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Allele-Specific Imbalance of Oxidative Stress-Induced Growth Inhibitor 1 Associates With Progression of Hepatocellular Carcinoma

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BACKGROUND & AIMS: Although there are a few highly penetrant mutations that are linked directly to cancer initiation, more less-penetrant susceptibility alleles have been associated with cancer risk and progression. We used RNA sequence analysis to search for genetic variations associated with pathogenesis of hepatocellular carcinoma (HCC). **METHODS:** We analyzed 400 paired HCC and adjacent nontumor tissues, along with clinical information, from patients who underwent surgery at Sun Yat-Sen University in Guangzhou, China. Total RNA was extracted from tissues and sequenced, and variations with allele imbalance were identified. Effects of variants on cell functions were investigated in HCC cell lines and tumor xenografts in mice. Variants were associated with patient outcomes. **RESULTS:** We found a high proportion of allele imbalance in genes related to cellular stress. A nucleotide variation in the Oxidative Stress-Induced Growth Inhibitor 1 (*OSGIN1*) gene (nt 1494: G–A) resulted in an amino acid substitution (codon 438: Arg–His). The variant form of OSGIN1 was specifically retained in the tumor tissues. Functional assays showed that the common form of OSGIN1 functioned as a tumor suppressor, sensitizing HCC cells to chemotherapeutic agents by inducing apoptosis. However, the variant form of OSGIN1 was less effective. It appeared to affect the translocation of OSGIN1 from the nucleus to mitochondria, which is important for its apoptotic function. The expression pattern and localization of OSGIN1 was altered in HCC specimens, compared with adjacent liver tissue. Levels of OSGIN1 messenger RNA were reduced in 24.7% of HCC specimens, and down-regulation was associated with shorter overall and disease-free survival times of patients. Patients with the OSGIN1 1494A variant had the shortest mean survival time (32.68 mo) among patient subgroups, and their tumor samples had the lowest apoptotic index. **CONCLUSIONS:** We identified *OSGIN1* as a tumor suppressor that is down-regulated or altered in human HCCs. Variants of *OSGIN1* detected in HCC samples reduce apoptosis and are associated with shorter survival times of patients.

Keywords: Apoptosis; Variation; Liver Cancer; Prognostic Factor.

Hepatocellular carcinoma (HCC) is one of the most frequent human malignances worldwide and causes more than half a million deaths every year.[1] The prevalence of HCC varies markedly in different regions, which suggests that multiple genetic and environmental factors play a role in the progression of this disease.[2] Over the past 2 decades, researchers have focused on identifying high-penetrance genes. However, mutations in these highly penetrant genes usually only affect a minority of families and cannot account for most of the cancer risk.[3] Similar to other solid tumors, it is widely accepted that HCC progression is affected by multiple genes; thus, a large number of less-penetrant genes may make a substantial contribution to HCC incidence.[4] Genotypic variations may directly affect the functions of genes relevant to carcinogenesis. Individuals carrying such alleles might suffer from an increased lifetime risk for the disease.[5]

The 2 alleles of a gene usually are expressed in equal amounts in a normal cell; however, high incidences of allele-specific imbalance often are observed in cancer patients.[6] Certain molecular mechanisms, such as allele-specific methylation, loss of heterozygosity, and allele-specific transcription factor binding, may account for this phenomenon.[7,8] The common trait is that the risk alleles usually are overrepresented in cancer cells as a result of selective retention or amplification, and the affected genes usually are critical oncogenes or tumor-suppressor genes that play roles in cancer development. Genomic instability is a major hallmark of human HCC. According to the comparative genomic hybridization data, chromosome segments 1q and 8q frequently are amplified, whereas 1p, 4q, 8p, 16q, and 17p frequently are lost in HCC patients.[9] Many well-known oncogenes and tumor-suppressor genes that have been associated with HCC initiation and progression, such as Cadherin 1 (*CDH1*), *p53, c-myc,* and others, are located within these chromosomal regions.[10] Genetic alterations accumulate during the long-term preneoplastic stages and often result in allele imbalance at these vulnerable loci.

Abbreviations used in this paper: **DFS, disease-free survival; GFP, green fluorescent protein; HCC, hepatocellular carcinoma; NT, nontumor; OS, overall survival; OSGIN, Oxidative Stress-Induced Growth Inhibitor 1; OSG-Wt, wild-type OSG; OSG-V, variant form of OSG; PCR, polymerase chain reaction; SNV, single-nucleotide variation.**

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Better characterization of the chromosomal regions with recurrent allele-specific imbalance during HCC progression may help identify novel risk alleles and critical genes that are related to cancer susceptibility and progression.

