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EXPRESSION OF AN N-TERMINALLY TRUNCATED FORM OF HUMAN FOCAL ADHESION KINASE IN BRAIN+

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We have cloned a novel tyrosine kinase that is widely expressed in human tissues using degenerate oligonucleotide primers. The cDNA clone was subsequently found to be the human homologue of the recently cloned chicken focal adhesion associated kinase (pp^{125}FAK). The homology between the chicken and human sequences is 95% at the amino acid level. By RT-PCR we have detected hFAK in human tonsillar T and B cells, several human lymphoid cell lines, a neuroblastoma cell line and HeLa cells. By Northern blot analysis we show that hFAK is expressed in all organs tested with the highest abundance in brain and the least in heart and skeletal muscle. An additional transcript of ca. 3.3 kb, encoding an N-terminally truncated form of hFAK, was observed only in brain.

Tyrosine phosphorylation of proteins is believed to play an important role in the signal transduction pathways regulating cell differentiation and proliferation [1]. Protein tyrosine kinases can be divided into two classes: (1) the receptor tyrosine kinases (RTK) that span the membrane and (2) the cytosolic tyrosine kinases. RTKs have an extracellular ligand-binding domain separated from the catalytic domain by a hydrophobic transmembrane region. A number of RTKs bind soluble growth and differentiation factors such as nerve growth factor (NGF) and epidermal growth factor (EGF) [2,3]. Cytosolic tyrosine kinases lack a transmembrane domain. They were often identified as cellular homologues of retroviral oncogenes like the members of the *src* subfamily [4]. Some tyrosine kinases are expressed in various forms that in some cases show a differential

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Abbreviations: I in nucleotide sequences, Inosine; M in nucleotide sequences, A or C; R in nucleotide sequences, A or G; S in nucleotide sequences C or G; W in nucleotide sequences A or T; Y in nucleotide sequences C or T; PCR, polymerase chain reaction; PTK, protein tyrosine kinase; RT-PCR, reverse transcription PCR; RACE, rapid amplification of cDNA ends.

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tissue distribution. The c-*src* oncogene for example, is differentially spliced generating an alternative form that is most highly expressed in the brain [5,6]. Alternative splicing may be in this case a mechanism to serve a special function of *src* in the brain. The RTK trkB is expressed in mouse brain as a full-length receptor that has PTK activity and as a C-terminally truncated form that lacks the kinase activity [7]. A role for such truncated receptors could be the regulation of activity via the formation of a heterodimer between truncated and non-truncated receptors [8]. The PDGFβ receptor is also expressed in two alternative forms. In embryonic stem cells the most abundant PDGFβ receptor mRNA encodes an N-terminally truncated protein that is believed to be a constitutively active RTK[9].

In this study we report the isolation and molecular characterization of a novel protein tyrosine kinase. This PTK seems to be expressed in alternative forms that may encode N-terminally as well as C-terminally truncated molecules. Subsequentially it was found that the novel kinase has high homology to the recently published chicken focal adhesion kinase which co-localizes with the focal adhesion protein tensin [10]. We therefore name this kinase hFAK. We show that hFAK is expressed in lymphoid cell lines, different human tissues but most abundantly in brain. There, we detected, in addition to the mRNA of 4.3 kb detected in other tissues, a transcript of 3.3 kb which lacks the first 1 kb of 5' sequence when compared to the chicken sequence.

MATERIALS AND METHODS

RNA preparation

Total RNA was prepared from several cell lines according to the method described [11]. Poly(A) RNA was purified from total RNA using the Poly(A)Quick mRNA isolation kit (Stratagene).

