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Identification and Characterization of Nine Novel Human Small GTPases Showing Variable Expressions in Liver Cancer Tissues

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Digestion and detoxification are the two most important functions of the liver, and liver cells always keep a high metabolism level and active vesicular traffic. The malfunction of the vesicular traffic system might be a cause of the abnormal biological behavior of cancerous liver cells. The Ras superfamily is known to regulate various steps of vesicular traffic in eukaryotic cells. It would be significant to determine the change of vesicular transport molecules such as the members of Ras superfamily in carcinogenesis of liver cells. In the present study, we have cloned nine novel genes encoding human small GTPases: *RAB1B, RAB4B, RAB10, RAB22A, RAB24, RAB25 ARL5, SARA1*, and *SARA2*, among which the former six belong to the RAB family and the latter three belong to the ARF/SAR1 family. The identification of these new genes has greatly enlarged the pool of the Ras superfamily. It is interesting to find that they are upregulated in most of the 11 hepatocellular carcinoma and 1 cholangiohepatoma cases. Furthermore, the expression in 16 normal human adult tissues, the chromosome loci, and the gene structures of the nine genes are also described. The above findings could be valuable for understanding the vesicular transport system and elucidating the molecular basis of liver cancer carcinogenesis.

RAB ARF/SAR1 Cloning Expression Liver cancer

THE liver plays a vital role in digestion and detoxification in vertebrates, and liver cells keep a high metabolism level and active vesicular traffic. However, canceration often impairs the function of liver cells. The malfunction of cancerous liver cells might be partially related to the abnormal vesicular traffic system in liver cancer. In fact, the structure of the membrane system in tumor cells often changes. For instance, in tumor cells, the amount, size, and structure of mitochondria differ from normal cells. The endoplasmic reticulum often enlarges and is abnormally arranged. The amount of lysosomes, as well as pinocytosis vacuole-like structure, obviously increases (10,23). Interestingly, accumulating data reveal that the genes encoding some endocytosis-related proteins are the target of chromosomal rearrangement in human hematopoietic malignancies, and abnormal expression or mutation of some endocytosis proteins has been reported in human cancers, suggesting a potential link between endocytosis proteins and cancer (11). Therefore, making a comprehensive depiction of the molecular basis of vesicular transport and its alternation in tumor genesis could be of value in understanding the pathology of liver cancer.

Many proteins are now known to be involved in vesicular transport, among which low molecular weight GTPases, collectively called the Ras superfamily, are a large class. They are important in mediating multiple signaling transduction pathways that are involved in the regulation of cellular proliferation, differentiation, and transformation (1). A great number of small GTPases are known to regulate vari-

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ous steps of vesicular traffic in eukaryotic cells (19). These small GTPases include the members of the RAB/Ypt and ARF/SAR1 families. The cycle between an active GTP-bound state and an inactive GDP-bound state of the proteins serves as a molecular switch that is involved in budding, targeting, and fusion of transport vesicles.

RAB proteins constitute the largest family of small GTPases. To date, 11 Ypt proteins in *Saccharomyces cerevisiae* (14) and over 40 RAB proteins (including isoforms) in mammalian cells have been found. Each protein is believed to function in one or more steps in vesicular traffic. In mammalian cells, for instance, RAB1A and RAB10 play a role in the transport of endoplasmic reticulum to Golgi and intra-Golgi, and RAB4A and RAB4B function in the recycling pathway from endosomes to plasma membranes (18). Unlike the initial notion that they are mainly implicated in vesicle docking and fusion, accumulating data indicate that RAB/Ypt proteins may be required for each process of vesicular transport, such as vesicle budding (18) and facilitating transport along the cytoskeleton (7,17).

ARF/SAR1 is another large family of small GTPases. The ARF proteins were initially recognized and purified according to their ability to stimulate the ADP-ribosyltransferase activity of the cholera toxin (CT) (13,22) and they are now known to be involved in the vesicle traffic and the activation of phospholipase D (PLD) (1,6,15). The best-characterized example is ARF1, which is localized to Golgi complex and is a regulator of nonclathrin and clathrin coat recruitment. ARL is a class of proteins that show significant similarity to ARFs. Despite their homology, it is believed that they cannot activate CT or PLD. However, Hong et al. found that human ARL1 has both activities under certain conditions (12). The biological functions of ARL proteins remain unclear. SAR1 GTPase plays a key role in initializing transport from the endoplasmic reticulum (ER) to Golgi apparatus in yeast (6,20,21), and in mammals, SAR1 is required for recruiting β-COP onto the ER and promoting vesical budding (2). SAR1 subfamily members are evolutionarily distant from the RAB/Ypt family and show slight homology with ARF proteins.

Although a large number of small GTPases have been proved or supposed to function in vesicular transport, the group is still growing continually. Because our knowledge of the molecular basis of vesicular transport is far from satisfying, the identification of additional members involved in the process will be of great importance in elucidating the molecular mechanisms and vesicular traffic alternation in tumor genesis. In this article, we report the identification of nine human small GTPases and their expression changes in human liver cancer. Furthermore, their chromosome loci, gene structures, and the expression patterns in normal human adult tissues are also depicted.

