Journal of Biochemistry and Molecular Biology, Vol. 34, No. 1, January 2001, pp. 33-38

JOURNAL OF
Biochemistry
and
Molecular Biology

The Human PTK6 Interacts with a 23-kDa Tyrosine-Phosphorylated Protein and is localized in Cytoplasm in Breast Carcinoma T-47D Cells

Joon-Seol Bae and Seung-Taek Lee*

National Research Laboratory of Cellular Biochemistry, Department of Biochemistry, College of Science, and Protein Network Research Center, Yonsei University, Seoul 120-749, Korea

Received 3 November 2000, Accepted 28 November 2000

The human PTK6 (also known as Brk) polypeptide, which is deduced from its full-length cDNA, represents a non-receptor protein tyrosine kinase (PTK). It contains SH3, SH2, and tyrosine kinase catalytic domains that are closely related to Src family members. We generated an anti-human PTK6 antibody by immunizing rabbits with a PTK6-specific oligopeptide conjugated to BSA, which corresponds to 11 amino acid residues near the C-terminus. An immunoblot analysis with the antibody detected an expected 52-kDa band in various mammalian transformed cell lines. Immunoprecipitation and immunoblot analyses demonstrated that PTK6 is phosphorylated on the tyrosine residue(s) and interacts with approximately a 23-kDa tyrosine-phosphorylated polypeptide (most likely a substrate of PTK6) in breast carcinoma T-47D cells. An immunofluorescence analysis demonstrated that PTK6 is localized throughout the cytoplasm of T-47D cells. These results support a possible role for PTK6 in the intracellular signal transduction through tyrosine phosphorylation.

Keywords: Antibody, Interacting protein, PTK6, Tyrosine kinase

Introduction

Protein tyrosine kinase-6 (PTK6, also known as Brk) is a non-receptor type protein tyrosine kinase (PTK), a partial cDNA, which was first identified during an extensive survey of PTK mRNAs that were expressed in human melanocytes by reverse transcription-PCR using degenerate oligonucleotides to invariant amino acid sequences of PTK catalytic domains (Lee *et al.*, 1993). The human full-length cDNA was cloned from breast carcinoma cells (Mitchell *et al.*, 1994) and the cDNA of its mouse homolog, Sik, which has an 80% identity to PTK6 in the amino acid sequence, was cloned from mouse intestinal crypt cells (Vasioukhin *et al.*, 1995). PTK6 and Sik are expressed at high levels in the normal gastrointestinal tract (Vasioukhin *et al.*, 1995; Lee *et al.*, 1998; Llor *et al.*, 1999). Although the expression of PTK6 was not detected in normal mammary epithelial cells and normal melanocytes (Lee *et al.*, 1993; Barker *et al.*, 1997), its elevated expression was reported in breast carcinomas (Mitchell *et al.*, 1994; Barker *et al.*, 1997), melanomas (Easty *et al.*, 1997), and colon carcinomas (Chen *et al.*, 1999; Llor *et al.*, 1999).

The PTK6 polypeptide deduced from the cDNA sequence contains a Src homology (SH) 3 domain, an SH2 domain, and a catalytic domain of tyrosine kinase (Mitchell *et al.*, 1994). PTK6 shows the strongest homology to Src family members such as Src, Yes, and Fyn (43-41% identity) (Lee *et al.*, 1998). However, PTK6 was not believed to be another Src family member for several reasons. (1) Homologies among the Src family members are significantly higher (55-75% identity) than those between the PTK6 and Src family members (Lee *et al.*, 1998). (2) PTK6 lacks a consensus sequence for myristoylation in the N-terminus (Mitchell *et al.*, 1994). (3) The exon-intron boundaries of the PTK6 gene, which was sublocalized to the human chromosome 20q13.3 by fluorescence *in situ* hybridization (Park *et al.*, 1997), were quite different from those of the Src family genes, which are evolutionarily conserved (Lee *et al.*, 1998). These results suggest that the human PTK6, along with mouse Sik, constitutes a new family of non-receptor type PTKs that are evolutionarily distinct from Src family members.

