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Suppression of Noncoding RNAs as Shared Early Genetic Events in Multistep Hepatocarcinogenesis

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Common genetic traits are not well defined for HCC because long-lasting necroinflammation facilitates various genetic errors in hepatocytes prior to hepatocarcinogenesis.

Case Presentation

A 73-year-old Japanese female who had chronic hepatitis C for decades was referred to our hospital due to multiple liver tumors. Contrast-enhanced MRI with gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid classified the tumors into three types based on the combinations of signal intensities in specific sequences and dynamic phases (Fig. 1). One of each type of tumor and surrounding nontumorous (NT) liver tissue was obtained under ultrasound guidance. Histological diagnoses of types 1, 2, and 3 revealed suspicion of dysplastic nodules (DNs), well-differentiated HCC (WEL), and moderately differentiated HCC (MOD), respectively.

Biotinylated sense-strand complementary DNA was generated and hybridized to GeneChip Human Transcriptome Array 2.0 (Affymetrix, Santa Clara, CA). The raw data of 913,605 signals covering 67,528 genes were collected for each sample and deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GSE 153565) after normalization.

In total, 2,855 genes showed at least a 2-fold increase and 3,206 at least a 2-fold decrease in expression in tumor samples compared to NT samples; 57.7%, 12.4%, and 3.9% were specific for MOD, WEL, and DN, respectively (Fig. 2A; transcriptome analysis console 4.0.1). Only 7.3% were up-regulated or down-regulated and shared among all tumor types. Down-regulation was more frequent among these shared genes than among those with expression changes specific for each tumor type ($P < 0.0001$, Fisher's exact test with Bonferroni correction). The dysregulated gene set shared in all tumor types mostly consisted of immune-related genes, noncoding genes including 94 small nucleolar RNAs, or genes with unknown function, with frequencies of 19.7%, 24.4%, or 50.5%, respectively (Supporting Table S1). The principal component analysis (PCA) incorporating all genes for distinguishing tumors from NT defined three dimensions of PCA1 to PCA3, in which MOD, WEL, and DN were radially plotted from NT tissue along almost each dimension (Fig. 2B). Hierarchical clustering of typical genes determining PCA1, PCA2, or PCA3 discriminated MOD, WEL, or NT from

Abbreviations: DN, dysplastic nodules; MOD, moderately differentiated HCC; ncRNA, noncoding RNA; NT, nontumorous; PCA, principal component analysis; WEL, well-differentiated HCC.

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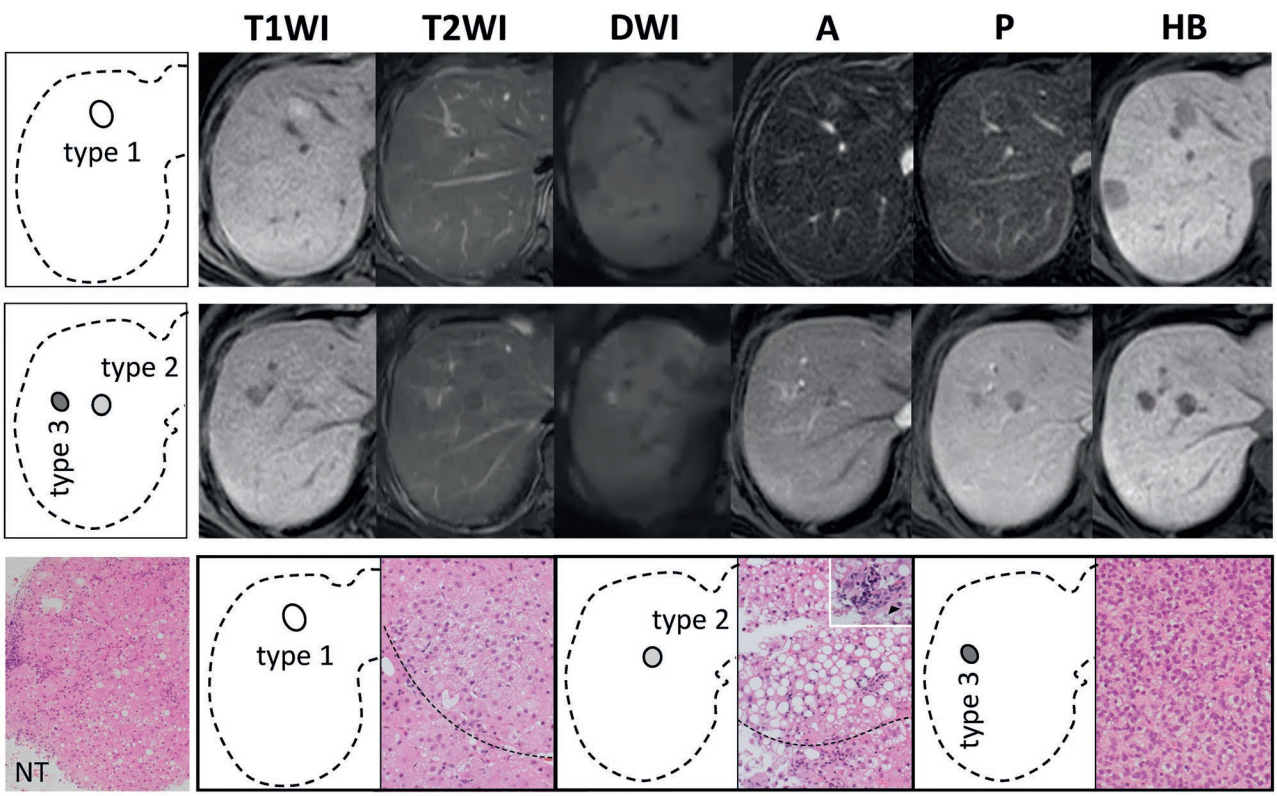