MATERIALS AND METHODS

Cancer Tissues and Total RNA Extraction

Surgical resection specimens were obtained from 12 Chinese male patients at the Institute of Liver Cancer Research at Qidong county, Jiangsu province in China. The age of the patients ranged from 32 to 47. Among them 11 patients were undergoing hepatocellular carcinoma (HCC) of differential class III. Another patient was suffering from poorly differentiated cholangiohepatoma (CH). All the patients were carriers of hepatitis B virus (HBV) and undergoing integument invasion (except case HCC06) and cancer embolus (except case HCC11), but were free from hepatitis C virus (HCV). The α-fetoprotein (AFP) value was over 1000 μg/L in three cases (HCC01, HCC05, and CH), 676.6 μg/L in one case (HCC04), 410.6 μg/L in one case (HCC06), and lower than 80 μg/L in the other cases. The cancer position was IV in one case (HCC02), II–IV in one case (HCC11), and V–VIII in all other cases. The size of the tumors was smaller than 6×6 cm in seven cases (CH, HCC03, HCC05, HCC06, HCC08, HCC09, HCC11) and equal to or larger than 6×6 cm in the other cases. The resected tumor specimens and the adjacent normal tissues were immediately snap-frozen in liquid nitrogen and stored at −80°C. Total RNA of tumor tissues and normal tissues was obtained under a protocol approved by the manufacturer (Trizol reagent, Gibco BRL) and then subjected to gel electrophoresis in 0.8× MOPS buffer under the electric field of 2 V/cm. The segregated total RNA was subsequently transferred to Nylon membranes, which was then dried at 80°C and subsequently kept at 4°C.

EST Selection and Management With Bioinformatics Methods

The amino acid sequences of 24 known members of the RAB family and 9 known members of the ARF/SAR1 family collected from GenBank database were aligned, respectively. Two highly conserved regions of each family were selected and the consensus sequences of these two regions were subsequently used for searching the EST database with tBLASTn algorithm. Homology was considered meaningful if ESTs shared at least 40% identity over 80 bp with

either one of the consensus sequences. The selected ESTs were then used to search against nonredundant division in GenBank using BLASTn algorithm. Those sharing 50–95% identity over at least 150 bp of DNA sequence with known genes were selected. The overlapping ESTs were then integrated into contigs, which were in turn used to search EST division in GenBank again to extend both the 5′ and 3′ ends.

cDNA Cloning

To confirm the sequences of the EST contigs, specific primers were synthesized according to the sequences flanking the putative ORFs in the contigs (Table 1) and PCR amplification was performed on the human cDNA libraries (Clontech). The PCR system was: 1 μl of template was amplified in a 25-μl volume mixture containing 2.5 μl of 10× PCR buffer, 0.5 μl of 0.02 mol/L dNTPs, 0.75 μl of 2.5×10^{-3} mol/L $MgCl_2$, and 1 unit of Taq polymerase (Promega) and 0.5 μl of 2.5×10^{-2} mol/L of each specific primer. PCR conditions were an initial denaturation step at 94°C for 5 min, followed by 29 cycles at 94°C for 1 min, 58–62°C for 30 s, and 72°C for 1 min. After an additional elongation step at 72°C for 10 min, the reactions were terminated by incubation at 4°C. After PCR products were purified and directly sequenced, the amplified fragments were recombined into vector pGEM-T and transformed into *E. coli* JM109. Four positive clones were selected from each kind of transformed clone and the plasmids were extracted and sequenced with the vector arm primers F_2 (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′) and R_2 (5′-TCA CAC AGG AAA CAG CTA TGA C-3′).

Northern Hybridization

The gene-specific PCR fragments of the small GTPases were labeled with [α-^{32}P]dATP by random primer kit (Amershan) to hybridize the MTN membranes carrying mRNA from 16 human tissues (Clontech) and nylon membrane carrying total RNA from 12 Chinese HCC or CH patients. The membranes were prehybridized in hybridization/prehybridization solution (50% formamide, 5× SSPE, 10× Denhardt's solution, 2% SDS, 100 mg/L calf thymus DNA) at 42°C for 24 h, followed by hybridization with the labeled probe for another 24 h, shaking continuously in a hybridization oven (Hybaid, UK). Then the membranes were washed orderly for three times in washing solution (2× SSC, 0.1% SDS; 0.5× SSC, 0.1% SDS; 0.1× SSC, 0.1% SDS) at 42°C before exposure to X-ray film at −70°C for 5 days. As a control, MTN I and MTN II were also hybridized with a 2.0 kb β-actin cDNA under the same condition as mentioned above. After hybridization, the membranes were exposed to X-ray film with an intensifying screen at −80°C for 4 h. The hybridized bands from autoradiography were scanned with GDS-800 (Bio-RAD) and Annutatin Grabber-1 T2.51 scanner software as well as UVP Gelworks ID Advanced Version 2.51 analysis software. The results of total RNA electrophoresis or β-actin were used as control and scanned similarly.

TABLE 1

PRIMERS USED IN ASSAYS

Gene Name	Primers Used in PCR (5′ to 3′)	Primers Used in RH Mapping (5′ to 3′)
RAB1B	A: CCGCCATGAACCCCGAATATGAC	RH1: CTGTGCTGTTGCCTCTAGGTGAC
	B: GGGACATCATCTGGAGAAGGTGC	RH2: GACTCCCTCCAGGCTCAAGACAC
RAB4B	A: ACCGAGTCATGGCTGAGACCTAC	RH1: CGTGTGGCTGCTGAGCTCTGTG
	B: GGAGCTCTTGGGATATGGTTAGG	RH2: ACTAGCAACAGCAGGGCCAGATG
RAB10	A: AGTGAGGAGTTGGCCGTAGTGAG	RH1: TGCTGCTGAGCATTCTCCTGTTC
	B: GGGTAGTGGATGGCAACTGATGG	RH2: GAATAGATTTGAAATGATTGCTATAGG
RAB22A	A: GGCCATGGCGCTGAGGGAGCTC	RH1: TCCATCCACTGACGCCAACCTG
	B: CAAGTCTGAGAGGCTGAGGTTCG	RH2: ATCTTGATTACAAAACTACTCAACAAC
RAB24	A: CCGAGCGGAGATCGGGGTTTGC	RH1: TGAGTCAGCACTCACCTGGCCTG
	B: AGAGGACCTGGGGTAGCTCAGA	RH2: GCAGACACAAGTGCTGTCCAGGG
RAB25	A: AACACACAGATTTGTCGCCTCTTG	RH1: CAAGCAGAGACAGAACAGCATC
	B: GCATGAAGACCTTAAGACCCTGAG	RH2: AGAGGTATTTGTGATAGGGCATG
SARA1	A: GATTGCGTTCCCTCCAGTCGCAG	RH1: TTAACACAAACTCACATTGGTTC
	B: CTGAGTAAGCCTGAACGTTGAGACC	RH2: TAACTGTACACTTAAGATAGGTG
SARA2	A: GCCGTAGTAAGCATTAATAATGTC	RH1: CACTGTTGGGAGGTAATGCTGCC
	B: ACAGAGACTCTTGGCTTCTCAAC	RH2: CAGTGGAAACCTGCCACAACTGC
ARL5	A: TCTTCACTAGAATATGGAGACTG	RH1: CTCAGGACATTGTGTAGCCTATG
	B: TCTATGAGAAGAGGTCAGTAGAG	RH2: AGCTCAGCGATTTGGGAGGTTG

