Biochemical and Biophysical Research Communications **266,** 81–87 (1999)
Article ID bbrc.1999.1733, available online at http://www.idealibrary.com on IDEAL®

A New Member of a Hepatoma-Derived Growth Factor Gene Family Can Translocate to the Nucleus

Kazuhiro Ikegame, Mitsunari Yamamoto, Yoshihiko Kishima, Hirayuki Enomoto, Kenya Yoshida, Masaki Suemura, Tadamitsu Kishimoto, and Hideji Nakamura[1]
Department of Molecular Medicine, Osaka University Medical School, Yamada-oka 2-2, Suita, Osaka 565-0871, Japan

Received October 7, 1999

Hepatoma-derived growth factor (HDGF) and HDGF-related proteins (HRP) belong to a gene family with a well-conserved amino acid sequence at the N-terminus (the *hath* region). A new member of the HDGF family in humans and mice was identified and cloned; we call it HRP-3. The deduced amino acid sequence from HRP-3 cDNA contained 203 amino acids without a signal peptide for secretion. HRP-3 has its 97-amino-acid sequence at the N-terminus, which is highly conserved with the *hath* region of the HDGF family proteins. It also has a putative bipartite nuclear localizing signal (NLS) sequence in a similar location in its self-specific region of HDGF and HRP-1. Northern blot analysis shows that HRP-3 is expressed predominantly in the testis and brain, to an intermediate extent in the heart, and to a slight extent in the ovaries, kidneys, spleen, and liver in humans. Transfection of green fluorescent protein (GFP)-tagged HRP-3 cDNA showed that HRP-3 translocated to the nucleus of 293 cells. GFP–HRP-3 transfectants significantly increased their DNA synthesis more than cells transfected with vector only. The HRP-3 gene was mapped to chromosome 15, region q25 by FISH analysis. These findings suggest that a new member of the HDGF gene family, HRP-3, may function mainly in the nucleus of the brain, testis, and heart, probably for cell proliferation. © 1999 Academic Press

Key Words: **HRP-3; nuclear translocation; HDGF family; *hath*.**

Hepatoma-derived growth factor (HDGF) was purified from the conditioned medium of human hepatoma-derived cell line, HuH-7, which proliferates autonomously in serum-free chemically defined medium, and its complementary DNA was cloned from the HuH-7 cell cDNA library (1). HDGF has growth stimulating activity for fibroblasts, hepatoma cells and endothelial cells (1–3). While screening the mouse testis cDNA library to identify a mouse homologue of human HDGF, we cloned not only a mouse homologue, but also two other genes encoding HDGF-related proteins (HRP-1 and HRP-2) (4). The messages of HDGF and HRP-2 were ubiquitously expressed in various normal tissues, and highly expressed in the testis, brain and skeletal muscle. On the other hand, HRP-1 shows testis-specific expression in mice by Northern blot analysis. Their deduced amino acid sequences revealed that HDGF forms a new gene family with a highly conserved 98-amino acid sequence at the N-terminus (we named it the *hath* region) (4). However, these three proteins have no homology in amino acid sequences of their self-specific regions other than the *hath* region. Each protein of these HDGF family proteins, has a putative nuclear localizing signal (NLS) in the self-specific region, other than the *hath* region.

Recently, by computer-aided screening of Expression sequence tag (EST) database, some genes which contain homologous regions of any gene family were searched out and identified (5). We tried to search few another gene containing a homologous sequence to the *hath* region of HDGF by searching the DNA database. By EST cDNA search for the homology to the *hath* region, we identified and cloned another new gene containing, not only the *hath* region in the N-terminus, but also the NLS sequence in its self-specific region other than the *hath* region, that we named the HRP-3 gene. HRP-3 has growth stimulating activity. In the present study, we report the character, chromosomal localization and putative function of a new member of the HDGF gene family.

MATERIALS AND METHODS

Isolation and cloning of human and mouse HDGF-related protein (HRP)-3 cDNA. We searched the EST database for sequences with similarities to a consensus amino acid sequence of the highly conserved HDGF N-terminal region (the *hath* region) using the tBLASTN algorithms (5). Then we obtained overlapping human

[1] To whom reprint requests should be addressed. Fax: 06-6879-3839. E-mail: nakamura@imed3.med.osaka-u.ac.jp.