In this study, RNA sequencing was used to identify novel single-nucleotide variations (SNVs) and allele-specific imbalances in 3 paired HCC tissues. A novel nucleotide variation was found in the coding region of the *OSGIN1* gene. An allele imbalance of *OSGIN1* has been observed frequently in a cohort of HCC patients, and the common form preferentially was lost in the tumor tissue. Functional assays showed that the variant allele of *OSGIN1* was less effective at inducing apoptosis than the common form, which might be owing to their different abilities to localize to the mitochondria. Furthermore, we found that HCC patients carrying the variant allele have poorer prognoses than the common form carriers. These findings suggested that *OSGIN1* might be an important tumor-suppressor gene that plays a role in the progression of HCC, and the retention of the variant allele might be a risk factor for HCC.

Material and Methods

Clinical Samples, RNA Sequencing, and Data Analysis

Studies using human tissues were reviewed and approved by the Committees for Ethical Review of Research involving Human Subjects of Sun Yat-Sen University and the University of Hong Kong. All patients provided written informed consent for the use of their clinical specimens for medical research. A total of 400 paired human HCC and adjacent nontumor (NT) tissues that were removed surgically and snap-frozen in liquid nitrogen were obtained from the Sun Yat-Sen University Cancer Centre. None of these patients received preoperative chemotherapy or radiotherapy. Total RNA was extracted, and Solexa (Illumina, San Diego, CA) RNA sequencing was performed and analyzed as indicated in a previous study.[11] Allele imbalance was defined as the reads of the variant allele account for at least 60% of the total reads in the complementary DNA library established from the tumor tissues. A full list of the novel nonsynonymous nucleotide variations with allele imbalance is shown in Supplementary Table 1.

In Situ Terminal Deoxynucleotidyl Transferase–Mediated Deoxyuridine Triphosphate Nick-End Labeling Assay and the Detection of Basal Apoptotic Index in Clinical Tissue Slides

Apoptotic cells were detected using an in situ cell death detection kit (Roche, Basel, Switzerland). The nuclei of apoptotic cells were labeled with green signals. Converter reagents were added to the slides and incubated at 37°C for 30 minutes. Finally, the slides were washed with phosphate-buffered saline, stained with diaminobenzidine (Dako, Glostrup, Denmark), and counterstained with hematoxylin. The nuclei of the apoptotic cells were stained with dark brown signals and could be analyzed using a light microscope. The basal apoptotic index was expressed as the ratio of positively stained tumor cells and bodies to all of the tumor cells, expressed as a percentage for each case, and was determined according to previous reports.[12] A minimum of 2000 cells was counted at a magnification of 400×.

Statistical Analysis

Statistical analysis was performed using SPSS version 16 (SPSS, Chicago, IL). A Pearson $\chi 2$ test was used for the categoric variables, and an independent Student t test was used for continuous data. Kaplan–Meier plots and log-rank tests were used for overall survival and disease-free survival analysis, respectively. The independent Student t test was used to compare the cell inhibition rate, cell growth rate, number of foci, colony formation, and the relative expression levels of target genes between any 2 preselected groups. The association of *OSGIN1* expression with the clinical pathologic features was examined using a Pearson $\chi 2$ test. A P value less than .05 was considered statistically significant.

A detailed description of the Materials and Methods can be found in the Supplementary Materials and Methods section.

Results

Identification of a Novel SNV in OSGIN1 and its Allele-Specific Imbalance in HCC Patients

Recent advances in high-throughput, deep-sequencing technology have provided a powerful tool to identify novel genetic changes in the transcriptomes and genomes of cancer cells. By sequencing the whole transcriptome of a tumor tissue and its paired nontumor tissue, information about novel single nucleotide variations, somatic mutations, transcript abundance, and allele imbalance can be discovered.[13] Recently, we sequenced the transcriptome of 3 paired HCC tumor tissues and their adjacent nontumor tissues.[11] After removing the noncoding SNVs and the synonymous SNVs, we found strong allele imbalance in the nonsynonymous coding SNVs in the tumor tissue (Supplementary Table 1). Overrepresentation of those variant alleles in the tumor tissue indicated their functional importance in cancer progression. Gene ontology analysis showed that the genes affected by the allele imbalance were enriched in cell signaling pathways, including cell-cycle regulation, cell death, and the cellular responses to stress and DNA damage (Supplementary Table 2).[14]

OSGIN1 is one gene of interest that showed a high proportion of allele imbalance. A G to A nucleotide substitution at nt 1494 was identified in the coding region of *OSGIN1* and resulted in an amino acid substitution at codon 438 (arginine to histidine). The variant form of *OSGIN1* was expressed dominantly in the tumor tissue (Figure 1*A*). We further checked the genotype of this position in the paired nontumor tissue for the same sample and examined the corresponding genomic DNA. The results showed that the heterozygous G/A form was present in the adjacent nontumor tissue; however, only the 1494A variant was retained in the tumor tissue at both the DNA and messenger RNA levels (Figure 1*B*). To ensure that the 1494A variant