Enhanced expression of mRNAs of antisecretory factor-1, gp96, DAD1 and CDC34 in human hepatocellular carcinomas

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Abstract

To identify differentially expressed genes in hepatocarcinogenesis, we performed differential display analysis using surgically resected hepatocellular carcinoma (HCC) and adjacent non-tumorous liver tissues. We identified four cDNA fragments upregulated in HCC samples, encoding antisecretory factor-1 (AF), gp96, DAD1 and CDC34. Northern blot analysis demonstrated that these mRNAs were expressed preferentially in HCCs compared with adjacent non-tumorous liver tissues or normal liver tissues from non-HCC patients. The expression of these mRNAs was increased along with the histological grading of HCC tissues. These mRNA levels were also high in three human HCC cell lines (HuH-7, HepG2 and HLF), irrespective of the growth state. We also demonstrate that sodium butyrate, an inducer of differentiation, downregulated the expression of AF and gp96 mRNAs, supporting in part our pathological observation. Immunohistochemical analysis revealed that gp96 and CDC34 proteins were preferentially accumulated in cytoplasm and nuclei of HCC cells, respectively. Overexpression of these genes could be an important manifestation of HCC phenotypes and should provide clues to understand the molecular basis of hepatocellular carcinogenesis. ß 2001 Elsevier Science B.V. All rights reserved.

Keywords: Hepatocellular carcinoma; Differential gene expression; Differential display; Northern blot analysis; Sodium butyrate; Immunohistochemistry

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world today. Persistent hepatitis B virus (HBV) and C virus (HCV) infections have been closely associated with the development of HCC [1,2]. Other causative factors including dietary consumption of aflatoxins, alcohol and an iron overload have also been reported [3–5]. Recent
advances in molecular biology have revealed various genetic abnormalities in HCC cells. HBV encodes a protein called X (HBx) that can transactivate several cellular genes containing a common DNA motif known as the kX site, and promote transformation of fibroblasts as well as liver cells [6–8]. Overexpression of a HCV core protein can also transform liver cells [9]. Furthermore, overexpression and allelic amplification of c-myc, mutation in codon 12 or 61 of H-, K- and N-ras, activation of c-met oncogenes, aberrations in p53 and Rb tumor-suppressor genes have been observed in some HCC cells [10–17]. However, these findings are, for the most part, depending on precedent knowledge based on gene analyses in viruses or other types of cancers. Comprehensive studies on genetic abnormalities accumulated in the development of HCC have been rare.

In order to study the wide variety of genetic events in HCC cells, we have been screening differentially expressed genes related with human liver carcinogenesis [18]. In this study, as an alternative approach, we used a newly developed differential display (DD) method [19] to identify differentially expressed genes between HCC and non-tumorous liver tissues. Eventually we identified four genes whose transcripts were upregulated specifically along with HCC progression.

2. Materials and methods

2.1. Human liver tissue samples and HCC cell lines

Surgically resected primary HCC and adjacent non-tumorous liver tissues from patients 1–11 and, as controls, normal liver tissues from patient N1 who suffered from liver abscess and from patient N2 who had died of pancreatic cancer, were used. The clinicopathological data of HCC patients are summarized in Table 1.

Three human HCC cell lines, HuH-7, HepG2 and HLF, were obtained from the Japanese Cancer Research Resources Bank.

2.2. Culture of HuH-7, HepG2 and HLF cells

Cells were seeded into 10 cm culture dishes containing Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum (FBS). Culture medium was changed every other day during the experiment. Total RNAs were extracted from these HCC cell lines for Northern blot analysis.

Table 1
Clinicopathological characteristics of HCC patients and the tissue samples