0006-291X/99 $30.00

(accession number: AA001015, AA053893, AA400940, AA400963, AA455020 and W38901) and mouse (accession number: AA039116 and 061110) ESTs, designated as HRP-3, which had the *hath* region, but was quite different from HDGF, HRP-1 and HRP-2 in the C-terminal. The nucleotide sequence of human HRP-3 is longer than that of mouse HRP-3 obtained from ESTs. In order to recheck the nucleotide sequence, we designed specific primers and sequenced the PCR products from Jurkat cells, human peripheral blood lymphocytes (PBL) and the human testis cDNA library, and the mouse testis cDNA library. Then, we acquired an identical nucleotide sequence with only a two-base alteration. Primers derived from human HRP-3 were 5′ primer: 5′-ACCGCTCGTCCGCCGGCTTG-3′ and 3′ primer: 5′-GGTAGTTAGGTCCCTTCACTGGTTTT-CT-3′.

Northern blot analysis. The HRP-3 specific probe, which did not contain the encoding nucleotide of the highly conserved *hath* region, was generated by PCR using the following primers; 5′-GGCTAC-CAGGCAATTCAGCAACA-3′ and 5′-TGGTAGTTAGGTCCCTTCACT-3′. After gel purification by standard techniques, the HRP-3 specific probe was labeled with [α-^{32}P]dCTP using a Megaprime DNA labeling kit (Amersham Life Science, Tokyo, Japan) and used for Northern hybridization. Multiple tissue Northern (MTN) blots (Clontech., Palo Alto, CA) were hybridized with a radiolabeled HRP-3 specific probe in QuikHyb hybridization solution (Stratagene LaJolla, CA) according to the protocol of the manufacturer.

Fluorescence in situ hybridization (FISH) mapping. Lymphocytes isolated from human blood were cultured in α-minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and phytohemagglutinin (PHA) at 37°C for 68–72 hr. The lymphocyte cultures were treated with bromodeoxyuridine (0.18 mg/ml, Sigma) to synchronize the cell population. Cells were harvested and slides were made by using standard procedures including hypotonic treatment, fix and air-dry. DNA probe (1.2 kb long) was biotinylated with dATP using Gibco BRL BioNick labeling kit at 15°C for 1 hr (6). The procedure for FISH detection was performed according to Heng *et al.* (6, 7). Briefly, slides were baked at 55°C for 1 hr. After RNase treatment, the slides were denatured in 70% formamide in 2× SSC, followed by dehydration with ethanol. The probes were denatured at 75°C for 5 min in a hybridization solution consisting of 50% formamide and 10% dextran sulfate. The probes were loaded on the denatured chromosomal slides. After overnight hybridization, the slides were washed and detected as well as ampilfied. FISH signals and the DAPI banding pattern were recorded separately by taking photographs, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI banded chromosomes (7).

GFP plasmid construction, gene transfection, and visualization. The expression vector for the chimera protein of human HRP-3 and green fluorescein protein (GFP) was constructed using pQBI-25 (Takara Co., Kyoto). pQBI-25 was digested with *Nhe*I, and ligated with cloned cDNA fragments of human HRP-3. DNA was transfected using the calcium phosphate co-precipitation method. Briefly, human 293 cells maintained in Dulbecco's modified Eagle's medium with 10% FCS and antibiotics were transiently transfected with a mixture of 6 μg of pQBI-25-HRP3 construct per 135 μl of double distilled water (DDW), 15 μl of 25 mM $CaCl_2$, and 150 μl of 2× HBS (280 mM NaCl, 50 mM HEPES and 1.48 mM Na_2HPO_4 adjusted to pH 7.05). The medium was changed 18 hr post-transfection. Transfected cells were evaluated at day 2 or day 3 using a microscope equipped for GFP visualization (488 nm excitation and FITC filter set).

Cell growth assay. GFP-tagged HRP-3-overexpressing 293 cells, cells transfected with vector pQBI only and parent 293 cells were seeded at 10^4 cells/well after geneticin selection for one week in DME supplemented with 10% FCS. After 24 hr-culture, the culture medium was changed to DME supplemented with 0%, 1% or 5% FCS. 88 hr later, 0.5 μCi of [H^3]thymidine (Thd) was added; the cells were then harvested.

Nucleotide accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession numbers AB029156 (human HRP-3) and AB029493 (mouse HRP-3).