Considering the primary structure and expression profile, PTK6 is thought to play a role in the regeneration or differentiation of gastrointestinal epithelium and development of epithelial carcinomas (Vasioukhin and Tyner, 1997; Lee *et al.*, 1998; Llor *et al.*, 1999). However, the intracellular function of PTK6 in these processes has been poorly understood. In an attempt to characterize the role of PTK6 in the signaling pathways, we generated the rabbit anti-human PTK6 antibody. Using the antibody, we analyzed the

*To whom correspondence should be addressed.
Tel: 82-2-2123-2703; Fax: 82-2-362-9897
E-mail: stlee@yonsei.ac.kr

expression in various cell lines, the presence of interacting proteins, and the subcellular localization of PTK6.

Materials and Methods

Preparation of anti-PTK6 polyclonal antibody Anti-PTK6 polyclonal antiserum was raised by immunization of the PTK6 oligopeptide-conjugated BSA in New Zealand white rabbits. A synthetic oligopeptide (Acetyl-CFKALRERLSS-amide; amino acid position 433-443) derived from the C-terminal region of PTK6 (Mitchell *et al.*, 1994) was synthesized by PeptidoGenic Research & Co. (Livermore, USA). The oligopeptide was coupled to BSA, which was activated with the *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce Chemical Co., Rockford, USA) (Green *et al.*, 1982). The oligopeptide-conjugated BSA (0.5 mg/injection) was initially injected with Freund's complete adjuvant (Sigma Chemical Co., St. Louis, USA) and subsequently with Freund's incomplete adjuvant (Sigma Chemical Co.) into rabbits intramuscularly (Freund, 1956; Lim *et al.*, 1998). Generation of the anti-PTK6 antibody was tested by a solid-phase enzyme-linked immunoadsorbent assay (ELISA) (Miles and Hales, 1968; Shin *et al.*, 2000). Anti-BSA antibody in the antiserum was removed by affinity chromatography using cyanogen bromide (CNBr)-activated Sepharose 4B beads (Pharmacia Biotech Inc., Piscataway, USA) coupled with BSA (Kohn and Wilchek, 1984). Removal of anti-BSA antibody in the flow-through fraction was confirmed by immuno-blot analysis of the nitrocellulose filter blotted with either BSA or the PTK6 oligopeptide.

Cell culture Human breast carcinoma cell lines, MCF-7, T-47D, SK-BR-3, MDA-MB-231, and ZR-75-1, human hepatocellular carcinoma cell lines, Hep G2 and Hep 3B, a transformed monkey kidney fibroblast cell line, COS-1, and a mouse embryonic fibroblast cell line, NIH3T3, were obtained from the American Type Culture Collection (Manassas, USA). These cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies Inc., Gaithersburg, USA), containing 100 unit/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM glutamine in the presence of 10% fetal bovine serum (FBS; Life Technologies Inc.) at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Western blot analysis Sub-confluent cells were fed with a fresh medium for 12 h, washed twice with ice-cold phosphate-buffered saline (PBS) containing 0.91 mM $CaCl_2$ and 0.49 mM $MgCl_2$ (PBS-complete), scraped off from the plate into PBS-complete, and centrifuged for 5 min at 1000 g. The cell pellets were solubilized by boiling for 5 min in a 2 × SDS gel sample buffer of the same volume of cell pellets. The cell lysates (5 μl) were separated on SDS-10% polyacrylamide gel (PAG) under reducing conditions. Proteins in the gel were transferred to a nitrocellulose membrane (Schleicher & Schuell Inc., Keene, USA). The western blot was reacted with an anti-PTK6 antiserum or the desired primary antibodies according to the method previously described (Jo *et al.*, 2000).

Immunoprecipitation analysis Sub-confluent T-47D cells were fed with a fresh medium for 12 h, washed twice with ice-cold PBS-complete. The cells on a 10-cm dish were lysed in 500 μl of an NP-40 lysis buffer [50 mM Tris-Cl, pH7.5, 150 mM NaCl, 1% NP-40, 0.2 mM sodium orthovanadate, 0.02% NaN_3, 5 mM EDTA, 10 μg/ml protease cocktail inhibitor (P-8340, Sigma Chemical Co.)] for 20 min at 4°C. The insoluble material was removed by centrifugation. The supernatant was pre-cleared by incubation with 20 μl of protein A-Sepharose (P-3391, Sigma Chemical Co.) for 10 min at 4°C and centrifugation. The pre-cleared supernatant was incubated with 20 μl of pre-immune rabbit serum, 20 μl of rabbit anti-PTK6 antiserum, or 3 μg of mouse anti-Src antibody (GD11, Sigma Chemical Co.) for 2 h at 4°C and subsequently with 20 μl of protein A-Sepharose for an additional 1 h at 4°C. The precipitates were washed three times by incubation with a washing buffer (5 mM Tris-Cl, pH7.5, 0.4 M NaCl, 1% NP-40, 0.1% SDS, 0.5% deoxycholate) for 5 min at 4°C and centrifugation. Proteins bound to the washed resins were solubilized by boiling for 2 min in 20 μl of a 2 × SDS gel sample buffer and were separated on SDS-10% PAG under reducing conditions (Kim *et al.*, 1999). Western blot analysis of the immunoprecipitated samples with the desired primary antibodies was performed as described above.

