Dynamic Patterns of PTK7 Protein Expression in Adult Mouse Tissues

Jihee Kim 1, Ho Yoon 2, Song-ee Lee 1, Won-Suk Kang 2, Iksoo Jeon 1, Da-Jeong Chang 1, Nayeon Lee 1, Taesun Hwang 2, Won-Sik Shin 3, Seung-Taek Lee 1, Nayeon Lee 1, Taesun Hwang 2, Han-Woong Lee 3, Seung-Taek Lee 3 and Jihwan Song 1,*

1CHA Stem Cell Institute, Pochon CHA Univ, 606-16 Yeoksam, Kangnam, Seoul 135-081, Korea,
2Dept of Anatomy, College of Medicine, Pochon CHA Univ, 222 Yatap, Bundang, Seongnam, Gyeonggi-do 463-070, Korea,
3Dept of Biochem, College of Life Sci and Biotech, Yonsei Univ, 134 Shinchon, Seodaemun, Seoul 120-749, Korea
(Received: Oct. 27th, 2008; Accepted: Oct. 31st, 2008)

Abstract: PTK7 belongs to a subgroup of receptor protein tyrosine kinases with inactive catalytic activity of the protein tyrosine kinase (PTK), which is known to be important for signal attenuation, cell adhesion, and regulation of planar cell polarity. In this study, to decipher possible biological roles of PTK7, we examined the tissue distribution and localization of PTK7 protein in adult mouse tissues. PTK7-positive signals were detected in variety of tissues and cells in multiple systems, including respiratory, digestive, urinary and reproductive organs, as well as liver and pancreas. These expression profiling data imply various physiological roles of PTK7 in multiple organ systems. Interestingly, among various tissues examined, no specific signal was detected in the heart and muscles. Taken together, dynamic patterns of protein expression in adult tissues strongly suggest PTK7 may play important roles during organogenesis and histogenesis.

Key words: PTK7, receptor protein tyrosine kinase, expression patterns, tissue distribution, organogenesis

1. Introduction

Cell interactions, which are important both during embryonic development and adult life, are mediated by receptors that bind ligands and transduce signals to the cell machinery. The kinase receptors form a large group of membrane receptors that respond to ligand binding by modulating the catalytic activity of their intracellular kinase domain. They are implicated in the control of a wide range of cellular processes, including cell cycle, metabolism, cell survival, specification of cell fate and differentiation. Alteration of their signaling ability is associated with many human diseases. PTK7, a subgroup of RPTKs (receptor protein tyrosine kinases), was first identified by the cloning of its cDNA fragment that was obtained from reverse transcription of normal melanocyte mRNAs and PCR with the degenerate primer pairs corresponding to the conserved subdomains VIb to IX of PTKs. The full-length cDNA of colon carcinoma kinase-4 (CCK-4), which is identical to PTK7, was cloned from a colon carcinoma tissue. The mouse PTK7 belongs to the PTK7 family, along with chick Klg, Hydra Lemon, and Drosophila Dtrk that were also proposed to be orthologues of the human PTK7. It was reported that the human PTK7 mRNA is highly expressed in fetal colon and that Hydra Lemon mRNA is upregulated in the developmental stages of spermatogenesis, oogenesis, and embryogenesis. Mouse embryos expressing a truncated form of CCK4 consisting of only the first 114 residues (which does not include the pseudokinase domain) die perinatally, with profound defects in neural tube closure and orientation of the stereociliary bundles in the inner ear, indicating that this pseudokinase is involved in the regulation of polarity within planes of epithelial cells.