Cloning by PCR of cDNA fragments derived from the catalytic domain of tyrosine kinases

Degenerate primers were designed to hybridize to the highly conserved region FVHRDLAAR- - -/ 53 aa /- - -DVWSFGV in the catalytic domain of tyrosine kinases (upstream primer: 5' TTY GTI CAY MGI GAY YTI GCI ACI MGI AA 3'; downstream primer: 5' AYI CCI ARI SWC CAI AYR TC 3'). Ten μg total tonsillar B cell RNA was converted into cDNA by Mu-MLV reverse transcriptase (Life Technologies) using the downstream primer. 4% of the cDNA was combined with 18 μl of the PCR mixture. Amplification was done through 35 PCR cycles with 37 °C annealing temperature. The PCR products were analysed by electrophoresis in 2.5 % agarose gels. DNA corresponding to a 200 bp fragment was eluted and reamplified by 30 more cycles of PCR with the same primers. The reamplified material was subcloned into Bluescribe plasmid (pBS$^+$, Stratagene) and the nucleotide sequences of 50 recombinant clones were determined.

Rapid Amplification of cDNA Ends (RACE)

PCR-based amplification of 3' terminal portions of cDNA sequences specific for hFAK was performed according to Frohman [12]. A set of target-specific primers was used. After the first round of PCR a second round of PCR was carried out with nested primers. Amplified material was separated by agarose gel electrophoresis, recovered and subcloned into pBS$^+$ vector (Stratagene) for sequence analysis.

Construction and screening of a cDNA library

A cDNA library was constructed in λ uniZAPII (Stratagene). RNA was extracted from purified human tonsillar B cells. Starting with 0.5 mg total RNA a poly (A)-rich fraction was prepared. cDNA synthesis was performed using Stratagene's cDNA synthesis kit according to the manufacturer's instructions. The primary library represented 2 x 10^6 independent clones. This library was screened using the PCR-generated PTK2 fragment as a probe.

Hybridization probes

For Northern blots, isolated DNA fragments of cloned cDNA were multiprime-labelled using reagents from Stratagene. For screening of libraries, PCR-generated DNA fragments were uniformly labelled as described [13]. Briefly, about 20 ng of DNA was heated to 95 °C for 3 min in a total volume of 50 µl of a reaction mixture containing restriction buffer #2 (Life Technologies), 25 pmoles each of the specific primers used for amplification of the DNA fragment, 0.5 mM each of dCTP, dGTP, dTTP and 50 µCi [α ^{32}P]-dATP (Amersham). After heating, the reaction mixture was chilled on ice and 6 units of DNA polymerase (Klenow fragment; Stratagene) were added. The reaction was carried out at 45 °C for 10 min and stopped by addition of EDTA to 20 mM final concentration. Labelled DNA fragments were purified through a spun column [14].

Northern blot hybridization

A human "Multiple Tissue Northern" blot (Clontech) containing 2 µg of poly(A)$^+$ RNA per lane was hybridized under stringent conditions with probes derived from different regions of the human cDNA clone.

Sequence analysis

The cDNAs and PCR fragments were subcloned into pBS vector (pBS$^+$, Stratagene) and sequenced by the dideoxy chain termination method [15,16] using T7 DNA polymerase and specific oligonucleotides. Sequences were assembled with the Staden-Plus software (supplied by Amersham) on a personal computer. The analysis was performed with programs from the university of Wisconsin, Genetic Computer Group.

RESULTS AND DISCUSSION

Isolation of cDNA clones encoding PTKs

The catalytic domain of PTKs contains sequence motifs that are highly conserved among the protein tyrosine kinases [17]. We used a PCR approach to clone from human tonsillar B cells novel receptor tyrosine kinases that may play a specialized role in B cell activation. By employing degenerate primers (Materials and Methods) and RT-PCR we amplified part of the kinase domain of various tyrosine kinases. This approach yielded a total of five different PTK-specific cDNAs. Most often we isolated a clone named PTK2 which shows highest homology to bovine FGF receptor (53%). In subdomain VIII of the catalytic domain [17] it exhibits the sequence motif WMAPES which is believed to indicate transmembrane kinases since it is present in PDGFR, cKIT, CSF1R and FLK-2 [18]. Because PTK2 appeared to be a novel member of the family of RTKs we attempted to clone the full length cDNA. Using PTK2 as a probe the human tonsillar cDNA library (Materials and Methods) was screened and one cDNA clone of 2.4 kb was isolated (cPTK2). This clone contained a poly(A) tail but only subdomains VI to XI of the kinase domain [17]. cPTK2 thus was unlikely to represent a full-length clone. From expression studies we learned that PTK2 is highly expressed in brain (see below). Therefore we

