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Expression Analysis and Modulation by HIV-Tat of the Tyrosine Phosphatase HD-PTP

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Abstract The human immunodeficiency virus type 1 Tat transactivates viral proteins and also affects the expression of eukaryotic genes. Since Tat is angiogenic, we assumed that the isolation of differentially expressed genes in Tat-treated endothelial cells would yield insights into the molecular mechanisms of the angiogenic process. By RNA fingerprinting, we found that Tat upregulates the tyrosine phosphatase HD-PTP mRNA in a human endothelial cell line. At the moment, little is known about HD-PTP. We here show that HD-PTP is highly conserved through evolution from yeast to man, and is ubiquitously distributed in adult and fetal tissues. HD-PTP is expressed in human cell lines derived from different tumors, but the mRNA levels do not appear to correlate with the malignant phenotype of the cells. HD-PTP mRNA was also detected in cell lines derived from tumors that develop in BKV/Tat-transgenic mice. Interestingly, a relation exists between the amounts of secreted Tat and the levels of HD-PTP mRNA. HD-PTP encodes a 185-kDa protein which is expressed in human endothelial from the umbilical cord and in human Kaposi-spindle cells. Tat-induction of HD-PTP mRNA parallels only with a slight increase of the protein, which occurs after 24 and 48 h of incubation in the presence of Tat. These results suggest that HD-PTP amounts might be regulated both at the transcriptional and post-transcriptional levels. J. Cell. Biochem. 98: 301–308, 2006.

Key words: Tat; AIDS; angiogenesis; HD-PTP

The human immunodeficiency virus transactivator of transcription HIV-1-Tat (Tat) is a small polypeptide essential for efficient transcription of viral genes and for viral replication. Tat, which is released from Tat expressing cells into the extracellular milieu, binds to specific receptors on the neighboring cell membrane, triggering different signal transduction pathways that lead to the transactivation of cellular genes.

Tat can function as a cytokine in the activation of endothelial cells [Hofman et al., 1993]. Moreover, it plays a role in the pathogenesis of Kaposi's sarcoma (KS), a highly vascularized skin lesion characterized by marked endothelial proliferation and migration, resulting in the formation of new capillaries. Accordingly, BKV/Tat transgenic mice develop highly vascularized lesions, which closely resemble KS as well as tumors of different histotypes. Indeed, Tat is angiogenic in vivo [Albini et al., 1995] and binds preferentially receptors such as vascular endothelial growth factor receptor (VEGFR)-2 [Albini et al., 1996] and, via the RGD motif, $\alpha_v\beta_3$, and $\alpha_5\beta_1$ integrins [Barillari et al., 1999] in endothelial cells. Tat induces transient activation of mitogen-activated protein kinase (MAPK) ERK(1/2) in endothelial cells [Rusnati et al., 2001] and modulates endothelial cell permeability acting on the MAPK pathway [Oshima et al., 2000]. Tat also modulates the activity of protein tyrosine phosphatases (PTP) in endothelial cells, leading to a decrease in the levels of phosphotyrosine-containing proteins such as Erk-2/MAPK kinase [Wu et al., 1998]. Moreover, Tat-dependent expression of MMP-9 in human monocytes is mediated by activation of PTPs [Kumar et al., 1999]. In addition, signal transduction mechanisms regulated by PTP activities are important for HIV-1 replication and PTP inhibitors increase HIV-1 gene expression [Ouellet et al., 2003].

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Since Tat modulates endothelial function, we assumed that the isolation of differentially expressed genes in Tat-treated endothelial cells would yield insights into the molecular regulatory mechanisms of early events in the angiogenic process in general and in the pathogenesis of KS in particular. By RNA fingerprinting, we found that Tat upregulates HD-PTP mRNA. Human HD-PTP, a member of the PTP superfamily, has been isolated and located on the short arm of chromosome 3 [Toyooka et al., 2000], an area frequently deleted in many types of human tumors [Hibi et al., 1994; Kok et al., 1997]. The rat homolog of HD-PTP was reported to inhibit activated Ha-ras-mediated NIH-3T3 transformation [Cao et al., 1998]. HD-PTP predicted sequence contains a PTP-like domain and a PEST motif (rich in proline, glutamic acid, serine, and threonine) in the C-terminal region. In the N-terminal region, it is homologous to a yeast protein, BRO1, involved in the MAPK signaling pathway. However, HD-PTP has not yet been characterized.

In this manuscript, we show for the first time that HD-PTP is evolutionary conserved and ubiquitously expressed. At the protein level, we demonstrate that human KS-derived spindle cells and endothelial cells, which are considered their precursors, produce HD-PTP. In addition, in endothelial cells, Tat induces HD-PTP mRNA but only slightly modulates the total amounts of the protein, even when we generate a pro-inflammatory environment by adding interleukin (IL)-1α.

