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Human SAK related to the PLK/polo family of cell cycle kinases shows high mRNA expression in testis

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Abstract. We identified the nucleotide sequence of a cDNA encoding a polypeptide with a kinase domain that is related to the catalytic region of *Drosophila melanogaster polo*, *Saccharomyces cerevisiae* CDC5 as well as human FNK and PLK. The novel gene seems to represent the human counterpart of the mouse gene *sak*. The sequence of SAK predicts a serine/threonine kinase of 970 aa. The distribution of SAK mRNA in adult organs is restricted to certain tissues such as testis and thymus. Northern analyses of tumor tissues (lung, breast, brain) and corresponding normal tissues from the same patient did not reveal SAK expression. Comparing the mRNA distribution of the proliferation-associated polo-like kinase (PLK) with the expression of SAK we observed distinct differences. Thus, we suggest that these kinases have unique physiological roles in different cells or in response to different signals.

Introduction

Phosphorylation plays a pivotal role in controlling the progression through the eukaryotic cell cycle, cellular differentiation and changes of cellular structures. The *Drosophila melanogaster polo* gene (1) and the *Saccharomyces cerevisiae* cell cycle gene CDC5 (2) are two conserved protein kinases which are required for progression through mitosis: Mutations in polo result in abnormal chromosome segregation in larval neuroblasts of *Drosophila* due to defective spindel formation in mitotic and meiotic cells (1,3). Deletion of CDC5 was lethal in *Saccharomyces cerevisiae* displaying an abnormal morphology of dividing cells with their nuclei almost divided but still connected (2). Recently, two human serine/threonine kinases have been identified which are homologous to polo: PLK (4-7) and FNK/PRK (8,9, Holtrich *et al*, unpublished). It could be demonstrated that PLK mRNA expression is regulated during terminal erythrodifferentiation and during the cell cycle (4). In our own studies several lines of evidence indicate that the levels of PLK-mRNA and -protein correlated with cellular proliferation (7,10). In addition we found that the prognosis of patients suffering from non-small cell lung cancer (NSCLC) correlates with PLK mRNA expression in lung tumor tissues. Thus, the determination of PLK mRNA expression helps to define subgroups of patients in clinical stages I and II, who have a bad prognosis because of an aggressively growing tumor (11).

Recent observations support the central role of PLK-related kinases for the control of mitosis: A serine/threonine kinase, named Plx1, was isolated from *Xenopus* egg extracts, which exhibits close homology of 80% to human PLK, indicating that it represents its *Xenopus* counterpart. Recombinant Plx1 phosphorylated Cdc25 and stimulated its activity in a purified system, suggesting that Plx1 controls Cdc2, the cyclin dependent kinase that triggers mitosis, by regulating the activity of Cdc25 (12).

FNK/PRK expression is limited to certain tissues such as placenta, ovaries and lung (9). Re-feeding of serum-deprived cell lines of hemopoietic origin activates FNK/PRK mRNA expression. The level of FNK/PRK transcripts seems to be downregulated in lung tumors compared to uninvolved lung tissues. Murine *fnk* was shown to be an immediate early gene whose expression is first detected at 30 min after addition of various growth factors such as FGF and platelet-derived growth factor-BB to quiescent cells (8).

In a search for protein kinases, which play a role in human lung cancer, we used a PCR-based approach to amplify kinase-related sequences. Here, we report the cloning and genetic analysis of a third human PLK-related gene, which encodes a putative serine/threonine kinase closely related to murine *sak* (13). Analysis of human SAK expression in adult tissues and primary cells revealed a tissue specific expression restricted to thymus and testis.

Materials and methods

RNA isolation and Northern blots. Tissues were homogenized in a guanidinium isothiocyanate solution (14). RNA

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was isolated by centrifugation through a 5.7 M CsCl cushion. For Northern blot analysis RNA was separated in a denaturing agarose/formaldehyde gel and transferred to nitrocellulose membranes (Amersham). Hybridization and washing were performed under high-stringency conditions (15). Multiple tissue blots were purchased from Clontech (Palo Alto, CA).