screened a human fetal brain cDNA library (Clontech) using cPTK2 as a probe. We isolated a 3.1 kb cDNA clone (cPTK3) encoding a protein of 850 amino acids. The size of clone cPTK3 corresponds well with the size of a 3.3 kb transcript found exclusively in brain by Northern hybridization (see below). The nucleotides surrounding the first ATG of the cDNA correspond to the consensus sequence for translation initiation [19]. The deduced amino acid sequence comprises the complete kinase domain but lacks a transmembrane region or myristylation sites. No SH2 and SH3 domains [20] are encoded by this cDNA clone. The sequence 5' of the catalytic domain does not show any significant homology to other known sequences. During our studies the sequence of a novel chicken PTK was published [10]. Because of its localisation close to focal contacts in fibroblasts this PTK was named FAK (pp^{125}FAK). The sequence identity between chicken FAK and our human clone cPTK3 is 85% at the nucleotide level and 95% at the amino acid level (Fig.1). In the kinase domain 98.5% of the amino acids are identical. We therefore conclude that clone cPTK3 represents a human homologue of FAK and we name it hFAK. cPTK3 lacks the first 157 amino acids found in chicken FAK. The hFAK clone has inserted additional 29 extra amino acids between Ala 447 and Leu 448 of the chicken sequence (Fig.1). This insertion comprises three cysteine and two tyrosine residues. Furthermore, amino acids corresponding to positions 809 to 829 of chicken FAK are absent in the hFAK clone. Further studies are necessary to determine wether these differences are due to neural-specific alternative splicing as has been seen for c-*src* for example [5, 6].

The 5' end of the hFAK transcript encoding the N-terminal 157 amino acids that are missing in clone cPTK3 was cloned from tonsillar mononuclear cell RNA using RT-PCR. The downstream primer was specific to the hFAK cDNA and the upstream primer was derived from the chicken FAK cDNA sequence. The size of the amplified PCR fragment was 0.8 kb. The sequence homology to the chicken FAK cDNA is 88% at the nucleotide level (data not shown).

Splicing variants of hFAK

Using the RACE protocol we isolated three different clones of hFAK varying at their 3' ends. Compared to the cPTK3, none of the RACE clones contained the complete 3' translated region (Fig. 2). PTK2-31 stops after kinase subdomain XI. It may represent a cDNA encoding a molecule that contains the amino terminal and the complete catalytic domain but lacks part of the C-terminal region. This cDNA contains a poly(A) tail. The last 30 amino acids encoded by this clone are different from the corresponding sequence of the 3.1 kb hFAK clone (Fig.1). The 3' untranslated region has 36% identity to the 3' untranslated region of cPTK3. Clones PTK2-32 and PTK2-33 do not encode the complete catalytic domain (Fig.2). Their open reading frames are fully identical to each other and to cPTK3 up to amino acid Lys426 followed by 5 non-identical amino acids as compared to cPTK3 (Fig.1). Their 3' untranslated regions are different, both from cPTK3 (38% identity to PTK2-32 and 42% identity to PTK2-33), and from each other (97% identity).

C-terminally truncated forms of PTKs have been described for different growth factor receptors such as bFGFR, EGFR and trkB. Regulation of protein tyrosine kinase