The cDNA expression levels were normalized with the total RNA levels or β-actin.

Radiation Hybrid Mapping and Gene Structures of the Small GTPases

At the 3′ UTR of the cDNA of each small GTPase, a pair of primers was designed (Table 1). The primers were used to type the GB4 radiation hybrid cell panel (Research Genetics). The PCR results were checked by electrophoresis and then used for statistical analysis at Sanger Centre (http://sanger.ac.uk/Rhserver/RHserver.shtml). To determine the gene organization of the nine small GTPases, their cDNAs were further used to search the NR and HTGS division in GenBank for matched human genomic sequences. The gene structure of each gene was determined by aligning the cDNA sequence with the corresponding genomic sequence(s).

RESULTS AND DISCUSSION

Identification of ESTs Homologous to RAB and ARF/SAR1 Families

By aligning the sequences of known human RAB proteins, two highly conserved regions in the family were selected and used to generate consensus sequences (Fig. 1). About 280 homologous ESTs were hit by screening the human EST database with the two consensus sequences of the RAB family. ESTs are considered part of known genes if they share at least 95% identity on BLAST search in NR division of GenBank and are therefore discarded. In contrast, the 59 ESTs that shared 50–95% identity with any known human RAB genes were supposed to represent potential novel RAB family members. The ESTs with overlapping regions were grouped and assembled into EST contigs. Six contigs were further extended toward 5′ and 3′ ends by searching overlapping ESTs in the human EST database, which ultimately generated intact putative open reading frames (ORFs) (Table 2).

The same method was applied to the ESTs found by searching the human EST database with the two consensus sequences of the ARF/SAR1 family. Among the 286 ESTs we obtained, 34 sharing 50–95% identity with the known ARF/SAR1 family members were assembled into 3 EST contigs, which were further extended to cover intact putative ORFs (Table 2).

Cloning of Nine Small GTPases and the Nomenclature

The reliability of the ORFs of the nine contigs was subsequently confirmed by PCR amplification with gene-specific primers (Table 2) and direct sequencing. Both the nucleotide and the deduced amino acid sequences of the ORFs share homology with the known RAB or ARF/SAR1 family members, and the regions corresponding to the two consensus probes used above exhibited high homology (Fig. 2). The nine novel cDNAs were therefore deposited in GenBank database and separately designated as *RAB1B, RAB4B, RAB10, RAB22A, RAB24, RAB25, ARL5, SARA1*, and *SARA2* according to their orthologs in mammals by HUGO Nomenclature Committee.

Amino Acid Sequence Analysis

Besides the sequence similarities, some other sequence characteristics were found in all of the deduced proteins. The deduced proteins all comprise four highly conserved motifs of GTPases, namely G1 [GxxxxGK(S/T)], G2 (T), G3 (DxxG), and G4 [(N/T)(K/Q)xD] (Fig. 2B). G1 motif form a loop in which main-chain amide hydrogens of amino acids and the e-amino group of lysine interact with the α- and β-phosphates of GDP and GTP. Besides, the serine/threonine in G1 interacts with a Mg^{2+} ion, which is coordinated to oxygens of the β- and γ-phosphates of GTP and interacts with a highly conserved threonine in motif G2 and, through an H_2O molecule, with an invariant aspartate in the motif G3. The G4 motif interacts with the guanine nucleotide ring. Thus, all four motifs are critical for guanine nucleotide binding and hydrolysis (3,4,19,24,25).

Most RAB proteins contain two adjacent cysteine residues within one of the following C-terminal se-

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Figure 1. Alignment of the amino acid sequences of known human RAB and SAR1 proteins. The highly conserved residues of the proteins are indicated in black-shaded boxes and similar residues are in gray-shaded boxes. (A) The two consensus sequences of RAB family are generated by aligning 24 family members including RAB1A (NM_004161), RAB2 (M28213), RAB3A (NM_002866), RAB3B (NM_002867), RAB4 (NM_004578), RAB5A (NM_004162), RAB5B (X54871), RAB5C (NM_004583), RAB6 (NM_002869), RAB7 (NM_004637), RAB7L1 (NM_003929), RAB8 (NM_005370), RAB9(NM_004251), RAB11A (NM_004663), RAB11B (NM_004218), RAB13 (NM_002870), RAB27A (NM_004580), RAB27B (NM_004163), RAB28 (NM_004249), RAB30 (NM_014488), RAB31 (NM_006868), RAB32 (NM_006834), and RAB35 (NM_006861). (B) The two consensus sequences of ARF/SAR1 family are generated by aligning nine family members including ARF1 (NM_001658), ARF3 (NM_001659), ARF4 (NM_001660), ARF5 (NM_001662), ARF6 (NM_001663), ARL1 (NM_001177), ARL2 (NM_001667), ARL3 (NM_004311), and ARL4 (NM_001661).