MATERIALS AND METHODS

Isolation of HD-PTP

RNA fingerprinting on 1 μg of total RNA from endothelial cells treated for 4 and 24 h with recombinant 100 ng/ml Tat (Intracel, Cambridge, MA) was performed as described [Dragoni et al., 1998]. The bands corresponding to differentially expressed genes were excised from the polyacrylamide gel, electroeluted, reamplified by PCR, sub-cloned in Bluescript, and sequenced. The 2.5 Kb fragment of HD-PTP was obtained by screening a human umbilical vein endothelial cell (HUVEC) cDNA library (Clontech) with the 700-bp fragment obtained by DNA fingerprinting. The DNA sequence was analyzed by the Analyze and Interpret programs of the Mac Molly Suite (Berlin). Deduced protein sequences were compared and aligned using BLASTX with ClustalW 1.7 programs made available by Baylor College of Medicine.

Cell Culture

HUVEC were cultured in M199 containing 10% fetal calf serum (FCS), endothelial cell growth supplement (ECGS) (150 μg/ml), and heparin (5 U/ml) on 2% gelatin coated dishes. KS-IMM cells, provided by Dr. A. Albini (Genova), were derived from a human KS lesion and can induce KS-like lesions when injected subcutaneously in nude mice [Marchio et al., 1999]. BKV/Tat transgenic mice develop tumors of different hystotypes [Corallini et al., 1993]. Cell lines were derived from these tumors: T53 and T61 from adenocarcinoma, T111 from a leiomyosarcoma, and TTB from cutaneous Kaposi-like lesions. KS-IMM and all the cells from the BKV/Tat-transgenic mice were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FCS.

Western Blot Analysis

A rabbit polyclonal antibody was generated by standard procedures immunizing rabbits with a peptide (N-SIRPPGGLESPVASLPG-PAEP-C) contained within the C-terminus (amino acids 1506–1526) of human HD-PTP. This epitope is not conserved in other species. The antibody recognizes a protein band of 185 kDa, which is consistent with the predicted size of human HD-PTP. Pre-immune serum does not recognize any band. IgGs against HD-PTP were purified on a protein A-Sepharose column. For Western blot, cell extracts (75 μg/lane) were resolved by 8% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose sheets at 150 mA for 16 h, and probed with anti-HD-PTP IgGs (10 μg/ml). Antibodies against actin were from Santa Cruz (Tebu-bio). Secondary antibodies were labeled with horseradish peroxidase (Pierce). The SuperSignal chemiluminescence kit (Pierce) was used to detect immunoreactive proteins. All the Western blots were repeated at least three times.

Purification of RNA and Northern Blot

Cells were rinsed with phosphate-buffered saline and lysed in 4 M guanidinium isothiocyanate. RNA was purified as described [Dragoni et al., 1998; Rusnati et al., 2001]. RNA was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, capillary-blotted onto

nylon membranes, and UV-cross-linked. HD-PTP and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were labeled with a random primer labeling kit (Ambion, Inc.). Filters were hybridized in 0.5 M sodium phosphate (pH7.2) containing 7% SDS, 1 mM EDTA, and 20% formamide at 65°C for 20 h and extensively washed at high stringency. mRNAs were visualized by autoradiography.

Zoo Blot

A Southern blot containing 4 μg of genomic DNA/lane from nine eukaryotic species was purchased from Clontech and hybridized as described above.

RESULTS

Evolutionary Conservation and Tissue Distribution of HD-PTP

To obtain cDNAs representing mRNAs regulated by Tat in human endothelial cells, we utilized a modified PCR-based differential screening approach commonly referred to as RNA fingerprinting [Consalez et al., 1996]. We identified several differentially expressed genes (Fig. 1) and four of them, temporarily denominated from C1 to C4, were significantly modulated. We pointed our attention to the C1 fragment. By sequence analysis and sequence homology search (NCBI-Genbank), C1 was shown to encode for the C-terminal region of HD-PTP, a tyrosine phosphatase located on human chromosome 3p21.3 [Toyooka et al., 2000]. Sequence comparison also showed a 70% identity at the nucleotide level to rat PTP-TD14 [Cao et al., 1998].

Using the 2.5 Kb cDNA, a Southern blot was performed on EcoRI cut genomic DNA from different species. Under conditions of high stringency hybridization, we observed strong specific signals in all the species examined, including yeast, reflecting the existence of orthologues of HD-PTP in each of these species (Fig. 2).

