

Identification of Genes Associated with Dedifferentiation of Hepatocellular Carcinoma with Expression Profiling Analysis

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To identify the genes associated with dedifferentiation of hepatocellular carcinoma (HCC), gene expression profiles of HCCs of well- and moderately differentiated grades were compared by means of oligonucleotide arrays. One tumor showed a nodule-in-nodule appearance (NIN), which is occasionally observed in the course of progression of HCC from well to less differentiated grade, when an inner, moderately differentiated tumor (MD) develops sequentially from the outer, well-differentiated tumor (WD). Seventy-six genes were identified to be up-regulated more than 3-fold and 33 genes were down-regulated in the inner nodule in NIN. By statistical analysis of the profiles from 10 individual additional liver tumors, 5 WDs and 5 MDs, we were able to identify 12 genes, *LAMA3*, *PPIB*, *ADAR*, *PSMD4*, *NDUFS8*, *D9SVA*, *CCT3*, *GBAP*, *ARD1*, *RDBP*, *CSRP2*, and *TLE1*, with significantly elevated expression, and 4 genes, *CP*, *IL7R*, *CD48*, and *PLGL*, with decreased expression in MD. These selected genes were further validated using another 12 tumors, 5 WDs and 7 MDs, with semi-quantitative RT-PCR. We also applied neighborhood analysis to list the genes with high predictability values as most closely correlated with WD-MD distinction. Seven genes, *ADAR*, *PSMD4*, *D9SVA*, *CCT3*, *GBAP*, *RDBP*, and *CSRP2*, whose expression was elevated and one gene, *IL7R*, whose expression was decreased, were included among the top 50 predictor genes. These genes are likely to be associated with dedifferentiation of HCC and their identification may help to elucidate the mechanism of liver cancer progression.

Key words: Multistep carcinogenesis — Oligonucleotide array — Nodule-in-nodule appearance — Liver cancer progression — Neighborhood analysis

Recent molecular studies have revealed that development of human cancers is a multistep process.¹⁾ In hepatocarcinogenesis, early stage hepatocellular carcinoma (HCC), which is small with indistinct margins and consists of well-differentiated cancerous tissues, occasionally gives rise to less differentiated cancerous tissues in the pre-existing well-differentiated tumor during its progression. The internal tumor continues to develop until it finally replaces the nodule.²⁾ As such dedifferentiation occurs as a step in multistep hepatocarcinogenesis, nodule-in-nodule appearance can be observed in the course of expansive tumor proliferation.³⁾ Thus, a single HCC with nodule-in-nodule appearance (NIN) is best suited to investigate dedifferenti-

ation of HCC, because the inner nodule of lower differentiation grade must have developed sequentially from the well-differentiated outer nodule on the same genetic background.

Thus, multistep hepatocarcinogenesis can be observed in a single tumor macroscopically, although the genetic alterations in each stage are still unknown. In this study, we analyzed the alterations in expression profile of approximately 5600 transcripts in the course of liver cancer progression, using an oligonucleotide array.⁴⁾ Genes which were selected as transcripts with altered expression levels in moderately differentiated hepatocellular carcinoma (MD), when compared to well-differentiated hepatocellular carcinoma (WD) in NIN, were further evaluated by analyzing the expression profiles of 10 independent tumors using oligonucleotide arrays, then validated by analyzing 12 additional tumors by RT-PCR. In addition, we performed neighborhood analysis⁵⁾ to list genes with high predictability values for classification between WD and MD, which included many of the genes we selected above. Our data mining procedure revealed several genes differentially expressed between WD and MD, which have not been reported previously to be associated with dedif-

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Abbreviations: HCC, hepatocellular carcinoma; WD, well-differentiated hepatocellular carcinoma; MD, moderately differentiated hepatocellular carcinoma; NIN, hepatocellular carcinoma with nodule-in-nodule appearance; RT-PCR, reverse transcription-polymerase chain reaction.

All the gene data are available at the following URL: <http://www2.genome.rcast.u-tokyo.ac.jp/database/index.asp>

ferentiation of HCC. Their possible involvement in the progression of HCC is discussed.