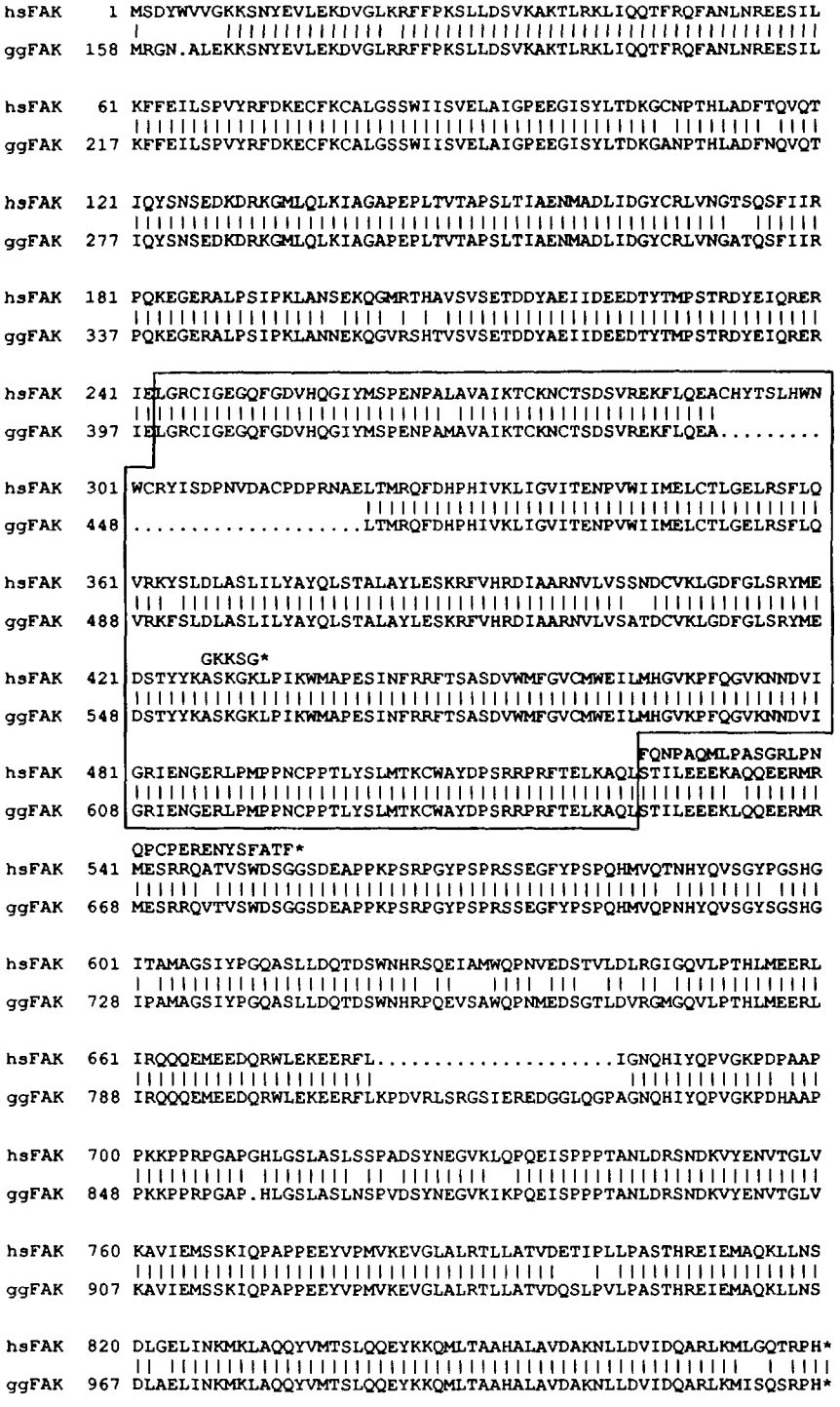

Fig.1. Comparison of the amino acid sequences of human FAK and chicken FAK cDNA. The numbers on the left indicate the positions relative to the putative start methionine. The catalytic domain is boxed. The amino acids encoded by the RACE clones that are not identical to cPTK3 are indicated. PTK2-31 is identical to cPTK3 up to amino acid 524, PTK2-32 and PTK2-33 are identical up to amino acid 426.