PCR. First strand cDNA synthesis and PCR were performed as described previously (16). Primers were synthesized using an Applied Biosystems 380A DNA synthesizer. Primer sequences are as follows: Eco-VHRDL (motif VHRDL), 5'-TTTGGAATTCGTNCAYMGNGAYYT-3'; Eco-P62[DEA] (motif DVWXXGM), 5'-TTGGAATTCATCCCNNNNNNC CACACATC-3'; P12(T+), 5'-GCAGAATTCGTGAACTGC GGCCGCA(dT)12-3'; EB 5'-CTGAATTCGGATCCGACT GGTCTGACTCG-P-CH_2-CHOH-CH_2-NH_2; EBcom 5'-CGA GTCAGACCAGTCGGATCCGAATTCAG-3', K3L2, 5'-TT CAGTCTGAGGTGTCGGGTCTGC-3'; K3L4, 5'-GCTTCA TTTCTGAGAAGGGTTTCAC-3'; K3U6, 5'-GTCAAAAA GAACTCTGATGCTTCTG-3'.

Labelling of probes. PCR was applied to obtain probes corresponding to aa 365-474 of SAK. Radiolabelling of the antisense strand was performed using primer K3L2 and 150 µCi of [α-^{32}P]dCTP (6000 Ci/mmol); 1 Ci=37 GBq.

Construction and screening of a cDNA library. Total RNA was isolated (14) from a human lung tumor (squamous cell carcinoma). Poly(A)$^+$ RNA was selected by using oligo(dT)-cellulose (17). The construction of the cDNA library followed the method of Gubler and Hoffmann (18). In summary, a Pharmacia kit was applied for synthesis and purification of cDNA, which was ligated to EcoRI-digested λgt 10 DNA. After packaging with Gigapack II Gold (Stratagene) and plating, 1.8×10^6 independent recombinant clones were screened under high stringency conditions (42˚C, 50% formamide) with the primary PCR product as a probe derived from the catalytic domain of SAK corresponding to aa 142-184.

5' and 3' elongation by the RACE technique. Since the longest cDNA clone which was obtained by screening of the lung tumor cDNA library did not encompass the entire open reading frame of SAK, a modified RACE (rapid amplification of cDNA ends) technique was applied (19,20). For this purpose specific primers from the 5' region of the SAK-PCR product were utilized for cDNA synthesis. The cDNA was subsequently ligated to a 3' modified oligonucleotide (EB) and amplified using primer EB (com) and the SAK specific primer K3L4 resulting in the extension of 478 bp of upstream sequences. Anchored PCR was performed to complete the missing 3' portion: a cDNA starting at the poly A-tail was synthesized with P12(T+). Subsequent PCR amplification with the specific primer K3U6 located at the 3' end of SAK and primer P12(T-) provided the complete open reading frame.

Sequencing. Sequencing of double-stranded DNA was performed by the dideoxy chain termination method with Taq polymerase using an ABI 373A DNA sequencer according to the protocols of the manufacturer (Applied Biosystems, Weiterstadt). Cycle sequencing on a DNA thermal cycler (Perkin Elmer Cetus) was performed with dye terminators.

Results

PCR-based isolation of a PLK related protein kinase cDNA from human tissues. cDNA from human embryonic tissues was amplified using primers corresponding to highly conserved amino acid motifs (VHRDL and DVWXXGM) from subdomains VI and IX within the catalytic domain of protein kinases (21) in order to identify novel members of the kinase family and to evaluate their role in cancer development. Resulting PCR products were ligated to the pBluescript KS(+) vector (Stratagene). Sequence determination of 280 inserts revealed various unknown sequences related to protein kinases: Specific extension of two unknown kinase related sequences yielded the complete open reading frames of the new protein-kinases PLK (7) and MO15 (22). Since this approach has been shown to provide new kinases which are of importance for lung tumors such as PLK, a marker for cellular proliferation with prognostic significance for NSCLC-patients, we have chosen an additional clone, named K3, representing a sequence related to PLK for further studies. A 200 bp-insert derived from K3 was used as a probe for the screening of a cDNA library based on RNA from a human lung tumor (squamous cell carcinoma). We obtained a clone of 1.3 kb. The RACE technique was applied for 5' and 3' elongation of this cDNA in order to obtain the complete open reading frame. The analysis of the elongated clone revealed a continuous sequence of 3.1 kb with a complete open reading frame exhibiting an ATG and stop codon. Using cDNA from normal lung tissues we verified the sequence of 3.1 kb by PCR-amplification and direct sequencing.

