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Gasdermin (*Gsdm*) localizing to mouse Chromosome 11 is predominantly expressed in upper gastrointestinal tract but significantly suppressed in human gastric cancer cells

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Abstract. Amplification of proto-oncogenes associated with their over-expression is one of the critical carcinogenic events identified in human cancer cells. In many cases of human gastric cancer, a proto-oncogene ERBB-2 is co-amplified with CAB1 genes physically linked to ERBB-2, and both genes are over-expressed. The amplified region containing ERBB-2 and CAB1 was named 17q12 amplicon from its chromosomal location. The syntenic region corresponding to the 17q12 amplicon is well conserved in mouse. In this study we isolated and characterized a novel mouse gene that locates telomeric to the mouse syntenic region. Northern blot analysis using the mouse cDNA and a cloned partial cDNA of human homolog disclosed a unique expression pattern of the genes. They are expressed predominantly in the gastrointestinal (GI) tract and in the skin at a lower level. Moreover, in the GI tract, the expression is highly restricted to the esophagus and stomach. Thus, we named the mouse gene Gasdermin (*Gsdm*). This is the first report of a mammalian gene whose expression is restricted to both upper GI tract and skin. Interestingly, in spite of its expression in normal stomach, no transcript was detected by Northern blot analysis in human gastric cancer cells. These data suggest that the loss of the expression of the human homolog is required for the carcinogenesis of gastric tissue and that the gene has an activity adverse to malignant transformation of cells.

Introduction

Recent progress in the molecular biology of oncogenesis has disclosed that qualitative and quantitative changes in genes controlling cell proliferation lead to neoplastic transformation of cells (Hunter 1997). Loss of tumor suppressor genes is known to be a critical event in the carcinogenesis of various types of cancer in a broad spectrum of tissues. On the contrary, the gain of extra copies of proto-oncogenes that are involved in cell proliferation and differentiation also results in a tumorigenic change of cells.

Somatically amplified regions of the genome in cancers, which are called amplicons, spread from hundreds to thousands of kilobases of genome DNA, and they often contain proto-oncogenes (Brookes et al. 1993; Tanigami et al. 1992). ERBB-2 is one of the proto-oncogenes of an EGF (epidermal growth factor) receptor-related tyrosine kinase type, and is mapped to human Chromosome (Chr) 17q12. This gene is amplified and over-expressed in about 25% of human breast cancers and in about 10% of gastric cancers (Stein et al. 1994; Yokota et al. 1988; Houldsworth et al. 1990; Ranzani et al. 1990). It was reported that four genes, A39, CAB1, C51, and GRB-7, were isolated from a 500-kb DNA of a yeast artificial chromosome (YAC) clone containing the ERBB-2 gene (Akiyama et al. 1997). These four genes were all co-amplified with ERBB-2 in TE6, an esophageal cancer cell line (Akiyama et al. 1997). In primary gastric cancers and gastric cancer cell lines, CAB1, GRB-7, and C51 were co-amplified with ERBB-2. Moreover, CAB1, GRB-7, and ERBB-2 were over-expressed in those cell lines (Kishi et al. 1997). Thus, the region containing CAB1, C51, and ERBB-2 was named 17q12 amplicon, and the GRB-7 is located telomeric to it. These facts suggest that in some cases of cancer, the amplicon is attributable to uncontrolled cell proliferation and deregulated differentiation of cells, which eventually lead to carcinogenesis.

In the process of positional cloning of a responsible gene for a mouse mutant *Rim3*, which is characterized by skin anomalies and is mapped to the region syntenic to the human 17q12 amplicon, we isolated one cDNA clone by cDNA selection with mouse bacterial artificial chromosome (BAC) clones corresponding to the 17q12 amplicon (Sato et al. 1998). Characterization of the clone revealed a unique expression pattern both in the alimentary tract, tightly restricted to the esophagus and stomach, and in the skin. Hence, we named the gene Gasdermin (*Gsdm*) for its expression pattern. We detected a substantial level of amplification of the human homolog of this gene in human gastric cancers and breast cancer cell lines. And we expected over-expression of *Gsdm* in gastric cancer cells because of both the gene location close to the amplified region and its expression in the stomach. However, contrary to our expectation, no transcript of human *Gsdm* gene was detected by Northern blot analysis in any human gastric cancer cell lines examined. This significant suppression of *Gsdm* expression in gastric cancer cells suggests that it has an activity of regulating cell proliferation and/or differentiation and that suppression of the gene is required for carcinogenesis of gastric tissue.

Materials and methods

Construction of BAC contig. With specific primers to GRB-7 (5′-AACATCCCCTAACCCTGTCC-3′ and 5′-AGCAGATCAACTGGCCTCTC-3′) and primers to D11Mit14 (Research Genetics, Inc., Huntsville, Ala., USA), Mouse bacterial artificial chromosome DNA pools release II (Research Genetics, Inc.) was screened by a PCR procedure. By screening with primers specific to the terminus sequences of the obtained

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two BAC clones, a clone bridging between them was also isolated. Finally, a BAC contig consisting of the three BAC clones that covers the critical region of *Rim3* mutation was constructed.

