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Primary research on MGSAD023, a new homologue of eIF4G

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Abstract

MGSAD023 (Mouse Gene Similar to AD023; Genbank accession AK150749) and its human compartment (AD023; Genbank accession AF225422) are new two genes with unknown functions. They were derived from high-throughput cDNA sequence analysis of the mouse genome project and Human Genome Project (HGP), respectively. MGSAD023 shows 88.6% identity to AD023 (NCBI Blast), and we decided to use MGSAD023 as a temple to explore the functions of these two compartment genes. With the NCBI Conserved Domain Database (CDD) and the gap program in GCG sequence analysis software, we found that the amino acids of MGSAD023 showed 58.3% similarity and 41.2% identity to eIF4G and also showed a good match to the middle domain of eIF4G.

We determined the transcription pattern of MGSAD023. RT-PCR results showed that MGSAD023 was expressed in all of the tissues checked, including cerebellum, cerebra, liver, kidney, tongue, testis, heart and spleen, without any significant differences. The results of *In Situ Hybridization* (ISH) showed that there was a very strong signal of mRNA of MGSAD023 on the seminiferous tubular basement membrane, where spermatogonia A and B are located. *In silico* exploration revealed homologous genes in various species, including chimpanzee, rat, mice, dog, zebra fish, fruit fly, xenopus, and cattle. These results hint that MGSAD023 is a conserved gene. It may act as a translation factor during cellular mitosis, especially in spermatogonia.

Key words: AD023; eIF4G; IRES.

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Introduction

eIF4G plays a pivotal role during the initiation of protein synthesis. Human eIF4G (accession No. AF104913)[1] may be divided into three distinct functional domains. The N-terminal one-third is from amino acids (aa) 1 to 634 and contains the binding site of eIF4E, a 25-kDa cap-binding phosphoprotein[2),3)]. The middle third, from 635 to 1039 aa, contains binding sites for eIF4A, a 46-kDa bi-directional ATP-dependent helicase[4)], and eIF3, a multi-subunit initiation factor (eIF3) as well as an RNA binding site[5),6)]. The C-terminal third, from 1040 to 1560 aa, contains a second eIF4A binding site and a Mnk1 binding site[7),8)]. Not only does the middle domain of eIF4G have an amino acid sequence that is highly homologous to all isoforms of eIF4G proteins, it is also highly conserved in human, yeast, mouse and plant eIF4Gs[9)]. This domain is essential for translational activity, such as to catalyze the cap-dependent and cap-independent binding of ribosome to RNA[6)].

The human gene AD023 and its mouse compartment MGSAD023 (Mouse Gene Similar to AD023, accession No. AK150749) are new homologues of eIF4G. They were originally identified by high-throughput cDNA sequence analysis of the human and mouse genome projects by Celera. MGSAD023 is located on mouse chromosome 11E2. Its complete cDNA has a full length of 1323 nt and gives a 222aa protein. MGSAD023 contains a middle domain of Eukaryotic translation Initial Factor 4 Gamma (MIF4G), as does its human counterpart. Amino acids of MGSAD023 show 58.3% similarity and 41.2% identity to eIF4G according the gap program of the GCG sequence analysis software package, version 10.3, gap creation penalty, 3.00; extension penalty, 1.0). Based on the presence of homologous genes in various species, including chimpanzee, rat, mice, dog, zebra fish, fruit fly, xenopus, frog and cattle, MSAD023 might be considered as a conserved gene.

Some proteins are homologous to eIF4G, such as NAT1/DAP5/P97, DUG, PAIP, etc. Most of them have been explored intensively and are related to protein synthesis. MGSAD023 is a new homologue of eIF4G, but its cellular function is totally unknown. Information on the temporal and spatial expression pattern of a gene often suggests its function under physiological and pathological conditions. To elucidate the cellular function of MGSAD023, we studied the cellular localization and expression level of MGSAD023 in various mouse tissues by in situ hybridization (ISH) and RT-PCR.