MATERIALS AND METHODS

Tissue samples Twenty patients with HCC undergoing hepatectomy in Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo were included in this study after having given their informed consent. Among the 20 hepatectomies, 7 were performed in patients with chronic hepatitis, and the remaining 13 were performed in patients with liver cirrhosis. Twenty-four tumors and corresponding non-cancerous liver tissues were obtained from 20 patients, as three patients had multicentric nodules and one patient had NIN. Serologically, 6 patients were hepatitis B surface antigen positive, and 14 patients were hepatitis C virus positive. On the basis of histological findings of the resected specimen, 11 tumors were diagnosed as WD (ON, W1–W10), which is characterized by increased cell density and indefinite nuclear atypia with or without fatty infiltration, and 13 tumors as MD (IN, M1–M12), which is characterized by increased cell density and definite nuclear and structural atypia. One tumor was diagnosed both macroscopically and histologically as NIN, where the differentiation grade was well-differentiated in the outer nodule and moderately differentiated in the inner nodule (Fig. 1). The specimens were immediately cut into small pieces after resection, snap-frozen in liquid nitrogen, and stored at -80°C in a freezer.

RNA extraction and cDNA synthesis Total RNAs were isolated from tissues with “ISOGENE” (Nippon Gene, Tokyo), according to the manufacturer’s protocol. Five micrograms of purified total RNA was reverse-transcribed into cDNA, using “SuperScript II” (Life Technologies, Palo Alto, CA) with oligo(dT) primer.

RT-PCR amplification Semi-quantitative RT-PCR amplification with *Taq* polymerase was carried out with a denaturing step at 94°C for 30 s, an annealing step at 63°C for 30 s, and an extension step at 72°C for 60 s. The number of PCR cycles for each gene was optimized so as to distinguish the difference of expression levels between WD and MD, i.e., it should be in the exponential phase before the amplification reaches the plateau level. The primer sequences and number of cycles are listed in Table I. Samples were subjected to electrophoresis on 2% agarose gels and stained with ethidium bromide.

GeneChip protocol Experimental procedures for “GeneChip” were according to the Affymetrix (Santa Clara, CA) GeneChip Expression Analysis Technical Manual, as was described in the previous report.⁶⁾ A single expression level for each gene (average difference) was calculated by averaging the differences between matched probes and mismatched probes. Absolute call is judgment of the pres-

ence or absence of gene expression, determined by the GeneChip Suite Software.

Identification of genes with expression change in dedifferentiation and statistical analysis We identified the genes whose expression levels were increased during dedifferentiation in three steps. First, in NIN (ON, IN), after we had eliminated all the genes with absolute call as absent in the inner nodule, we selected over-expressed genes whose expression levels were increased more than 3-fold in the inner nodule, compared with the outer nodule, and were not changed between the outer nodule and the non-cancerous liver tissue. Genes that were decreased were also identified: after eliminating the genes with absolute call as absent in the outer nodule, we selected decreased genes whose expression levels were reduced to less than one-third in the inner nodule. Second, the genes thus selected were evaluated by Mann-Whitney *U* test by comparing the average differences in 10 liver tumors, five WDs (W1–5) and five MDs (M1–5). Differences were considered as significant at $P < 0.05$. Third, validation of these genes with altered expression in MD was carried out by semi-quantitative RT-PCR using 12 additional liver tumors, five WDs (W6–10) and seven MDs (M6–12).

Distinction of MD from WD by neighborhood analysis In order to select the predictor genes which distinguish MD from WD, neighborhood analysis was carried out with 6 WDs (ON, W1–5) and 6 MDs (IN, M1–5), as was described in the previous report.⁵⁾ Briefly, approximately 5600 genes were sorted by their degree of correlation with the histological groups to list 100 genes (predictor genes) with significant predictive values for classification between WD and MD. For each gene, all expression values among samples were calculated according to the following algorithm as *P* values.

$$P \text{ value} = (\mu_1 - \mu_2) / (\sigma_1 + \sigma_2),$$

where μ_1 and μ_2 are the average of each group, and σ_1 and σ_2 are standard deviation of each group. The *P* value is the difference between the two means divided by the sum of the standard deviations. According to the *P* values, the top 50 genes and bottom 50 genes were selected.