We also determined the distribution of HD-PTP transcript in adult and fetal human tissues. A 5.8 kb mRNA was detected in all the adult human tissues examined and was particularly abundant in heart, placenta, and skeletal muscle, while it was almost undetectable in liver (Fig. 3A). HD-PTP is also expressed in all the fetal tissues analyzed (Fig. 3B), being most abundant in the brain and in the kidney.

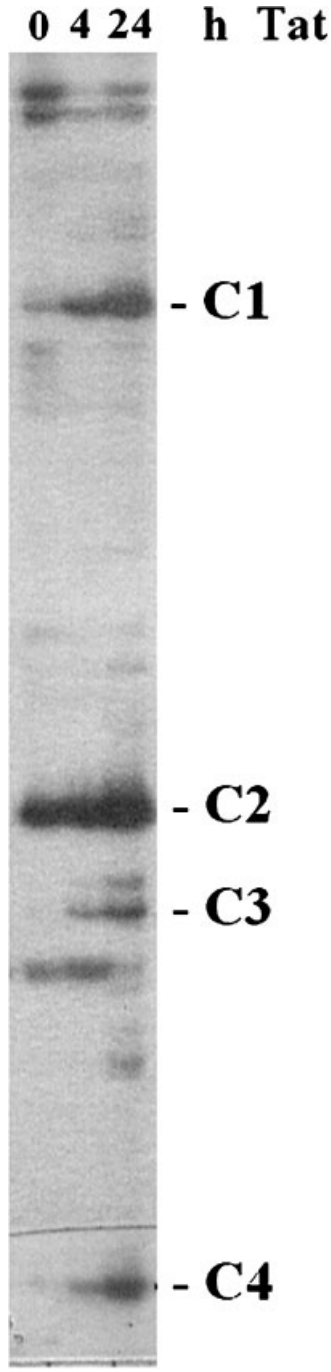

Fig. 1. RNA fingerprinting analysis of Tat-modulated mRNA in endothelial cells. Cells were treated with 100 ng/ml Tat for 4 and 24 h and differential display was performed as described in the Materials and Methods. The fragments, which were significantly modulated by Tat, were indicated as C1, C2, C3, and C4.

Expression of HD-PTP RNA in Different Cell Lines

Since *HD-PTP* gene has been located on human chromosome 3p21.3, a region suspected to play an important role in tumorigenesis [Hibi

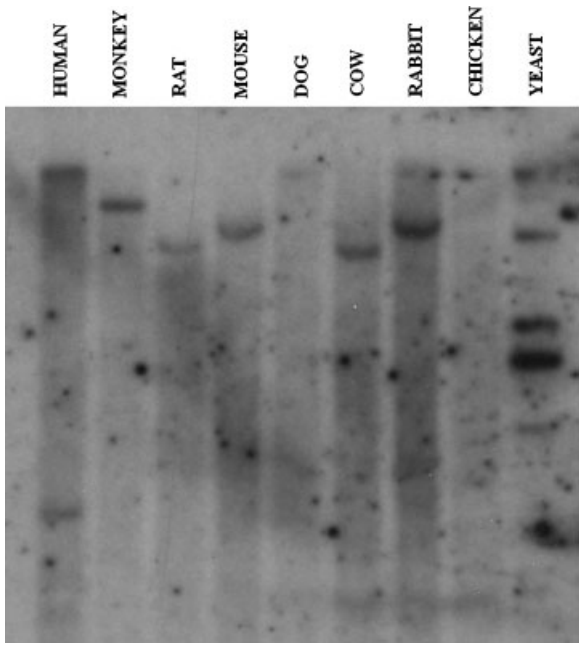

Fig. 2. Southern blot analysis of HD-PTP. The zoo blot was hybridized at high stringency to HD-PTP cDNA and visualized by autoradiography.