1. Materials and methods

1.1 Animals and tissue preparation

Eight-week-old male ICR mice were decapitated under diethylether anesthesia. The fresh brains, hearts, testis, livers, kidneys, tongues and spleens were quickly removed and immediately frozen on powdered dry ice. A set of these organs was then stored at –80° C until RNA extraction. Another set of organs was used for ISH. Frontal sections (20 mm thick) were cut in a cryostat, thaw-mounted onto gelatin-coated slides, and stored at –80°C until ISH.

1.2 Oligonucleotide probes

To improve the specificity of ISH, we designed one sense probe and two antisense probes. The sense probe for MGSAD023 was complementary to bases 42-81 of MGSAD023 full-length mRNA, (–CGCGGGGTGC GACCTGGCAA GACCAACAAC TTCACGCAAA –), antisense 1 probe was complementary to bases 945-995 (–GCCAGGAGAC AAGTCCAGAT TGAGGGAAGG GAAGACGGCA GAACCACAGCA–), and antisense 2 probe was complementary to bases 1015-1066 (–CTGTCAGGCC CAGGAGGAAG GTGGTGGGAA GATAACAGGA CTTGAGGGGA GA–). A BLAST search in Genbank revealed no significant homology with these probes.

Primers for RT-PCR were selected by using the program Genetyx.

F1 TTCAGCAAGGAAGCAGGAC (from 340~358), R1 TGCTGCTGGAGATGATTGAG (from 792~770).

1.3 RT-PCR

Total RNA was extracted (with Trizol; Bioprobe Systems, Montreuil Sous Bois, France) from various ICR mouse tissues according to a one-step procedure. One-step reverse transcription (RT)-PCR was performed as a single-tube reaction using primers specific for MGSAD023 cDNA in the coding region. RT-PCR of 1 mg extracted RNA (from various ICR mouse tissues) was performed in a 50 ml reaction system (BD TITANIUM™ One-Step RT-PCR Kit) in a 200 ml thin-walled Hot Start Tube containing 5ml 10×One-Step Buffer, 1ml 50× dNTP Mix, 0.5 ml Recombinant RNase Inhibitor (40 units/ml), 25 ml Thermostabilizing Reagent, 10 ml GC-Melt, 1 ml Oligo (dT) Primer, 1ml 50× RT-TITANIUM Taq enzyme mix, 2 ml PCR primer mix (25 mM), and 1 mg total RNA, made up to 50 ml with DEPC DDW. All reactions were performed in an ASTEC PC808 PCR System with the following program: 1 cycle of 60 min at 50°C, followed by 5 min at 94°C and 32 two-step cycles of 94°C for 30 s and 58°C for 30 s, followed by 1 min at 68°C. ß-actin amplification was performed to minimize differences in DNA and RNA yield among samples according to the procedure for the BD TITANIUM™ One-Step RT-PCR Kit.

1.4 Sequence analysis of amplified products

Nucleotide sequences of RT-PCR products were determined by double-strand DNA cycle sequencing using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA) according to the manufacturer's instructions. RT-PCR products were inserted into T-vector after electrophoretic analysis. Electrophoresis and an analysis of DNA sequence reactions were then performed with an automatic DNA sequencer (Model 310 A, Applied Biosystems, Roissy Charles de Gaulle, France). The sequencing primers were T7 and Sp6 matched to T-vector. Before they were inserted into T-Vector, the PCR products were purified using a Qiaquick gel extraction kit (QIAGEN, Germany). Both strands of the DNA fragments were sequenced. The sequence data were then analyzed automatically using the Usedit, Sequence Navigator (Applied Biosystems) and Mac DNAsis (V3.5, Hitachi Software) programs.