Immunofluorescence microscopy T-47D cells were plated on coverslips and sub-confluent cells were fed with a fresh medium for 12 h. Indirect double immunofluorescence staining was performed by the method described by Lee *et al.* (1992), but with minor modifications. Cells on coverslips were washed with ice-cold PBS-complete, fixed with acetone at -20°C for 20 min, and washed again with PBS-complete. Washed cells were pre-blocked for 1 h with PBS-complete containing 1% BSA and then primary antibodies applied in the same buffer for 1 h. Concentrations of primary antibodies were 1:50 dilution of rabbit anti-PTK6 antiserum, 1 : 50 dilution of mouse anti-Src antibody, 1 : 100 dilution of mouse anti-caveolin antibody (C13620, Transduction Laboratories, Lexington, USA) and 1:50 dilution of mouse anti-actin antibody (AC-40, Sigma Chemical Co.). Cells were extensively washed with PBS-complete containing 1% BSA, and incubated with a mixture of tetramethyl rhodamine isothiocyanate-conjugated goat anti-rabbit IgG antibody (1 : 200 dilution) and fluorescein isothiocyanate-conjugated sheep anti-mouse IgG antibody (1 : 200 dilution) for 1 h. Cells were washed with PBS-complete without BSA, treated with 1 mg/ml *p*-phenylenediamine (Sigma Chemical Co.) in PBS-complete, mounted on glass slides and viewed by a fluorescence microscope (Model Axioskop; Zeiss, Esslingen, Germany).

Results

Generation of anti-PTK6 antibody Polyclonal rabbit anti-human PTK6 antiserum was obtained using an antigen, which is a synthetic oligopeptide corresponding to the C-terminal region of the PTK6 polypeptide (Fig. 1) coupled to BSA. By dot blot analysis, the antiserum recognized both the PTK6 oligopeptide and BSA. To remove the anti-BSA antibody, the antiserum was applied to affinity chromatography using BSA-conjugated Sepharose 4B beads. Dot blot analysis

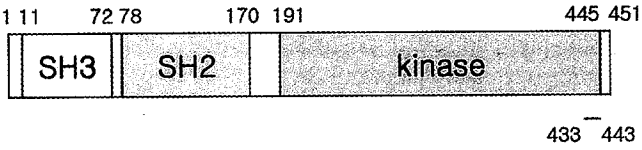

Fig. 1. Relative position of the synthetic oligopeptide used for the production of the anti-PTK6 antibody. The block diagram represents a schematic structure of PTK6 polypeptide. The SH2, SH3, and catalytic (marked as kinase) domains are shown as shaded boxes. The 11-amino acid oligopeptide (acetyl-CFKALRERLSS-amide; amino acid position 433-443) near to the C-terminus, which was used for antibody production, is shown as a black bar. The numbering of the PTK6 polypeptide is followed by Mitchell *et al.* (1994).