RESULTS

Nucleotide and Translated Amino Acid Sequence Analysis

Human HRP-3 nucleotide sequence and translated amino acid sequence are shown in Fig. 1A. The sequence around a putative translational initiation site was compatible with Kozak's consensus sequence, and the stop codon resides at the 101 base pair upstream to this putaitve initiation site (8, 9). Thus, we designated this +1ATG as a translational initiation site. The stop codon was shown at the 609 base pairs downstream from this initiation site, and the cDNA of HRP-3 was sequenced and found to contain the 609 bp open reading frame (ORF) encoding a protein with a calculated M.W. of 22.6 kDa and a pI of pH 8.39. The nucleotide sequences we acquired from the PCR products from the Jurkat cell, human perypheral blood lymphocytes (PBL) and testis cDNA were identical to the sequence from the EST database, with only a two-base alteration (G103 and C116) resulting in a two-amino acid change (G35 and P39, respectively). HRP-3 lacks a signal peptide sequence, as do other HDGF family protein and consists of 203 amino acids; it is the shortest protein among the HDGF family proteins previously reported (4). The nucleotide sequence of mouse cDNA in the coding region and translated amino acid sequences were highly conserved to 94.7% and 98.0% of the human homologue, respectively. Two changed bases in human HRP-3 from the EST database were conserved in the mouse homologue, too. In the deduced amino acid sequence, mouse HRP-3 lacks only one amino acid and three amino acids are different from human homologue (Fig. 1B). The N-terminal amino acid sequence of HRP-3 was highly conserved to the *hath* region of HDGF (Fig. 2). The N-terminal 97 amino acid sequence of HRP-3 was 81.4% identical to the *hath* region of HDGF. However, the amino acid sequence in the self-specific region, other than the *hath* region, in the C-terminal region from G98 in HRP-3 was quite different from three other family proteins, including HDGF, except for the conservation of the consensus sequence for NLSs and the putative N-glycosylation site. HRP-3 has a basic motif, KRKNEKAGSKRKK (residues 136–148, basic residues underlined), homologous to the reported consensus sequences for bipartite NLSs in the self-specific region other than the *hath* region (10, 11). The location of putative NLS in the HRP-3 molecule was almost similar to the position of the NLSs in HDGF and HRP-1, and this putative NLS in HRP-3