1.5 In situ hybridization

After being warmed to room temperature,

slide-mounted sections were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 15 min (all steps were performed at room temperature unless otherwise indicated), rinsed three times (5 min each) in 4 ×SSC (pH 7.0) (4×SSC contained 0.15 M sodium chloride and 0.015 M sodium citrate), and dehydrated through a graded ethanol series (70–100%). Sections were then defatted with chloroform for 5 min, and immersed in 100% ethanol (twice for 5 min each time) before being subjected to hybridization. Hybridization was performed by incubating the sections with a buffer [4xSSC, 50% deionized formamide, 0.12 M phosphate buffer (pH 7.2), Denhardt's solution, 2.5% tRNA, 10% dextran sulfate] containing $^{[35\ S]}$dATP (1000–1500 Ci/ mmol)- labeled probes (1.0×10^7 cpm/ ml, 0.2 ml/ slides) for overnight at 41. After hybridization, the sections were rinsed in 1×SSC (pH 7.2) for 10 min at 55°C, and then rinsed three times in 1×SSC at 55°C for 20 min each time. The sections were then dehydrated through a graded ethanol series (70–100%). After film exposure for 3 days at room temperature, the sections were coated with Kodak NBT-2 emulsion diluted 1:1 with water. Sections were then exposed at 4°C for 2 weeks in a tightly sealed dark box. After being developed in D-19 developer (Kodak), fixed with photographic fixer, and washed with tap water, the sections were counterstained with thionin solution to allow morphological identification. In situ hybridization sections were analyzed on a Leica photomicroscope equipped with bright field and dark field, and computer images were obtained with an Olympus OLY-200 digital camera (Olympus, Melville, NY).

2. Results and Discussion

2. 1 Results of RT-PCR

We first investigated if MGSAD023 was expressed in various mouse tissues by RT-PCR. A clear band of about 400bp was detected in all of the tissues examined, including cerebellum, cerebra, liver, kidney, tongue, testis, heart and spleen. The signal of the band for the testis seemed to be the strongest. To further confirm the band containing the sequence expected, we purified the bands for the testis and heart from gel, inserted them into T-vector and then sequenced them in an ABI310A

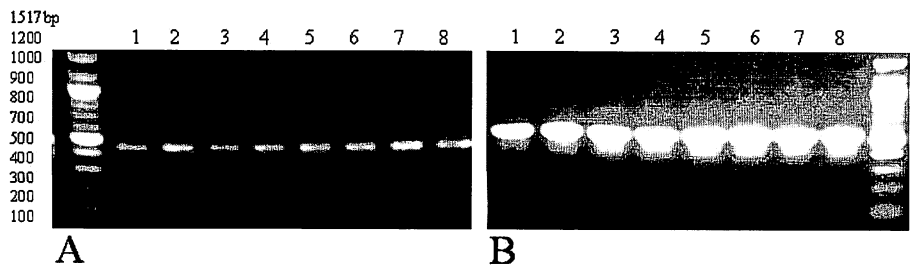

Fig. 1. Detection of MGSAD023 expression in various mouse tissues. Panel A: standard RT-PCR amplification of MGSAD023 performed as described in the Materials and Methods: line 1-heart, 2- testis, 3-cerebellum, 4-cerebra, 5-liver, 6-kidney, 7-tongue and 8-spleen. Panel B: ß-actin amplification was performed to minimize differences in DNA and RNA yield among samples. A representative experiment is shown.

sequence machine. The sequence results matched the fragments expected.

2.2 Results of ISH

We used one sense probe and two antisense probes to examine the expression pattern of MGSAD023 in various tissues of mouse, including cerebra, cerebellum, liver, kidney, tongue and testis. Signals were homogeneous in the cerebellum, cerebrum, liver, kidney, heart and tongue. In testis, the signal of MGSAD023 mRNA was mainly located on the sub-peripheral zone, which corresponded to the location of differentiating spermatogonium. The absence of signal in sections hybridized with the sense probe confirmed the specificity of the signal (Fig. 2 A-D).