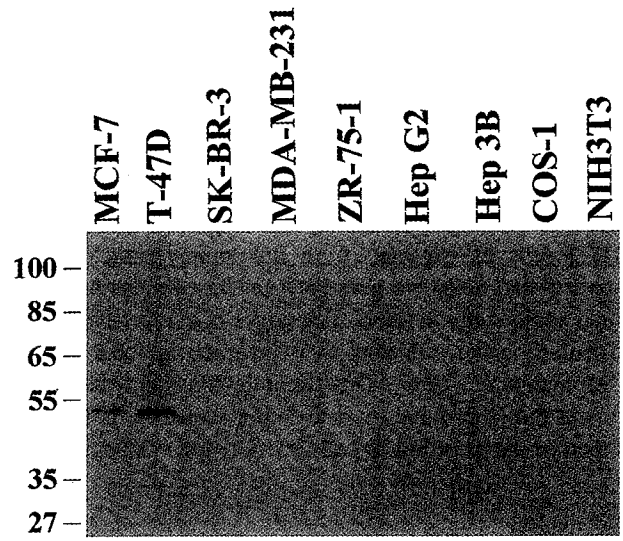

Fig. 2. Western blot analysis to detect PTK6 in various cell lines. Cell lysates from breast carcinoma cell lines, MCF-7, T-47D, SK-BR-3, MDA-MB-231, and ZR-75-1, hepatocellular carcinoma cell lines, Hep G2 and Hep 3B, a monkey kidney fibroblast cell line, COS-1, and a mouse embryonic fibroblast cell line, NIH3T3, were loaded in 10% SDS-PAG and blotted on a nitrocellulose membrane. The blot was incubated with a 1:100-diluted anti-PTK6 antibody and 1 : 5000-diluted HRP-conjugated anti-rabbit antibody and was developed by the ECL system.

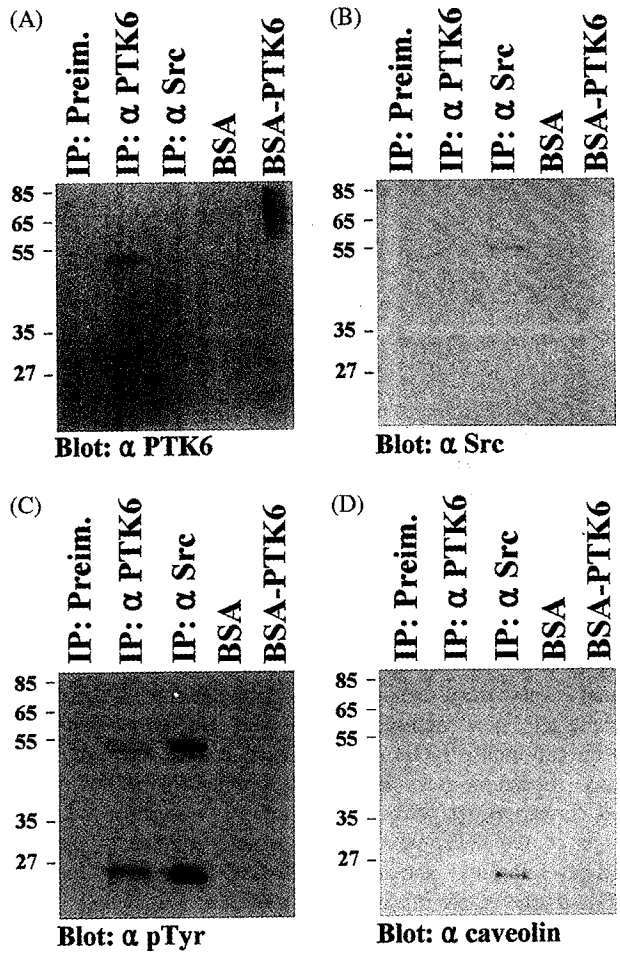

Fig. 3. Immunoprecipitation and western blot analyses to detect phosphorylation of tyrosine residues of PTK6 and proteins interacting with PTK6. The T-47D cell lysate was immunoprecipitated with preimmune serum, anti-PTK6 antibody, or anti-Src antibody. The precipitates were applied to 10% SDS-PAG and blotted on a nitrocellulose membrane. The western blot reacted with the anti-PTK6 antibody (A), anti-Src antibody (B), anti-phosphotyrosine antibody (C), or anti-caveolin antibody (D).

demonstrated that the anti-BSA antibody was efficiently removed in the flow-through fraction without loss of the anti-PTK6 antibody (data not shown). The flow-through fraction was thus used as an anti-PTK6 antibody in the following experiments.

Western blot analysis of PTK6 in various mammalian cell lines To examine whether or not the anti-PTK6 antibody specifically recognizes denatured PTK6 polypeptide, and which cells express PTK6, lysates from various cell lines were analyzed by western blot analysis using the anti-PTK6 antibody. The PTK6 was detected as a single 52-kDa band (Fig. 2) in western blot, which is similar to its calculated molecular weight, 51,834 Da. This result demonstrates that the anti-PTK6 antibody specifically recognizes the denatured PTK6 polypeptide in the cell lysate.