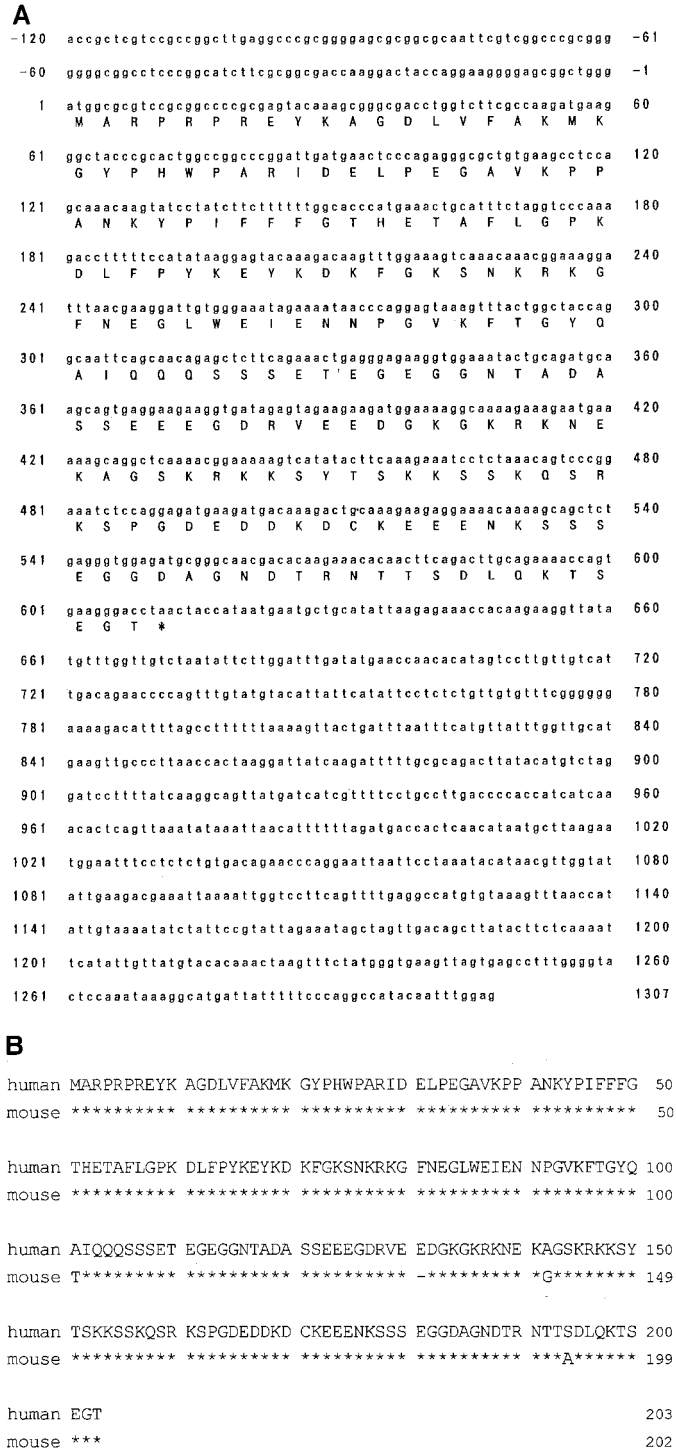

FIG. 1. Nucleotide and translated amino acid sequences of human HRP-3 cDNA. (A) The nucleotide sequence of HRP-3 cDNA is shown along with the amino acid sequence of human HRP-3 beginning with the first ATG codon. The amino acid sequence is shown in single-letter codes. (B) The homology of the translated amino acid sequences of HRP-3 in human and mouse genes. Asterisks denote identical amino acid residues. Deletions are indicated by dashes.

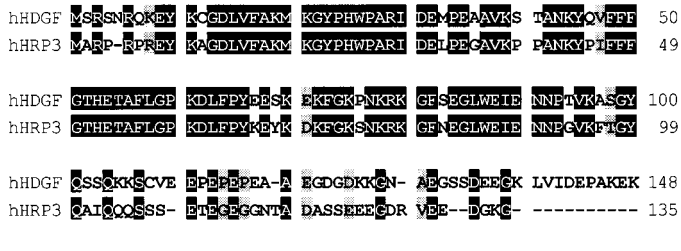

FIG. 2. The homology of the translated amino acid sequences between human HRP-3 and HDGF. Human HRP-3 and HDGF show significant homology in the *hath* region. Amino acid residues that are identical in HRP-3 and HDGF are highlighted by the black background, and conserved amino acid residues are indicated by the shaded background. Gaps introduced to generate this alignment are represented by dashes. Sequences are shown in single-letter code. Amino acid residues for each protein are numbered from the initiation methionine. Conserved amino acids are grouped as follows: (P, G), (S, T), (Q, N), (E, D), (K, R), (M, C), (V, L, I, A), and (F, Y, W, H).

should function as a potential nuclear translocation signal (1, 4). Thus, HRP-3 is a new member of the HDGF gene family.

HRP-3 Can Translocate to the Nucleus

In order to clarify the intracellular localization of HRP-3, Human embryonic kidney cell line, 293 cells were transfected of an expression plasmid encoding a chimera protein of human HRP-3 and GFP (pQBI-HRP3). As shown in Fig. 3, the green fluorescence images were detected in the nucleus, not in the cytoplasm nor on the membrane in the 293 cells that were transfected. Thus, HRP-3 can translocate into the nucleus and may function in the nucleus.

HRP-3 Gene Expression in Human Tissues

We examined the tissue distribution of the HRP-3 gene in humans. As shown in Fig. 4A, the messages of HRP-3 show one band about 2.5 kb in humans. HRP-3 is predominantly expressed in the brain and testis, to an intermediate extent in the heart, and to a minimal extent in the ovary, kidney, spleen and liver in humans. HRP-3 was ubiquitously expressed in the central nervous system, and predominantly expressed in the cerebral cortex and occipital, frontal and temporal lobes, and to a slight extent in the thalamus (Fig. 4B). However, which types of cells among the central nervous system predominantly express HRP-3 remains to

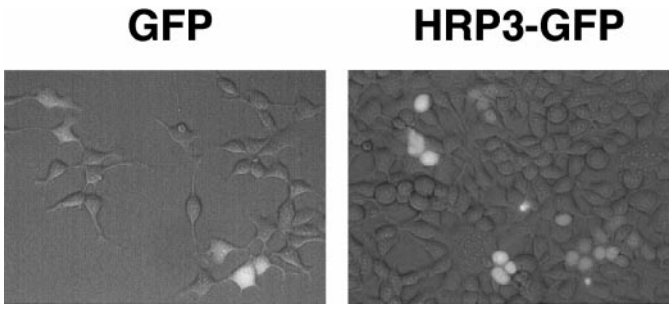

FIG. 3. Nuclear localization of HRP-3. Human 293 cells were transiently transfected with the expression vector encoding GFP-tagged HRP-3 cDNA. In the condition excited by 488-nm waves, 293 cells were imaged microscopically. Green fluorescence was determined in the nucleus.

be investigated using in situ hybridization methods or immunohistocytochemistry. And also in mice, HRP-3 is predominantly expressed in the brain and testis, and moderately in the heart and kidney (data not shown). These results show that HRP-3 is highly expressed in the brain and heart as well as in the testis as compared to HDGF, HRP-1 and HRP-2. It may play an important role, mainly in these organs, as compared to other family proteins.