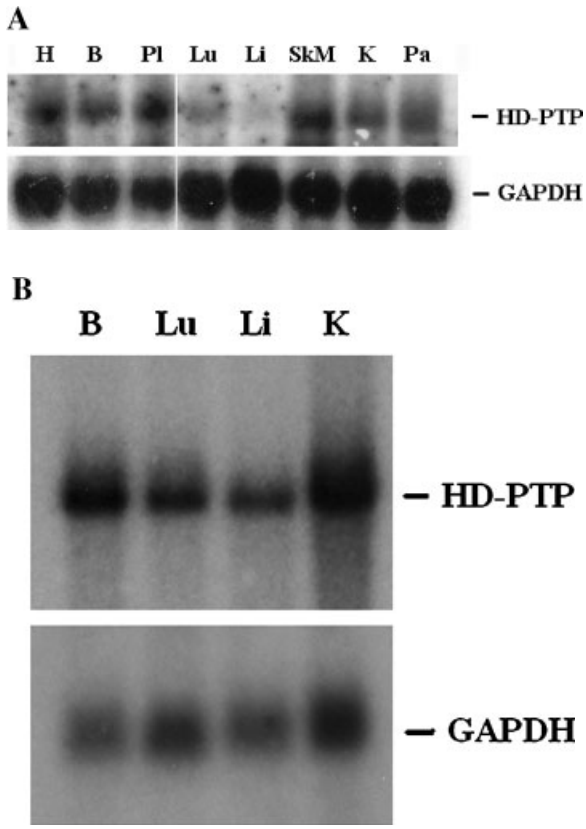

Fig. 3. Tissue distribution of HD-PTP. **A**: A human adult multiple-tissue Northern blot was purchased from Clontech. Northern blotting was performed at high stringency using the human HD-PTP cDNA probe. H, heart; B, brain; Pl, placenta; Lu, lung; Li, liver; SkM, muscle; K, kidney; Pa, pancreas. **B**: A human fetal multiple-tissue Northern blot was purchased from Clontech. Northern blotting was performed as described above. B, brain; Lu, lung; Li, liver; K, kidney. In A and B, hybridization to GAPDH indicates that similar amounts of RNA were used per lane.

et al., 1994; Kok et al., 1997], we evaluated HD-PTP expression in a panel of human cell lines derived from different tumors. Figure 4A shows the amounts of HD-PTP transcript in several breast cancer cell lines. Among these, only MDA-MB-486 and MDA-MB-578 showed increased levels of mRNA, but no relation exists with the extent of the malignant phenotype of the cells. We extended our study to a panel of cell lines derived from different tumors, but we did not find relevant differences by Northern blot (Fig. 4B). These data suggest that, despite the indications about a possible tumor suppressor function of HD-PTP, the mRNA levels do not appear to be a direct marker of transformation.

Modulation of HD-PTP RNA by Tat

The upregulation of HD-PTP by Tat detected by RNA fingerprinting was confirmed by Northern blot analysis in HUVEC exposed to Tat (100 ng/ml) up to 24 h. Tat increased HD-PTP mRNA at 4 h with a maximal stimulation observed after 24 h stimuli (Fig. 5A).

Since endothelial cells upregulate HD-PTP mRNA in response to Tat, we evaluated the levels of HD-PTP transcript in cell lines isolated from tumors that develop in BKV/Tat transgenic mouse. T53 and T61 cells derive from skin adenocarcinoma and T111 from a leiomyosarcoma [Corallini et al., 1993]. Kaposi-like spindle TTB cells were isolated from highly vascularized lesions that closely resemble human KS [Corallini et al., 1993]. Northern blot was performed utilizing the 2.5 kb human HD-PTP cDNA fragment which is highly homologous to the murine cDNA. After normalization, we show that TTB cells expressed less HD-PTP mRNA than T53, T61, and T111 cells (Fig. 5B). The different amounts of HD-PTP in adenocarcinoma-derived T53 versus T61 cells might reflect the different amount of Tat secretion. Indeed, T53 cells secrete much higher amounts of Tat than T61, while TTB cells do not express Tat at all [Campioni et al., 1995].

We therefore suggest that extracellular Tat plays a role in sustaining the expression of HD-PTP.

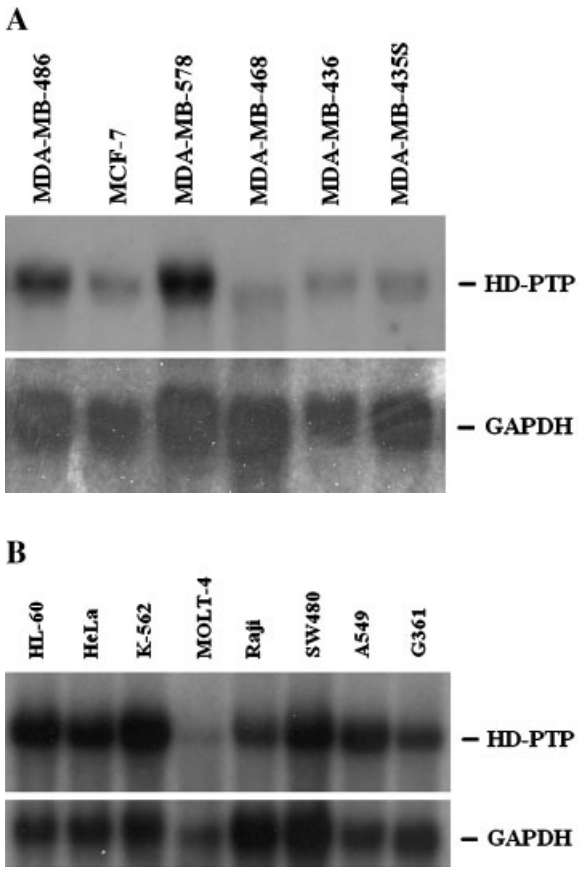

Fig. 4. Expression of HD-PTP in cell types derived from different tumors. Total RNA from breast cancer (**A**) and different tumor cell lines (**B**) was analyzed by Northern blot and normalized for GAPDH amounts as described in the Materials and Methods.