Expression of PTK6 was found at relatively high levels in human breast carcinoma cell lines, T-47D and MCF-7, which were reported to express PTK6 (Mitchell *et al.*, 1994) and at a very low level in a transformed monkey kidney fibroblast cell line, COS-1 (Fig. 2). PTK6 was not detected in the other breast carcinoma cell lines, SK-BR-3, MDA-MB-231, and ZR-75-1, in hepatocellular carcinoma cell lines, Hep G2 and Hep 3B, and in the mouse embryonic fibroblast cell line, NIH3T3. Thus, PTK6 is expressed in some breast carcinoma cell lines as reported previously (Mitchell *et al.*, 1994; Barker *et al.*, 1997), but not in the hepatocellular carcinoma cell lines tested.

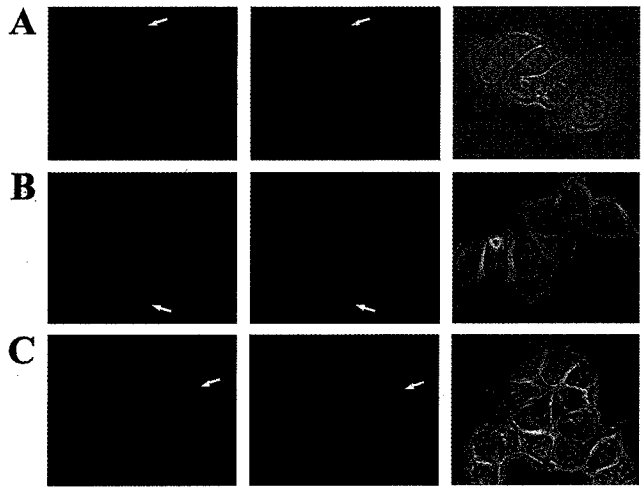

Fig. 4. Indirect double immunofluorescent staining of PTK6, Src, caveolin, and actin in T-47D cells. T-47D cells on coverslips were fixed with acetone and pre-blocked with 1% BSA in PBS-complete. Pre-blocked cells reacted with rabbit anti-PTK6 antibody (left panel) and mouse anti-Src (A), mouse anti-caveolin (B), or mouse anti-actin (C) antibody (center panel). They were then stained with a mixture of rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated sheep anti-mouse IgG antibodies. Left, center, and right panels show rhodamine and fluorescein immunofluorescent images and corresponding phase-contrast images, respectively. Leading edges of the cells, which were strongly stained by anti-Src, anti-caveolin, and anti-actin antibodies but not by the anti-PTK6 antibody, are marked by arrows.

Immunoprecipitation of PTK6 in T-47D cells To examine whether or not the anti-PTK6 antibody recognizes soluble PTK6, the lysate of T-47D cells was immunoprecipitated with the anti-PTK6 antibody and the precipitate was analyzed by western blot analysis using the anti- PTK6 antibody. As controls for the immunoprecipitation experiment, the same lysate was immunoprecipitated with preimmune rabbit serum and mouse anti-human Src antibody. Then western blot of the precipitates was examined using the anti-PTK6 antibody and anti-Src antibody. PTK6 was detected in the immunoprecipitate by the anti-PTK6 antibody and in PTK6 oligopeptide-conjugated BSA. However, it was not detected in either the immunoprecipitates by the preimmune serum and anti-Src antibody or in BSA (Fig. 3A). Similarly, c-Src was identified only in the immunoprecipitate by the anti-Src antibody (Fig. 3B). This result demonstrates that the anti-PTK6 antibody is able to specifically bind soluble PTK6 as well as denatured PTK6.