Identification of a human serine/threonine kinase gene related to the mouse gene sak. Determination of the nucleotide sequence exhibited a single open reading frame of 2910 nt extending from an ATG codon at position 141 to an in-frame stop codon at position 3051, which predicted a 109-kDa polypeptide of 970 aa (Fig. 1). The 5' untranslated sequence has a length of 140 bp with a putative start codon which is in agreement with Kozak's rule for the initiation of translation (23). The 3' untranslated sequence is 39 bp in length containing a potential polyadenylation signal (AATAAA). The predicted K3-polypeptide contains an aminoterminal kinase domain which shows the characteristics of protein serine/threonine kinases. Interestingly, a lysine residue located within the motif HRDLK, which is conserved in almost all members of this family, is substituted by a threonine residue (aa 138) in the putative K3-amino acid sequence. A computer analytic comparison of the 970 aa-polypeptide with other known proteins (Swiss-Prot and the Protein Identification Resource, September 1996) confirmed the first observation that it belongs to the family of serine/threonine kinases. In particular it is homologous to polo-related kinases: Comparing the kinase domains of the new protein to those of human PLK, human FNK/PRK and

Figure 1. Nucleotide and amino acid sequence of human SAK (K3) including partial untranslated sequences. The start codon is in bold type. The stop codon is underlined. The highly conserved motifs of the kinase domain (shaded) are shown in inverted letters: the invariant residues (DFG) implicated in ATP-binding and the consensus sequence (GXGXXA) of serine/threonine kinases related to *cdc2* (21,26).

murine *snk* (24) the similarity was determined to be 37%, 41% and 39% respectively. The closest overall homology of 82.3% has been detected to a murine gene, which was named *sak* (13). Comparing the kinase domain and the carboxyterminal portions of K3 and *sak* we observed similarities of 94.4% and 77.2%. The relationship of both genes (K3/*sak*) was confirmed by the homologies of 62% (140 bp) and 66% (40 bp) within the 5' and 3' untranslated regions. K3 exhibits the same topology as the members of the polo-family (polo, PLK, FNK and *snk*) with the kinase domain located at the aminoterminal portion of the protein. In contrast to these proteins K3 does not contain the polo-homology 2 (PH2) domain, which is a motif of approximately 30 aa within the carboxyterminal region of polo-related kinases. Due the the high degree of homology it seems likely that the 910 aa-polypeptide is the human counterpart of the murine gene *sak* (Fig. 2). Thus, we suggest to name it also SAK.

Fig. 3 shows the evolutionary relationship of the kinase domain of SAK to the rest of human serine/threonine kinases. In a phylogenetic tree based on sequence homologies within the kinase domain the split of SAK occurred before the evolution of the family of polo related kinases.

SAK exhibits tissue specific expression. The Northern blot analysis of RNAs isolated from various human tissues with a specific probe from the carboxyterminal portion of the open reading frame showed SAK transcripts of 4.0 kb to be most abundant in testis and thymus (Fig. 4). In all other tissues examined SAK mRNA was below the limit of detection under high stringency conditions. Furthermore, we carried out a Northern blot analysis of RNAs isolated from a variety of different human malignant tissues. Human tumors of various origin such as lung, breast and brain and corresponding normal tissues from the same patient did not exhibit SAK mRNA expression (data not shown).