cDNA selection. cDNA selection was performed by following the method of rapid isolation of cDNA by hybridization (RICH), which was recently developed (Hamaguchi et al. 1998). Total RNAs were extracted from the skin of C57BL/10J (B10) newborn mice by using the acid guanidinium-phenol-chloroform (AGPC) method (Chomczynski et al. 1987) and were further purified to isolate poly(A)$^+$ RNAs with QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia, Uppsala, Sweden). cDNAs were synthesized from the poly(A)$^+$RNAs with the TimeSaver cDNA Synthesis Kit (Amersham Pharmacia). Both the BAC DNAs and the skin cDNAs were digested with *Sau*3A I followed by adaptor-ligation (Hamaguchi et al. 1998). Upon incubation for 16 h at 67°C, half a microgram of each of the three BAC DNAs was hybridized with 0.5 μg of the skin cDNAs digested with *Sau*3A I. After selection of adaptor-ligation, the genome-cDNA hybrids were amplified by PCR and transcribed to RNA strands by MEGAscript™ (Ambion, Texas, USA). The RNA strands were amplified by a reverse transcription-coupled PCR (RT-PCR) with hybrid-specific primers. The products were size-fractionated by electrophoresis through 1% agarose gel, cloned into pCR2.1 vector with Original TA Cloning Kit (Invitrogen, Groningen, Netherlands), and sequenced.

Cloning of a full-length Gasdermin cDNA. cDNAs were synthesized from the poly(A)$^+$RNAs of B10 newborn mouse skin as described above. The cDNAs were ligated to λ ZAP II *Eco*RI-predigested/CIAP-Treated Vector (Stratagene, Calif.). The ligated cDNAs were subjected to in vitro packaging with Gigapack III Gold Packaging Extract (Stratagene). A probe for cDNA library screening was prepared by utilizing sequence information of the expressed sequence tags (EST) which contained the sequence obtained in the cDNA selection. The DNA fragments corresponding to a 443-bp region of the nucleotide position 976–1419 of Gasdermin were isolated by RT-PCR and cloned into the pCR2.1 vector. Excised fragments were labeled with α-^{32}P dCTP with BcaBEST Labeling Kit (Takara Biomedicals, Kyoto, Japan). The cDNA clone was isolated by a plaque hybridization procedure.

Preparation of probes for Northern and Southern blot analyses. The mouse probe for Northern and Southern blot analyses was the 1003-bp *Eco*RI–*Xba*I fragment spanning the nucleotide position from 1 to 1003 of the full-length Gasdermin cDNA. The human probe was prepared from a nucleotide sequence of the human EST clone (GenBank Accession No. AI795843) that contained the sequence corresponding to the 5′ side of mouse Gasdermin cDNA spanning the amino acid position from 1 to 58 with an amino acid similarity of 96%. With the primer set (5′-TCCCAGAGACAATGACCATG-3′ and 5′-TGTAGTCGGTGCGGACG-TAC-3′) designed to amplify a 186-bp fragment corresponding to the nucleotide position 72–257 of mouse Gasdermin cDNA, RT-PCR was performed with RNA extracted from adult human stomach as a template. The amplified fragments were labeled with α-^{32}P dCTP.

Northern blot analysis for normal expression. Multiple Choice Northern Blots (OriGene Technologies, Inc., USA) contained 20 μg of total RNAs per lane, which were extracted from 12 tissues, brain, heart, kidney, spleen, thymus, liver, stomach, small intestine, skeletal muscle, lung, testis, and skin, of Swiss Webster mice. Total RNAs for expression analysis of the GI tract were isolated from the distal esophagus, gastric fundus, gastric cardia, pylorus, and small intestine of B10 mice. The total RNAs were size-separated by electrophoresis through a gel containing formaldehyde, and transferred to a nylon membrane. The blots were incubated in a hybridization buffer containing the ^{32}P-labeled cDNA and subjected to autoradiography after washing.

Coupled in vitro transcription/translation reaction. The full-length cDNA of mouse Gasdermin was subcloned into a multi-cloning site of an expression vector pcDNA3.1. Another construct whose translation initiation site was replaced by a Kozak optimum consensus, ACCATGG, was also prepared. Proteins encoded by the constructs were produced by using TNT Quick Coupled Transcription/Translation Systems (Promega, Madison, Wis., USA) following the manufacturer's protocol. The product was labeled with Redivue L-[^{35}S]methionine (Amersham Pharmacia). Their molecular weight was estimated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography.

Detection of intracellular localization of Gasdermin protein by using a blue fluorescent protein (BFP) fusion protein. The coding region of Gasdermin cDNA was subcloned between *Sac*III and *Nhe*I sites of a BFP-expression vector pQBI50 (Takara Biomedicals, Tokyo, Japan) in-frame to produce a BFP fusion protein. The construct was prepared by using EndoFree Plasmid Maxi Kit (QIAGEN, Tokyo, Japan) and transfected to 293 cells (human embryonic kidney cell line) with Effectene Transfection Reagent (QIAGEN). Transfected cells were observed with a fluorescent microscopy 36 h after transfection.

Southern blot analysis of cancer cell lines and human gastric cancer tissues. DNA samples were extracted from eight surgical specimens of human gastric cancer. After digestion with *Eco*RI, 10 μg of each DNA sample was transferred to a nylon membrane after electrophoresis and hybridized with the 1003-bp mouse Gasdermin cDNA probe. The DNAs were also extracted from nine gastric cancer cell lines, T5, T16, T31, T32, T44, T48, T82, T83, and MKN7; an ovarian cancer cell line, SKOV3; an esophageal cancer cell line, TE6; and four breast cancer cell lines, BT474, SKBR3, UACC-893, and UACC-812. The DNA samples were subjected to the same procedure described above, except those were hybridized with the ^{32}P-labeled partial cDNA of the human homolog of the mouse *Gsdm* gene.