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Viral status</th>
<th>Liver histology</th>
<th>Tumor size (cm)</th>
</tr>
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<tbody>
<tr>
<td>P1</td>
<td>67</td>
<td>M</td>
<td>C</td>
<td>CH</td>
<td>3.0</td>
</tr>
<tr>
<td>P2</td>
<td>71</td>
<td>M</td>
<td>C</td>
<td>CH</td>
<td>7.0</td>
</tr>
<tr>
<td>P3</td>
<td>55</td>
<td>M</td>
<td>B</td>
<td>LC</td>
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</tr>
<tr>
<td>P4</td>
<td>66</td>
<td>F</td>
<td>NBNC</td>
<td>Normal</td>
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</tr>
<tr>
<td>P5</td>
<td>69</td>
<td>F</td>
<td>C</td>
<td>LC</td>
<td>4.0</td>
</tr>
<tr>
<td>P6</td>
<td>65</td>
<td>M</td>
<td>C</td>
<td>LC</td>
<td>7.5</td>
</tr>
<tr>
<td>P7</td>
<td>71</td>
<td>M</td>
<td>C</td>
<td>LC</td>
<td>5.5</td>
</tr>
<tr>
<td>P8</td>
<td>57</td>
<td>M</td>
<td>C</td>
<td>LC</td>
<td>3.0</td>
</tr>
<tr>
<td>P9</td>
<td>63</td>
<td>F</td>
<td>NBNC</td>
<td>LC</td>
<td>1.6</td>
</tr>
<tr>
<td>P10</td>
<td>38</td>
<td>F</td>
<td>NBNC</td>
<td>CH</td>
<td>8.0</td>
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<tr>
<td>P11</td>
<td>79</td>
<td>F</td>
<td>C</td>
<td>LC</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*M, male; F, female.

*C, anti-HCV antibody positive; B, HBV surface antigen positive; NBNC, HBV surface antigen and anti-HCV antibody negative.

*Non-tumor, non-tumorous liver tissue; LC, liver cirrhosis; CH, chronic hepatitis.

*Poor, poorly differentiated; Mod, moderately differentiated; Well, well differentiated HCCs.

*Represented as the largest diameter.

*A mixture of well-to-moderately and poorly differentiated HCC.

*A mixture of moderately and poorly differentiated HCC.
2.3. Sodium butyrate treatment

HuH-7 cells were seeded in DMEM supplemented with 10% FBS and various concentrations of sodium butyrate (0, 0.5, 3 mM). After a 24 h incubation, cells were harvested, and total RNA was extracted.

2.4. Differential display of mRNAs

DD was performed using 50 μg of total RNAs from HCC and non-tumorous liver tissues of patient 5 as described [19]. Briefly, total RNA was extracted from HCC and non-tumorous liver tissues using Iso-gen (Nippon Gene, Tokyo, Japan). Double-stranded cDNA was synthesized using oligo(dT)-latex beads (Takara, Tokyo, Japan). The cDNA was digested with either NlaIII or Sau3AI and ligated with specific adapters. Next, this cDNA was digested crosswise with Sau3AI or NlaIII and ligated with the same adapter pairs. The ligation product was amplified by polymerase chain reaction (PCR) in the presence of [α-32P]dCTP using 0.5 U of Taq DNA polymerase (Pharmacia, Uppsala, Sweden) with 16 pairs of primer sets, 5’-CGA ATG TAC AGG ATA CGC CAT GN-3’ and 5’-CAT AGT CAG TTG CGA CAC GAT CN-3’, where N represents either G, A, T, or C. The cycling parameters were as follows: 96°C for 30 s, 56°C for 30 s, 72°C for 1 min for 25 cycles, followed by 72°C for 10 min. The amplified cDNAs were separated on a 6% polyacrylamide sequencing gel and transferred to Whatman 3MM paper for autoradiographic exposure. The cDNA fragments showing a differential intensity between HCC and non-tumorous liver samples were recovered and re-amplified under the same conditions as in the DD except that no labeled nucleotide was added. PCR products were run on a 6% polyacrylamide gel, stained with ethidium bromide, eluted and used as probes for Northern blot analyses. Amplified cDNA fragments were also cloned into a pUC118 vector for the sequencing analysis.

2.5. Northern blot analysis

Total RNA was denatured and fractionated on a 1.2% agarose gel containing 0.66 M formaldehyde, transferred to a nylon membrane, then cross-linked at 80°C for 2 h. Probe labeling and hybridization were performed as described previously [20]. Specific band intensity was quantitated using a Fuji imaging analysis system, BAS-2000 (Fuji Film, Tokyo, Japan). We used the Wilcoxon signed rank test to compare the band radiointensities of Northern blot analysis. Significance was judged at P < 0.05.

2.6. DNA sequencing and data analysis

Sequence analysis of cDNAs was performed in both directions using M13 forward and reverse primers with a fluorescent automated 377XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were analyzed via the BLAST program for matches in the GenBank database.

