Mol. Cells, Vol. 10, No. 5, pp 598–600

Communication

Molecules and Cells

Human Fas Associated Factor 1, *hFAF1*, Gene Maps to Chromosome Band 1p32

Seung-Wook Ryu, Hae-Suk Kim[1], Sungjoo Kim Yoon[1], V. V. V. S. Murty[2], and Eunhee Kim*

Research Center for Biomedical Resources and Division of Life Science, PaiChai University, Taejon 302-735, Korea;
[1] Catholic Research Institutes of Medical Science, Catholic University of Korea, Seoul 137-040, Korea;
[2] Department of Pathology, P&S 15-408 College of Physicians and Surgeons of Columbia University, New York 10032, USA.

(Received on June 1, 2000)

Human Fas associated factor 1 protein (hFAF1) is involved in the positive regulation of Fas signaling even though it can not initiate the signal for itself. By chromosomal assignment using somatic cell hybrids (CASH), the *hFAF1* gene was located on human chromosome 1 between markers D1S443 and D1S197. The *hFAF1* gene was mapped to human chromosome band 1p32 by FISH utilizing a genomic PAC clone containing the gene. In genomic Southern analysis using *hFAF1* cDNA as a probe, several bands appeared in three different restriction enzyme digestions. The single band appearance in FISH analysis compared to several bands in Southern blots implies that the *hFAF1* gene would be rather big or that an additional *hFAF1* gene isotype(s) might be present in close vicinity.

Keywords: Chromosome; FAF1; Mapping; 1p32.

Introduction

Fas (also known as APO-1 or CD95) is a cell-surface protein mediating apoptosis. Unlike TNFR (tumor necrosis factor receptor) which activates NF-κB as well as inducing apoptosis, Fas seems to be dedicated solely to apoptosis (Nagata, 1997). FADD (Fas associating protein with a death domain), Daxx and FAP-1 (Fas associated phosphatase-1) have been discovered as proteins downstream from the Fas signaling pathway (Boldin *et al*., 1995; Chang *et al*., 1998; 1999; Chinnaiyan *et al*., 1995; Yang *et al*., 1997). FADD and Daxx activate two independent Fas pathways. FAP-1 is the only protein that associates with Fas and inhibits Fas-induced apoptosis (Yanagisawa *et al*., 1997). Also, FLASH (a Flice-associated huge protein) and SODD (a silencer of death domains) have been discovered as a caspase activator and a repressor, respectively, in the Fas signaling pathway (Ashkenazi and Dixit, 1998; Imai *et al*., 1999; Jiang *et al*., 1999; Porter, 1999).

To date, *FAF1* has been cloned in human (hFAF1), in mouse (mFAF1) and in quail (qFAF1) (Chu *et al*., 1995; Frohlich *et al*., 1998; Ryu *et al*., 1999). FAF1 is a Fas associating molecule known to enhance but unable to initiate apoptosis (Frohlich *et al*., 1998). The hFAF1 amino acid sequence showed 96% and 85% amino acid homologies with those of mFAF1 and qFAF1, respectively. Human FAF1 was expressed abundantly in testis, skeletal muscle, and heart as a 2.8 kb mRNA and was detected as a 74 kDa protein with a polyclonal antibody against hFAF1 (Ryu *et al*., 1999). A short form of FAF1 cDNA, with a 456 bp internal in-frame deletion corresponding to amino acids 189–340 of hFAF1, hFAF1(s), has been detected in HeLa and human liver cDNA libraries (Ryu *et al*., 1999). However such a shortened cDNA form has not been detected in other species. The deleted region includes a downstream ubiquitin conjugating enzyme-like domain (Ub domain) and a nuclear localization signal (NLS), which implies that intracellular localization of hFAF1(s) might be different from that of hFAF1. However, the functional characteristics of hFAF1(s) are hardly known.