Northern blot analysis of normal human tissues and human cancer cell lines. MTN™ blots (CLONTECH, Heidelberg, Germany) which contained total RNA extracted from 23 human tissues, brain, spinal cord, trachea, lung, heart, stomach, small intestine, colon, liver, pancreas, skeletal muscle, thyroid, adrenal gland, leukocyte, bone marrow, lymph node, spleen, thymus, kidney, prostate, placenta, testis, and ovary, were used for normal expression. Total RNAs were extracted from eight breast cancer cell lines, MCF7, T47D, 2R75-1, BT20, SKBR3, BT474, UACC-812, and UACC-893; 14 gastric cancer cell lines, OKAJIMA, TMK1, MKN74, MKN28, MKN1, MKN7, HSC44, HSC59, HSC60, HSC39, HSC43, KATOIII, OCUM1, and OCUM2M; and two ovarian cancer cell lines, SKOV3 and OVCAR3. THe RNA samples were subjected to Northern blot analysis. The blots were hybridized to the ^{32}P-labeled partial cDNA fragments of the human gene. The autoradiography was performed with a relatively long exposure time of up to 2 weeks.

Results

Mouse BAC contig containing ERBB-2 and GRB-7 genes and rapid isolation of cDNAs by hybridization (RICH). We constructed a BAC contig in the process of positional cloning of a responsible gene for a mouse mutation *Rim3*. By three BAC clones, 227J19, 372A19, and 321H6, the critical region of the *Rim3* was covered. PCR amplifications with primers specific to ERBB-2 and GRB-7 genes revealed that the BAC clone 227J19 contained both the genes.

To isolate genes located in the region containing GRB-7, we performed the RICH procedure using the three BAC clones and newborn mouse skin cDNAs. As a result, we obtained several clones, including a partial cDNA fragment of GRB-7. A 27-bp fragment designated as H312E was repeatedly isolated from the procedure. We searched a computerized EST data base, the EST Extractor at TigemNet (http://gcg.tigem.it/BLASTEXTRACT/estextract.html), for the ESTs containing a sequence of the H312E. Seven hundred base pairs of the DNA sequence of the total span were obtained by two ESTs, GenBank Accession Nos. AA763214 and AA930013, both of which contained the H312E sequence and were expressed in mouse skin.

Isolation of a full-length cDNA clone and its sequence. A cDNA library of newborn mouse skin was constructed and screened by hybridization with the labeled 443-bp fragment which was included in the 700-bp span of the ESTs. By the screening, we