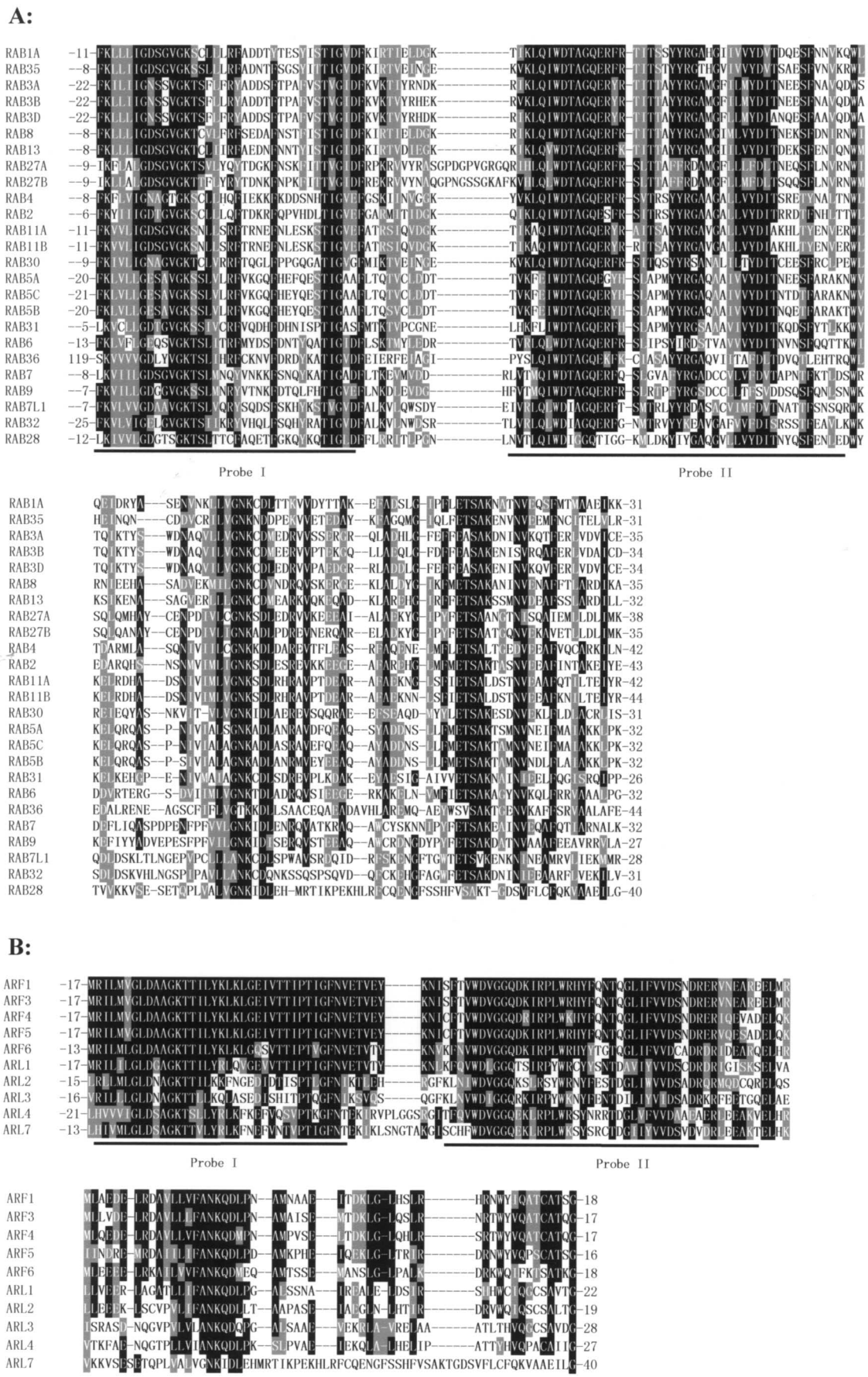
A:
RAB1A
RAB35
RAB3A
RAB3B
RAB3D
RAB8
RAB13
RAB27A
RAB27B
RAB4
RAB2
RAB11A
RAB11B
RAB30
RAB5A
RAB5C
RAB5B
RAB31
RAB6
RAB36
RAB7
RAB9
RAB7L1
RAB32
RAB28
Probe I
Probe II
B:
ARF1
ARF3
ARF4
ARF5
ARF6
ARL1
ARL2
ARL3
ARL4
ARL7
Probe I
Probe II

TABLE 2

PROFILES OF THE NINE SMALL GTPases

Gene	Acc. No.	cDNA Length (bp)	Deduced Protein Length (aa)	Chromosome Loci	BAC Clone	Number of Exons	Gene Length (kb)
RAB1B	AF092437	1038	201	11q13*	AP000630	6	>28.5
RAB4B	AF087861	1193	212	19q13.2-q13.3	AC008537	8	>20.0
RAB10	AF086917	1071	200	2p22.3-p23.1	AC011742	5	>36.7
RAB22A	AF125104	966	194	20q13.3*	HS20_2798	9	>50.1
RAB24	AF087904	911	203	5q35	—	—	—
RAB25	AF274025	1128	217	1q12-1q21.2	AL355388	5	>9.4
SARA1	AF087850	1229	198	5q23-q31.1	AC016284	8	>60.0
SARA2	AF274026	1112	198	10q21.3-q22.1	AC067966	6	>81.7
ARL5	AF087889	1299	179	2q23q24.1	AC069477	7	>26.2

—: There is no information given in the current human genome database about the structure of the gene.
*The cytogenic location of the genes is referred to, or refined by the entry information of the corresponding genomic clones.

quence motifs: -XXCC, -XCXC, or -CCXX, which can be geranylgeranylated by a unique RAB-specific geranylgeranyltransferase (9). This structure feature is also possessed by RAB1B, RAB4B, and RAB10. However, the geranylgeranylation of human RAB24 may be inefficient because it shares the same C-terminus with mouse RAB24 that was reported to be inefficiently geranylgeranylated, which may be partly due to the presence of two histidines distal to the target cysteines (8). Unlike the common C-terminal sequence motif, RAB22A and RAB25 possess unconventional C-termini (-CCISL and -CCISL, respectively).