It was recently reported that five BH1H binding sites, SP1-binding site, and TCF/LEF-binding site within the human PTK7 5’-promoter region are conserved in chimpanzee, rhesus monkey, mouse, and rat PTK7 genes. PTK7, also known as CCK4, is upregulated in colorectal cancer. Because the canonical WNT signaling pathway is frequently activated in colorectal cancer, conserved TCF/LEF-binding site within the PTK7 promoter region explains the mechanism of PTK7
Figure 1. Immunohistochemical detection of PTK7-positive cells in digestive, respiratory, endocrine, urinary and reproductive systems of the adult mouse. Expression patterns of PTK7 protein are described in the esophagus (A), stomach (B-D), intestine (E-G), colon (H, I), bronchus (J), lung (K), liver (L), kidney (M), pancreas (N) and testis (O). Arrows and/or asterisks in figures indicate the site of PTK7 expression: MBL (A), SEC and CS (B), CS (C), SEC (D), AC (E), EEC (F), AC (G), EEC (H), NMP and NSP (I), BC (J), 2oP (K), H (L), RT, TE and P (M), EC (N), and SG and ST (O). Abbreviations: L, lumen; LM, lamina; SM, submucosa; M, muscularis; MBL, middle and basal layer of epithelium; SEC, surface epithelial cell (mucus-secreting cell); CS, chief cell; EB, exterior of bowel; AC, absorptive cell; IV, intestinal villi; IG, intestinal gland; EEC, enteroendocrine cell; LP, lamina propria; G, goblet cell; E, epithelium; NMP, neurons in myenteric plexus; NSP, neurons in submucosal plexus; BC, bronchial cells, including ciliated cells, basal cells, small granule cells, etc.; AL, alveolar lumen; 2o P, type II pneumocytes (giant alveolar cells); HS, hepatic sinusoid; V, blood vessel; H, hepatocytes; RT, renal tubules (uriniferous tubules); TE, tubular epithelium; P, podocytes; G, glomerulus; PI, pancreatic island; EC, endocrine cells; A, acinus; ST, seminiferous tubules; SG, spermatogonium; SD, spermatids; AC, acrosome. Scale bar, 50 µm.
Dynamic Patterns of PTK7 Expression in Adult Mouse Tissues

upregulation in colorectal cancer.\(^{11}\)

We have previously shown that, by Northern blot analysis, mouse \textit{PTK7} mRNA was expressed at high levels in the lung and un-pregnant uterus, at moderate levels in the epididymis and colon, and at low levels in the others among adult tissues. Among the embryonic tissues, high levels of the \textit{PTK7} mRNA expression were detected in the head, chest and abdomen, as well as in the whole embryo. Using whole-mount \textit{in situ} hybridization, we had also described more detailed patterns of \textit{PTK7} mRNA expression during mouse embryonic development, showing high levels of expression in the tail, limbs, somites, gut and craniofacial regions.\(^{12}\) In this study, we carried out detailed immunohistochemical analyses on adult mouse tissues using polyclonal antibodies raised against mouse \textit{PTK7} proteins. \textit{PTK7}-positive signals were detected in multiple systems, including respiratory, digestive, urinary and reproductive organs, as well as liver and pancreas, implying diverse physiological roles of \textit{PTK7} during organogenesis.

2. Materials and Methods

2.1 Tissue Preparation

For expression study, various organs including heart, stomach, intestine, testis, etc were collected in cold PBS and fixed overnight in 4% formaldehyde(\(\text{4°C}\)). After fixation, organs were cryoprotected with 30% sucrose for 24 h, were frozen in Tissue-Tek(Sakura Finetek USA, Torrance, CA, USA) solution and were then sectioned at 10 \(\mu\text{m}\) using a cryostat(Microtome Cryostat HM 525, MICROM International GmbH, Walldorf, Germany).

2.2 Immunohistochemical Staining

For immunohistochemical staining, sections were blocked with 3.0%(\(\text{v/v}\)) normal horse serum(Vector Lab, Burlingame, CA, USA) in PBS containing 0.1% triton X-100(Sigma) for 1 h. The primary antibody used was rabbit anti-\textit{PTK7}(1:1000), and the secondary antibody used was biotinylated anti-rabbit IgG antibody(1:200, Vector Lab). Specificity of the immunoreactive signals were determined using the control sections, in which primary antibody was omitted. Reaction product was visualized with DAB(Sigma) at room temperature for 3 min. Sections were rinsed with tap water, immediately dehydrated by sequential immersion in gradient ethanol and histoclear, then mounted with Permount on coverslips. Sections were examined with a Nikon light/fluorescence microscope(DXM1200F) equipped with a Nikon DS-L1 camera.