Several principal protein domains involved in programmed cell death have been identified; DD (death domain), DED (death-effector domain), CARD (caspase activation and recruitment domain), TIR (Toll-interleukin-receptor domain), CART (cysteine-rich motif associated with RING and TRAF), MATH (meprin and the TRAF-homology domain) and BIR (baculovirus IAP repeat domain) (see Aravind *et al*., 1999 for a review). FAF1 does not contain such domains. Instead FAF1 contains two Ub domains (ubiquitin conjugating enzyme-like domains), a X domain (homologous with

* To whom correspondence should be addressed.
Tel: 82-42-520-5463; Fax: 82-42-520-5463
E-mail: ehkim@mail.paichai.ac.kr

Caenorhabditis elegans ORF C281.1), a UX domain (homologous with proteins involved in the ubiquitination pathway), a CAF (homologous with human chromatin assembly factor p150 subunit) and a novel NLS. The NLS of FAF1 has no homology with either the classical NLS motif or the characteristics of the M9 domain (Chu *et al*., 1995; Frohlich *et al*., 1998). However functional verification of the FAF1 domains needs further investigation.

In the present report, we mapped a hFAF1 gene by FISH and CASH, and analyzed the *hFAF1* gene by Southern blotting.

Materials and Methods

Radiation hybrid mapping Radiation hybrid mapping was carried out using a Genebridge 4 panel (Research Genetics, Huntsville, AL). This panel is composed of 93 hybrid cell lines that allow the whole genome to be mapped. Primer sequences were derived from a 3′ UTR of *hFAF1* and a 115 bp amplified product represents nt 1,942 to nt 2,056 of the cDNA (Ryu *et al*., 1999). The sequence of the sense primer was 5′-GCAAAAGAGTAAACACGGCCC-3′ and that of the antisense primer was 5′-TGAATTGAGTGACACAAACT-GT-3′. The PCR reactions were performed in a total volume of 20 μl containing 50 ng of template DNA, 1× *Taq* Buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM of each primer and 1 U of *Taq* polymerase (Perkin-Elmer, Foster City, CA). Initial denaturation was at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 3 min. The amplified products were separated on a 2% agarose gel and visualized by ethidium bromide staining.

Fluorescence *in situ* hybridization (FISH) FISH was performed according to a published protocol (Mathew *et al*., 1994). Briefly, normal metaphase chromosome spreads were hybridized with PAC DNAs labeled with biotin using a Nick translation Kit (Life Technology, USA). The probe was denatured and prehybridized with Cot-1 for 1 h and then allowed to hybridize to a denatured slide overnight. The slide was then washed in 50% formamide for 15 min at 37°C and then in 2× SSC for 8 min at 37°C. Hybridization signals were visualized by fluorescent-avidin antibody.

Southern blot analysis For Southern blot analysis, the digested DNAs with *Eco*RI, *Pst*I, or *Bgl*II were individually extracted using phenol/chloroform. Then, the solution was precipitated with 100% ethanol and washed with 70% ethanol. The pellet was resuspended in TE buffer [10 mM Tris.Cl (pH 7.4), 1 mM EDTA (pH 8.0)]. The genomic DNAs digested were separated on a 1% agarose gel and transferred onto a Hybond^{TM}-N membrane (Amersham, UK). The probes for hFAF1 were 1.9 kb (complete coding region). After hybridization, the filters were washed twice for 15 min each at room temperature in 0.24 M sodium phosphate buffer (pH 7.0) – 0.1% SDS and then twice for 30 min at 65°C in 0.4 M sodium phosphate buffer (pH 7.0) – 0.1% SDS. The filters were dried and autoradiographed at −70°C with X-ray film (Kodak, USA) and an intensifying screen.

Results and Discussion

For radiation hybrid-panel mapping, we used the Genebridge 4 Radiation Hybrid screening panel. The primer sequences were derived from the putative 3′-UT of *hFAF1*. Out of 93 clones, 9 gave a 115 bp PCR product specific to human *hFAF1*, 66 were negative and 18 inconclusive. The data vector was 000010000-2022000100220100202000000000100000201000000000-0000200001020201012000000000202000002 and the position of *hFAF1* was determined using a RH mapper (Whitehead Institute for computational and statistical analysis). Using RH mapper software, the position of *hFAF1* was determined to be 2.43 cR_{3000} proximal to D1S2134 (CHLC.GATA72H07) and 7.26 cR distal to D1S417 (lod 2.90), placing *hFAF1* at 166 cR_{3000} in the human chromosome 1 framework map. Thus the suggested order of this region is D1S443–D1S2134–*hFAF1*–D1S197–D1S417. The localization of *hFAF1* is consistent with its mapping result reported by others (Genemap 1999) because the interval between D1S2843 and D1S417 completely encompasses the region between D1S443 and D1S417.