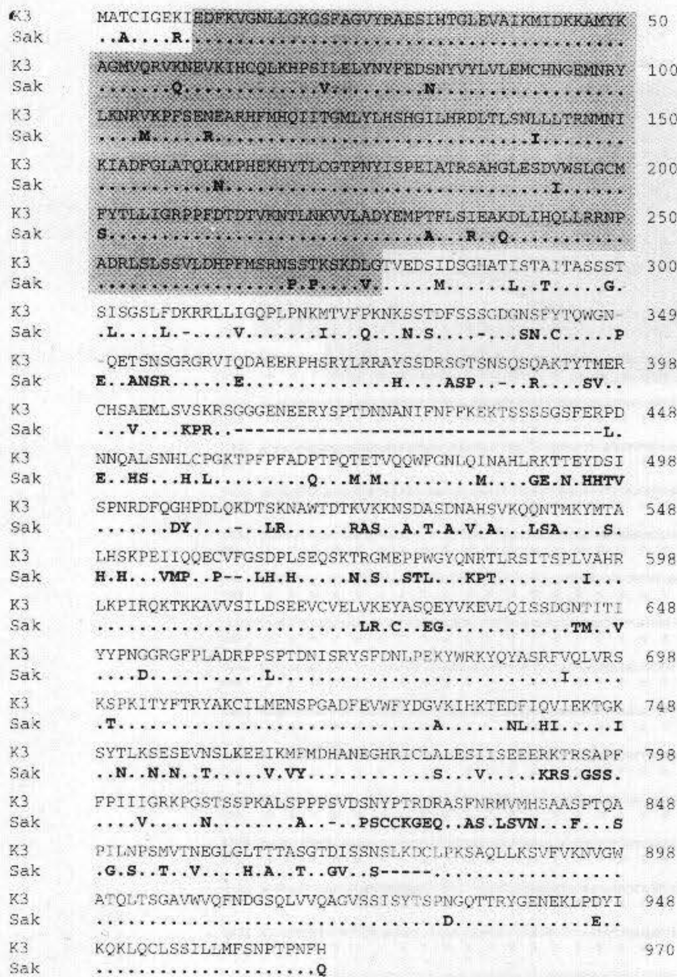

Figure 2. Sequence alignment of the predicted human SAK (K3) and murine *sak* amino acid sequences. Numbers to the right refer to the last amino acid in this line. Identical residues in the murine sequence are substituted by dots. Gaps represented by dashes were inserted to maximize the alignment. The shaded region represent the kinase domain.

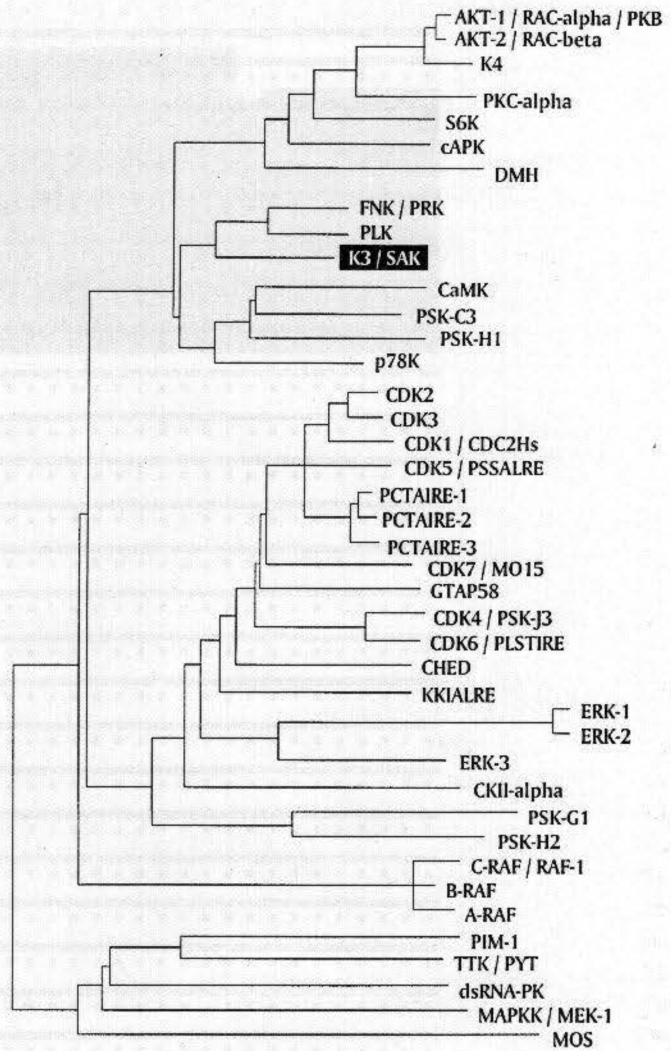

Figure 3. Phylogenetic relationship of SAK to other human serine/threonine kinases. The amino acid sequences of the catalytic domains of human serine/threonine kinases were used for calculation of a phylogenetic tree with the Tree program of HUSAR (Heidelberg Unix Sequence Analysis Resource, DKFZ, Heidelberg). It is based on the progressive alignment method of Feng and Doolittle (27) in a multiple sequence alignment.