2.7. Immunohistochemistry

Paraffin-embedded sections were deparaffinized. HuH-7 cells were grown on slide grasses, and fixed with 4% paraformaldehyde. Immunohistochemistry for albumin, gp96 and Cdc34 proteins was performed using the avidin-biotin-peroxidase complex method. Sections or plates prepared by this method were treated with 0.3% H2O2 in methanol to block endogenous peroxidase. After blocking with 1% skim milk, the sections were incubated with anti-albumin (1:200) (Dako Japan, Kyoto, Japan), anti-gp96 (1:100) (Santa Cruz, CA, USA) or anti-Cdc34 (1:50) (Transduction Laboratories, Lexington, KY, USA) antibodies overnight at 4°C, then washed in phosphate-buffered saline solution. Biotinylated anti-rabbit, anti-goat or anti-mouse IgG, used as secondary antibodies, were added and allowed to react for 60 min at room temperature, followed by strept-avidin-biotinylated peroxidase complex (Vector, Burlingame, CA, USA) for 30 min at room temperature. The sections were incubated with diaminobenzidine-hydrogen peroxide to visualize the reaction products.

3. Results

3.1. Isolation of differential cDNAs between human HCC and non-tumorous liver tissues

DD was performed to identify differentially ex-
pressed genes between HCC and adjacent non-tumorous liver tissues from patient 5, as described previously [19]. Isolated cDNAs were further examined by Northern blot analysis using several HCC samples. Consequently, we focused on antisecretory factor-1 (AF) (GenBank accession No. U24704), gp96 (X15187), DAD1 (D15057) and CDC34 (L22005) mRNAs, which were overexpressed significantly in HCC compared with non-tumorous liver tissues.

3.2. Comparison of mRNA expression in surgically resected human liver tissues

To confirm differential expression of mRNAs, Northern blot analysis using total RNAs from 11 HCC and two non-HCC patients was performed. As shown in Fig. 1A, the expression of AF, gp96, DAD1 and CDC34 mRNAs was significantly higher in at least eight or nine HCC tissues than in adjacent non-tumorous tissues. Although the levels of gp96 and DAD1 in patient N2 were relatively high compared with those of patient N1, these fluctuations of mRNA levels in normal liver tissues were within the range of those in non-tumorous liver tissues from HCC-bearing patients.

In order to analyze these mRNA levels quantitatively, the radiointensity of each mRNA was measured and the HCC/non-tumorous liver (H/L) ratios relative to that of β2-microglobulin (β2m) were plotted in Fig. 1B. Overexpression of these four mRNAs in HCC tissues was statistically significant (P < 0.05). We investigated the correlation between H/L ratios and histological grading. Pathological information indicated that the HCC tissue from patient 1 is a...
mixture of well-to-moderately and poorly differentiated HCC, and from patient 2 is a mixture of moderately and poorly differentiated HCC (Table 1). We tentatively classified them as poorly differentiated HCC, since the expression of mRNAs associated with dedifferentiation would be attributable to the higher grade portion of HCC tissues. As shown in Fig. 1B, the average H/L ratios for AF, gp96, CDC34 and β2m were relatively higher in poorly and moderately-to-poorly differentiated HCCs (patients 1, 2, 6 and 7), lower in well and well-to-moderately differentiated HCCs (patients 3 and 9) and intermediate in most of the moderately differentiated HCCs (patients 5, 8 and 11), though the ratios for these cDNAs remained at basal levels in a moderately differentiated HCC of patients 4 and 10.

These results demonstrate that high expression of AF, gp96, DAD1 and CDC34 might be associated with HCC progression. These mRNA levels seemed to be well correlated with the histological grading.

3.3. Expression of mRNAs in HCC cell lines

Then we examined the expression of these four mRNAs in human HCC cell lines, HuH-7, HepG2 and HLF. Northern blot analysis using total RNAs showed that the levels of these four mRNAs in all three HCC cell lines were higher than in normal liver tissues, consistent with the results using HCC tissues (Fig. 2A). In the previous study, we demonstrated that some differential cDNAs in HCC cells are regulated depending on growth conditions of tumor cells [18]. The expression of the four cDNAs was therefore examined in HepG2 cells in different growth conditions (Fig. 2B). HepG2 cells grew rapidly in 10% FBS until they formed a confluent monolayer. The level of cyclin A mRNA, as an indicator of proliferating HCC cells [21], was high in rapidly growing HepG2 cells in 10% serum (lane 1), whereas it was significantly suppressed in growth-inhibited cells at higher cell density (lane 2). In subconfluent cultures containing 0.1% serum, cells grew slowly, but the growth rate was not as slow as that of overcrowded cells (not shown). In these cells, cyclin A mRNA was not markedly reduced (lane 3). In contrast, the expression of AF, gp96, DAD1 and CDC34 mRNAs was constitutively high in both
rapidly growing and growth-arrested cells in 10% and 0.1% serum, respectively. We also performed these experiments using HuH-7 cells and obtained the same results (not shown).