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   1  CACCCTATAGACAGAACGAGCTCTGGTTCCTCACCACACACCAGCTGCCAGACAGGATCC  60
  61  AGCATCACCCTTCCCTGAGACAATGACTATGTTTGAGAATGTCACCCGGGCCCTGGCTAG 120
                          M  T  M  F  E  N  V  T  R  A  L  A  R
 121  ACAGCTGAACCCTCGAGGGGATCTGACACCCCTAGACAGCCTCATCGACTTCAAACGCTT 180
       Q  L  N  P  R  G  D  L  T  P  L  D  S  L  I  D  F  K  R  F
 181  CCATCCCTTCTGCCTGGTGCTGAGGAAGAGGAAGAGCACACTGTTCTGGGGAGCCCGCTA 240
       H  P  F  C  L  V  L  R  K  R  K  S  T  L  F  W  G  A  R  Y
 241  TGTGCACACCGACTACACTCTCCTGGATGTGCTGGAGCCGGGCAGCTCCCCCTCAGATCC 300
       V  H  T  D  Y  T  L  L  D  V  L  E  P  G  S  S  P  S  D  P
 301  GACAGACAGTGGCAACTTTAGCTTTAAGAATATGCTGGATGCTCGAGTAGAGGGAGATGT 360
       T  D  S  G  N  F  S  F  K  N  M  L  D  A  R  V  E  G  D  V
 361  GGATGTGCCAAAGACAGTGAAGGTAAAGGGGACTGCGGGTCTGTCACGGAGCAGCACACT 420
       D  V  P  K  T  V  K  V  K  G  T  A  G  L  S  R  S  S  T  L
 421  GGAGGTGCAGACGCTCAGCGTGGCTCCCACGGCTCTGGAGAACTTGCACAAGGAGAGGAA 480
       E  V  Q  T  L  S  V  A  P  T  A  L  E  N  L  H  K  E  R  K
 481  ACTGTCAGCAGACCACCCATTCCTGAAGGAGATGCGGGAACGCGGGGAGAACCTCTATGT 540
       L  S  A  D  H  P  F  L  K  E  M  R  E  R  G  E  N  L  Y  V
 541  GGTGATGGAGGTGGTGGAAACCCTACAGGAAGTCACTCTGGAGCGAGCCGGCAAGGCAGA 600
       V  M  E  V  V  E  T  L  Q  E  V  T  L  E  R  A  G  K  A  E
 601  GGGCTGCTTCTCTCTCCCCTTCTTTGCCCCACTGGGACTACAGGGATCCGTGAACCACAA 660
       G  C  F  S  L  P  F  F  A  P  L  G  L  Q  G  S  V  N  H  K
 661  GGAGGCTGTAACCATCCCCAAGGGCTGTGTTCTGGCCTATCGAGTGAGACAACTGATGGT 720
       E  A  V  T  I  P  K  G  C  V  L  A  Y  R  V  R  Q  L  M  V
 721  CAACGGCAAAGATGAGTGGGGCATTCCACACATTTGCAATGACAGCATGCAAACCTTCCC 780
       N  G  K  D  E  W  G  I  P  H  I  C  N  D  S  M  Q  T  F  P
 781  TCCTGGAGAAAAGCCAGGAGAAGGGAAGTTCATATTGATCCAGGCATCTGATGTTGGGGA 840
       P  G  E  K  P  G  E  G  K  F  I  L  I  Q  A  S  D  V  G  E
 841  GATGCACGAAGACTTCAAGACATTAAAGGAAGAGGTTCAGCGAGAGACTCAGGAAGTGGA 900
       M  H  E  D  F  K  T  L  K  E  E  V  Q  R  E  T  Q  E  V  E
 901  GAAGTTAAGTCCAGTGGGGCGAAGCTCACTACTCACTTCCCTCAGCCATCTCCTAGGAAA 960
       K  L  S  P  V  G  R  S  S  L  L  T  S  L  S  H  L  L  G  K
 961  GAAGAAAGAGCTCCAGGACCTTGAGCAGACGCTTGAAGGGGCTCTAGACAAGGGACACGA 1020
       K  K  E  L  Q  D  L  E  Q  T  L  E  G  A  L  D  K  G  H  E
1021  AGTGACCCTGGAAGCACTCCCCAAAGATGTCCTGCTGTCAAAGGACGCTATGGACGCCAT 1080
       V  T  L  E  A  L  P  K  D  V  L  L  S  K  D  A  M  D  A  I
1081  CCTTTACTTCCTCGGGGCTCTGACAGTGCTAAGTGAAGCCCAACAGAAGCTTCTAGTAAA 1140
       L  Y  F  L  G  A  L  T  V  L  S  E  A  Q  Q  K  L  L  V  K
1141  ATCCTTGGAGAAAAAGATCCTACCGGTGCAACTGAAGCTGGTTGAAAGCACCATGGAGAA 1200
       S  L  E  K  K  I  L  P  V  Q  L  K  L  V  E  S  T  M  E  K
1201  GAACTTCCTGCAAGATAAAGAGGGTGTTTTCCCCCTGCAACCTGATCTGCTCTCCTCCCT 1260
       N  F  L  Q  D  K  E  G  V  F  P  L  Q  P  D  L  L  S  S  L
1261  CGGGGAGGAGGAACTGATCCTAACAGAAGCACTGGTGGGACTAAGCGGCCTGGAAGTCCA 1320
       G  E  E  E  L  I  L  T  E  A  L  V  G  L  S  G  L  E  V  Q
1321  GAGATCAGGCCCCCAGTACACGTGGGATCCGGACACGCTCCCCCACCTTTGTGCCCTCTA 1380
       R  S  G  P  Q  Y  T  W  D  P  D  T  L  P  H  L  C  A  L  Y
1381  TGCTGGCCTCTCCCTCCTTCAACTGCTAAGCAAGAATTCCTAATGCACCTTCTTTGCCTG 1440
       A  G  L  S  L  L  Q  L  L  S  K  N  S  stop
1441  CTGCCCTAAAGCCTTCCCAGCCTTACTGTGCTCCATCTGTAACACTGCAAGACACTACAG 1500
1501  AGCCTCCAGGCTGAGGACAACTGAAATGCCAGCTCAAAAATCAGTCTCCAAGTTCCTTCT 1560
1561  GCCTCACCATCTATCTCTCTCCTTCTGTCCTCCAGCTCAGCACCCACTAAGCCCATCTCC 1620
1621  TGATGCTTAAATCCTGAAGATACAGAATCATTCAAACCCTTACTACTTTGAGTCACTTCA 1680
1681  TTAAGAGGGGTTGGGGGGAGGCTTGGGGTGGTGATGGTGGTGGTTATGGTGTGAGGCAGA 1740
1741  GGCAGGTGAATCTCTTAAGTTCGAGGCCAGCCTGGTCTGCAGAGTGAACTCCAGGACAGC 1800
1801  CAGGGCAACACAGAGAAACCTTGTCTTGAAAACAACAAAACCACATTTATTTCCTAGTCT 1860
1861  GATCCTTCCCATGTGTGCCTCA
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Fig. 1. Nucleotide sequence of mouse Gasdermin (*Gsdm*) cDNA and the predicted amino acid sequence. The ORF encoding 446 amino acids starts at the 83rd nucleotide and ends at the 1423rd. No motif of significance was identified in the ORF by searching a computer retrieval.

obtained five independent clones. The result of sequencing revealed that three of them had 1882 bp of length, excluding poly(A)$^+$ tail, with the same 5′-end sequence, and that the other two were partial. We inferred that the three were corresponding to the full-length cDNA (Fig. 1). It was supported by the genomic structure of its promoter region in which TATA box is identified 37-bp upstream from the 5′ end of the three cDNA clones (Aoki et al., unpublished data). The full-length cDNA included the sequences of the corresponding two ESTs. Analysis with a computer retrieval, ORF Finder, disclosed that the cDNA had several possible open reading frames (ORFs), one of which was 1341 bp in length, and all the others were less than 200 bp. The longest ORF encoded 446 amino acids and had no Kozak consensus in the proximity of the first methionine, a supposed translation initiation site, but in the proximity of the methionine at 371st. To confirm the exact position of the translation initiation site, the approximate molecular weight of the coded product was determined by the coupled in vitro transcription/translation reaction followed by SDS-PAGE (Fig. 2). The result indicate that the molecular weight of the product is about 50 kDa, which is consistent with the estimated value if the translation initiates from the first methionine in the longest ORF. This was again confirmed by the fact that the protein produced from the recombinant construct, in which the initiation site was replaced by the Kozak consensus, had the same molecular weight (data not shown). Searching for known motifs in the coded protein was performed using a motif retrieval system, MOTIF. No motif of significance was detected except a leucine-zipper that located between the 283rd and 304th amino acids of the