Unlike most other families of small GTPase, the ARF and SAR proteins have no posttranslational prenyl modifications. There is no cysteine in the C-termini of ARL5, SARA1, or SARA2. Instead, their C-terminal parts were found to match the consensus signature patterns of ARF ([HRQT]-x-[FYWI]-x-[LIVM]-x(4)-A-x(2)-G-x(2)-[LIVM]-x(2)-[GSA]-[LIVMF]-x-[WK]-[LIVM]) and SAR1 (R-x-[LIVM]-E-V-F-M-C-S-[LIVM](2)-x-[KRQ]-x-G-Y-x-E-[AG]-[FI]-x-W -[LIVM]-x-Q-Y) subfamilies, respectively. The corresponding segment is H^{150}QWHIQACCALTGEGLCQGLEWM^{172} in ARL5, R^{171}PLEVFMCSVLKRQGYGEGFRWMAQY^{196} in SARA1, and R^{171}PMEVFMCSVLKRQGYGEGFRW LSQY^{196} in SARA2.

Altered Expression Levels of the GTPases in HCC

Recent research supposed a potential link between endocytosis proteins and cancer (2). Considering the critical roles of members of the RAB and ARF/SAR families in vesicular traffic and high incidence of liver cancer in China, we determined the expression changes of the nine small GTPases at the transcriptional level in liver cancer (Table 3, Fig. 3). The [α-^{32}P]dATP-labeled gene-specific probes were used to hybridize the nylon membranes blotting the samples of total RNA extracted from tumor as well as the adjacent normal tissues. The expression levels of the genes were normalized by the densities of the electrophoresis bands, for β-actin is reported to be overexpressed in liver cancer (5). In each case, overexpression of the small GTPases in liver cancer is defined as exceeding 1.5-fold of expression levels in the adjacent normal tissues. The expression of most small GTPases were either upregulated or remained unchanged in tumor samples. These genes include *RAB1B* (overexpressed in 9/11 cases), *RAB4B* (4/11), *RAB10* (8/11), *RAB22A* (7/10), *RAB24* (9/11), and *ARL5* (5/11). A point worth mentioning is, although the overexprssion of some genes seems limited in a few cases, it is to a large extent due to the strict standard we set. In fact, all seven genes were upregulated with various amounts in almost all the tumor cases, and their overexpression was statistically significant when checked with *t*-test ($p = 0.01$). The overexpression of the small GTPases, however, seems unrelated with the pathological grading of the cases, such as size of cancer, AFP value, or necrosis. It may reflect the pathological requirements of cancer, such as the fast and unlimited growth of cells. Noticeably, *RAB1B, RAB24*, and *SARA1* were overexpressed in 9 out of 11 cancer cases, which suggests that these genes may be markers of HCC.

The two exceptions were *SARA2* and *RAB25*.