Discussion

In this study we identified a novel member of the family of serine/threonine kinases which is related to the *Drosophila melanogaster* gene polo. We describe the cloning of the new gene during a screen of a cDNA library based on human RNA designated to isolate new protein kinase genes which might participate in the development of lung cancer. The closest relationship of 82.3% has been found to murine *sak*. Maximal alignment required the introduction of several gaps (Fig. 2). Within the family of kinases required for the progression through the cell cycle we compared the open reading frames of human/murine counterparts and determined the homology to be 93.7% for PLK/plk, 91.8% for FNK/*fnk* and 95.1% and human/murine MO15. This comparison revealed that SAK is not as well conserved as other members of the polo-family. Still, the homologies of

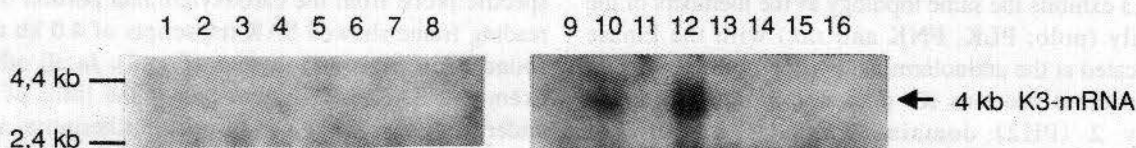

Figure 4. Expression of SAK (K3) mRNA in adult tissues. Each lane contained 2 µg of poly(A)$^+$ from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (lanes 1-8) as well as spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes (lane 9-16). Hybridization was done under high stringency with an antisense probe corresponding to aa 365-474. Size markers on the left are in kilobases.

the open reading frames and the untranslated parts of both cDNAs as well as the patterns of mRNA expression of murine/human SAK are quite similar. Thus we suggest that the new polo-related gene represents the human counterpart of murine *sak*.

An analysis of SAK expression by Northern blot hybridization has shown that out of 16 adult tissues SAK transcripts are restricted to testis and thymus. A detailed study of various tumor tissues and adjacent normal tissues from the same patient revealed that human SAK transcripts are not detectable in those specimens. These data indicate that human SAK is not expressed in the proliferative active tissues examined. This observation differs in some way from a study of *sak* expression in murine tissues which has shown that in the embryonic central nervous system *sak* transcripts are restricted to the ventricular zones, where neuroblasts are dividing. Murine *sak* transcripts are not detected in zones, where the postmitotic neurons are located (13). In adult mice, *sak* is expressed in tissues with a mitotic component, including hemopoietic tissues and the stem cells of the intestinal crypt. Furthermore, high levels of murine *sak* mRNA were found in meiotic spermatocytes and oocytes (13).

Expression of human SAK differs also from PLK, which belongs to the family of polo-related kinases. PLK was shown to be regulated during the cell cycle in NIH3T3 cells (4). In addition, PLK mRNA is highly expressed in rapidly dividing cell populations found in fetal and newborn tissues and adult hemopoietic tissues (25). Therefore, PLK mRNA expression is strongly correlated with the mitotic activity of cells and tissues. In our own studies on the function of PLK most human tumors of various origins were found to express high levels of PLK mRNA and protein, although its expression was not detectable in normal tissues, indicating that the expression of PLK is associated with cell proliferation (7,10,11).

In addition to the diverging pattern of mRNA expression the architecture of the putative SAK protein differs clearly from the related human polypeptides PLK and FNK/PRK: Close relatives of *Drosophila melanogaster polo* such as *Saccharomyces cerevisiae* CDC5 and human PLK, FNK/PRK exhibit a common domain, named polo-homology-(PH2) domain, with an unknown function. SAK lacks this motif of polo-related kinases. Thus, despite different structural similarities of SAK and polo-related kinases, the tissue distribution patterns of their mRNAs as well as the molecular design of the putative proteins are distinct, suggesting that these kinases have special physiological roles in different cells.

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