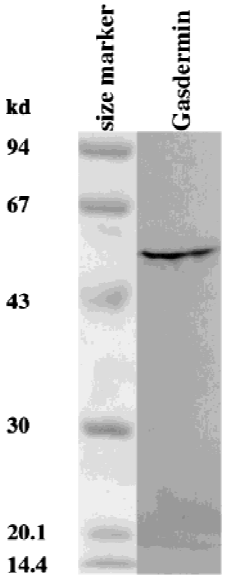

Fig. 2. SDS-PAGE analysis of Gasdermin protein. The protein encoded by the Gasdermin cDNA was synthesized by the coupled in vitro transcription/translation reaction, and applied to 10% polyacrylamide gel containing SDS. After the electrophoresis, the gel was dried and followed by autoradiography. The translated product was estimated to be about 50 kDa in weight.

product. Amino acid sequence of the leucine-zipper is: Leu-Leu-Thr-Ser-Leu-Ser-His-Leu-Leu-Gly-Lys-Lys-Lys-Glu-Leu-Gln-Asp-Leu-Glu-Gln-Thr-Leu. The full-length cDNA has 82 bp of the 5′ untranslated region (UTR) and 459 bp of the 3′ UTR. To con-

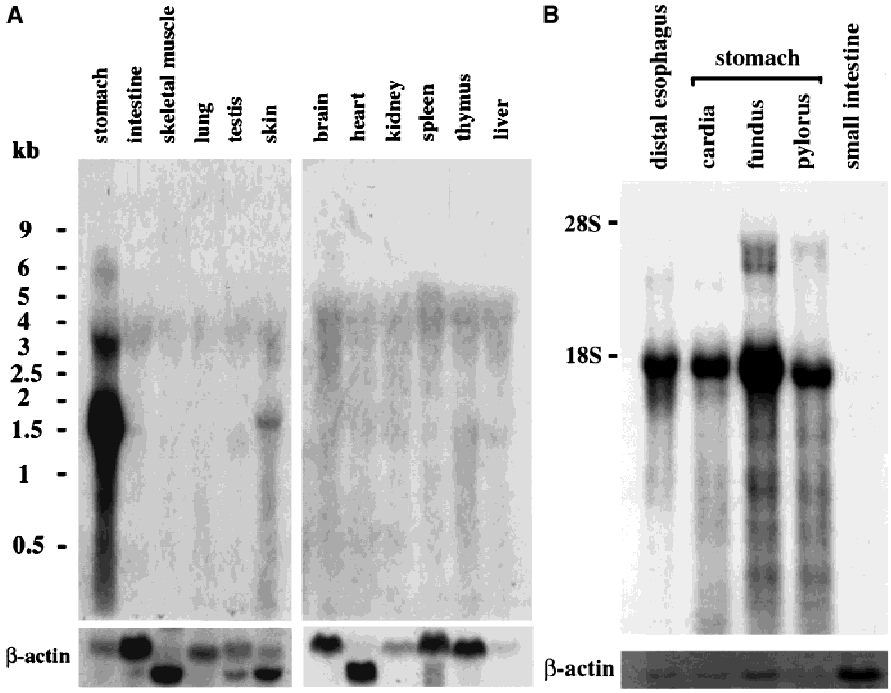

Fig. 3. Northern blot analysis of *Gsdm* gene. **A,** *Gsdm* expression in various mouse organs. Twenty micrograms of total RNAs extracted from mouse organs were loaded in each lane by the manufacturer. The RNAs were hybridized with labeled 1003-bp fragments of partial *Gsdm* cDNA. Note that a significant amount of the expression was detected in the stomach. The transcript was also observed in the skin. **B,** Expression in the gastrointestinal tract. Ten micrograms of total RNAs were loaded in each lane. The transcript was detected in the esophagus and stomach, but not in the small intestine. The relative positions of 28S and 18S ribosomal RNA were indicated as size markers. Northern blot of β-actin with a relatively short exposure time is also shown.

firm that the gene was contained by the BAC DNA covering the critical region of *Rim3*, we carried out both PCR with the several primer sets which were designed based on the cDNA sequence and dot blot hybridization with cDNA probe. The result disclosed the gene was contained by one of the three BAC clones, 321H6 (data not shown).

Northern blot analysis in mouse normal tissues. Northern blot analysis was carried out with total RNAs extracted from a variety of normal tissues of mice. By using labeled partial fragments of the cDNA, the expression at a significantly high level was detected in the stomach, and a much smaller amount of the expression was detected in the skin (Fig. 3A). A single band with a length of more than 1.5 kb was observed in both of the tissues, suggesting that only a single transcript is synthesized in the stomach and skin. To obtain more precise information on the gastrointestinal expression, we extracted total RNAs from the esophagus, gastric cardia, fundus, pylorus, and small intestine, and carried out Northern blot analysis. The transcript was detected only in the esophagus and in the entire stomach, but not in the small intestine (Fig. 3B). For this highly restricted expression pattern of the gene in gastric tissue and skin, we designated it as Gasdermin (*Gsdm*).