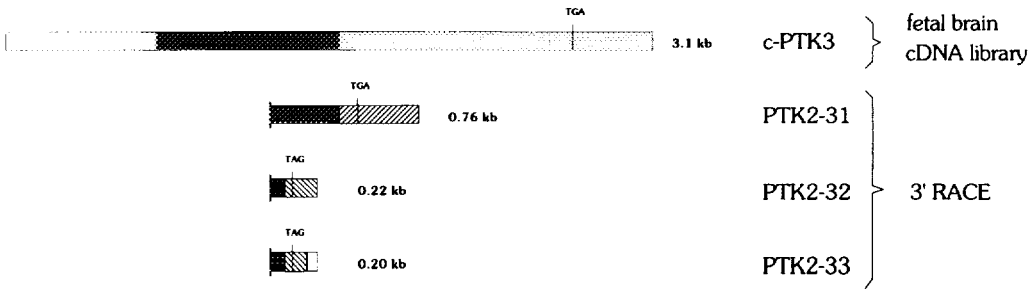

Fig.2. Schematic representation of the different hFAK clones. The filled area indicates the catalytic domain. Non-identical parts of the compared sequences are differently marked.

activity of RTKs through formation of heterodimers between the full-length receptor and truncated receptors is a possible role for such truncated molecules [8].

Expression of hFAK in different cell lines and organs

We tested whether hFAK was expressed in other tonsillar cells in addition to B cells. By RT-PCR we detected the hFAK message in tonsillar mononuclear cells, tonsillar T cells and germinal center cells. Different lymphoid cell lines (RPMI8866, RPMI8226, U266, Jurkat, Molt-4 and LAD) as well as HeLa cells and the neuroblastoma cell line SK-N-SH (ATCC) were tested in the same way for the expression of hFAK. Only in a LAD (lymphocyte adhesion deficient) cell line no hFAK-specific PCR product was detectable after 40 cycles of PCR (data not shown).

By Northern blot analysis we detected hFAK transcripts of different sizes, 4.3 kb, 3.3 kb and 2.4 kb (Fig. 3). The 4.3 kb transcript was expressed in all organs tested. It is most abundant in brain and lung but barely detectable in placenta, liver and kidney. The 2.4 kb transcript was found in lung, placenta and heart. A transcript of 3.3 kb is present only in brain. We used a human probe specific for the 5' end of FAK which does not overlap the 3.1 kb cDNA clone cPTK3 to hybridize the multiple tissue northen blot. This probe only hybridized to the 4.3 kb message (Fig.3) and not to the 3.3 kb message. This supports the idea that the 3.1 kb clone cPTK3 corresponds to the 3.3 kb transript detected by Northern analysis. Since we were unable to isolate from the fetal brain library a cDNA clone that contains the long 5' variant it is possible that the distribution of the two transcripts in fetal brain differs from that in the adult brain sample analysed by Northern hybridization. An interesting parallel has been reported for another PTK, the PDGFβ receptor. In embryonic stem cells the predominant PDGFβ mRNA encodes an N-terminally truncated protein [9]. This protein comprises the whole catalytic domain and the transmembrane region but lacks the extracellular domain. Based on the fact that v-*erb* is an N-terminally truncated form of EGFR, the 5' truncated form of PDGFβR is believed to represent a constitutively active receptor expressed early in development [8]. Whether the activation of hFAK also occurs via the N-terminal part remains to be shown. The model however would predict that the phosphorylation of hFAK in stable transformants