2.3 Discussion

Various proteins show homology to eIF4G, such as NAT1/DAP5/P97, DUG, PAIP etc[10), 11), 12)]. All of them can bind eIF4A *in vitro* or *in vivo* while NAT1/DAP5/P97 can bind both eIF4A and eIF3. NAT1/DAP5/P97 also suppresses cap-dependent and cap-independent translation while the other two proteins do not enhance cap-independent translation. Another study based on translation assays in a cell-free system suggested that NAT1/DAP5/P97 could act as a positive mediator of cap-independent translation, at least through its own IRES[13)]. Sivan et al. further indicated

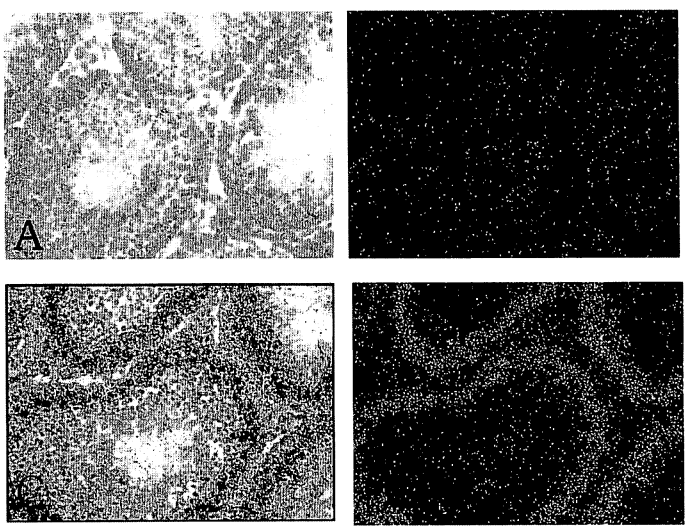

Fig.2. In situ hybridization for MGSAD023 in 8-week-old mouse testis. Slide-mounted sections (20 μm) were hybridized with ^{35}S-labeled oligoprobe of MGSAD023 as described in the Materials and Methods. A) Light field micrograph of a cross-section hybridized with sense MGSAD023. B) Dark field micrograph of a cross-section hybridized with sense MGSAD023. C) Light field micrograph of a cross-section hybridized with antisense MGSAD023. D) Dark field micrograph of a cross-section hybridized with antisense MGSAD023. The original magnification was 100X.

that caspase-cleaved NAT1/DAP5/P97 supported the translation of c-Myc, XIAP, Apaf-1 and NAT1/DAP5/P97 itself by an IRES mechanism under apoptotic conditions[14]. These data show that almost all homologues of eIF4G are involved in the process of protein translation.

Since it shows 58.3 % similarity and 41.2 % identity to the middle domain of eIF4G (according to the gap program of the GCG sequence analysis software package, version 10.3, gap creation penalty, 3.00; extension penalty, 1.0), MGSAD023 might bind to eIF4A and eIF3. Since the binding sites of eIF4A and eIF3 in the eIF4G middle domain could not mapped to a defined stretch of amino acids, we can not compare how MGSAD023 matches the eIF4A and eIF3 binding sites of the eIF4G middle domain (4). MGSAD023 may mimic the function of the C-terminal fragment of eIF4G in cells.

Many genes that are transcribed in a particular tissue, including testis, are also transcribed in other tissues. Some of these genes encode products that are required for normal processes in all cells. However, one or more of these housekeeping genes may be transcribed primarily or exclusively in testis. This is a type of tissue-specific transcription. A characteristic of the proliferation pattern in spermatogonia is its mitosis division pattern; it differs from other spermatogenesis cells such as primary spermatocyte and secondary spermatocyte, which show meiosis during cell division. Until now, there has been no evidence that the eIF4G homologues mentioned above are involved in protein synthesis during the G2/M phase of mitosis[15),16),17)]. As a new homologue of eIF4G, MGSAD023 is highly expressed in spermatogonia. It would be worth exploring if MGSAD023 acts as a scaffold in protein synthesis during the G2/M phase during spermatogonia division.