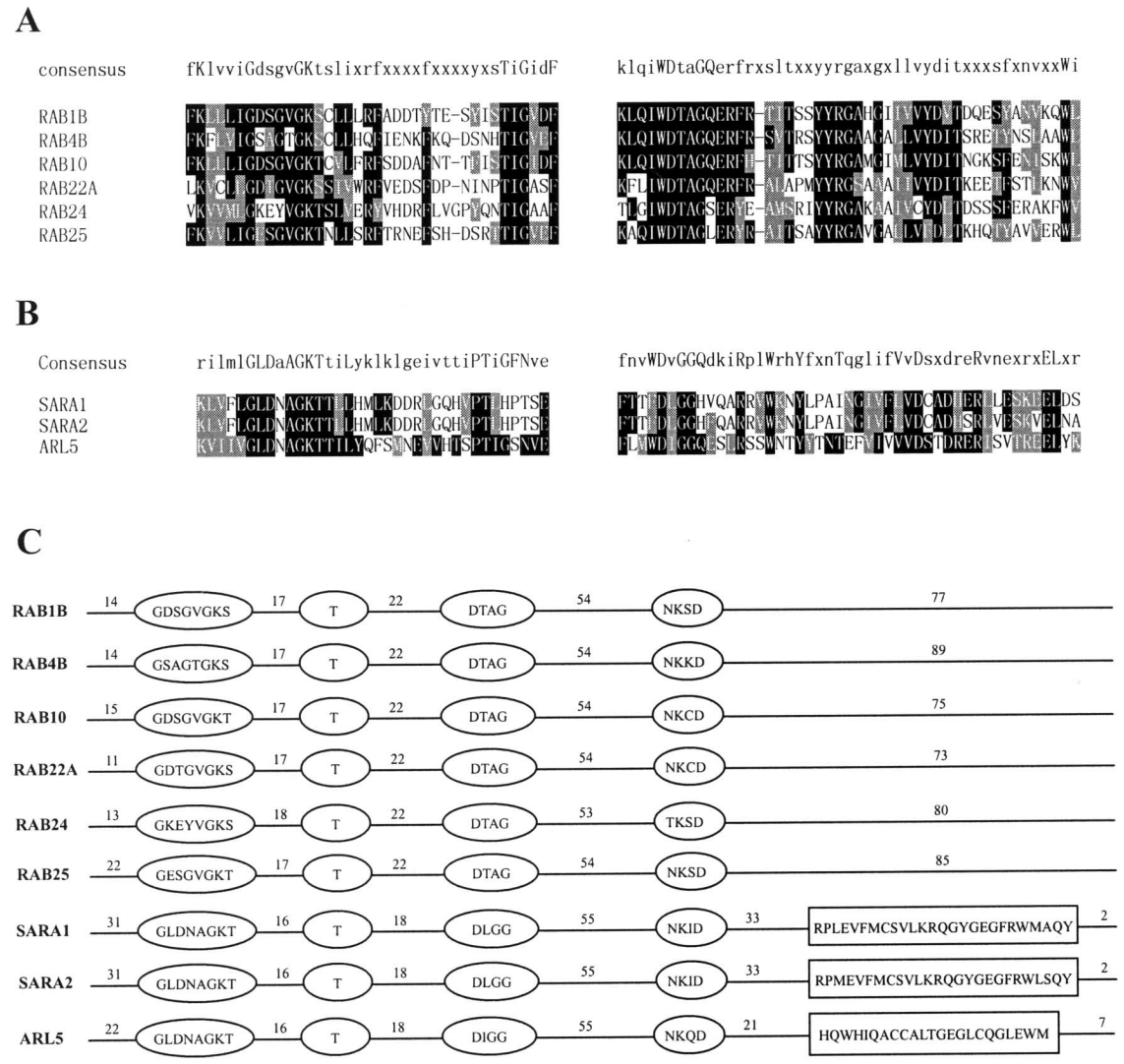

Figure 2. Representation of conserved regions of the small GTPases. The regions corresponding to the two consensus sequences used are shown in (A, RAB subfamily) and (B, ARF/Sar1 subfamily). The upper lanes in each figure show the two consensus sequences of the subfamilies. The lower lanes show the alignment of the novel small GTPases of the two subfamilies. The highly conserved residues of the proteins are indicated in black-shaded boxes and similar residues are in gray-shaded boxes. (C) Schematic shows the conserved domains in the nine small GTPases.

SARA2 was downregulated in three cases (density ratio of tumor/normal < 0.67), upregulated in one case, and remained unchanged in others. Though currently we failed to identify any relationships between its expression level and pathological background of the cases, the inconsistent behavior of the expression of *SARA2* may reflect the in-depth difference between the cases, and further investigation is required. As for *RAB25*, it was highly expressed in cholangiohepatoma, but was downregulated in tumorous liver samples. Therefore, it will be intriguing to study if there is any relationship between the expression of *RAB25* and cholangiohepatoma.

Tissue Expression Patterns of the Small GTPases

Previous researches show that most small GTPases are widely distributed in normal human tissues. However, some tissue-specific or cell type-specific RABs have been identified, often in highly differentiated cells, so it is meaningful to determine the tissue expression patterns of the small GTPases reported here. Northern hybridization with the same probes mentioned above was carried out on MTN membranes (Fig. 4A). The expression levels of the genes were normalized by β-actin and are also schematically shown (Fig. 4B). Two transcripts of different lengths

TABLE 3

EXPRESSION CHANGES OF THE NINE SMALL GTPases IN LIVER CANCER

Genes	Cases											
	HCC01	HCC02	CH	HCC03	HCC04	HCC05	HCC06	HCC07	HCC08	HCC09	HCC10	HCC11
RAB1B	+	+	+	+	n	n	+	+	+	+	+	+
RAB4B	n	+	+	n	n	+	n	n	+	n	n	+
RAB10	+	+	+	+	n	+	+	n	n	+	+	+
RAB22A	+	+	n	+	n	n	n	n	+	+	+	+
RAB24	+	+	+	+	+	n	+	n	+	+	+	+
RAB25	−	−	+	−	−	−	−	−	−	−	−	−
SARA1	+	+	n	n	+	+	+	n	+	+	+	+
SARA2	−	n	−	n	n	n	n	n	n	n	+	−
ARL5	+	+	n	n	n	n	+	n	+	n	n	+

+: The expression level is greater than 1.5-fold of that in the surrounding normal tissues.
−: The expression level is less than 0.66-fold of that in the surrounding normal tissues.
n: The expression level is between 0.66- and 1.5-fold of that in the surrounding normal tissues.