RESULTS

To list the genes associated with the dedifferentiation step in liver cancer progression, gene expression profiling analysis was carried out with 12 surgical HCC specimens, that is, the outer and inner nodules in NIN (ON, IN), five WDs (W1–5) and five MDs (M1–5). Semi-quantitative RT-PCR was performed in the outer nodule and inner nodule in NIN (ON, IN) to confirm the results from the second step selection, and five WDs (W6–10) and seven MDs (M6–12) for validation in the third step.

Genes up-regulated in dedifferentiation of HCC

First step: selection of over-expressed genes in NIN (ON, IN) by GeneChip: We selected 2366 genes with absolute call present in the inner nodule in NIN. Among the 2366 genes, we found that expression levels of 94 genes were increased more than 3-fold in the inner nodule, compared with the outer nodule in NIN. Out of these genes, 18 were ruled out because their expression levels in the outer nodule were altered from the non-cancerous tissue, one increased and 17 decreased. Seventy-six genes, therefore, were selected as 'over-expressed genes' in dedifferentiation from WD to MD.

Second step: selection of over-expressed genes using five WDs (W1-5) and five MDs (M1-5) by GeneChip: Using average difference values of the 76 selected genes in the first step, we applied the Mann-Whitney *U* test for statisti-

cal analysis, and identified the following 12 genes as significantly increased in a group of WDs, as shown in Fig. 2; *LAMA3* (L34155), laminin, α 3; *PPIB* (M63573), peptidylprolyl isomerase B (cyclophilin B); *ADAR* (U10439), adenosine deaminase, RNA specific; *PSMD4* (U24704), proteasome 26S subunit 4; *NDUFS8* (U65579), NADH dehydrogenase Fe-S protein 8; *D9SVA* (U95006), D9 splice variant A; *CCT3* (X74801), chaperonin containing TCP1, subunit 3; *GBAP* (J03060), glucosidase β acid, pseudogene; *ARD1* (X77588), N-acetyltransferase; *RDBP* (L03411), RD RNA-binding protein; *CSRP2* (U46006), cysteine and glycine-rich protein 2; *TLE1* (M99435), transducin-like enhancer of split 1. The GeneChip analysis data for the selected 12 genes were confirmed by semi-quantitative RT-PCR for the two nodules in the NIN tumor. Their expression levels from RT-PCR analysis

