b*) α-difluoromethylornithine (1%, w/v) via drinking water, (*c*) daily i.p. injections (0.2 mL) of HIV-Tat (1 μg/μL), or (*d*) α-difluoromethylornithine (1%, w/v) and daily i.p. injections (0.2 mL) of HIV-Tat (1 μg/μL). Animals were sacrificed, and tumor mass was determined after 18 days of treatment.

Results

HIV-Tat Peptide Is an Efficient Inhibitor of Polyamine Uptake in Human Carcinoma Cells

Previous work investigated the binding kinetics of growth-promoting polyamines to heparan sulfate, demonstrating K_d of 37 and 110 μmol/L for the interaction of the polyamine spermine with high-sulfated heparan sulfate and low-sulfated heparan sulfate, respectively (30). As a comparison, K_d of HIV-Tat binding to high-sulfated heparan sulfate was reported to be in the low-nanomolar range (31). Accordingly, Tat peptide displayed stronger binding to a heparin (high-sulfated heparan sulfate) affinity

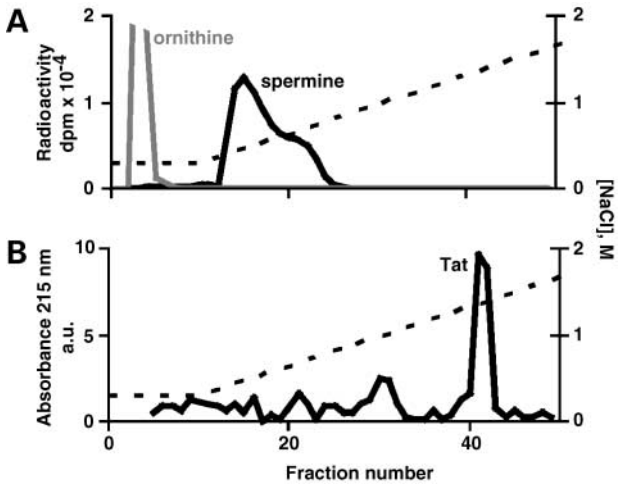

Figure 1. Tat peptide and polyamine binding to heparin. Spermine (0.5 mg) supplemented with 0.1 μCi [^{14}C]spermine or ornithine (0.5 mg) supplemented with 0.1 μCi [^{3}H]ornithine (**A**) or 100 μg Tat peptide (**B**) was added to a heparin-substituted HiTrap column, which was eluted with an NaCl gradient, as described in Materials and Methods. Liquid scintillation counting (**A**) or online UV detection at A_{215} (**B**) was done to determine the elution profile of the respective molecules. Data of two independent experiments.

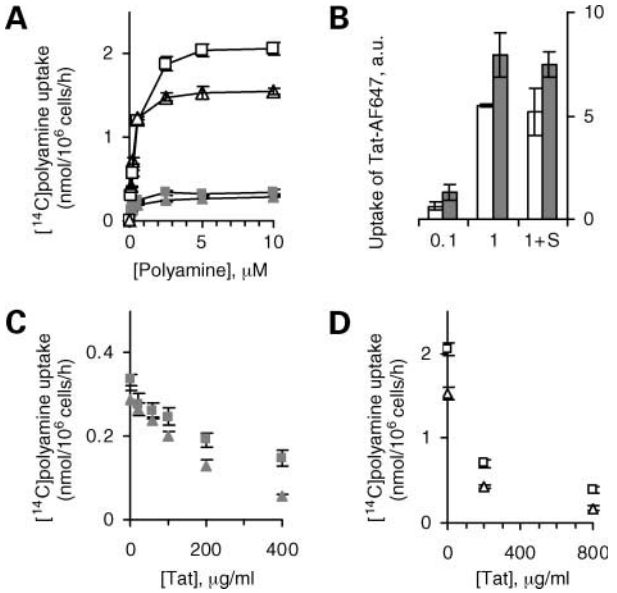

Figure 2. HIV-Tat efficiently inhibits polyamine uptake in mammalian cells. **A,** dose-dependent uptakes of [^{14}C]spermidine (*triangles*) and [^{14}C]spermine (*squares*) were determined in T24 cells after preincubation in the absence (*filled symbols*) or presence (*open symbols*) of 5 mmol/L α-difluoromethylornithine for 3 d. **B,** uptake of AlexaFluor-647–labeled Tat peptide (0.1 or 1 μg/mL) in either untreated (*open columns*) or α-difluoromethylornithine–treated (*gray columns*) T24 cells as in **A**. In some cases, spermine was added at a 1000-fold (w/w) excess (*1 + S*). **C,** the effect of Tat peptide on the uptake of 5 μmol/L [^{14}C]spermine or 5 μmol/L [^{14}C]spermidine in T24 cells. **D,** same experiment as in **C** after pretreatment of cells with 5 mmol/L α-difluoromethylornithine for 3 d. *Points,* mean ($n = 5$); *bars,* SD.