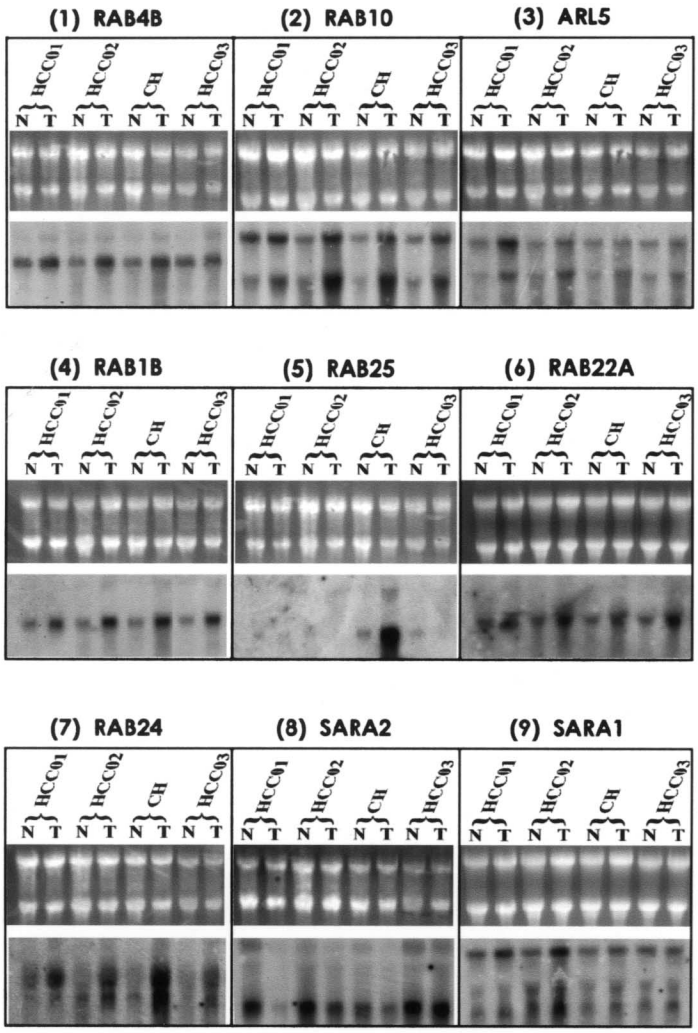

Figure 3. Expressional changes of the nine small GTPases in human liver cancer. Each figure shows the Northern hybridization result of three human liver cancer cases (HCC01–HCC03) and one cholangiohepatoma case (CH) with gene-specific cDNA probes that were marked from 1 to 9. N: normal surrounding tissue, T: tumor tissue. The electrophoresis figures of total RNA extracted from the tissues were used as controls.

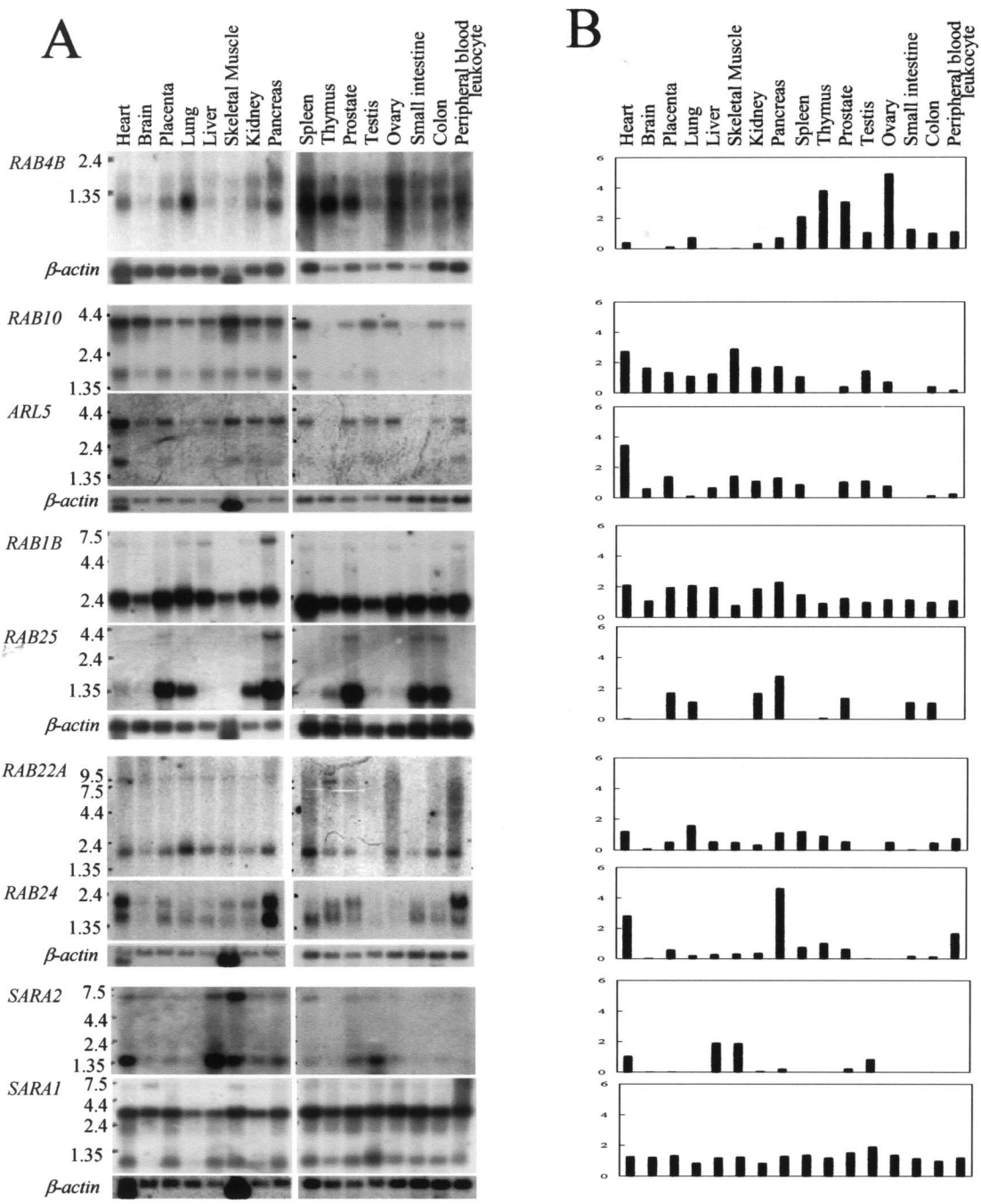

Figure 4. Northern hybridizations of nine small GTPases in human adult tissues. The isotope-labeled probes were used to hybridize MTN membranes. Each lane contains 2 μg poly(A)$^+$ RNA isolated form adult human tissues. The human β-actin cDNA was used as control probe to hybridize the same membrane. (A) Results of the radioautograph of the Northern hybridization. The expression level in each lane was normalized by β-actin and schematically represented in (B).

were observed in all of the nine Northern hybridizations, a phenomenon common to members of small GTPase family. The result also showed that *RAB1B, RAB4B, RAB10, RAB22A, ARL5*, and *SARA2* were expressed ubiquitously, albeit with different amounts, in almost all of the 16 tissues examined.