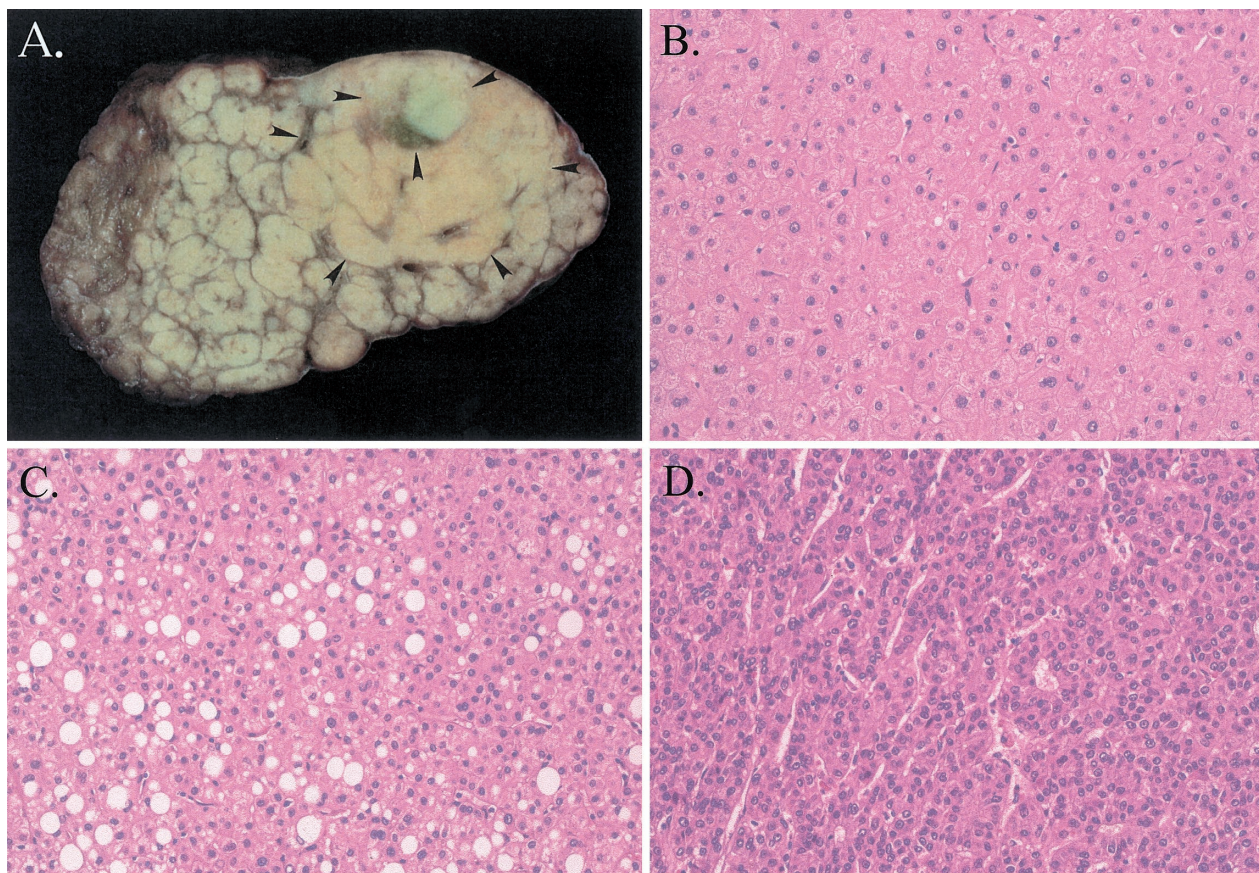


Fig. 1. Pathological findings of the hepatocellular carcinoma with nodule-in-nodule appearance. (A) Macroscopic findings of the tumor. The tumor appeared to be whitish-yellow without capsule formation (arrow), including a green nodule with bile production (arrow). (B) Non-tumor area. (C) Histological findings of the outer nodule, well-differentiated hepatocellular carcinoma. Tumor cells showed resemblance to normal hepatic cells, but decreased cytoplasm, unremarkable nuclear atypia and fatty infiltration. (D) Histological findings of the inner nodule, moderately differentiated hepatocellular carcinoma. Tumor cells showed larger and hyperchromatic nuclei with atypia. The nuclei occupy a relatively greater proportion of the cell.

were apparently concordant with those from GeneChip analysis, although there were relatively subtle differences in *ARD1* and *RDBP* (Fig. 3).

Third step: validation of over-expressed genes using additional WDs (W6–10) and MDs (M6–12) by semi-quantitative RT-PCR: The twelve genes selected above were further examined for validation by semi-quantitative RT-PCR using RNA prepared from 12 additional tumors, five WDs (W6–W10) and seven MDs (M6–M12) (Fig. 3). The intensity of RT-PCR products was higher in a majority of MD cases than in WD cases, which is compatible with the data obtained by GeneChip analysis.

Genes down-regulated in dedifferentiation of HCC Similarly, among 1912 genes with absolute call as present in the outer nodule, 33 were reduced in expression to less than one-third in the inner nodule as compared with the outer nodule of NIN. Among the selected ‘decreased genes’ in the inner nodule, only four were statistically sig-

nificantly decreased in expression levels when compared between five WDs (W1–W5) and five MDs (M1–M5), i.e., *CP* (M13699), ceruloplasmin; *IL7R* (M29696), inter-