3.4. Expression of mRNAs in HuH-7 cells treated with sodium butyrate

Our results in Fig. 1B suggest that the expression
Fig. 4. Sections of moderately differentiated HCC (B,D,F) and surrounding non-tumorous liver (NL) (A,C,E) from patient 8. Sections of well-to-moderately differentiated HCC (G) and moderately-to-poorly differentiated HCC (H, right) and adjacent non-tumorous liver (H, left). Sections A and B were stained with hematoxylin and eosin (HE); magnification, ×150. Sections C, D, G and H were immunostained with polyclonal antibody against human gp96; E and F were immunostained with monoclonal antibody against human CDC34; magnification, ×300.
of AF, gp96, DAD1 and CDC34 mRNAs could be correlated with the differentiation status of HCC cells. To evaluate whether the mRNA expression is correlated with liver cell differentiation, we performed Northern blot analysis using total RNAs from HuH-7 cells treated with sodium butyrate, an inducer of differentiation of HCC cells [22]. As already reported [22], the growth rate of HuH-7 cells was markedly reduced by sodium butyrate dose-dependently (the doubling time was reduced from 32 h to 41 h by 0.5 mM and to 590 h by 3 mM of sodium butyrate; data not shown). Accordingly, the expression of cyclin A was suppressed by sodium butyrate in HuH-7 cells (Fig. 3A,B). We further demonstrated that the mRNA expression of α-fetoprotein (AFP) was decreased (Fig. 3A,B), while cytoplasmic albumin immunostaining was markedly increased (Fig. 3C) in HuH-7 cells after butyrate treatment, suggesting that the agent successfully induced differentiated phenotypes in the cells. The mRNA levels of AF and gp96 were reduced by butyrate in a dose-dependent manner, similar to that of AFP mRNA (Fig. 3A,B). However, in contrast with the expression of cyclin A, the expression of AF and gp96 mRNAs was constitutively high irrespective of the growth state in HuH-7 cells (Fig. 2B). We also confirmed that the expression of AFP mRNA was constitutively high in growth-arrested HuH-7 cells (not shown). Therefore, the reduced expression of AF and gp96 mRNAs in butyrate-treated HuH-7 cells was not simply associated with the cell growth rate. On the other hand, the expression of DAD1, CDC34 and β2m, as a control, was unaffected by butyrate. These results suggest that the high levels of AF and gp96 mRNAs are correlated with dedifferentiation of HCC cells.

3.5. Immunohistochemistry of gp96 and Cdc34 proteins

To see the protein level and in vivo manifestation, we performed an immunohistochemical analysis for gp96 and Cdc34 to determine their localization in liver tissue from patient 8. As shown in Fig. 4, cytoplasmic gp96 immunostaining was markedly increased in HCC cells (Fig. 4D) in comparison with that in adjacent non-tumorous liver cells (Fig. 4C). On the other hand, nuclear staining for Cdc34 was observed in some HCC cells (Fig. 4F, arrows), but not in non-tumorous liver cells (Fig. 4E), whereas the reactions for both proteins were negative in fibroblasts in the stroma and in the infiltrated lymphocytes.

To compare the immunoreactivity for gp96 among HCCs of different histological grades, we performed immunostaining of paraffin-embedded HCC tissues from 13 other patients. All of these HCC tissues were more immunostained with gp96 antibody than adjacent non-tumorous liver tissues. Representative results of well-to-moderately differentiated and moderately-to-poorly differentiated HCCs were shown (Fig. 4G,H, respectively). Similar to the result of patient 8 (Fig. 4C,D), cytoplasmic gp96 immunostained granules were markedly increased in number in HCC cells (Fig. 4H, right) in comparison with non-tumorous liver cells (Fig. 4H, left). Furthermore, we observed the tendency that poorly or moderately-to-poorly differentiated HCCs (Fig. 4H, right) exhibited a larger and rougher granular pattern in immunostaining for gp96 in comparison with moderately (Fig. 4D), well or well-to-moderately differentiated (Fig. 4G) HCCs.