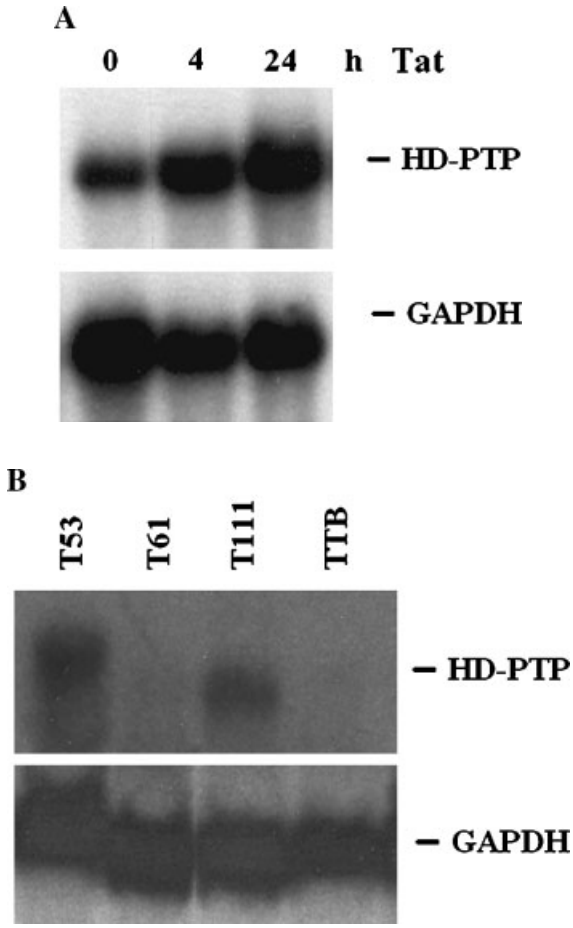

Fig. 5. Modulation of HD-PTP mRNA by Tat. **A**: Subconfluent cultures of HUVEC were exposed to 100 ng/ml Tat for 4 and 24 h. Northern Blot was performed as described. **B**: Murine cell lines from BKV/Tat transgenic mice were analyzed by Northern blot. T53 and T61 cells derive from adenocarcinomas; T111 from a leiomyosarcoma; Kaposi-like spindle TTB cells derive from highly vascularized lesions similar to human KS. In A and B, GAPDH was used to verify equal amounts of RNA loading among the lanes.

HD-PTP Protein Levels in Endothelial and KS-Spindle Cells

We developed a rabbit polyclonal antibody against HD-PTP by immunizing against a hydrophilic C-terminal epitope of the human HD-PTP protein. The antibody specifically recognized a ~185 kDa protein which corresponds to the predicted molecular mass of HD-PTP. Using our antibody, human endothelial and human Kaposi-spindle cells (KS-IMM) were tested for the total amount of HD-PTP by Western blot analysis. KS are particularly intriguing because they show a unique phenotype and originate from endothelial precursors [Cavallaro et al., 1996]. Figure 6 shows that the total amounts of HD-PTP are slightly higher in KS-IMM cells than in HUVEC.

To evaluate whether Tat induction of HD-PTP translated into an accumulation of the protein, we performed Western blots on HUVEC treated with Tat for different times. We observed a modest but reproducible increase of HD-PTP levels in cells exposed to Tat for 24 and 48 h (1.5- and 1.7-fold, respectively) (Fig. 7A). On the bases of previous results [Albini et al., 1995], we reasoned that a pro-inflammatory environment might enhance endothelial cell response to Tat. We treated HUVEC for 24 and 48 h with Tat in the presence or in the absence of IL-1α. The pro-inflammatory cytokine IL-1α alone did not affect the total amounts of HD-PTP, whereas the coincubation with both Tat and IL-1α slightly induced HD-PTP (Fig. 7B).