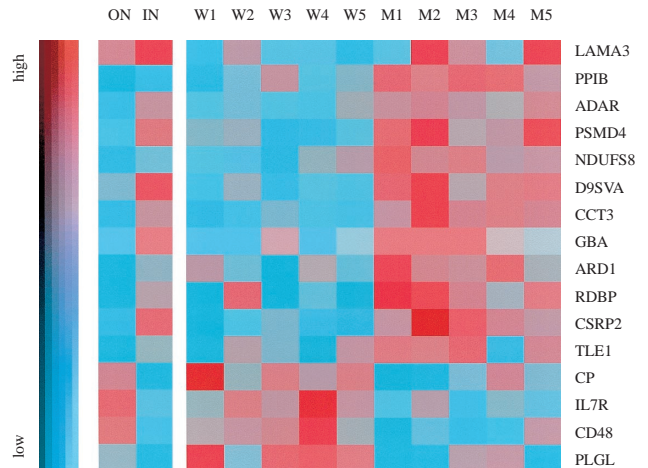


Fig. 2. Genes associated with dedifferentiation of HCC from WD to MD. The 12 genes elevated and the 4 genes decreased in moderately differentiated hepatocellular carcinoma, compared to well-differentiated hepatocellular carcinoma are shown. Each row corresponds to a gene, with the columns corresponding to expression levels in different sample tissues. Expression levels for each gene were normalized across the samples such that the mean is 1 and are shown by relative scales, e.g., expression levels greater than the mean are shown in red, and those below the mean are shown in blue. The scale indicates relative expression above or below the mean. ON, outer nodule in NIN; IN, inner nodule in NIN.

Table I. Oigonucleotide Pairs Used in RT-PCR Experiments

Gene	Sequence	Cycles
<i>LAMA3</i>	5'-AGGGTGCCATTTCTTCAGCCTC-3'	35
	5'-GGGTTCTGGTTTATGCAGTCTCC-3'	
<i>PPIB</i>	5'-CGCAACATGAAGGTGCTCCTTG-3'	25
	5'-CGGTCACCTCAAAGAAAGATGTCCC-3'	
<i>ADAR</i>	5'-CCCATCCATTTCAAGGCTATGAG-3'	27
	5'-CCAGACAGATCATGTGCTGTGG-3'	
<i>PSMD4</i>	5'-CGAATGGAGACTTCTTACCCAC-3'	23
	5'-CCACCTTACCAGCCAAAATC-3'	
<i>NDUFS8</i>	5'-GATCCCGAGATGGACATGAAGTC-3'	23
	5'-CAAGTAGTCAGCCTGGATGTTGG-3'	
<i>D9SVA</i>	5'-GACAAGACCAAAGAAGCAGCAGTC-3'	25
	5'-ATCGCATGGCTGGAAAGGTC-3'	
<i>CCT3</i>	5'-ATTCAAGTCCAGCATCCAGCGG-3'	23
	5'-AATCCAGCAGCACAATGCGAGG-3'	
<i>GBAP</i>	5'-CAAGTCCTTCCAGAGAGGAATGTC-3'	25
	5'-TCAGGGGTGTCTGCATAGGTGTAG-3'	
<i>ARD1</i>	5'-GGACGAGAATGGGAAGATTGTGG-3'	27
	5'-TTGACATCTGTGCTCTCTGTGGTC-3'	
<i>RDBP</i>	5'-TGGTGAAGTCAGGAGCCATCAG-3'	25
	5'-CGCCGTTACAGGAATGAATC-3'	
<i>CSRP2</i>	5'-ACGCAGAAGAGGTGCAGTGTGATG-3'	25
	5'-ATGAACAAGAGCCCTGCTCCTTG-3'	
<i>TLE1</i>	5'-ACAAGAAGCACCAGATGCAG-3'	30
	5'-TTAACGAGGGGTCTATGGCTG-3'	
<i>CP</i>	5'-CCACTGAAGAACAAGTCCTGGG-3'	25
	5'-CACTCCTGGACCTGGAAAAAGG-3'	
<i>IL7R</i>	5'-CACTGACCTGTGCTTTTGAGGAC-3'	30
	5'-CAAGATGACCAACAGAGCGACAG-3'	
<i>CD48</i>	5'-GAAGCATGTGCTCCAGAGGTTG-3'	30
	5'-TGCCATTCTGTGCTCACAG-3'	
<i>PLGL</i>	5'-CAACAACATCCTGGGATTGGGAC-3'	28
	5'-GCATGGATTTGGTAGCCACAGG-3'	