HRP-3 Gene Mapped to Human Chromosome 15 by FISH Analysis

Next we investigated the chromosomal localization of HRP-3 in human by FISH analysis. Under the conditions used, FISH detection efficiency was approximately 71% for the HRP-3 cDNA probe (1.2 kb long); among 100 mitotic figures that were checked, 71 of them showed signals on one pair of chromosomes. Since the DAPI banding was used to identify the specific chromosome, the assignment between signals from the probe and the long arm chromosome 15 was obtained. Figure 5 shows the FISH signals on chromosome 15. The detailed position was further determined in the diagram based on the summary of 10 photos (Fig. 5B). Thus, HRP-3 is mapped to human chromosome 15, region q25.

Growth Stimulating Activity

Human embryonic kidney cell line 293 cells were transfected of an expression vector containing GFP-tagged human HRP-3 cDNA (pQBI-HRP3). After about 1-week of geneticin selection, GFP-HRP-3 transfected cells were obtained. After confirming their green fluorescence in the nucleus by microscopy, their thymidine uptake was measured. As shown in Fig. 6, the DNA synthesis of the 293 cells overexpressing GFP-HRP-3 significantly increased compared to that of the 293 cells transfected of the pQBI vector only or parent cells. These findings suggested that HRP-3 may function as an intracrine factor in the nucleus for cell proliferation. Whether HRP-3 acts on the cells outside and stimulates the DNA synthesis of the 293 cells has not yet been investigated. The mechanism of HRP-3 stimulating cell growth in the nucleus still remains to be clarified in the future.

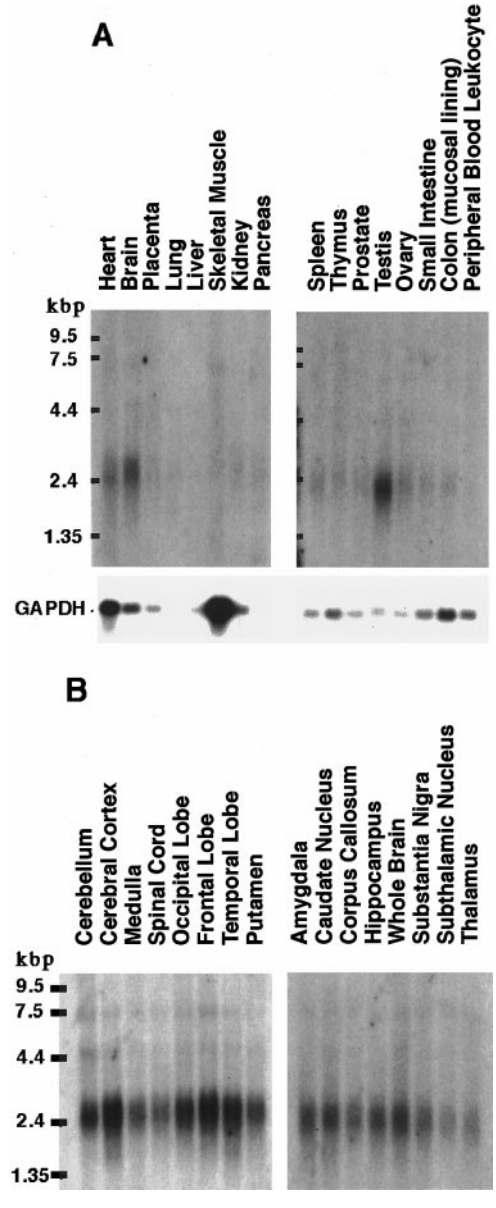

FIG. 4. The tissue distribution of HRP-3 mRNA expression in humans. (A) Northern blot analysis of HRP-3 mRNA in various human organs. Human multiple tissue Northern blots were used. Each lane contains 2 μg of highly pure poly(A)$^+$ RNA from various human tissues. Numbers on the left indicate RNA size markers (in kilobases). (B) Northern blot analysis in human brain.