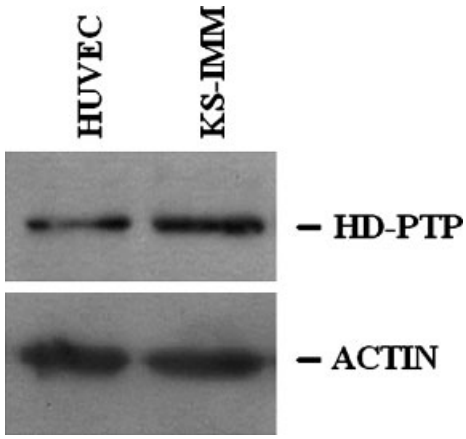

Fig. 6. Expression of HD-PTP in endothelial and KS cells. Western blot was performed on cell extracts (50 μg) from HUVEC and KS-IMM cells using immunopurified IgGs against HD-PTP.

DISCUSSION

PTPs regulate fundamental cellular processes and are now recognized as critical regulators of signal transduction under normal and pathophysiological conditions [Stoker, 2005]. Indeed, rather than simply scavenging phosphotyrosine, the PTPs specifically regulate a wide range of signaling pathways controlling diverse processes such as focal adhesion dynamics, cell–cell adhesion, and insulin signaling. Little is known about HD-PTP, a tyrosine phosphatase isolated a few year ago. Since we found HD-PTP mRNA upregulated in response to Tat in endothelial cells, we have performed some studies to characterize it. We found that HD-PTP is highly conserved through evolution. Its evolutionary conservation suggests that HD-PTP is involved in basic cellular functions, since it has been detected from yeast to mammals. In addition, HD-PTP is expressed in all adult and fetal tissues as well as in different cell types. Due to its ubiquitous distribution, it is reasonable to propose that HD-PTP is involved in modulating cell functions in different tissues.

PTPs are of interest in the pathogenesis of AIDS, since (i) PTPs are important for HIV-1 replication and (ii) PTP inhibitors increase *HIV-1* gene expression [Ouellet et al., 2003]. Tat, a

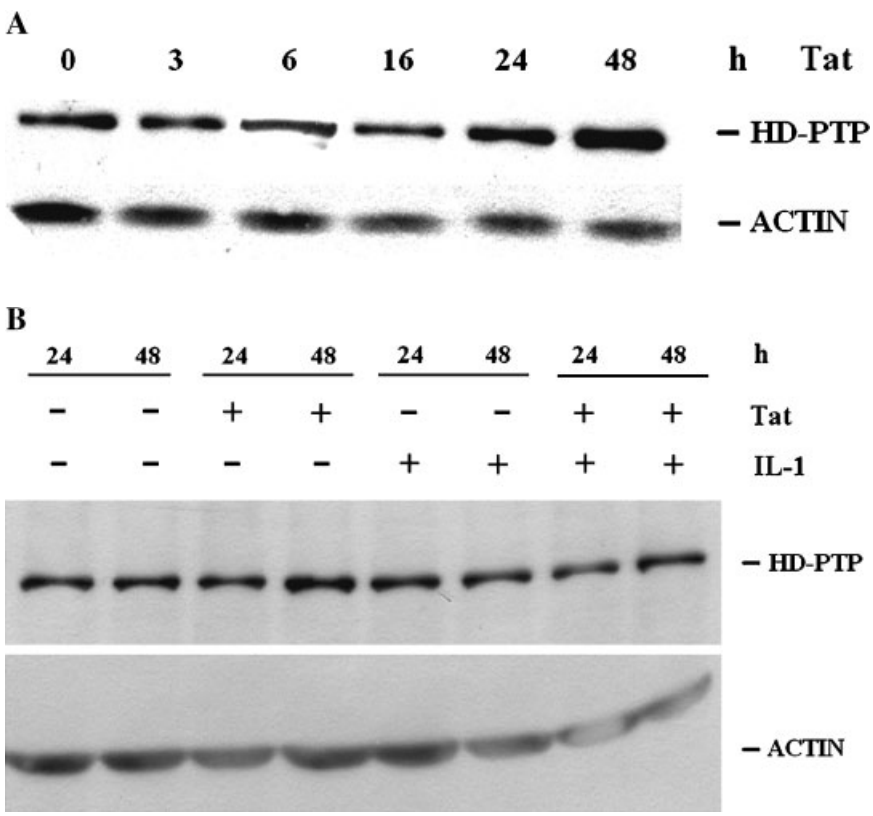

Fig. 7. Expression of HD-PTP in endothelial cells in response to Tat. **A**: Western blot on HUVEC exposed to Tat for different times. **B**: Western blot on HUVEC exposed to Tat and IL-1α alone or in combination, for 24 and 48 h. In A and B, actin was used to show that similar amounts of protein were loaded per lane.