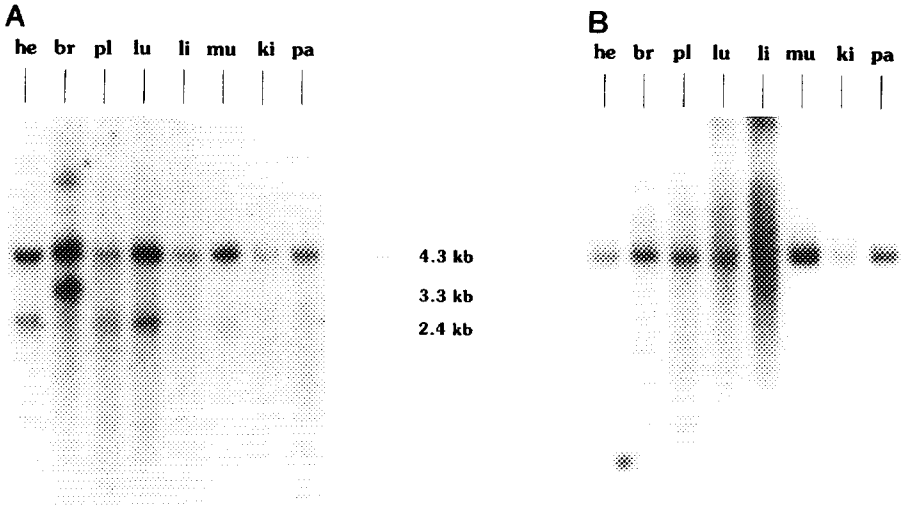

Fig.3. Hybridization of human "Multiple Tissue Northern" blots (Clontech) with 2 different hFAK probes. The lanes were loaded with 2 μg poly(A)$^+$ RNA from heart (he), brain (br), placenta (pl), lung (lu), liver (li), skeletal muscle (mu), kidney (ki) and pancreas (pa). (A) Autoradiography (24h exposure) after hybridization to cPTK3. (B) Autoradiography (3 days exposure) after hybridization to the hFAK-specific PCR fragment corresponding to amino acids 1 - 150 of the chicken sequence which are not encoded by cPTK3.

expressing only the 5' truncated hFAK could not be regulated via *src* and/or fibronectin as it was shown for chicken FAK expressing fibroblasts [21].

While the preparation of this manuscript was in progress the sequence of the mouse homologue of chicken FAK was published (FadK) [22]. The homology between mouse and human FAK is 93% at the amino acid level.

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REFERENCES

1. Hunter, T. and Cooper, J.A. (1985) Ann. Rev. Biochem. 54, 897-930.
2. Ullrich, A. and Schlessinger, J. (1990) Cell 61, 203-212.
3. Yarden, Y. and Ullrich, A. (1988) Annu. Rev. Biochem. 57, 443-478.
4. Cooper, J.A. (1990), CRC press, Boca Raton p. 85-113.
5. Brugge, J.S., Cotton, P.C., Queral, A.E., Barrett, J.N., Nonner, D., and Keane, R.W. (1985) Nature 316, 554-557.
6. Ross, C.A., Wright, G.E., Resh, M.D., Pearson, R.C.A., and Snyder, S.H. (1988) Proc. Natl. Acad. Sci. USA 85, 9831-9835.

7. Klein,R., Conway, D., Parada, L.F., and Barbacid, M. (1990) Cell 61, 647-656.
8. Middlemas, D.S., Lindberg, R.A., and Hunter, T. (1991) Mol. Cell. Biol. 11, 143-153.
9. Vu, T.H., Martin, G.R., Lee, P., Mark, D., Wang, A., and Williams, L.T. (1989) Mol. Cell. Biol. 9, 4563-4567.
10. Schaller, M.D., Borgman, C.A., Cobb, B.S., Vines, R.R., Reynolds, A.B., and Parsons, J.T. (1992) Proc. Natl. Acad. Sci. USA, 89, 5192-5196.
11. Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156-159.
12. Frohman, M. (1990) Amplifications 5, 11-15.
13. Becker-André, M., Hooft van Huijsduijnen, R., Losberger, C., Whelan, J., and Delamarter, J.F. (1992) Eur. J. Biochem. 206, 401-411.
14. Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.
15. Chen, Y.E. and Seeburg, P.H. (1985) DNA 4, 165-170.
16. Sanger, F., Nicklen, S., and Coulsen, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
17. Hanks, S.K., Quinn, A.M., and Hunter, T. (1988) Science 241, 42-52.
18. Matthews, W., Jordan, C.T., Wiegand, G.W., Pardoll, D., and Lemischka, I.R. (1991) Cell 65, 1143-1152.
19. Kozak, M. (1991) J. Cell Biol. 115, 887-903.
20. Koch, C.A., Anderson, D., Moran, M.F., Ellis, C., and Pawson, T. (1991) Science 252, 668-674.
21. Guan, J.-L. and Shalloway, D. (1992) Nature 358, 690-692.
22. Hanks, S.K., Calalb, M.B., Harper, M.C., and Patel, S.K. (1992) Proc. Natl. Acad. Sci. USA 89, 8487-8491.