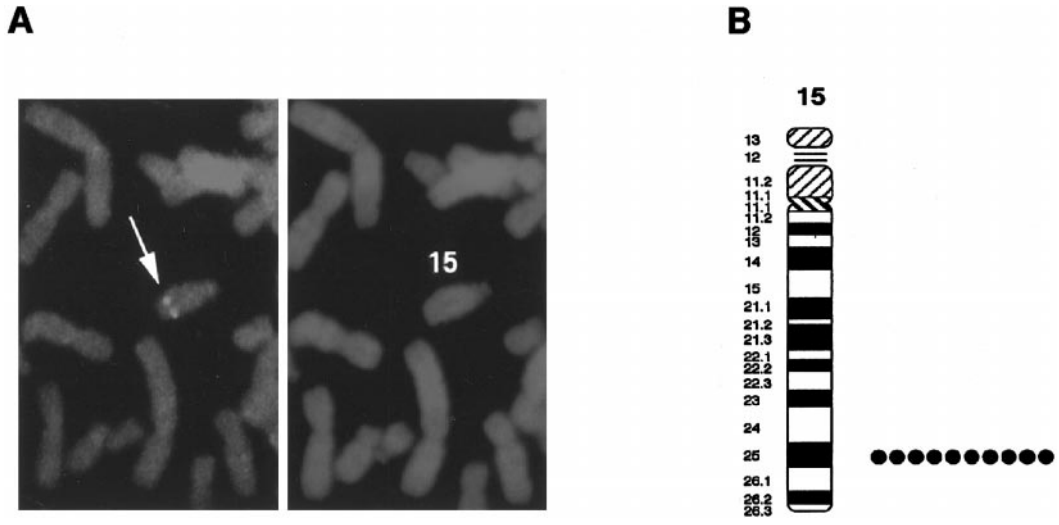

FIG. 5. Chromosomal localization by FISH analysis. (A) Photography of FISH mapping of probe HRP-3. The left panel shows the FISH signals on a chromosome, and the right panel shows the same mitotic figure stained with DAPI to identify chromosome 15. (B) Diagram of FISH mapping results for probe HRP-3. Each dot represents the double FISH signals detected on chromosome 15.

DISCUSSION

HRP-3 is the third member of HDGF-related proteins and its nucleotide and amino acid sequences are highly conserved in humans and mice. Although human homologue of other HDGF family protein (HRP-1 and HRP-2) have not yet been cloned, the nucleotide and amino acid sequences of HDGF are highly conserved between humans and mice, too. Thus, we consider that the HDGF family proteins may be highly conserved in mammalian species. HRP-3 also has an N-terminal amino acid sequence consisting of 97 amino acids highly homologous to the *hath* region conserved in the HDGF family proteins. Among the four members of the HDGF family proteins, the homology of the *hath* region is 80~92% in the amino acid sequences. We estimate that the *hath* region of these proteins might function as the critical region and a domain for protein-protein interactions or protein-DNA interactions in the nucleus. However, the mechanism of how the highly conserved region, which consists of about 100 amino acids in the N-terminus of the HDGF family proteins, should function for the activities of these proteins has not yet been made clear. The function of the *hath* region will be clarified in the future.

It is interesting that HRP-3 has a bipartite NLS in a self-specific region other than the *hath* region, and can translocate into the nucleus. HDGF, HRP-1 or HRP-3 has a putative NLS in a similar position, about 60–70 amino acids upstream from the C-terminus of each molecule. HRP-2 has several charge clusters in its self-specific amino acid sequence other than the *hath* region, which are characteristics of eukaryotic regulatory proteins, including transcription and replication factors functioning in the nucleus (4). Immunohistochemical studies showed that HRP-1 was present in the nucleus of germ cells in mouse testis (12). Thus, the