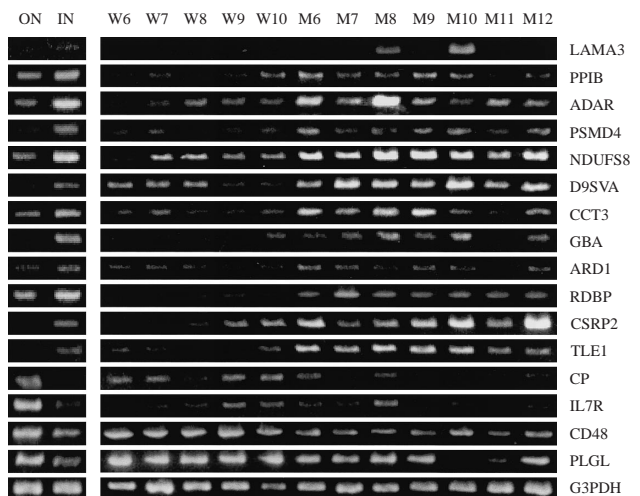


Fig. 3. Semi-quantitative RT-PCR of over-expressed or decreased genes in dedifferentiation from WD to MD. cDNA was synthesized and specific gene segments amplified as indicated in “Materials and Methods.” ON, outer nodule in NIN; IN, inner nodule in NIN.

Table II. Top 30 Up-regulated Genes Distinguishing MD from WD

Predictor genes	P value
proteasome 26S subunit, ATPase, 5	2.774
cytochrome <i>c</i> oxidase subunit VIa polypeptide 1	1.992
chaperonin containing TCP1, subunit 3	1.983
prohibitin	1.803
human D9 splice variant B mRNA	1.753
proteasome subunit, β , type 4	1.733
hydroxyacyl-coenzyme A dehydrogenase, type II	1.697
peptidylprolyl isomerase A	1.662
adenosine deaminase, RNA-specific	1.654
GCN5-like 1	1.591
mitochondrial ribosomal protein L12	1.493
proteasome 26S subunit, non-ATPase, 4	1.473
ribosomal protein S10	1.455
cytochrome <i>c</i> oxidase subunit VIII	1.452
small nuclear ribonucleoprotein D2 polypeptide	1.435
cysteine and glycine-rich protein 2	1.331
laminin receptor 1	1.325
human cell adhesion protein mRNA	1.315
protein kinase C substrate 80K-H	1.312
eukaryotic translation initiation factor 3, subunit 9	1.311
interferon γ receptor 2	1.311
glucosidase, β; acid, pseudogene	1.291
proteasome subunit, β type, 3	1.291
transforming growth factor, β 3	1.286
H3 histone, family 3A	1.286
ubiquitin A-52 residue ribosomal protein fusion product 1	1.257
neural precursor cell expressed	1.248
RD RNA-binding protein	1.236
SMC1-like 1	1.207
uropalakin 1A	1.203

leukin 7 receptor; *CD48* (M37766), CD48 antigen (B-cell membrane protein); *PLGL* (M93143), plasminogen-like (Fig. 2). Semi-quantitative RT-PCR confirmed the difference in expression of the four genes between the outer and inner nodules in NIN (Fig. 3). Then, these four genes were further validated with semi-quantitative RT-PCR using another set of 12 tumors. The RT-PCR data were well correlated with the GeneChip data (Fig. 3).

Selection of the genes distinguishing WD from MD by neighborhood analysis Using neighborhood analysis on the basis of the expression pattern of about 5600 genes, we found the 100 predictor genes, 50 up-regulated genes and 50 down-regulated genes in MD compared to WD, as being most highly correlated with the WD-MD class distinction. Among these predictor genes, 60 genes including the top 30 genes and the bottom 30 genes are listed in Tables II and III with their *P* values.