FIG. 1. Contrast-enhanced MRIs using gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid and histological findings. Images and histological findings revealed a suspicion of DN (type 1), WEL (type 2), and MOD (type 3). Upper and middle panels: MRI classified multiple liver tumors into three groups based on combinations of signal intensities in T1-weighted, T2-weighted, and diffusion-weighted b800 images and in arterial, portal, and hepatobiliary phases. In this order, the intensities are high-iso-iso-high-iso-low, low-low-low-low-low-low, and low-high-high-high-low-low in tumors of types 1, 2, and 3, respectively. In terms of arterial and portal in the upper panel, original images were subtracted from images obtained under the same sequence before injection of contrast medium. Lower panel: Microscopic observations of liver biopsy specimens that were obtained from NT liver tissue and type 1, 2, and 3 tumors and stained with hematoxylin and eosin (original magnification ×200 except for NT [×100]). A type 1 tumor involves cells with minimal nuclear atypia and an increase in the nuclear-to-cytoplasmic ratio, as shown above the dotted line. A type 2 tumor that is demarcated by the dotted line comprises cells smaller than those in the surrounding area with mild nuclear atypia and a noticeable change in fat content. Tumor cell infiltration into the portal area was suspected, as shown in the white box (original magnification ×400). The closed arrow indicates an interlobular artery located in the vicinity of the invaded area. A type 3 tumor revealed a thickened trabecular pattern consisting of cells with distinct nucleoli, eosinophilic cytoplasm, and a high nuclear-to-cytoplasmic ratio. Abbreviations: A, arterial; DWI, diffusion-weighted image; HB, hepatobiliary; P, portal; T1WI, T1-weighted image; T2WI, T2-weighted image.

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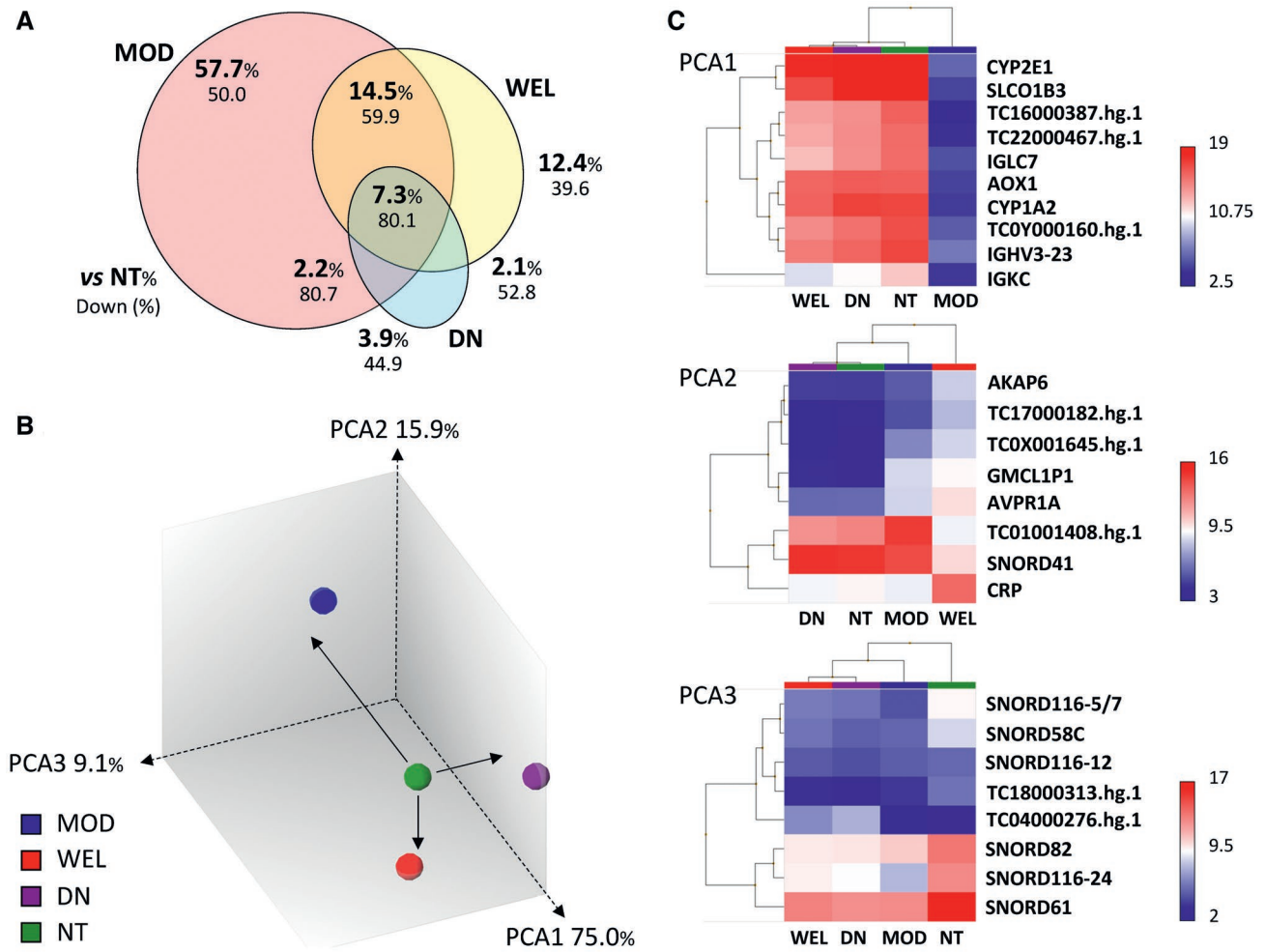