column compared with spermine (Fig. 1). Tat eluted as a major peak at ~1.4 to 1.5 mol/L NaCl, which is in agreement with previous studies (31, 32). Independent studies have implicated a role for heparan sulfate proteoglycan in the uptake of polyamines and HIV-Tat peptide in CHO cells (1); CHO cell mutants deficient in heparan sulfate proteoglycan showed reduced uptake of the polyamine spermine and HIV-Tat (24, 33). We next investigated whether these structurally unrelated polybasic compounds share the same route of entry in human carcinoma cells. Polyamine biosynthesis inhibition by α-difluoromethylornithine results in a severalfold induction of polyamine uptake in tumor cells, as has been previously shown (Fig. 2A; ref. 34). Intriguingly, α-difluoromethylornithine–treatment also increased HIV-Tat uptake (Fig. 2B). Internalization of both spermidine and spermine was efficiently inhibited by HIV-Tat peptide in a dose-dependent manner (Fig. 2C and D). HIV-Tat was a more efficient inhibitor of spermidine uptake than of spermine uptake (Fig. 2C), which may be expected due to the fact that spermidine and spermine have three and four positive charges, respectively, at physiologic conditions. Importantly, HIV-Tat peptide also showed substantial inhibition of polyamine uptake in α-difluoromethylornithine–treated cells (Fig. 2D). Consistent with the heparin binding data (Fig. 1), spermine was a poor inhibitor of HIV-Tat peptide uptake (Fig. 2B).

The well-established polyamine uptake–deficient CHO-MGBG cell line was isolated as a CHO cell subclone insensitive to methylglyoxal bis(guanylhydrazone) (a highly toxic polyamine analogue that enters cells via the polyamine transport pathway; refs. 18, 35). CHO-MGBG cells showed no alterations in cell-associated proteoglycan production compared with wild-type CHO cells, neither in terms of overall charge density (cf. *left* and *right panels* in Fig. 3C) nor in polyamine affinity (cf. *left* and *right panels* in Fig. 3D) as determined by ion exchange and spermine affinity chromatography, respectively. There were no obvious differences in the relative amounts of intact proteoglycans and low molecular weight oligosaccharides (cf. *left* and *right panels* in Fig. 3A). Moreover, CHO-MGBG cells displayed intact HIV-Tat peptide internalization compared with wild-type cells (Fig. 3A). Together with the fact that polyamine uptake and HIV-Tat uptake are reduced in proteoglycan-deficient cells (24, 33), our data suggest that HIV-Tat inhibits polyamine uptake via competition for proteoglycan binding sites rather than a putative polyamine-specific transporter that is defective in CHO-MGBG cells.

