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LETTER TO THE EDITOR

Somatic Mutation of *ARHI* Gene in Hepatocellular Carcinomas

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To the Editor:

Hepatocellular carcinoma (HCC) is an aggressive form of cancer, is the third leading cause of cancer mortality worldwide [1]. Although several novel therapeutic modalities have been developed in recent years, prognosis of advanced HCC remains poor status. The 5-year relative survival rate is about 7 % and causes more than 600,000 deaths annually worldwide [2]. Until now, little is known about the molecular genetic events in the development and progression of HCC.

A Ras homologue member I (ARHI) gene is a maternally imprinted tumor suppressor gene that encodes a 26 kDa GTP-binding protein. It shares 50–60 % amino acid homology with Ras and Rap and is located at human chromosome 1p31 [3]. In contrast to Ras, ARHI inhibits cell growth, motility, and invasion [4, 5]. Recent studies have shown that ARHI is a negative regulator of tumor cell growth in a large number of human cancers [6–8]. For instance, reexpression of *ARHI* gene into cancer cells that truncates signaling through down-regulation of the cyclin D1 and induction of $p21^{WAF1/CIP1}$ [3]. Previous studies have also shown that the expression of ARHI is downregulated in many cancers, including breast carcinomas, ovarian cancers, pancreatic cancer and a few other cancers [9–11]. Furthermore, loss of ARHI expression occur through genetic and/or epigenetic events, including gene mutations, loss of heterozygosity, DNA methylation and histone acetylation [12–15]. All of these findings strongly imply that *ARHI* is a possible candidate tumor suppressor gene that it may contribute to carcinogenesis.

Until now, the important role of ARHI gene in hepatocarcinogenesis has been emphasized. ARHI has pro-apoptotic effects on HCC cell lines [16]. In addition, ARHI overexpression inhibits tumor growth and angiogenesis in HCC xenografts [17]. Furthermore, hypermethylation in ARHI promoter is an important event for the downregulation of HCCs [12]. However, somatic inactivation of the *ARHI* gene have not been reported in HCC. As a possible inactivation mechanism for *ARHI* in HCCs, we analyzed somatic mutation of *ARHI* gene in a series of 38 sporadic HCC tissues from Korean patients.

HCC samples and their corresponding non-cancerous liver tissues of 38 patients were evaluated. This study was approved by the Institutional Review Boards at the Ulsan University Hospital. Frozen tissue samples were ground to a very fine powder in liquid nitrogen. Genomic DNA was prepared using a procedure based on a protocol described previously [18]. Genomic DNA samples from cancer cells and corresponding non-cancerous liver tissues were amplified with 4 sets of primers covering the entire coding region (exon 2) of the *ARHI* gene (Table 1). Numbering of DNA of the *ARHI* was done in respect to the ATG start codon according to the genomic sequence of Genbank accession No. NM_004675. All PCR products in exons 2A–2D of the ARHI gene were screened by single strand conformation polymorphism (SSCP) analysis (Mutation Detection Enhancement; FMC BioProducts, Rockland, ME, USA) with 10 % glycerol and sequencing analysis. After detection of a mutant allele by mobility shifts on SSCP gel, direct DNA sequencing was performed.

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Table 1 Primer sequence for amplifying the coding region of the *ARHI* gene

Name of primer	Nucleotide sequence	Product size (bp)
E2A-F	5′-ACG TAT CTC CCC TCC GAA TC-3′	192
E2A-R	5′-TGT GCA GCA GCG TAC TTT TC-3′	
E2B-F	5′-AAA AGT ACG CTG CTG CAC AA-3′	225
E2B-R	5′-CAG GGT TTC CTT CTT GGT GA-3′	
E2C-F	5′-GAA GGA AAC CCT GGA AGA GC-3′	217
E2C-R	5′-GAA CAG CTC CTG CAC ATT CA-3′	
E2D-F	5′-CCA AGA CCG ATG TGA ATG TG-3′	200
E2D-R	5′-CCA CGT TTT CTA CAC GCT ACA G-3′	

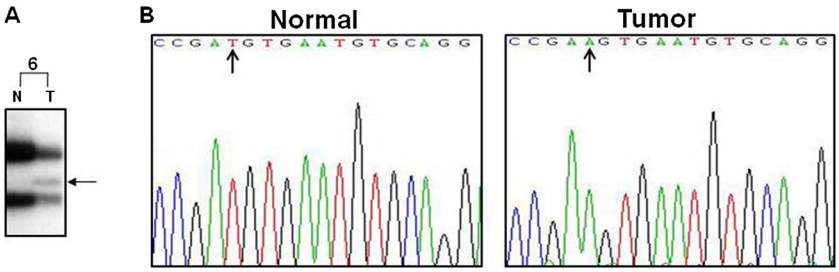

Fig. 1 Representative SSCP and DNA sequencing of ARHI gene mutation. SSCP (**a**) and DNA sequencing analysis (**b**) of ARHI gene from a HCC (Lane T) and its normal tissue (Lane N). (**a**) SSCPs of DNAs from cancer cells (case no. 6) shows one aberrantly migrating band (*arrow*) with wild-type bands. (**b**) Direct DNA sequencing analysis shows T to A transition at nucleotide 555. There is a nucleotide change (*arrow*) in cancer tissue as compared with normal tissue (N, normal; T, tumor)

Finally, we found one (2.6 %) mutation in 38 HCC cases. The mutation was missense mutation: a GAT to GAA transition (Asp → Glu) at codon 185 in exon 2C, which lies out of GTP-binding domain (Fig. 1a and b). The mutation was found in a patient with a HBV positive, cirrhotic background. There was no mutation in corresponding normal DNAs of these cases, indicating that the mutation detected in the cancer cells had risen somatically. We repeated the experiments three times, including PCR, SSCP and sequencing analysis to ensure the specificity of the results, and found that the data were consistent (data not shown).

The present study, we first report that somatic mutation of *ARHI* gene is HCC, however, detected only one *ARHI* mutation. Our data indicate that somatic mutation in a *ARHI* may be rare in HCCs and suggest that somatic mutational events in *ARHI* may not contribute to development of HCCs. Additional studies of large patient populations are need to verify these initial observations and functional analysis of the mutation identified in this study will broaden our understanding of the pathogenesis of HCCs.

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