To examine the presence of tyrosine-phosphorylated proteins co-precipitated with PTK6, western blot of the immunoprecipitates was analyzed with the anti-phosphotyrosine antibody. In the immunoprecipitate by the anti-PTK6 antibody, two bands (one superimposes to the PTK6 band in Fig. 3A and the other is a 23-kDa tyrosine-phosphorylated polypeptide) were detected (Fig. 3C, lane IP: α PTK6). Similarly, two bands corresponding to the c-Src and 22-kDa tyrosine-phosphorylated polypeptide were detected in the immunoprecipitate by the anti-Src antibody (Fig. 3C, lane IP: α Src). Since the 22-kDa caveolin is known to be coimmunoprecipitated with and phosphorylated by Src family tyrosine kinases, which includes c-Src (Rothberg *et al.*, 1992; Li *et al.*, 1996), the western blot was reacted with the anti-caveolin antibody. The 22-kDa band, which was coimmunoprecipitated with c-Src, was also detected by the anti-caveolin antibody, but the 23-kDa band coimmunoprecipitated with PTK6 was not detected (Fig. 3D). These results demonstrate that PTK6 is phosphorylated in tyrosine residue(s) and the 23-kDa tyrosine-phosphorylated polypeptide interacting with PTK6 is likely to be a substrate of PTK6.

Subcellular localization of PTK6 In order to examine subcellular localization of PTK6, T-47D cells were examined by indirect double immunofluorescence staining using the anti-PTK6 antibody as well as anti-Src, anti-caveolin, and anti-actin antibodies as controls. As shown in Fig. 4 (left panels), PTK6 is present throughout most of the cytoplasm of T-47D cells as expected from its primary structure. Src, caveolin, and actin are also localized in cytoplasm, but are found additionally in the leading edges of cell periphery (Fig. 4, center panels) reflecting their presence in focal contacts (Chapman *et al.*, 1999). This result demonstrates that PTK6, without the N-terminal myristoylation sequence, is localized in the cytoplasm of T-47D cells but not in the cell surface.

Discussion

We generated a polyclonal rabbit anti-human PTK6 antibody using a synthetic oligopeptide corresponding to the C-terminal region of PTK6 polypeptide. The antibody recognized both denatured and non-denatured PTK6 polypeptide. Also, its usefulness was demonstrated by western blot analysis, immunoprecipitation, and immunofluorescence staining. The anti-PTK6 antibody detected a 52-kDa band in western blot analysis, as expected from the polypeptide deduced from the cDNA. PTK6 was expressed in some of the breast carcinoma cell lines, such as T-47D and MCF-7, and a transformed monkey fibroblast COS-1 cell line, but not in hepatocellular carcinoma cell lines. In addition, PTK6 was not detected in a mouse embryonic fibroblast NIH3T3 cell line. However, the anti-PTK6 antibody is unlikely to detect a mouse homolog of the human PTK6, Sik (Vasioukhin *et al.*, 1995), because 5 amino acid residues (among the 11-amino acid region of the PTK6 oligopeptide used for the antigen) are different in Sik. Therefore, it is unclear whether or not the NIH3T3 cell line expresses Sik.

By immunoprecipitation of the T-47D cell lysate with the anti-PTK6 antibody and western blot analysis with anti-phosphotyrosine antibody, we found that PTK6 is phosphorylated with the tyrosine residue(s) and interacts with

a 23-kDa tyrosine-phosphorylated polypeptide. Since we detected a known substrate of c-Src, caveolin, in a control experiment using the anti-Src antibody, the 23-kDa polypeptide is likely to be a substrate of PTK6. However, it was unsuccessful for identification since we could not obtain enough of the 23-kDa polypeptide by immunoprecipitation. While preparing this manuscript, Mitchell *et al.* (2000) reported a substrate of BRK, BKS (BRK kinase substrate), and Derry *et al.* (2000) reported a substrate of BRK and Sik, Sam68 (Src associated during mitosis, 68 kDa). The cDNAs for BKS were isolated by yeast two-hybrid screening and encode polypeptides of 449 (BKS-l) and 403 (BKS-s) amino acids containing pleckstrin homology (PH)-like and SH2-like domains, characteristics of an adaptor protein. Sam68 was identified by colocalization of BRK in distinct nuclear dots, termed Sam68 nuclear bodies (SNBs) (Huang, 2000) by immunofluorescence confocal microscopy (Derry *et al.*, 2000). Sam68 is an RNA binding protein that was first identified as a major substrate of Src during mitosis (Fumagalli *et al.*, 1994; Taylor and Shalloway, 1994). Although the function of Sam68 is unclear, it may play a role in signaling cascades by Src family tyrosine kinases to RNA metabolism.