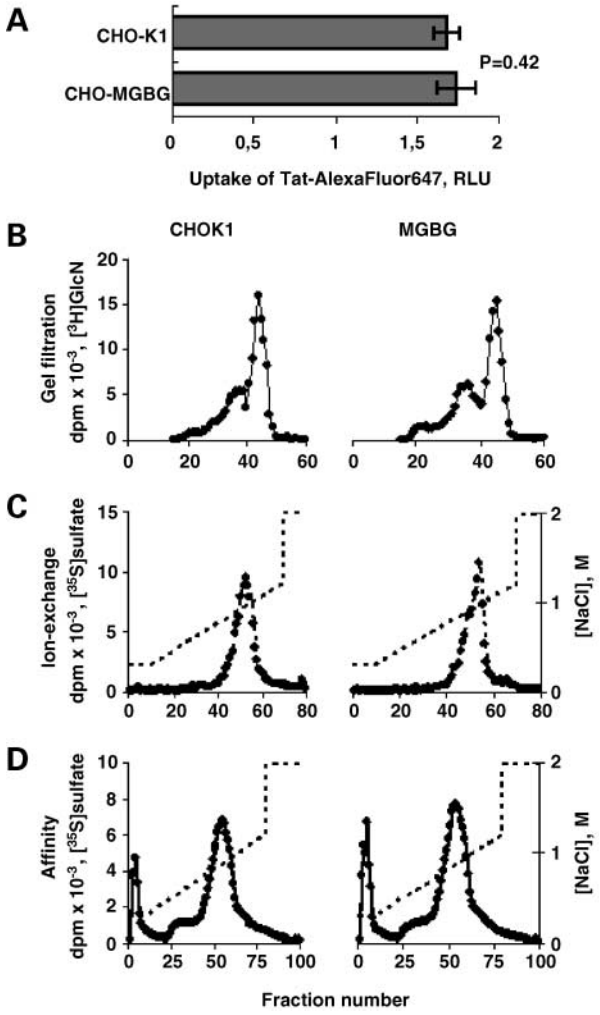

Figure 3. Polyamine uptake–deficient CHO-MGBG cells exhibit normal proteoglycan production and unperturbed HIV-Tat peptide uptake. **A**, wild-type CHO-K1 cells and mutant and polyamine uptake–deficient CHO-MGBG cells were grown to subconfluence. After rinsing, cells were incubated for 20 min with AlexaFluor-647-Tat (1 μg/mL). Intracellular AlexaFluor-647-Tat fluorescence was determined by flow cytometry. *Columns*, mean ($n = 4$); *bars*, SD. **B-D**, proteoglycans were labeled with [^{3}H]glucosamine (*GlcN*) and [^{35}S]sulfate in CHO-K1 cells (*left panel*) or CHO-MGBG cells (*right panel*), and proteoglycan material was analyzed by gel filtration chromatography on a Superpose 6 column (**B**). Fractions (18–30) corresponding to the heparan sulfate proteoglycan material were pooled and analyzed by ion exchange chromatography on a MonoQ column eluted with 0.3–1.2 mol/L NaCl gradient (**C**). Alternatively, [^{35}S]sulfate-labeled proteoglycan material was analyzed by affinity chromatography on a spermine-substituted HiTrap column eluted with 0.3 to 1.2 mol/L NaCl gradient (**D**). In all cases, aliquots of collected fractions were analyzed for radioactivity by liquid scintillation.

HIV-Tat Inhibits the Bioavailability of Extracellular Polyamines

It is well established that depletion of intracellular polyamines by α-difluoromethylornithine treatment results in inhibition of tumor cell proliferation, unless cells are provided with extracellular polyamines, such as in the *in vivo* situation where polyamines are provided in the diet, from bacteria in the gut and from a constant efflux of intracellular polyamines (13–15). As expected, T24 carcinoma cells showed decreased DNA synthesis in the presence of α-difluoromethylornithine, and this inhibition could be overcome by restituting the growth medium with polyamine (Fig. 4A). Whereas HIV-Tat peptide per se had no effect on DNA synthesis, the peptide counteracted the rescue by extracellular polyamine in α-difluoromethylornithine–treated cells. The data suggest that the HIV-Tat peptide competes for the bioavailability of extracellular polyamines and that the peptide is unable to replace the intracellular activities of polyamines. Consistent with this notion, the Tat peptide efficiently prevented intracellular accumulation of exogenous spermidine in α-difluoromethylornithine–treated cells (Fig. 4B). The fact that the spermidine level was even lower in cells treated with α-difluoromethylornithine and Tat peptide than with α-difluoromethylornithine alone suggests that HIV-Tat also interferes with the reuptake of polyamines that have leaked into the cell medium.

Tumor Growth Attenuation by Combined α-Difluoromethylornithine and HIV-Tat Peptide Treatment

We next investigated the combined effect of α-difluoromethylornithine and HIV-Tat peptide on *in vivo* tumor growth (Fig. 5). A relatively low α-difluoromethylornithine dose (1% w/v in the drinking water) had no significant effect on T24 carcinoma cell tumor formation under these conditions. Previous studies have shown that i.p. administration of HIV-Tat peptide has high bioavailability at the systemic level (36). Daily injections of HIV-Tat peptide (10 mg/kg) via this route had no significant effect on tumor formation. However, combined treatment with α-difluoromethylornithine and HIV-Tat peptide dramatically inhibited tumor growth. Average tumor mass of the control group was 51.9 ± 23.4 mg, whereas average tumor mass in the α-difluoromethylornithine/Tat group was 4.4 ± 2.2 mg (i.e., a 91.5% reduction in tumor mass). Importantly, no