Intracellular localization of Gasdermin protein. Because *Gsdm* contains a sequence similar to the leucine-zipper motif, it seemed to encode a DNA-binding protein. We analyzed the intracellular localization of the Gasdermin product. For this purpose, the coding region of *Gsdm* cDNA with its 5′UTR was ligated in-frame to the 5′ side of BFP of the expression vector to produce a Gasdermin-BFP fusion protein. By the lipofection procedure, the construct was introduced to 293 cells. Under a fluorescent microscopy, the fusion protein was detected only in the cytoplasm, but not in the nucleus (Fig. 4).

Gene amplification of the human homolog of Gsdm in human cancer cells. Southern blot analysis with a labeled partial cDNA of the human homolog of the *Gsdm* gene as a probe revealed the amplification of the human gene, which occurred in the human

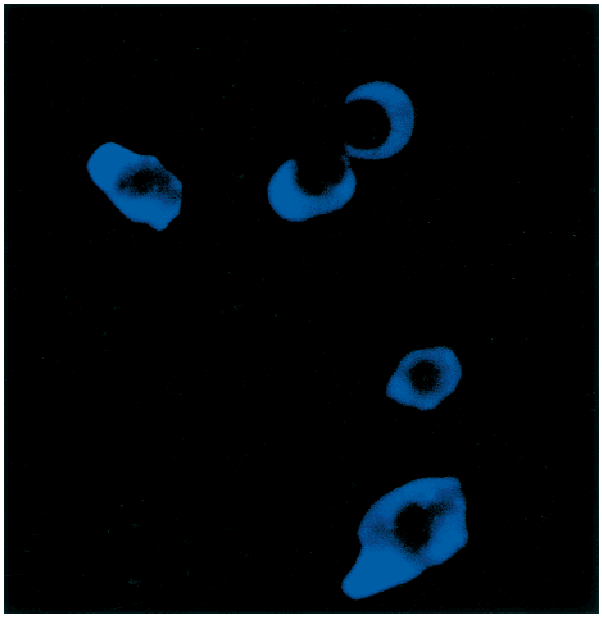

Fig. 4. Cytoplasmic localization of the Gasdermin-BFP fusion protein. The entire coding region of *Gsdm* cDNA was inserted in-frame to the 5′ side of BFP cDNA of an expression vector pQBI50. The construct was introduced to 293 cells by lipofection. The fluorescent-microscopic observation disclosed localization of the fusion protein (bluish fluorescent) in the cytoplasm.

gastric cancer tissues and in breast cancer cell lines (Fig. 5A and B). The cancer tissues and cell lines analyzed in this study contained an amplified ERBB-2 gene, which was previously demonstrated (Kishi et al. 1997). We observed one signal band in the tissues and cell lines, suggesting no other homolog or pseudogenes exist in the human genome. In two of the eight gastric cancer tissues with the amplification of ERBB-2 gene, the human gene was also substantially amplified (lanes 3 and 5 in Fig. 5A). Restriction-fragment length polymorphism (RFLP) was also ob-

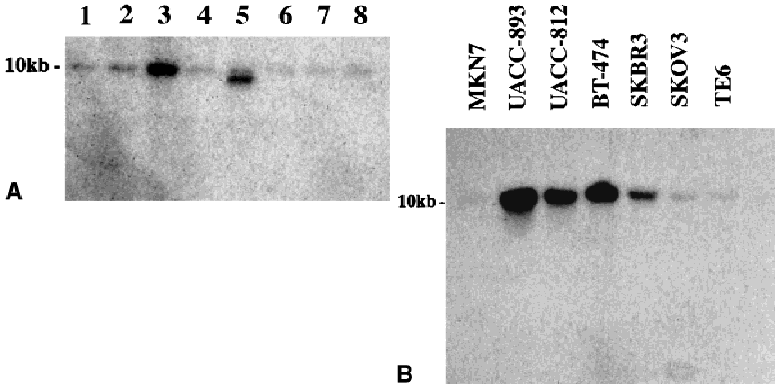

Fig. 5. Gene amplification of the human homolog of mouse *Gsdm* gene in human cancer cells. **A,** Southern blot analysis on human gastric cancers. DNA samples extracted from eight surgical specimens of human gastric cancer were blotted to a nylon membrane and hybridized with labeled 1003-bp fragments of mouse *Gsdm* cDNA. The gene amplification was detected in two samples (lanes 3 and 5). The signal of a single copy of the gene was also observed in the other samples. The amplification of ERBB-2 gene was previously demonstrated in all the DNA samples (Kishi et al. 1997). **B,** Southern blot analysis on human cancer cell lines. DNAs were extracted from seven cancer cell lines and hybridized with a partial cDNA of the human *Gsdm* homolog after blotting. MKN7, gastric cancer cell line; UACC-893, UACC-812, BT-474, and SKBR3, breast cancer cell lines; SKOV3, ovarian cancer cell line; TE6, esophageal cancer cell line. Note the amplification of the human homolog in the four breast cancer cell lines. The other cell lines had a single copy of the gene. The amplification of ERBB-2 was previously observed in all the cancer cell lines (data not shown).

served (lane 5 in Fig. 5A). The human homolog gene was amplified in all the examined cell lines of breast cancer origin, UACC-893, UACC-812, BT-474, and SKBR3, which contained the amplified ERBB-2 gene, but not in the nine gastric cancer cell lines, the ovarian cancer cell line, and the esophageal cancer cell lines, in spite of the fact that they contained the amplified ERBB-2 (Fig. 5B, data are not shown for gastric cancer cell lines except for MKN7).