By immunofluorescence microscopy using the anti-PTK6 antibody, we found that most of the PTK6 is localized throughout the cytoplasm of the T-47D cells. Derry *et al.* (2000) reported that BRK is present in the SNBs of MCF-7 cells and colon carcinoma HT29 cells, and we found the presence of BRK in the cytoplasm of their data. However, small nuclear dots were barely detectable in the T-47D cells that were stained with the anti-PTK6 antibody (Fig. 4, left panels) and anti-Src antibody (Fig. 4A, center panel). It is unclear whether or not this discrepancy resulted from the difference of cell types or antibodies. Nevertheless, our results suggest that PTK6 functions mainly in cytoplasm rather than in nucleus at least in the T-47D cells.

The expression of PTK6 mRNA was detected at high levels in normal epithelia, such as the colon and intestine (Lee *et al.*, 1997) and in breast carcinomas (Mitchell *et al.*, 1994; Barker *et al.*, 1997), colon carcinomas (Chen *et al.*, 1999; Llor *et al.*, 1999), and melanomas (Easty *et al.*, 1997). Although roles of PTK6 in signal transduction pathways have not been elucidated, the overexpression of PTK6 appears to be involved in the development of epithelial tumors. The deduced PTK6 polypeptide shows the highest homology to Src family tyrosine kinases, such as c-Src, c-Fyn, and c-Yes. Despite a strong homology to Src family members, it was suggested that PTK6, along with Sik, constitutes a distinct family of non-receptor protein tyrosine kinases because it lacks the N-terminal myristoylation domain. Also, its gene organization is quite different from those of Src family members (Lee *et al.*, 1998). Moreover, this study demonstrates the additional differences between PTK6 and c-Src in interacting proteins and the intracellular localization pattern. These results suggest that PTK6 plays distinct roles in intracellular signal transduction through tyrosine phosphorylation in the proliferation of normal epithelial cells and the development of epithelial tumors.

Acknowledgments We thank Mr. Kwang-Min Bae for proofreading this manuscript and for the illustrations. This work was supported by a grant from the Genetic Engineering Research Fund (997-019-D0010) of the Ministry of Education, Republic of Korea.

References

Barker, K. T., Jackson, L. E. and Crompton, M. R. (1997) BRK tyrosine kinase expression in a high proportion of human breast carcinomas. *Oncogene* **15**, 799-805.

Chapman, H. A., Wei, Y., Simon, D. I. and Waltz, D. A. (1999) Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb. Haemost.* **82**, 291-297.

Chen, W.-S., Kung, H.-J., Yang, W.-K. and Lin, W.-c. (1999) Comparative tyrosine-kinase profiles in colorectal cancers: enhanced arg expression in carcinoma as compared with adenoma and normal mucosa. *Int. J. Cancer* **83**, 579-584.

Derry, J. J., Richard, S., Valderrama Carvajal, H., Ye, X., Vasioukhin, V., Cochrane, A. W., Chen, T. and Tyner, A. L. (2000) Sik (BRK) phosphorylates Sam68 in the nucleus and negatively regulates its RNA binding ability. *Mol. Cell. Biol.* **20**, 6114-6126.

Easty, D. J., Mitchell, P. J., Patel, K., Florenes, V. A., Spritz, R. A. and Bennett, D. C. (1997) Loss of expression of receptor tyrosine kinase family genes PTK7 and SEK in metastatic melanoma. *Int. J. Cancer* **71**, 1061-1065.

Freund, J. (1956) The mode of action of immunologic adjuvants. *Adv. Tuberc. Res.* **7**, 130-148.

Fumagalli, S., Totty, N. F., Hsuan, J. J. and Courtneidge, S. A. (1994) A target for Src in mitosis. *Nature* **368**, 871-874.

Green, N., Alexander, H., Olson, A., Alexander, S., Shinnick, T. M., Sutcliffe, J. G. and Lerner, R. A. (1982) Immunogenic structure of the influenza virus hemagglutinin. *Cell* **28**, 477-487.

Huang, S. (2000) Review: perinucleolar structures. *J. Struct. Biol.* **129**, 233-240.

Jo, Y., Yeon, J., Kim, H.-J. and Lee, S.-T. (2000) Analysis of tissue inhibitor of metalloproteinases-2 effect on pro-matrix metalloproteinase-2 activation by membrane-type 1 matrix metalloproteinase using baculovirus/insect-cell expression system. *Biochem. J.* **345**, 511-519.