D1S443 and D1S197 are genetically mapped at 75 cM and 78 cM of the Genethon genetic map, respectively. Since *hFAF1* is positioned between D1S443 and D1S197, *hFAF1* mapped to the region between 75 cM and 78 cM of the Genethon genetic map. Cytogenetically, *hFAF1* is localized at ch1p32 because both D1S2134 and D1S197 are also localized at ch1p32 (GDB map viewer 2). This result is consistent with the position of the mouse homolog of *hFAF1*, *mFAF1*, which is localized at ch4 band c6, the syntenic region to human chromosome 1p32. In addition to *hFAF1*, *POU3F1*, *COL9A2* and *HCRTR1* are also localized in the same region and to mouse counterparts on the syntenic region.

To confirm the cytogenetic location of *hFAF1*, FISH was performed using a genomic PAC clone containing *hFAF1*. We screened a 3× genome equivalent RPCI-1 PAC library by Southern blot hybridization with a 372 bp DNA fragment as a probe (data not shown). This probe represents 579 nt to 950 nt of hFAF1 cDNA (Ryu *et al*., 1999). Two genomic PAC clones, 282I3 and 412C9, were identified and used for FISH mapping. Metaphase chromosome spreads were hybridized with PAC DNAs labeled with biotin and all 20 metaphases examined yielded signals exclusively at 1p32 and no nonspecific signals were seen at other regions. Metaphases counterstained with DAPI showed hFAF1 hybridization signals. Arrows indicate the hybridization signals. Both PACs localized to 1p32 (Fig. 1).

Southern blot analysis was carried out with human genomic DNA. A full-length cDNA probe of *hFAF1* was labeled with [α-^{32}P] dATP. In Southern blot analysis of genomic DNAs digested with *Eco*RI, *Pst*I, or *Bgl*II

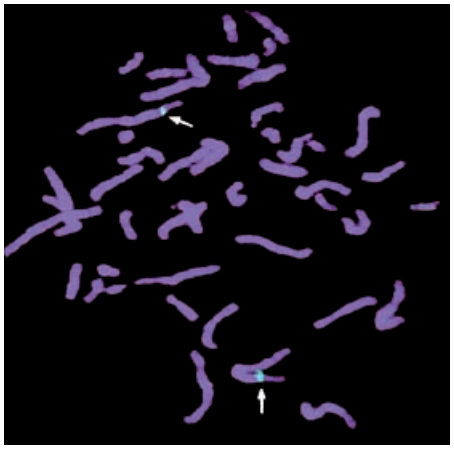

Fig. 1. The chromosomal localization of *hFAF1* on human chromosome 1p32. A biotinylated *hFAF1* PAC clone was used as a probe. Arrows indicate the FITC signals specific for *hFAF1* in the short arm of chromosome 1, namely 1p32.

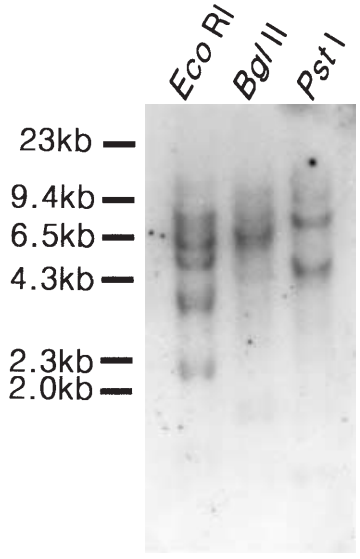

Fig. 2. Southern blot hybridization of *hFAF1* cDNA to genomic DNA isolated from human tissue. Numbers at the left indicate the DNA molecular size marker.

enzymes, several bands with different intensities appeared (Fig. 2). hFAF1 cDNA has no site for *Eco*RI, and only 1 and 2 sites for *Pst*I and *Bgl*II, respectively. The size summed up to >50 kb in the band pattern digested by each *Eco*RI, *Pst*I, *Bgl*II enzyme. This implies that the *hFAF1* gene might be discontinuous and its size would be rather large. Also another possibility is that functional and/or nonfunctional hFAF1 isotypes might exist in close vicinity considering that the single signal in the FISH analysis cannot be ruled out.