Loss of expression of the human homolog in human cancer cells. To analyze the expression of the human homolog of the *Gsdm* gene in human cancers, we carried out Northern blot analysis with a partial cDNA clone of the human *Gsdm* gene as a probe. We examined several human cancer cell lines in which the ERBB-2 gene was amplified. By Northern blot analysis in human normal tissues, the transcript was detected only in the stomach (Fig. 6A). In the analysis of the human cancer cells, no expression of the human homolog was observed in 14 gastric cancer cell lines. In addition, no transcript was detected in eight breast cancer cell lines, including four cell lines, UACC-893, UACC-812, BT-474, and SKBR3, in which amplification of the human *Gsdm* gene was observed (Fig. 5B), and in two ovarian cancer cell lines (Fig. 6B). This result suggests that its expression was suppressed in human gastric cancers and that, even with the amplification of the gene, it is not expressed in breast cancer cells.

Discussion

The most striking feature of Gasdermin (*Gsdm*) is its unique expression pattern. It is expressed in the upper gastrointestinal (GI) tract with a boundary between the stomach and small intestine. Several genes are known to be expressed mainly in the GI tract of developing and/or adult mice. For instance, trefoil peptides, mucins, gob-5, galectin-2, and adrenomedullin are expressed in the alimentary tract. Their expression is, however, diffused in the GI tract or spreads to extra-GI tract organs (Labouvie et al. 1999; Reiss et al. 1999; Komiya et al. 1999; Oka et al. 1999; Sakata et al. 1998). In contrast, the expression of *Gsdm* in the GI tract is restricted to the esophagus and stomach, and no transcript was detected in any other tissues, except in the integumental system where a very small amount of *Gsdm* gene is expressed. This rigid restriction of the expression domain is conserved in human as well. In human, no transcript was detected in the brain, spinal cord, trachea, lung, heart, small intestine, colon, liver, pancreas, skeletal muscle, thyroid, adrenal gland, leukocyte, bone marrow, lymph node, spleen, thymus, kidney, prostate, testis, and ovary (Fig. 6A). The promoter responsible for the highly restricted expression in the upper GI tract would be a useful spatial control device in constructing a virus vector for gene therapy of diseases of the upper GI tract.

In mammals, no other gene whose expression is restricted to the GI tract and skin has been reported so far. In the newt, the genes encoding EP37 family proteins, non-lens members of the β γ-crystallin superfamily, are expressed in the integumental system. Interestingly, several genes of the family are also expressed in the GI tract (Ogawa et al. 1998). For example, the *ep37A1* gene is expressed in the esophagus as well as in the integument, and expression of the *ep37L1* gene was detected in the stomach by Northern blot analysis. A newt gene, *gep*, which is considered as an *ep37* homolog, is expressed exclusively in the esophagus and stomach, but not in the integument. In the digestive system, the esophagus has a stratified squamous epithelium, which is also characteristic of skin epithelium, while the epithelium of the stomach is a simple columnar type. At the junction of the esophagus and cardia of the stomach, there is an abrupt transition from a stratified squamous to a simple columnar epithelium. The esophagus is considered to have characteristics of both ectodermal and endodermal origin. Considering the unique expression pattern of the mouse *Gsdm* gene and the newt ep37 family genes, it is possible that these genes are involved in the development of the upper GI tract. A combination of these genes or coordination with unknown gene(s) might specify the fine segmentation, especially the epithelium, of the rostral portion of the gut tube as the esophagus and stomach. Although we have not obtained enough information on the expression profile of the gene to speculate on its function in developing mouse and human embryos, the gene may play some roles in achieving and/or maintaining the final differentiation of cells in the GI tract. Preliminary experiments indicated that the *Gsdm* is expressed in 16.5-dpc mouse embryos (unpublished data). At present, it is also not clear which cell types in the stomach express the *Gsdm* gene. However, a significant amount of the transcript was detected by Northern analysis in gastric mucosa, almost all of whose cells are epithelial (unpublished data). The identification of the cell types expressing the gene remains open for future study.

The chromosomal region that contains the *Gsdm* gene is highly conserved between mouse and human. As in the mouse genome, the human homolog is located telomeric to the GRB-7 in Chr 17q that is syntenic to mouse Chr 11 (Y. Kuwahara and H. Sasaki, unpublished data). It is known that the human genomic region containing CAB1, C51, and ERBB-2 constitutes a core amplified region in stomach cancer as well as in breast cancer, and that the GRB-7 was also co-amplified (Stein et al. 1994; Akiyama et al. 1997; Kishi et al. 1997; Tomasetto et al. 1995). The expression of these genes was detected in gastric cancer cell lines, MKN-7 and KATO III, and in esophageal cancer cell lines TE2, TE6, and TE11. In particular, their expression was enhanced in the MKN-7