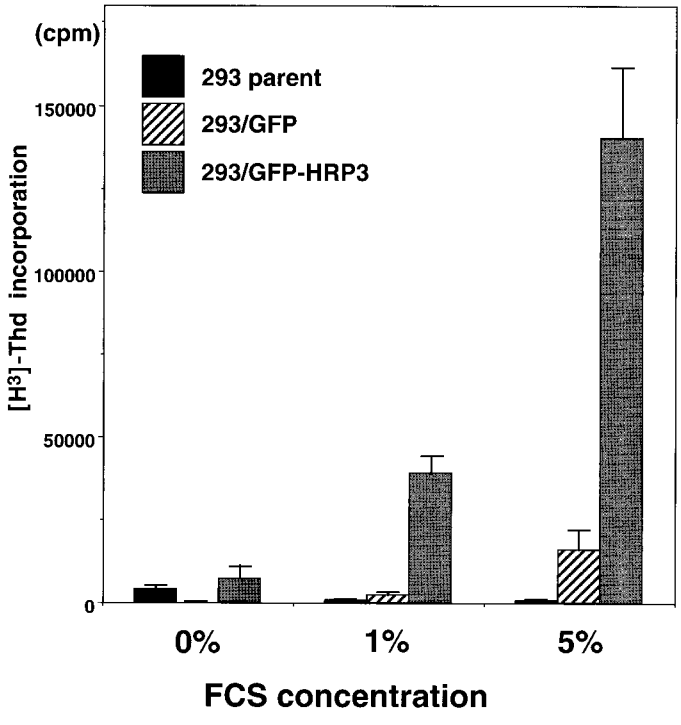

FIG. 6. Growth of HRP-3-overexpressing cells. The DNA synthesis of HRP-3-overexpressing 293 cells was significantly elevated. Plasmid pQBI containing GFP-tagged HRP-3 cDNA (pQBI-HRP3) was transfected into 293 cells. After 1-week selection with geneticin, viable cells were seeded and [H^3]Thd incorporation into DNA was measured as described under Materials and Methods. Error bars correspond to SEM using quadricate cultures.

HDGF family proteins, including HRP-3, may function mainly in the nucleus.

Among the HDGF family proteins, HDGF and HRP-2 are ubiquitously expressed, but HRP-1 is specifically expressed in the testis by Northern blot analysis in mice suggesting that HRP-1 may play an important role in meiosis in mouse spermatogenesis (1, 4, 10, 12). In humans, HDGF is highly expressed in the testis and skeletal muscle, moderately in the liver and kidney, and mildly in the heart, brain and lung (1). In comparison to HDGF, HRP-3 is predominantly expressed in the testis, brain and heart, however, it is expressed very little in other human organs. The expression of HRP-3 in muscle was very weak. The tissue distribution of HRP-3 is quite different from that of HDGF in humans except for high expression in the testis. These results suggest that in humans, HRP-3 may play an important role in cell proliferation or differentiation in the brain and heart than HDGF. HDGF has been reported to be expressed in mesenchymal cells (3). What kinds of cell types predominantly express HRP-3 remains to be investigated in the brain and heart.

HDGF stimulates the cell proliferation of fibroblasts, endothelial cells and hepatoma cells. HRP-3-overexpressing cells also show significant stimulation of their DNA synthesis. Several growth factors, such as FGF-1,2,9 and Schwanomma-derived growth factor, were reported to stimulate the DNA synthesis after nuclear translocation. Although it is not clear how HRP-3 works for the cellular growth, the nuclear translocation ability suggests that HRP-3 mainly functions in the nucleus, possibly in an intracrine and/or autocrine fashion. On the other hand, it was recently reported that p52/p75 containing the *hath* region, was a transcriptional factor or a transcriptional cofactor, which mediated functional interactions between upstream sequence-specific activators and the general transcription apparatus (13). HRP-3 might function as a transcriptional factor or a transcriptional cofactor for the cellular growth. Further studies must be done to make clear of the functional mechanism of HRP-3 in the nucleus.

The HRP-3 gene was mapped to chromosome 15, region q25 in humans. The human HDGF gene was reported to be mapped to the X chromosome (14). Therefore, HRP-3 is quite different from HDGF in humans, although the chromosomal localization of other HRP genes, HRP-1 and HRP-2, have not been demonstrated yet. The genes that have been reported to be mapped to human chromosome 15, q25 are the human Bloom's syndrome protein (BLM) gene, human aggrecan gene, human DNA-PKcs interacting protein (KIP) gene, the tyrosine kinase receptor (TRK) C, human homologue to the apoptosis associated-murine mammary protein (MFG-E8) and c-fes oncogenes (15–20). On the other hand, a gene for osteosclerosis was reported to be localized in the area of chromosome15, q25 by linkage analysis (21). Craniosynostosis was associated with partial duplication of chromosome15, q25-qter (22). By comparative genomic hybridization of adenocarcinomas developed from gastric mucosa and hepatocellular carcinomas, recurrent high level amplification or recurrent loses could be determined in this region of chromosome15, q25 (23, 24). Thus, HRP-3 might be related to these diseases or carcinogenesis in stomach and liver.

In summary, we cloned and characterized a new member of HDGF family, HRP-3. We have demonstrated that HRP-3 translocates to the nucleus and induces cellular growth. Further investigations including the targetted disruption or transgenic models will reveal the precise mechanism and function of the HDGF family proteins.