Table III. Top 30 Down-regulated Genes Distinguishing MD from WD

Predictor genes	P value
B lymphocyte signal transduction gene	-1.699
Id1—also represents	-1.524
inositol polyphosphate-5-phosphatase	-1.481
clock (mouse) homolog	-1.403
stromal cell-derived factor 1	-1.373
integrin, α X	-1.339
matrilin 1, cartilage matrix protein	-1.336
zinc finger protein 38	-1.329
fatty-acid-coenzyme A ligase, long-chain 2	-1.324
nuclear factor-like 2	-1.318
complement component 9	-1.306
phosphodiesterase 6B, cGMP-specific, rod, β	-1.276
immunoglobulin κ constant	-1.275
matrix metalloproteinase 15	-1.272
ephrin-B1	-1.263
protein kinase, cAMP-dependent, catalytic, α	-1.26
mitogen-activated protein kinase kinase 3	-1.252
sex hormone-binding globulin	-1.252
<i>H. sapiens</i> mRNA for zinc finger protein	-1.229
caldesmon 1	-1.218
thrombospondin 1	-1.217
small inducible cytokine subfamily A, member 14	-1.207
purinergic receptor P2X, ligand-gated ion channel, 5	-1.201
cyclin 1	-1.194
mannose-binding lectin 2, soluble	-1.189
integrin, α 1	-1.179
transcription factor 4	-1.167
KIAA0033 protein	-1.165
RAB1, member RAS oncogene family	-1.161
regulator of G-protein signalling 1	-1.157

In this study, in order to identify the genes regulated during dedifferentiation of HCC, we applied two methods described above, that is, the former method based on progression of HCC and the latter by neighborhood analysis. Seven up-regulated genes, *ADAR*, *PSMD4*, *D9SVA*, *CCT3*, *GBAP*, *RDBP* and *CSRP2*, and one down-regulated gene, *ILR7* were selected in common.

DISCUSSION

A model of multistep tumorigenesis was first proposed by Vogelstein *et al.* in colorectal cancer,¹⁾ i.e., genetic alterations accumulate in each stage of colorectal carcinogenesis, such as polyp, atypical polyp, early-stage cancer and advanced-stage cancer. Hepatocarcinogenesis has also multiple steps to advanced HCC, including adenomatous hyperplasia, WD, MD and poorly differentiated HCC, although the genetic alterations in each stage are not fully

understood.³⁾ In this study, we performed gene expression profile analysis on WDs and MDs by GeneChip, and to classify liver tumors, we initially applied hierarchical clustering, as described by other investigators.⁷⁻¹⁰⁾ However, hierarchical clustering was not very effective to discriminate MDs from WDs in our data (data not shown). Because gene expression profiles are regulated by many factors other than dedifferentiation, unknown conditions not correlated with the WD and MD distinction might have affected the clustering pattern, e.g., lot variation of arrays among the analysis, or hypoxic stress before surgical resection. We, therefore, attempted to identify genes correlated with dedifferentiation by using neighborhood analysis and the analysis of a tumor with NIN in which the inner nodule of NIN must have sequentially developed from the outer nodule. By the latter analysis, we identified 12 genes increased and four genes decreased in MD in comparison to WD, which have not been previously described in the process of dedifferentiation of HCC. These 16 genes were examined by semi-quantitative RT-PCR analysis of 12 additional tumors, five WDs and seven MDs, to validate the selection of genes with altered expression in dedifferentiation of HCC. Furthermore, seven up-regulated genes, *ADAR*, *PSMD4*, *D9SVA*, *CCT3*, *GBAP*, *RDBP* and *CSRP2* and one down-regulated gene, *IL7R*, were listed among the top 50 genes with high predictability value as most closely correlated with WD-MD distinction when used for neighborhood analysis⁵⁾ (Tsutsumi, S., unpublished data).

Up-regulation of the nuclear protein TLE1 is intriguing, because TLE1 acts as a co-repressor of AML1, which plays pivotal roles as a transcriptional repressor in myeloid differentiation. TLE1 was also found to influence LEF-1, which is a target of the Wnt signaling pathway by interacting with β -catenin. Another molecule that interacts with TLE1 is hepatic nuclear factor 3b, which is responsible for the basal expression of many liver-specific genes.¹¹⁾ Further study would be required to understand the transcriptional regulation by TLE1.