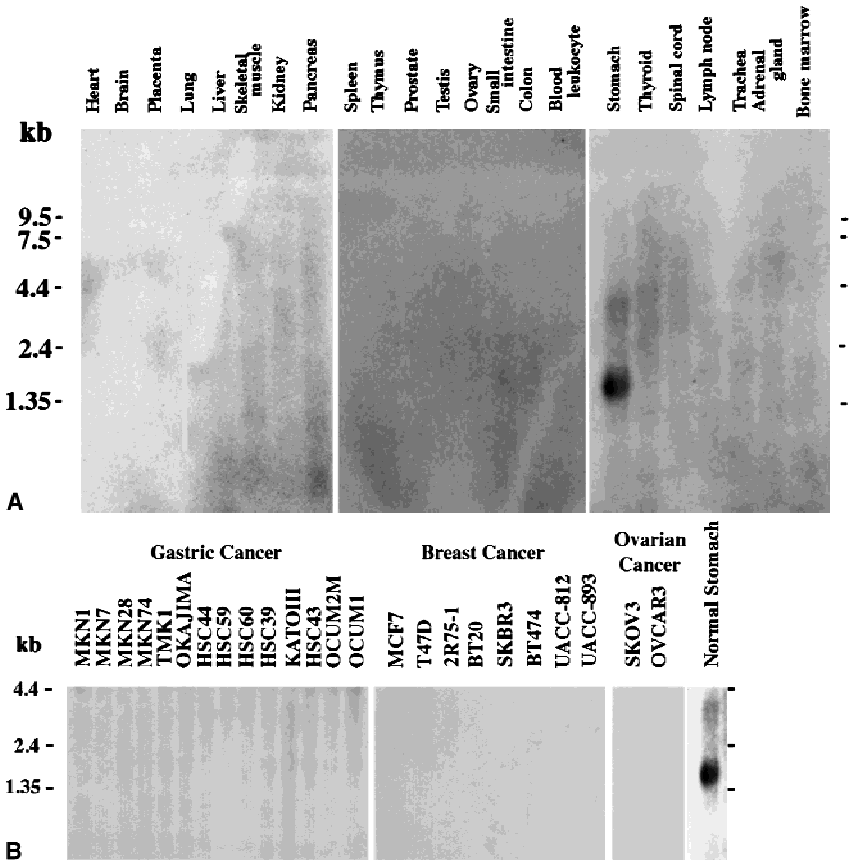

Fig. 6. Loss of the expression of the human *Gsdm* homolog in cancer cell lines. **A,** Normal expression. RNA samples were extracted from 23 human tissues, heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, blood leukocyte, stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow. Note that a single transcript was seen only in the stomach. **B,** Loss of the expression of human *Gsdm* homolog in gastric, breast, and ovarian cancers. Total RNAs were extracted from cancer cell lines; gastric cancer, MKN1, MKN7, MKN28, MKN74, TMK1, OKAJIMA, HSC44, HSC59, HSC60, HSC39, KATOIII, HSC43, OCUM2M, and OCUM1; breast cancer, MCF7, T47D, 2R75-1, BT20, SKBR3, BT474, UACC-812, and UACC-893; and ovarian cancer, SKOV3 and OVCAR3. No transcript was observed in any type of cancer, even in four breast cancer cell lines, UACC-893, UACC-812, BT-474, and SKBR3, in which the amplification of the human *Gsdm* gene was detected (Fig. 5B). It was observed that all the cell lines contained the amplified ERBB-2 gene (data not shown).

and the TE6, reflecting the level of their amplification (Akiyama et al. 1997).

It was observed that, in 15 samples of human gastric cancer containing the amplified ERBB-2, all the samples had co-amplified CAB1 and C51, and 11 samples (73%) had co-amplified GRB-7, which is located telomeric to the three genes. By contrast, only two samples (13%) showed co-amplification of the V-6 gene, which is located in the telomeric region of GRB-7 (Y. Kuwahara, and H. Sasaki, unpublished data). These data suggest that the genes located in the core-amplified region and the GRB-7 gene have an essential function of driving the carcinogenic process. On the other hand, gene(s) located in the region telomeric to GRB-7 may have no effect or, rather, adverse effects on carcinogenesis. The human homolog of the *Gsdm* gene is located to the region telomeric to the GRB-7 and centromeric to the V-6 gene (Y. Kuwahara and H. Sasaki, unpublished data). In all the gastric cancer cell lines examined in this study, Northern blot analysis showed significant reduction of the transcript of the human *Gsdm* gene, which is expressed in normal stomach at an extremely high level (Fig. 6). No expression of the human gene was also detected in breast cancer cell lines, even with amplification of the *Gsdm* gene, as well as in ovarian cancer cell lines. Thus, the suppression of *Gsdm* in human cancer is independent of both tissue origin and amplification of the gene. All these data support the possibility that the chromosomal region telomeric to GRB-7 contains gene(s) with some adverse activities to carcinigenesis, and that the human *Gsdm* homolog is a candidate for it.

Subcellular localization of the *Gsdm* product in a gastric epithelial cell is unknown so far. Analysis using the Gasdermin-BFP fusion protein demonstrated that Gasdermin has no nuclear localization signal (NLS) that drives the protein to move into the nucleus, suggesting that it is probably a cytoplasmic protein. Since no motif characteristic of any known protein and NLS is identified in Gasdermin, it is too early to speculate on the function of this protein. So far, many genes encoding transcription factors and regulatory proteins involved in the cell cycle check points or DNA repair have been classified into the category of tumor suppressor. Another type of protein, like E-cadherin, a membrane protein, is also thought to have tumor-suppressor activity (Christofori and Semb 1999; Semb and Christofori 1998). If Gasdermin is cytoplasmic, the detailed mechanism of the adverse effect to carcinogenesis, will have to remain to be proven in future studies.

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