ACKNOWLEDGMENT

We thank Dr. Hiroyasu Yamamoto (Department of Molecular Medicine, Osaka University Medical School) for technical support and for providing vector plasmids.

REFERENCES

1. Nakamura, H., Izumoto, Y., Kambe, H., Kuroda, T., Mori, T., Kawamura, K., Yamamoto, H., and Kishimoto, T. (1994) *J. Biol. Chem.* **269,** 25143–25149.
2. Nakamura, H., Kambe, H., Egawa, T., Kimura, Y., Ito, H., Hayashi, E., Yamamoto, H., Sato, J., and Kishimoto, S. (1989) *Clin. Chim. Acta* **183,** 273–284.
3. Oliver, J. A., and Al-Awqati, Q. (1998) *J. Clin. Invest.* **102,** 1208–1219.
4. Izumoto, Y., Kuroda, T., Harada, H., Kishimoto, T., and Nakamura, H. (1997) *Biochem. Biophys. Res. Commun.* **238,** 26–32.
5. Pearson, W. R., and Lipman, D. J. (1998) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
6. Heng, H. H. Q., Squire, J., and Tsui, L-C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9509–9513.
7. Heng, H. H. Q., and Tsui, L-C. (1993) *Chromosoma* **102,** 325–332.
8. Kozak, M. (1984) *Nucleic Acids Res.* **12,** 857–872.
9. Kozak, M. (1987) *Nucleic Acids Res.* **15,** 8125–8148.
10. Dingwall, C., and Laskey, R. A. (1991) *Trends Biochem. Sci.* **16,** 478–481.
11. Robbins, J., Dilworth, S. M., Laskey, R. A., and Digwall, C. (1991) *Cell* **64,** 615–623.
12. Kuroda, T., Tanaka, H., Nakamura, H., Nishimune, Y., and Kishimoto, T. (1999) *Biochem. Biophys. Res. Commun.* **262,** 433–437.
13. Ge, H., Si, Y., and Roeder, R. G. (1998) *EMBO J.* **17,** 6723–6729.
14. Wanschura, S., Schoenmakers, E. F., Huysmans, C., Bartnitzke, S., Van de Ven, W. J., and Bullerdiek, J. (1996) *Genomics* **32,** 298–300.
15. Woodage, T., Prasad, M., Dixon, J. W., Selby, R. E., Romain, D. R., Columbano-Green, L. M., Graham, D., Rogan, P. K., Seip, J. R., Smith, A., and Trent, R. J. (1994) *Am. J. Hum. Genet.* **55,** 74–80.

16. Just, W., Klett, C., Vetter, U., and Vogel, W. (1993) *Hum. Genet.* **92,** 516–518.
17. Seki, N., Hayashi, A., Abe, M., Araki, R., Fujimori, A., Fukumura, R., Hattori, A., Kozuma, S., Ohhira, M., Hori, T., and Saito, T. (1998) *J. Hum. Genet.* **43,** 275–277.
18. Valent, A., Danglot, G., and Bernheim, A. (1997) *Eur. J. Hum. Genet.* **5,** 102–104.
19. Collins, C., Nehlin, J. O., Stubbs, J. D., Kowbel, D., Kuo, W. L., and Parry, G. (1997) *Genomics* **39,** 117–118.
20. Harper, M. E., Franchini, G., Love, J., Simon, M. I., Gallo, R. C., and Wong-Staal, F. (1983) *Nature* **304,** 169–171.
21. Tomek, M. S., Brown, M. R., Mani, S. R., Ramesh, A., Srisailapathy, C. R., Coucke, P., Zbar, R. I., Bell, A. M., McGuirt, W. T., Fukushima, K., Willems, P. J., Van Camp, G., and Smith, R. J. (1998) *Hum. Mol. Genet.* **7,** 285–290.
22. Van Allen, M. I., Siegel-Bartelt, J., Feigenbaum, A., and Teshima, I. E. (1992) *Am. J. Med. Genet.* **43,** 688–692.
23. Van Dekken, H., Geelen, E., Dinjens, W. N., Wijnhoven, B. P., Tilanus, H. W., Tanke, H. J., and Rosenberg, C. (1999) *Cancer Res.* **59,** 748–752.
24. Zimonjic, D. B., Keck, C. L., Thorgeirsson, S. S., and Popescu, N. C. (1999) *Hepatology* **29,** 1208–1214.