RDBP is a component of negative elongation factor (NELF) which is a protein factor required for DRB, a classic inhibitor of transcription elongation by RNA polymerase II, although the function of RDBP is unknown.¹²⁾

The N-terminal of the catalytically active 20S proteasome β -subunits appears to be susceptible to inactivation by N-acetylation. Ard1, together with Nat1, comprises the major N-acetyltransferase in yeast.¹³⁾ Up-regulation of ARD1, therefore, might result in inactivation of proteasome activity. PSMD4, a subunit of the 26S protease, binds to ubiquitin polymers and is involved in ubiquitin-dependent and proteasome-mediated protein degradation pathway.¹⁴⁾ Taken together, the results suggest that a protein degradation system acting on a certain substrate is altered in the process of liver cell differentiation.

LAMA3 is α -3 subunit of laminin 5, which, in epithelial basement membranes, forms a complex that functions as a cell adhesion ligand for integrins.¹⁵⁾ CCT3 plays a role in actin and tubulin folding.¹⁶⁾ CSRP2 contains 2 LIM motifs that are implicated in specific protein-protein interactions, particularly involving cytoskeletal components.¹⁷⁾ Presumably, CSRP2 might play a role in liver cell differentiation through interaction with other transcription factors. Altered expression of these genes might be caused through the interaction between liver cancer cells and surrounding cells, then might affect the histological phenotypic patterns of cancer tissues.

PPIB, which enhances the immunosuppressive activity of cyclosporin,¹⁸⁾ plays a role in folding of proteins. Expression levels of PPIB may be increased in accordance with accelerated translation in dedifferentiation of HCC.

ADAR is involved in site-selective RNA editing that changes adenosine residues of target substrate RNAs to inosine.¹⁹⁾ It is critical for embryonic erythropoiesis in the liver, as most ADAR1+/- chimeric embryos died before embryonic day 14 with defects in the hematopoietic system,²⁰⁾ although its role in hepatocarcinogenesis remains unclear. NDUFS8, coding for the TYKY subunit of the human mitochondrial NADH:ubiquinone oxidoreductase, contains two consensus motifs including four cysteines (CxxCxxCxxxCP) that are presumed to ligand two [4Fe-4S] iron-sulfur clusters. This feature makes the TYKY subunit a prominent subunit directly involved in the electron transfer process.²¹⁾ The mechanism of the altered expression of GBAP²²⁾ and D9SVA is unknown.

As for down-regulated genes, expression of CP might be decreased in the course of dedifferentiation, as CP is produced in normal hepatocytes.²³⁾ Cancer-mediated proteolysis of plasminogen generates angiostatin, which is a potent endogenous inhibitor of angiogenesis.²⁴⁾ PLGL, which has a homologous sequence to plasminogen, showed down-regulation. On the other hand, the association of liver cell dedifferentiation and decreased expression of IL-7 and CD48, B-cell membrane protein, remains unclear.^{25, 26)} The present study analyzed the bulk cancerous tissues, which contain many different cell types other than liver cancer cells. Further experiments, such as expression profiling using microdissected tissues, would provide more accurate information about which cell types express those transcripts.

In summary, this study has identified genes with altered expression in dedifferentiation from WD to MD, by means of gene expression profiling analysis. By applying two different data mining methods, we identified genes that showed significantly altered expression, 12 increased and four decreased, in association with dedifferentiation from WD to MD. Further evaluation of those genes would be required to elucidate their possible involvement in dedifferentiation of HCC.

ACKNOWLEDGMENTS

We thank Yoshitaka Hippo and Sarah Kim for critical reading of the manuscript, and Hiroko Meguro and Erio Ashihara for valuable technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research (B) 09557014 and

10470131 and Scientific Research on Priority Areas (C) 12217031 from the Ministry of Education, Culture, Sports, Science and Technology (H. A.)

(Received February 18, 2002/Revised April 2, 2002/Accepted April 5, 2002)

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