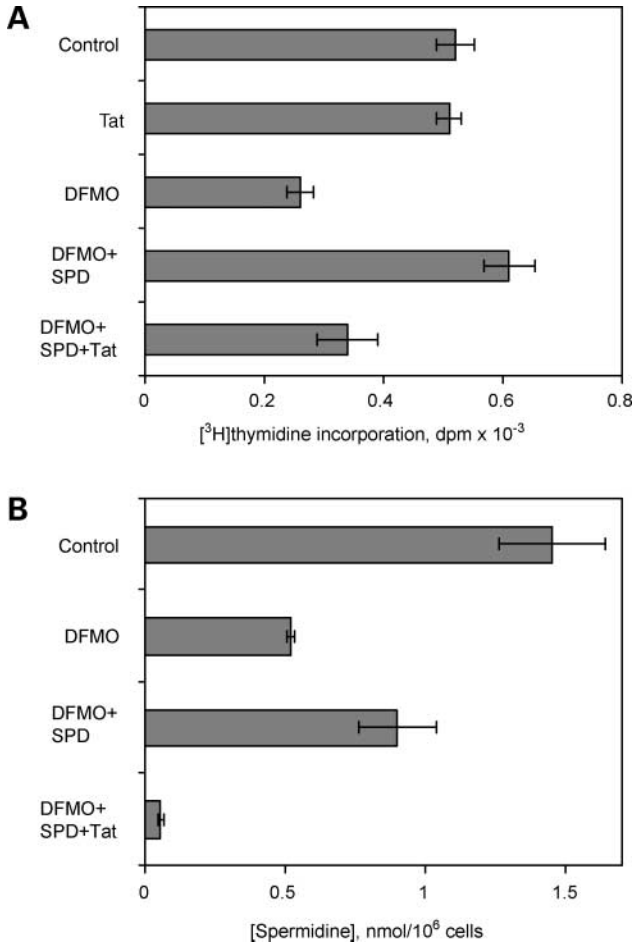

Figure 4. HIV-Tat peptide inhibits the bioavailability of extracellular polyamines. **A,** T24 cells were grown in the absence (*Control*) or presence of 5 mmol/L α-difluoromethylornithine (*DFMO*) for 72 h. The growth medium was then supplemented with 0.5 μmol/L spermidine (*SPD*) and 100 μg/mL HIV-Tat, and cells were incubated for another 16 h in the presence of 3 μCi/mL [^{3}H]thymidine. DNA synthesis was determined by measurement of incorporated [^{3}H]thymidine. **B,** T24 cells were grown in the absence or presence of 5 mmol/L α-difluoromethylornithine for 72 h. The growth medium was then supplemented with 0.5 μmol/L spermidine and 100 μg/mL HIV-Tat, and cells were incubated for another 16 h. Finally, cells were analyzed for spermidine content, as described in Materials and Methods. *Columns,* mean (n = 3–6); *bars,* SD.

signs of serious adverse effects of the α-difluoromethylornithine/HIV-Tat peptide combination were observed (e.g., the animal weight was unaltered compared with the controls). Only a few of the tumors in the group receiving combined treatment exceeded a diameter of 1 to 2 mm, and the tumors seemed as avascular pale nodes as opposed to the tumors in the other treatment groups that displayed rich vascularization upon gross examination. These observations were supported by very limited CD31 staining in tumors from animals receiving combined treatment.[4]

[4] K. Mani and M. Belting, unpublished observation.

Discussion

We show that a protein transduction peptide derived from HIV-Tat protein efficiently competes with polyamine uptake in mammalian cells and that combined treatment with the polyamine synthesis inhibitor α-difluoromethylornithine and HIV-Tat peptide inhibits carcinoma cell growth *in vitro* and attenuates tumor formation *in vivo*.

We and collaborators have previously shown that proteoglycans facilitate polyamine uptake in mammalian cells; mutant CHO cells deficient in proteoglycan formation were also deficient in polyamine uptake compared with wild-type cells (24). Moreover, proteoglycan-deficient cells were hypersensitive to α-difluoromethylornithine treatment and insensitive to rescue by extracellular polyamines, both *in vitro* and *in vivo* (24). Indeed, it has been shown that α-difluoromethylornithine–induced polyamine uptake is associated with structural alterations of heparan sulfate proteoglycan that favor polyamine binding and internalization (23, 37). The fact that α-difluoromethylornithine also seems to up-regulate Tat peptide internalization (Fig. 2B) suggests a common internalization pathway for polyamines and HIV-Tat peptide. The CHO-MGBG cell line that does not accumulate extracellular polyamines and