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References

1. Bovee ML, Lamphear BJ, Rhoads RE, Lloyd RE: Direct cleavage of eIF4G by poliovirus 2A protease is inefficient in vitro. Virology.1998; 245:241-9
2. Lamphear BJ, Kirchweger R, Rhoads R E: Mapping of Functional Domains in Eukaryotic Protein Synthesis Initiation Factor 4G (eIF4G) with Picornaviral Proteases. Implications for cap-dependent and cap-independent translational initiation. J Biol Chem. 1995; 270: 21975 -83
3. Mader S, Lee H, Pause A, Sonenberg N: The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. Mol Cell Biol. 1995; 15: 4990-7
4. Imataka H, Sonenberg N: Human eukaryotic translation initiation factor 4G (eIF4G) possesses two separate and independent binding sites for eIF4A. Mol Cell Biol. 1997; 17: 6940-7
5. Goyer C, Altmann M, Sonenberg N: TIF4631 and TIF4632: two yeast genes encoding the high-molecular-weight subunits of the cap-binding protein complex (eukaryotic initiation factor 4F) contain an RNA recognition motif-like sequence and carry out an essential func-

tion. Mol Cell Biol. 1993; 13: 4860-74

6. Pestova T V, Shatsky I N, Hellen C U T: Functional dissection of eukaryotic initiation factor 4F: the 4A subunit and the central domain of the 4G subunit are sufficient to mediate internal entry of 43S preinitiation complexes. Mol Cell Biol. 1996; 16: 6870-8
7. Pyronnet S, Imataka H, Sonenberg N: Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. EMBO J. 1999; 18: 270-9
8. Waskiewicz A J, Johnson J C, Cooper J A: Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. Mol Cell Biol. 1999; 19: 1871-80
9. Metz A M, Browning K S: Mutational analysis of the functional domains of the large subunit of the isozyme form of wheat initiation factor eIF4F. J Biol Chem. 1996; 271: 31033-6
10. Imataka H, Olsen HS, Sonenberg N: A new translational regulator with homology to eukaryotic translation initiation factor 4G. EMBO J. 1997; 16: 817-25
11. Goke A, Goke R, Knolle A, Trusheim H, Schmidt H, Chen YH: DUG is a novel homologue of translation initiation factor 4G that binds eIF4A. Biochem Biophys Res Commun. 2002; 297: 78-82
12. Craig AW, Haghighat A, Yu AT, Sonenberg N: Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. Nature. 1998; 392: 520-3
13. Henis-Korenblit S, Stumpf NL, Goldstaub D, Kimchi A: A novel form of DAP5 protein accumulates in apoptotic cells as a result of caspase cleavage and internal ribosome entry site-mediated translation. Mol Cell Biol. 2000; 20: 496-506
14. Stoneley M, Chappell S A, Jopling C L, Dickens M, MacFarlane M, Willis A E: c-Myc protein synthesis is initiated from the internal ribosome entry segment during apoptosis. Mol Cell Biol. 2000; 20: 1162– 9
15. Bonneau, Sonenberg N: Involvement of the 24-kDa cap-binding protein in regulation of protein synthesis in mitosis. J Biol Chem. 1987; 262: 11134–9
16. Pyronnet S, Pradayrol L, Sonenberg N: A cell cycle-dependent internal ribosome entry site Mol Cell. 2000; 5:607-16
17. Stoneley M, Paulin FEM, Quesne JPC Le, Chappell S A, Willis A E: C-myc 5′ untranslated region contains an internal ribosome entry segment. Oncogene. 1997; 16: 423–8

Footnotes: MGSAD023, mouse gene similar to AD023; eIF4G, eukaryotic initiation factor 4 gamma; IRES, internal ribosome entry site.