transactivator of both viral and host gene, modulates some cellular functions through the activation of PTPs. In endothelial cells, Tat decreases the levels of phosphotyrosine-containing proteins such as Erk-2/MAPK kinase by activating PTPs [Wu et al., 1998]. It is therefore intriguing that we found that Tat induces HD-PTP mRNA in endothelial cells. Interestingly, a discrepancy exists between the induction of HD-PTP at the mRNA and protein level, since the marked induction of HD-PTP mRNA by Tat was paralleled only by a slight increase of the total amounts of the protein. To this purpose, it is noteworthy that in the endothelial ECV cell line Tat induced HD-PTP only at the mRNA and not at the protein level, while another angiogenic protein, that is, fibroblast growth factor (FGF)-2, stimulated HD-PTP degradation via the proteasome system [Mariotti et al., in press]. We therefore propose that also in HUVEC post-transcriptional events might be involved in regulating HD-PTP levels. Interestingly, it has been shown that Tat affects the subunit composition of proteasomes and increases the proteolytic activities [Gavioli et al., 2004]. In addition, HD-PTP possesses a PEST domain at the C-terminal and many PEST-containing proteins are degraded through the proteasome [Spencer et al., 2004]. The involvement of other intracellular degradation enzymes in shortening HD-PTP half-life is also possible.

At the moment, we have no data about the enzymatic activity of HD-PTP. When the rat homolog PTP-TD14 was tested in non-specific PTPase assays, no phosphatase activity was detected [Cao et al., 1998]. Nevertheless, expression of PTP-TD14 in NIH-3T3 cells decreased Ha-ras mediated transformation, while a mutant, in which the putative catalytic domain was mutated, did not. These results suggest that PTP-TD14 may have restricted substrate specificity.

HD-PTP is located on human chromosome 3p21.3 [Toyooka et al., 2000], a region suspected to play an important role in tumorigenesis [Hibi et al., 1994; Kok et al., 1997]. We therefore evaluated HD-PTP mRNA in different carcinoma cell lines and suggest that no relation exists between HD-PTP mRNA and the rate of cell proliferation or cell transformation. HD-PTP has previously been proposed as a candidate tumor suppressor gene [Cao et al., 1998]. At the moment, our results argue against this possibility. However, further studies at the protein level are necessary to address the possible role of HD-PTP in tumors. By Western blot, we found slightly higher amounts of HD-PTP in KS-IMM than in HUVEC. Since KS-IMM proliferate faster than endothelial cells (not shown) and are tumorigenic in vivo, our results underscore that no relation may exist between HD-PTP total amounts and an aggressive phenotype. Indeed, a rather complicated picture is emerging in relation to the possible role of tyrosine phosphatases in cancer. For instance, overexpression of PTPα causes persistent activation of the PTK Src, with concomitant cell transformation [Zheng et al., 1992]. Accordingly, increased PTPα mRNA levels were demonstrated in late-stage colorectal tumors [Tabiti et al., 1995], and PTPα protein levels were elevated in one-third of primary breast cancer [Ardini et al., 2000].

Our future challenge is to define the substrate specificities of HD-PTP in endothelial cells, with the ultimate goal of applying our knowledge to a therapeutic setting in any situation characterized by dysregulated angiogenesis.

REFERENCES

Albini A, Barillari G, Benelli R, Gallo RC, Ensoli B. 1995. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. Proc Natl Acad Sci USA 92:4838–4842.

Albini A, Soldi R, Giunciuglio D, Giraudo E, Benelli R, Primo L, Noonan D, Salio M, Camussi G, Rockl W, Bussolino F. 1996. The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. Nat Med 1996; 2(12):1371–1375.

Ardini E, Agresti R, Tagliabue E, Greco M, Aiello P, Yang LT, Menard S, Sap J. 2000. Expression of protein tyrosine phosphatase alpha (RPTPalpha) in human breast cancer correlates with low tumor grade, and inhibits tumor cell growth in vitro and in vivo. Oncogene 19:4979–4987.

Barillari G, Sgadari C, Fiorelli V, Samaniego F, Colombini S, Manzari V, Modesti A, Nair BC, Cafaro A, Sturzl M, Ensoli B. 1999. The Tat protein of human immunodeficiency virus type-1 promotes vascular cell growth and locomotion by engaging the alpha5beta1 and alphavbeta3 integrins and by mobilizing sequestered basic fibroblast growth factor. Blood 94(2):663–672.

Campioni D, Corallini A, Zauli G, Possati L, Altavilla G, Barbanti-Brodano G. 1995. HIV type 1 extracellular Tat protein stimulates growth and protects cells of BK virus/tat transgenic mice from apoptosis. AIDS Res Hum Retroviruses 11(9):1039–1048.

Cao L, Zhang L, Ruiz-Lozano P, Yang Q, Chien KR, Graham RM, Zhou M. 1998. A novel putative protein-tyrosine phosphatase contains a BRO1-like domain and

suppresses Ha-ras-mediated transformation. J Biol Chem 273:21077–21083.

