SHORT COMMUNICATION

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Isolation and chromosomal assignment of a human gene encoding protein inhibitor of activated STAT3 (PIAS3)

Received: January 4, 1999 / Accepted: February 13, 1999

Abstract Mouse PIAS3 (protein inhibitor of activated STAT3) is a specific inhibitor of STAT3 that downregulates its signaling pathway. Here we report the isolation and chromosome mapping of the human *PIAS3* gene. Human PIAS3 cDNA encoded a predicted protein of 619 aa which has 83% overall amino acid identity to the mouse counterpart. Based on polymerase chain reaction assisted analysis of a human/rodent mono-chromosomal hybrid cell panel and a radiation hybrid mapping panel, the human *PIAS3* gene was mapped to the chromosome 1q21 region. Mapping of a crucial gene in modulating the STAT3 signaling pathway may provide new clues to the understanding of malignancies or genetic disorders caused by this chromosome region.

Key words STAT3 · PIAS3 · Cytokine · Transcription factor · Hematopoiesis · 1q21

Introduction

STAT (signal transducer and activator of transcription) proteins are latent cytoplasmic transcription factors that become activated by tyrosine phosphorylation in response to cytokine stimulation (Ihle and Kerr 1995). Tyrosine phosphorylated STATs undergo dimerization and translo-

cate into the nucleus to activate specific set of genes. Seven members of the STAT protein family have been isolated, which have distinct functions in cytokine signaling (Leonard and O'Shea 1998). STAT3 is activated by cytokines such as interleukin-6 (IL-6), leukemia inhibitory factor (LIF), oncostatin M, granulocyte colony-stimulating factor (G-CSF), epidermal growth factor (EGF), and leptin (Akira et al. 1994, Zhong et al. 1994, Vaisse et al. 1996). Recently, a family of proteins named PIAS (protein inhibitor of activated STAT), which can bind to STATs and inhibit their transcriptional activity, has been identified. Mouse PIAS1 and PIAS3 can specifically inhibit STAT1 and STAT3 signaling, respectively (Liu et al. 1998, Chung et al. 1997). In humans, PIAS1, as well as additional members PIASx α , PIASx β , and PIASy, have also been identified (Liu et al 1998). However, the function of these new members still remains elusive. Here we report the isolation of human PIAS3 cDNA and its chromosomal location.

Source and isolation of the human *PIAS3* gene

A partial cDNA clone (initially called HFB096) which had a sequence highly homologous to mouse PIAS3 was identified by a screening system for nuclear proteins (Ueki et al. 1998). Specific primers were designed according to the HFB096 sequence, and the 5' portion of the cDNA was obtained from a human fetal brain library, using GeneTrapper (GIBCO BRL, Gaithersburg, MD, USA). The nucleotide sequences of both strands were determined by a primer walking method and using an ABI377 sequencer (Perkin Elmer, Norwalk, CT, USA). The resultant cDNA was 2802 bp in length, with a poly A at the 3' end, and contained an open reading frame (ORF) of 619 amino acids. The nucleotide sequence of the cDNA will appear in GenBank/EMBL/DDBJ databases under the accession number AB021868. The sequence analysis of the cDNA clone revealed that the predicted protein had an overall high homology to mouse PIAS3, and thus we renamed this clone human PIAS3. The alignments of the amino acid

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Fig. 1. Alignment of the PIAS family of proteins. The predicted amino acid sequences of human PIAS1 (hPIAS1; accession number, AF077951), mouse PIAS1 (mPIAS1; accession number, AF077950), human PIAS3 (hPIAS3; accession number, AB021868), mouse PIAS3 (mPIAS3; accession number, AF034080), human PIASxα (hPIASxα;

accession number, AF077953), human PIASxβ (hPIASxβ; accession number, AF077954) and human PIASy (hPIASy; accession number, AF077952) are shown. Identities are indicated by black background and similar residues are shadowed. Asterisks denote the termination codons

sequences of hPIAS3, mPIAS3, hPIAS1, mPIAS1, hPIASxα, hPIASxβ, and hPIASy are shown in Fig. 1. The amino acid identity of human and mouse PIAS3 proteins was 83%, while hPIAS3 and hPIAS1, hPIASxα, hPIASxβ, and hPIASy were 56%, 53%, 55%, and 39% identical, respectively.

Chromosomal mapping of the gene

Chromosomal assignment of the human *PIAS3* gene was done by polymerase chain reaction (PCR) analysis of a human/rodent somatic cell hybrid panel (National Institute

Fig. 2. Chromosomal placement of the human *PIAS3* gene at a relative distance to framework markers on the WICGR (Whitehead Institute for Biomedical Research/MIT Center for Genome Research) radiation hybrid map of the human genome (<http://www.genome.wi.mit.edu/>). The approximate corresponding cytogenetic location of the gene on the 1q21 region is indicated. Distances of the markers are in centirays (cR) and centimorgans (cM) from the top of the chromosome 1 linkage group

of General Medicine Service, Coriell Cell Repositories, Camden, NJ, USA) and a radiation hybrid panel (Genebridge 4, Research Genetics, Huntsville, AL, USA), as described previously (Seki et al, 1997). The hPIAS3-specific PCR primers were designed at the 3'-untranslated region of the gene (5'-GAT TGG GAA GGA GGG CAC AGG-3', 5'-ACT TCC CCT GCC TCC TAC TCC-3') which would give rise to a PCR product with a size of 240 bp. The specific amplified product for humans was detected only from the hybrid containing human chromosome 1 (data not shown). Then, we performed further mapping analysis, using a radiation hybrid panel with the same primer set. Statistical analysis of the radiation hybrid data was performed using the RHMAPPOR software package (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>). The data vector for the *hPIAS3* gene was 0001010001 0110000110 0000110110 0101000110 1011101000 1001000000 0000101010 0101000001 1100000101 011 and the consequent report indicated that the gene was placed to 7.36 cR distal from D1S442 (lod>3.0). The region including the marker was cytogenetically mapped to the 1q21 region (Lioumi et al. 1996) (Fig. 2).

Discussion

STAT3 is an essential transcription factor for the growth and development of hematopoietic cells (Minami et al. 1996). Thus, dysregulation of the STAT3 signaling cascade

may lead to uncontrolled hematopoiesis. In fact, *STAT3* is found to be constitutively activated in acute leukemia (Gouilleux-Gruart et al. 1997). However, no mutation in the *STAT3* gene associated with human diseases has been found to date. Since *PIAS3* is an inhibitor of *STAT3*, it is conceivable that dysregulation of *PIAS3* may modulate *STAT3* signaling. In this respect, it is noteworthy that the 1q21 region where hPIAS3 is mapped is associated with hematopoietic malignancies such as multiple myeloma (Keung et al. 1998) and large B-cell lymphoma (Rao et al. 1998). Our precise chromosomal positioning of the *hPIAS3* gene may contribute toward the ongoing positional candidate approaches for the above-mentioned diseases linked to the chromosomal locus.

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