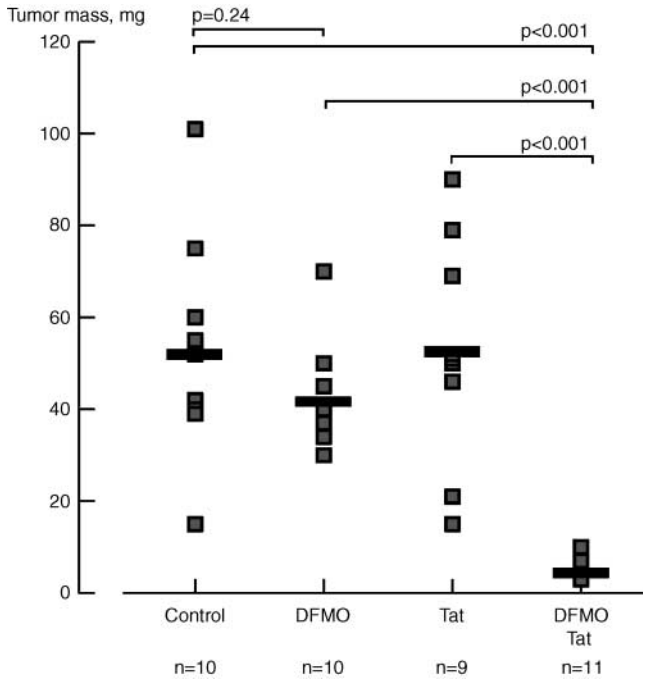

Figure 5. Combined treatment with α-difluoromethylornithine and Tat peptide attenuates *in vivo* tumor growth. T24 cells were inoculated s.c. on the dorsal side of female SCID mice. The animals were then randomly divided into control and treatment groups receiving (*a*) no additives, (*b*) α-difluoromethylornithine (1%, w/v) via the drinking water, (*c*) daily i.p. injections (0.2 mL) of HIV-Tat (1 μg/μL), or (*d*) α-difluoromethylornithine (1%, w/v) and daily i.p. injections (0.2 mL) of HIV-Tat (1 μg/μL). After 18 d of tumor inoculation, animals were sacrificed and tumor mass was determined. *Horizontal bars,* average tumor mass of respective groups. Data were obtained from two independent experiments. *P* values were calculated using Student's *t*-test.

displays normal proteoglycan production when compared with wild-type CHO cells showed unperturbed uptake of Tat peptide (Fig. 3). The data suggest divergent internalization pathways for HIV-Tat and polyamines downstream of the proteoglycan-dependent internalization step and that additional, yet unknown, transport molecule/s are necessary for efficient polyamine uptake.

It is clear from our data that the Tat peptide is unable to mimic the growth-promoting function of polyamines in α-difluoromethylornithine–treated cells. The HIV-1 Tat protein, reportedly, has effects that are unrelated to its interaction with the target TAR HIV sequence, and both inhibitory and stimulatory effects of exogenously administered Tat on cellular proliferation have been reported (38–42). In our study, Tat efficiently inhibited growth of α-difluoromethylornithine–treated cells and prevented intracellular accumulation of exogenous polyamine, suggesting that Tat specifically interferes with the bioavailability of extracellular polyamines.

We show that combined α-difluoromethylornithine and Tat treatment has profound effects on tumor formation *in vivo* (Fig. 5). Although the Tat peptide per se did not inhibit tumor formation, it is still possible that the Tat peptide, in the context of polyamine deprivation, exhibits effects in addition to inhibition of polyamine uptake. Because heparan sulfate proteoglycans are known to act as coreceptors for major proangiogenic growth factors, most notably vascular endothelial growth factor and fibroblast growth factor 2 (25), it is conceivable that the Tat peptide exerts antiangiogenic effects via competition with growth factors for heparan sulfate proteoglycan binding, thus, leading to blockage of tumor expansion. This would explain the relatively greater *in vivo* effects of the α-difluoromethylornithine/Tat peptide combination compared with the *in vitro* situation. Notably, it has been shown that in ornithine decarboxylase–overexpressing mice that develop spontaneous skin tumors due to elevated polyamine levels, treatment with α-difluoromethylornithine resulted in tumor regression through an antiangiogenic effect (43). Ongoing studies in our laboratory aim at investigating the role of polyamines in angiogenesis in more detail.

Interestingly, systemic administration of HIV-Tat peptide shows wide tissue distribution, including to the central nervous system (36), which should make gliomas that have overall poor prognosis and have shown partial response to α-difluoromethylornithine treatment (17, 44) an interesting target for combined α-difluoromethylornithine and HIV-Tat peptide treatment.

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