Cavallaro U, Gasparini G, Soria MR, Maier JA. 1996. Spindle cells isolated from Kaposi's sarcoma-like lesions of BKV/Tat-transgenic mice co-express markers of different cell types. AIDS 10(11):1211–1219.

Consalez GG, Corradi A, Ciarmatori S, Bossolasco M, Malgaretti N, Stayton CL. 1996. A new method to screen clones from differential display experiments prior to RNA studies. Trends Genet 12(11):455–456.

Corallini A, Altavilla G, Pozzi L, Bignozzi F, Negrini M, Rimessi P, et al. 1993. Systemic expression of HIV-1 *tat* gene in transgenic mice induces endothelial proliferation and tumors of different histotypes. Cancer Res 53:5569–5575.

Dragoni I, Mariotti M, Consalez GG, Soria MR, Maier JA. 1998. EDF-1, a novel gene product down-regulated in human endothelial cell differentiation. J Biol Chem 273. 31119–31124.

Gavioli R, Gallerani E, Fortini C, Fabris M, Bottoni A, Canella A, Bonaccorsi A, Marastoni M, Micheletti F, Cafaro A, Rimessi P, Caputo A, Ensoli B. 2004. HIV-1 tat protein modulates the generation of cytotoxic T cell epitopes by modifying proteasome composition and enzymatic activity. J Immunol 173(6):3838–3843.

Hibi K, Yamakawa K, Ueda R, Horio Y, Murata Y, Tamari M, Uchida K, Takahashi T, Nakamura Y, Takahashi T. 1994. Aberrant upregulation of a novel integrin alpha subunit gene at 3p21.3 in small cell lung cancer. Oncogene 9:611–619.

Hofman FM, Wright AD, Dohadwala MM, Wong-Staal F, Walker SM. 1993. Exogenous tat protein activates human endothelial cells. Blood 82:2774–2780.

Kok K, Naylor SL, Buys CH. 1997. Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. Adv Cancer Res 71:27–92.

Kumar A, Dhawan S, Mukhopadhyay A, Aggarwal BB. 1999. Human immunodeficiency virus-1-tat induces matrix metalloproteinase-9 in monocytes through protein tyrosine phosphatase-mediated activation of nuclear transcription factor NF-kappaB. FEBS Lett 462(1-2): 140–144.

Marchio S, Primo L, Pagano M, Palestro G, Albini A, Veikkola T, Cascone I, Alitalo K, Bussolino F. 1999. Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. J Biol Chem 274(39):27617–27622.

Oshima T, Flores SC, Vaitaitis G, Coe LL, Joh T, Park JH, Zhu Y, Alexander B, Alexander JS. 2000. HIV-1 Tat increases endothelial solute permeability through tyrosine kinase and mitogen-activated protein kinase-dependent pathways. AIDS 14(5):475–482.

Ouellet M, Barbeau B, Tremblay MJ. 2003. Protein tyrosine phosphatases in T cell activation: Implication for human immunodeficiency virus transcriptional activity. Prog Nucleic Acid Res Mol Biol 73:69–105. Review

Rusnati M, Urbinati C, Musulin B, Ribatti D, Albini A, Noonan D, Marchisone C, Waltenberger J, Presta M. 2001. Activation of endothelial cell mitogen activated protein kinase ERK(1/2) by extracellular HIV-1 Tat protein. Endothelium 8(1):65–74.

Spencer ML, Theodosiou M, Noonan DJ. 2004. NPDC-1, a novel regulator of neuronal proliferation, is degraded by the ubiquitin/proteasome system through a PEST degradation motif. J Biol Chem 279:37069–37078.

Stoker AW. 2005. Protein tyrosine phosphatases and signalling. J Endocrinol 185(1):19–33.

Tabiti K, Smith DR, Goh HS, Pallen CJ. 1995. Increased mRNA expression of the receptor-like protein tyrosine phosphatase alpha in late stage colon carcinomas. Cancer Lett 93:239–248.

Toyooka S, Ouchida M, Jitsumori Y, Tsukuda K, Sakai A, Nakamura A, Shimizu N, Shimizu K. 2000. HD-PTP: A novel protein tyrosine phosphatase gene on human chromosome 3p21.3. Biochem Biophys Res Commun 278:671–678.

Wu Z, Cavallaro U, Marchisio PC, Soria MR, Maier JA. 1998. Fibronectin modulates the effects of HIV-1 Tat on the growth of murine Kaposi's sarcoma-like cells through the downregulation of tyrosine phosphorylation. Am J Pathol 152:1599–1605.

Zheng XM, Wang Y, Pallen CJ. 1992. Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 359:336–339.