FIG. 2. Genetic traits of suspicious DN, WEL, and MOD. (A) Scheme of the frequencies of genes with 2-fold or more expression changes in tumor versus NT liver tissue and ratios of down-regulated genes in each tumor or their combinations. (B) A plot of the gene expression signature of each tumor and NT in a virtual three-dimensional space structured by three axes of PCA1, PCA2, and PCA3, which were deduced by PCA. The percentage of each axis revealed a ratio of variabilities captured by each axis. (C) The major genes that comprise PCA1, PCA2, or PCA3 were selected (R Project, http://www.r-project.org) and subjected to hierarchical clustering. The color represents the signal intensities of expression on a linear scale. Abbreviations: AKAP6, A-kinase anchoring protein 6; AOX1, aldehyde oxidase 1; AVPR1A, arginine vasopressin receptor 1A; CRP, C-reactive protein; CYP, cytochrome P450; GMCL1P1, germ cell-less 1P1; IGHV3-23, immunoglobulin heavy variable 3-23; IGKC, immunoglobulin kappa constant; IGLC7, immunoglobulin lambda constant 7; SLCO1B3, solute carrier organic anion transporter family member 1B3; SNORD, small nucleolar RNA.

others (Fig. 2C). Functional targets of typical genes include xenobiotics, immunity, and anion transport for PCA1; energy metabolism and complement for PCA2; and noncoding RNAs (ncRNAs) for PCA3.

Discussion

Information for common genetic codes causing each disorder potentiates the development of a specific molecular targeting therapy such as imatinib mesylate for chronic myeloid leukemia. Unfortunately, evaluation of classical HCCs from various patients reveals large genetic variations.[1] Actually, a path from NT tissue through DN and WEL to MOD for each tumor in our case was not observed by PCA; instead, the expression profiles of each tumor deviated from NT tissue along each axis, suggesting that multicentric HCCs may not dedifferentiate by following steps in a common pathway. Overall, minimizing

genetic variations unconnected to carcinogenesis should be critical to elucidate a common genetic event in hepatocarcinogenesis. Comparison of numerous expression profiles from DN, WEL, and MOD with consecutive evaluation of a single liver from the very beginning of hepatocarcinogenesis would be the least biased approach.

Recently, pan-cancer analysis of whole genomes revealed that 25% of cancers carry at least one putative noncoding driver mutation.[2] Consistently, this brief report suggests that approximately one-fourth of expression changes shared with all types of tumors involve ncRNAs, which were major constituents of PCA3. The ncRNAs comprise >90% of the RNAs made from the human genome and play a critical role in cancer. Because small nucleolar RNAs were the most abundant dysregulated ncRNAs that were shared in all tumor types, they were further annotated using the web-based database snoRNA in Cancers (http://bioinfo.life.hust.edu.cn/SNORic).[3] Consequently, several small nucleolar RNAs revealed consistent results of significant repression in HCCs irrespective of histologic grades and pathologic stages (Supporting Fig. S1). The results suggest that errors suppressing expression of noncoding regions may play a key role in the early stage of hepatocarcinogenesis. Although observations in one case may result in an inadequate assessment of biological variabilities and should be confirmed in a larger cohort, molecular targeting of these ncRNAs has the potential to pave a new path for preventing HCC development by overcoming the current limitation of targeted therapies for HCCs at advanced stages, at which genetic traits are highly heterogenous.[1]

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.32077/suppinfo.