Unlike those widely expressed genes, the expression levels of *RAB24* were high in pancreas, heart, and peripheral blood leukocyte. Expression of *RAB25* was only detected in placenta, lung, kidney, pancreas, prostate, small intestine, and colon. An interesting point is that, although *SARA1* and *SARA2* share high

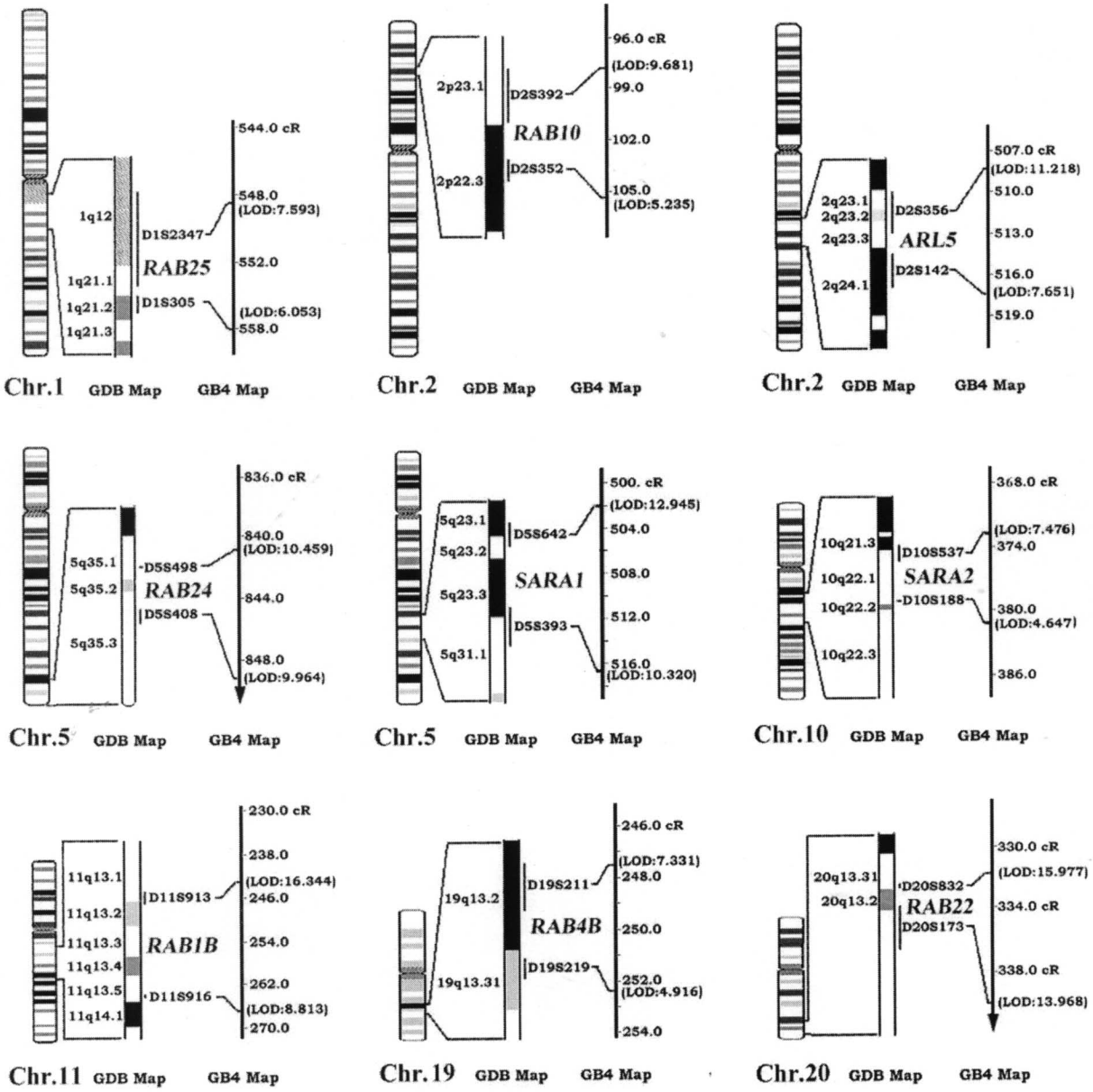

Figure 5. RH mapping of the nine small GTPase genes. Each gene was located between two markers by using multipoint RHMAPPER algorithm. The cytogenetic location of the markers refers to the comprehensive map in GDB database.

sequence similarity at the amino acid level (89.4%), their expression patterns are distinct. *SARA2* was expressed ubiquitously, while *SARA1* was only expressed moderately in heart, liver, skeletal muscle, and testis, and very lowly expressed in other tissues. This fact indicates their different roles in distinct tissues. The tissue-specific distributions of *RAB24, RAB25*, and *SARA1* suggest these proteins not only are involved in different steps of vesicular trafficking, but also play specific roles in various tissues.

Chromosome Loci and Genomic Structures of the Genes

The chromosome loci of the nine small GTPase genes were determined by RH mapping method. After being validated by amplifying the genomic library generated from a normal Chinese adult, each pair of gene-specific primers was used to type GB4 panel. The PCR results were submitted to Sanger Centre for statistic analysis. Figure 5 schematically shows the mapping results of the nine genes.

To determine the genomic structures of the genes we cloned, the cDNA sequences were used to screen the HTGS division in GenBank. Each cDNA was then aligned with the corresponding genomic sequence(s). Table 2 outlines the data of gene structures and chromosome loci of the nine novel small GTPases.

In summary, we identified nine novel human genes that belong to the RAB or ARF/SAR1 subfamily of small GTPases. These genes were found to be upregulated or remain unchanged in most human HCC cases tested by Northern hybridization. The identification of these small GTPases and their altered expression in liver cancer might be valuable for us to understand the vesicular transport system in both normal and tumorous liver tissue.

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