Acknowledgments This work was supported financially by the Korean Ministry of Science and Technology and by the Korea Science and Engineering Foundation through the Research Center for Biomedical Resources in PaiChai University, Korea.

References

Aravind, L., Dixit, V. M., and Koonin, E. V. (1999) The domains of death: evolution of the apoptosis machinery. *Trends Biochem. Sci.* **24**, 47–53.

Ashkenazi, A. and Dixit, V. M. (1998) Death receptors: signaling and modulation. *Science* **281**, 1305–1308.

Boldin, M. P., Varfolomeev, E. E., Pancer, Z., Mett, I. L., Camonis, J. H., and Wallach, D. (1995) A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J. Biol. Chem.* **270**, 7795–7798.

Boldin, M. P., Goncharov, T., Goltsev, Y. V., and Wallach, D. (1996) Involvement of MACH, a novel mort1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* **85**, 803–815.

Chang, H. Y., Nishitoh, H., Yang, X., Ichijo, H., and Baltimore, D. (1998) Activation of apoptosis signal regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* **281**, 1860–1863.

Chang, H. Y., Yang, X., and Baltimore, D. (1999) Dissecting Fas signaling with an altered-specificity death-domain mutant: requirement of FADD binding for apoptosis but not Jun N-terminal kinase activation. *Proc. Natl. Acad. Sci. USA* **96**, 1252–1256.

Chinnaiyan, A. M., O'Rourke, K., Tewari, M., and Dixit, V. (1995) FADD, a novel death domain-containing protein interacts with the death domain of Fas and initiates apoptosis. *Cell* **81**, 505–512.

Chu, K., Niu, X., and Williams, L. (1995) A fas-associated protein factor, FAF1, potentiates Fas-mediated apoptosis. *Proc. Natl. Acad. Sci. USA* **92**, 11894–11898.

Frohlich, T., Risau, W., and Flamme, I. (1998) Characterization of novel nuclear targeting and apoptosis-inducing domains in FAS associated factor 1. *J. Cell Sci.* **111**, 2353–2363.

Imai, Y., Kimura, T., Murakami, A., Yajima, N., Sakamaki, K., and Yonehara, B. (1999) The CED-4-homologous protein FLASH is involved in Fas-mediated activation of caspase-8 during apoptosis. *Nature* **398**, 777–785.

Jiang, Y., Woronicz, J. D., Liu, W., and Goeddel, D. V. (1999) Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* **283**, 543–546.

Mathew, S., Murty, V. V., Cheifetz, S., George, D., Massague, J., and Chaganti, R. S. (1994) Transforming growth factor receptor gene TGFBR2 maps to human chromosome band 3p22. *Genomics* **20**, 114–115.

Nagata, S. (1997) Apoptosis by death factor. *Cell* **88**, 355–365.

Porter, A. G. (1999) Protein translocation in apoptosis. *Trends Cell Biol.* **9**, 394–401.

Ryu, S. W., Chae, S. K., Lee, K. J., and Kim, E. (1999) Identification and characterization of human Fas associated factor 1, *hFAF1*. *Biochem. Biophys. Res. Commun.* **262**, 388–394.

Yang, X., Khosravi-Far, R., Chang, H. Y., and Baltimore, D. (1997) Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* **89**, 1067–1076.

Yanagisawa, J., Takahashi, M., Kanki, H., Yano-Yanagisawa, H., Tazunoki, T., Sawa, E., Nishitoba, T., Kamishohara, M., Kobayashi, E., Kataoka, S., and Sato, T. (1997) The molecular interaction of Fas and FAP-1. *J. Biol. Chem.* **272**, 8539–8545.