3. Results

Among various tissues and organs examined, we have summarized the expression patterns of \textit{PTK7} protein as follows:

3.1 Digestive System

We found that \textit{PTK7} is strongly expressed at the middle and basal layer of the esophagus(Fig 1A). In stomach, \textit{PTK7} is highly expressed in the chief cells(Fig 1B and C), as well as in the mucus-secreting surface epithelial cells(Fig 1B and D). In the case of intestine, some of the absorptive cells located in the intestinal villi were immunoreactive for \textit{PTK7}(Fig 1E and G), but no expression was detected in the goblet cells(Fig 1G). Interestingly, some of the enteroendocrine cells(EEC) in the intestinal glands were specifically positive for \textit{PTK7}(Fig 1F). \textit{PTK7}-positive expression patterns in EEC region continue to be found in the colon(Fig 1H). In the colon, \textit{PTK7} immunoreactivity was also detected in the neurons in submucosal plexus, as well as myenteric plexus(Fig 1I).

3.2 Respiratory System

In the bronchus, strong expression was detected in the bronchial cells, which include ciliated cells, basal cells, small granule cells, etc(Fig 1J). In the lung, \textit{PTK7} is shown to be expressed specifically in the type II pneumocytes, also known as great alveolar cells(Fig 1K).

3.3 Liver

Interestingly, we found that small portions of hepatocytes are immunoreactive for \textit{PTK7} antibody(Fig 1L). In this case, staining was restricted to the cytoplasm.

3.4 Urinary System

In the kidney, \textit{PTK}-positive cells were detected in various regions, including renal tubules, tubular epithelial cells, and podocytes of glomerulus(Fig 1M).

3.5 Pancreas

Endocrine cells, secretory cells in the pancreatic islet, were shown to be positive for \textit{PTK7} immunoreactivity(Fig 1N). By contrast, acinar cells were negative for \textit{PTK7} immunoreactivity in the pancreas(Fig 1N).

3.6 Reproductive System

In the testis, both spermatogonium and spermatids were
positive for PTK7 expression (Fig 1O). However, no expression was detected in spermatocytes. Interestingly, PTK7 signals were also detected in the acrosomal region of spermatids (Fig 1O). By contrast, no specific signals were detected in the ovary.

4. Discussion

PTK7 belongs to a subgroup of receptor protein tyrosinase kinase with inactive catalytic activity of the PTK, which is known to be important for signal attenuation, cell adhesion, and regulation of planar cell polarity. Mouse embryos expressing a truncated form of PTK7 die perinatally, with profound defects in neural tube closure and orientation of the stereociliary bundles in the inner ear. It is also known that PTK7 is upregulated in colorectal cancer, due to a misregulation of canonical WNT signaling pathway. In this study, we carried out detailed immunohistochemical analyses on adult mouse tissues using polyclonal antibodies raised against mouse PTK7 proteins. PTK7-positive signals were detected in multiple systems, including respiratory, digestive, urinary and reproductive organs, as well as liver and pancreas.

In the digestive organ systems, we found that PTK7 is strongly expressed at the middle and basal layer of the esophagus. In stomach, it is highly expressed in the chief cells, as well as in the mucus-secreting surface epithelial cells. In the intestine, PTK7 was expressed at some of the absorptive cells located in the intestinal villi, but no expression was detected in the goblet cells. Interestingly, some of the enteroendocrine cells (EEC) in the intestinal glands were specifically positive for PTK7. Expression of PTK7 is also detected in the colon, where it is also expressed in the neurons in submucosal plexus, as well as myenteric plexus. As for the respiratory system, PTK7 is strongly expressed in the bronchial cells, as well as in the type II pneumocytes of the lung. In the liver, we found that only small portions of hepatocytes are immunoreactive for PTK7 antibody. In the kidney, PTK expression was in various regions, including renal tubules, tubular epithelial cells, and podocytes of glomerulus. In the pancreas, only endocrine cells were shown to be positive for PTK7 immunoreactivity, and acinar cells were negative. In the testis, both spermatogonium and spermatids were positive for PTK7 expression, but no expression was detected in spermatocytes. By contrast, no specific signals were detected in the ovary. Interestingly, among various tissues examined in this study, no specific signal was detected in the heart and muscles. Taken together, dynamic patterns of protein expression in adult tissues strongly suggest PTK7 may play important roles during organogenesis and histogenesis.

Acknowledgements: This work was supported by the Korea Research Foundation Grant (KRF-2004-042-C00086).

References