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RESEARCH HIGHLIGHT

Allele imbalance in the transcriptome of human hepatocellular carcinoma: stress-induced gene plays a role

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The two alleles of a gene are usually expressed equally in a normal cell, however, high incidence of allele-specific imbalance is frequently observed in cancer cells. Chromosomal regions with recurrent allele-specific imbalance usually harbor risk alleles and critical genes associated with cancer susceptibility and progression. With the development of large scale transcriptome sequencing technology, systematic analysis of the allele imbalance in the cancer transcriptome could be achieved at the single nucleotide resolution. In the April 2014 issue of *Gastroenterology*, we reported that the allele-specific imbalance of Oxidative Stress Induced Growth Inhibitor 1 (OSGIN1) can significantly contribute to the progression of HCC. OSGIN1 is a stress-induced pro-apoptotic protein. By validating the sequencing results in a cohort of HCC patients, we found the variant 438H form of OSGIN1 was specifically overrepresented in the tumor tissues. Functional studies indicated that OSGIN1 has strong tumor suppressive function in HCC both in vitro and in vivo. The pro-apoptotic function of the variant form of OSGIN1 was found to be less potent than the wild-type form, and the functional defects might be due to its poor efficiency to localize to the mitochondria. Clinical pathological analysis further revealed that the expression and genotype of OSGIN1 are closely associated with the prognosis of HCC patients. Taken together, our study linked the stress-induced genes with allele imbalance in HCC transcriptome, and proposed OSGIN1 to be an important tumor suppressor gene in the progression of HCC. Further characterization of OSGIN1 might help predict the prognosis of HCC patients and their responses to chemotherapeutic drugs.

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The development of hepatocellular carcinoma (HCC) is a multi-step process, which involves accumulation of genetic and epigenetic alterations ^[1]. Both individual genetic background and environmental factors interplay in the pathogenesis of HCC ^[2]. Increasing evidences suggested that small changes in gene expression, which were regarded to be less-penetrant, might substantially contribute to cancer incidence and patient prognosis during cancer progression ^[3]. The alleles with functional defects were usually overrepresented in cancer cells, which resulted in the allele-specific imbalance in the cancer transcriptome, and confer risk to the patients ^[4]. In the April 2014 issue of *Gastroenterology*, we reported the allele-specific imbalance

of Oxidative Stress Induced Growth Inhibitor 1 (OSGIN1) can significantly contribute to the progression of HCC. In this study, RNA sequencing technology was used to analyze the transcriptome of HCC tissues compared with their matched non-tumor liver tissues. High proportion of allele imbalance in genes related to cellular stress was identified. A novel nucleotide variation was found in the coding region of the gene OSGIN1. OSGIN1 was identified as a cell growth inhibitor in response to oxidative stress ^[5]. The allele imbalance of OSGIN1 was frequently observed in a cohort of HCC patients, and the wild type allele was preferentially lost in the tumor tissue. Further functional assays indicated that the variant form of OSGIN1 might have functional defects,

and contributed to the poor prognosis of HCC patients [6]. This study first linked the stress-induced genes with allele imbalance in HCC transcriptome, and proposed OSGIN1 to be an important tumor suppressor gene in the progression of HCC.

Allele imbalance is a major hallmark of cancer. In a normal cell, the two alleles of a gene are usually expressed equally, however, high incidence of allele-specific imbalance is frequently observed in cancer patients [7]. Loss of the wild-type MLH1 gene was found to be a feature of hereditary nonpolyposis colorectal cancer [8]. In breast and ovarian cancer patients carrying BRCA1 mutation, the wild-type allele was also found to be selectively lost [9]. Allele-specific deletions of chromosome3p were found to occur at the early stage in the pathogenesis of lung carcinoma [10]. In colorectal cancer, LOH preferentially affects the normal allele of the well-known tumor suppressor APC, and the risk allele linked to disease is usually retained [3]. Allele-specific expression analysis of APC has been proposed to be an important indicator of pathogenicity of familial adenomatous polyposis patients without APC mutations [11]. In addition to APC, allele-specific expression of TGFBR1 which results in reduced expression of the gene also confers an increased risk of colorectal cancer [12]. In addition to the increased cancer risk, the allele-specific imbalance also affects the prognosis and outcome of cancer patients. Selective loss of the Arg72 allele and retention of the Pro72 allele of p53 was frequently observed in breast cancer and associated with poor overall survival of the patients, and the Arg72 p53 has been proved to be able to induce apoptosis at least five times better than the Pro72 p53 [13, 14]. The monoallelic expression of TP53 and IDH1 has also been found to determine the oncogenic progression and outcome in benign and malignant brain tumors [15]. Multiple molecular mechanisms such as allele-specific methylation (ASM), loss of heterozygosity and allele-specific transcription factor binding (ASTF) may explain the observed allele imbalance in the cancer transcriptome [16, 17].

With the development of large scale transcriptome sequencing, systematic analysis of the allele imbalance in the cancer transcriptome could be achieved at the single nucleotide resolution [18, 19]. This technology greatly contributed to the identification of novel genetic alterations as well as the allele imbalance events in cancer patients. By sequencing the transcriptome of 3 paired HCC tissues, high proportion allele imbalance of the newly identified non-synonymous single nucleotide variations (SNVs) was found in the tumor tissues. Gene ontology analysis revealed that most of the affected genes are associated with signaling pathways related to cell cycle progression and cellular response to stress conditions. This indicated the importance

of stress-induced genes in the pathogenesis of HCC. A nucleotide substitution from G to A at nt1494, which results in an amino acid substitution R438H, was identified in the coding region of OSGIN1. OSGIN1 was first identified as an oxidative stress induced growth inhibitor in mammary gland [5]. Further studies indicated that the expression of OSGIN1 could be significantly up-regulated upon DNA damage, and the activated OSGIN1 can enhance the permeability of mitochondria, thus inducing cell apoptosis [20, 21]. OSGIN1 can directly recruit p53 to mitochondria and the C terminal sequence of OSGIN1 is critical to its sub cellular localization [22]. In this study, the genotype of OSGIN1 was examined in 400 paired HCC tissues. The preferential retention of the variant form in the tumor tissues strongly indicated that it might have functional defects. Further in vitro functional assays and in vivo tumorigenic assays supported that the tumor suppressive functions of the variant form is less potent than the wild-type OSGIN1. The functional defect of the variant form might be caused by its poor efficiency to localize to the mitochondria. Interestingly, the staining of OSGIN1 showed a dot-scattered pattern in the healthy liver or para-tumor liver tissues. However, in the tumor tissues, the expression of OSGIN1 significantly decreased or restricted to the nucleus. The observation of OSGIN1 staining in the clinical samples is in accordance with that in the cell line models. These indicated the localization of OSGIN1 is important for its function. In this study, OSGIN1 was also linked to the chemo resistance to therapeutic drugs. The expression and genotype of OSGIN1 also significantly correlated with the prognosis of HCC patients. These findings suggested that further detection the expression or genotyping of OSGIN1 might help predict the outcome and the response to chemotherapeutic drugs of HCC patients.

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