Kim, M. J., Hwang, J. I., Chang, J. S., Ryu, S. H. and Suh, P. G. (1999) The SH3 domain of phospholipase C-gamma1 associates with Shc. *J. Biochem. Mol. Biol.* **32**, 119-126.

Kohn, J. and Wilchek, M. (1984) The use of cyanogen bromide and other novel cyanylating agents for the activation of polysaccharide resins. *Appl. Biochem. Biotechnol.* **9**, 285-304.

Lee, S.-T., Lee, S., Peters, D. P., Hoffman, G. G., Stacey, A. and Greenspan, D. S. (1992) Deletion of the pro-alpha 1(I) N-propeptide affects secretion of type I collagen in Chinese hamster lung cells but not in Mov-13 mouse cells. *J. Biol. Chem.* **267**, 24126-24133.

Lee, H., Kim, M., Lee, K.-H., Kang, K.-N. and Lee, S.-T. (1998) Exon-intron structure of the human PTK6 gene demonstrates

that PTK6 constitutes a distinct family of non-receptor tyrosine kinase. *Mol. Cells* **8**, 401-407.

Lee, S.-T., Strunk, K. M. and Spritz, R. A. (1993) A survey of protein tyrosine kinase mRNAs expressed in normal human melanocytes. *Oncogene* **8**, 3403-3410.

Li, S., Seitz, R. and Lisanti, M. P. (1996) Phosphorylation of caveolin by src tyrosine kinases. The alpha-isoform of caveolin is selectively phosphorylated by v-Src *in vivo*. *J. Biol. Chem.* **271**, 3863-3868.

Lim, Y. M., Sung, J. Y. and Lee, M. H. (1998) Polyclonal antibody against the active recombinant *Helicobacter pylori* urease expressed in *Escherichia coli*. *J. Biochem. Mol. Biol.* **31**, 240-244.

Llor, X., Serfas, M. S., Bie, W., Vasioukhin, V., Polonskaia, M., Derry, J., Abbott, C. M. and Tyner, A. L. (1999) BRK/Sik expression in the gastrointestinal tract and in colon tumors. *Clin. Cancer Res.* **5**, 1767-1777.

Miles, L. E. and Hales, C. N. (1968) Labeled antibodies and immunological assay systems. *Nature* **219**, 186-189.

Mitchell, P. J., Barker, K. T., Martindale, J. E., Kamalati, T., Lowe, P. N., Page, M. J., Gusterson, B. A. and Crompton, M. R. (1994) Cloning and characterization of cDNAs encoding a novel non-receptor tyrosine kinase, brk, expressed in human breast tumors. *Oncogene* **9**, 2383-2390.

Mitchell, P. J., Sara, E. A. and Crompton, M. R. (2000) A novel adaptor-like protein which is a substrate for the non-receptor tyrosine kinase, BRK. *Oncogene* **19**, 4273-4282.

Park, S.-H., Lee, K.-H., Kim, H. and Lee, S.-T. (1997) Assignment of the human PTK6 gene encoding a non-receptor protein tyrosine kinase to 20q13.3 by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* **77**, 271-272.

Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y. S., Glenney, J. R. and Anderson, R. G. (1992) Caveolin, a protein component of caveolae membrane coats. *Cell* **68**, 673-682.

Shin, S. Y., Park, J.-H., Lee, M. K., Jang, S. Y. and Hahm, K.-S. (2000) Characterization of KI-24, a novel murine monoclonal antibody with specific reactivity for the human immunodeficiency virus-1 p24 protein. *J. Biochem. Mol. Biol.* **33**, 92-95.

Taylor, S. J. and Shalloway, D. (1994) An RNA-binding protein associated with Src through its SH2 and SH3 domains in mitosis. *Nature* **368**, 867-871.

Vasioukhin, V., Serfas, M. S., Siyanova, E. Y., Polonskaia, M., Costigan, V. J., Liu, B., Thomason, A. and Tyner, A. L. (1995) A novel intracellular epithelial cell tyrosine kinase is expressed in the skin and gastrointestinal tract. *Oncogene* **10**, 349-357.

Vasioukhin, V. and Tyner, A. L. (1997) A role for the epithelial-cell-specific tyrosine kinase Sik during keratinocyte differentiation. *Proc. Natl. Acad. Sci. USA* **94**, 14477-14482.