4. Discussion

Our results demonstrated that AF, gp96, DAD1, and CDC34 mRNAs were preferentially expressed in HCC tissues relative to non-tumorous liver tissues. We have reported previously that the expression of some mRNAs was differentially regulated depending on cell-cell contact, serum growth factors, or other growth conditions [18]. However, the present results demonstrated that higher expression of AF, gp96, DAD1 and CDC34 mRNAs in HCC cells was not simply reflecting growth states of the HCC cells. As mentioned above, the expression of these mRNAs in a HCC sample from patients 4 and 10 were very low compared to that in other moderately differentiated HCC samples. This may be associated with the extraordinary case history of these patients; the primary tumor had developed without virus infection and cirrhosis compared to other HCCs. Therefore, constitutive upregulation of these mRNAs in moderately differentiated HCCs may be correlated specifically with the chronic process of HCC development. Further upregulation of AF, gp96, DAD1 and
CDC34 mRNAs was observed in poorly and moderately-to-poorly differentiated HCCs, suggesting that the expression is also associated with dedifferentiation of HCC cells. This is, in part, supported by the fact that the expression of AF and gp96 mRNAs was downregulated in HuH-7 cells treated with sodium butyrate. However, inconsistent with the pathological observation, sodium butyrate did not affect the levels of DAD1 and CDC34 mRNAs, suggesting that it may be a possible agent inducing some, but not all, of the differentiating properties. Furthermore, immunohistochemical analysis confirmed the upregulation of gp96 and Cdc34 at the protein levels and demonstrated that they were preferentially located in the cytoplasm and nuclei of HCC cells, respectively. We also observed the tendency that poorly differentiated HCCs exhibited a larger and rougher granular pattern in immunostaining for gp96 in comparison with that of moderately or well differentiated HCCs. Therefore, these manifestations could be a useful marker for the histological diagnosis of HCCs.

gp96, also known as GRP94, is a member of the heat shock protein family localized predominantly in the endoplasmic reticulum (ER) and, in part, the cell surface [23,24]. The expression is induced by glucose starvation, radiation, interferons (INFs), and interleukin 6 (IL-6) [25–28]. In the normal liver from patient N2, who had died of pancreatic cancer, a relatively higher level of gp96 mRNA was observed. This may be caused by adjuvant irradiation therapy which is, in general, recommended after resection of pancreatic cancer. It has been hypothesized that gp96 is a chaperone like GRP78/Bip [29], which is also highly expressed in HCCs (M. Shuda, in preparation). Both genes have several regulatory elements in common in the promoter region, and are induced in coordination by the presence of malfolded proteins in ER [30,31]. Interestingly, gp96 closely resembles tumor-associated heparanase, which has a key role in tumor progression and metastasis [32,33]. This may be associated with the fact that IL-6 can induce gp96 in metastatic breast cancer, but not in the primary cells, and also that tumorigenicity of colon carcinoma cells is increased by gp96 [28,34]. In addition, gp96 has anti-apoptotic activity [35]. Paradoxically, immunogenic peptide-bound gp96, rather than gp96 itself, could be a tumor-rejection antigen [36–38]. Therefore, gp96 could have pleiotropic effects capable of rendering malignant potential to tumor cells while evoking an anti-tumor immune response.

CDC34, also called UBC3, encodes a ubiquitin conjugating enzyme that participates in the ubiquitin-mediated proteolysis of several cyclins or cyclin-dependent kinase (CDK) inhibitors including p27Kip1, and has been shown to play an important role in the regulation of cell cycle progression [39–42]. Since reduced expression of p27Kip1 is correlated with the poor prognosis of several carcinomas including HCC [43–48], upregulation of CDC34 could promote tumor progression by degrading of p27Kip1 in HCC cells. CDC34 is also involved in the degradation of IκB-α, an inhibitor of transcription factor NFκB [49]. Since activated NFκB blocks Fas-associated apoptosis in liver cells, CDC34 may exert anti-apoptotic activity by reducing the level of functional IκB-α [50]. It has been known that CDC34 is localized in both the nucleus and the cytoplasm [51]; however, our results demonstrated that CDC34 was preferentially accumulated in some nuclei of HCC cells. This may be in line with the observation that CDC34 co-localizes with the mitotic spindle in anaphase cells of lower populations [51]. The other possibility is that our monoclonal antibody for CDC34 may not effectively detect the antigen in formalin fixed tissue samples and only a few cells which expressed higher levels of CDC34 were immunostained. Therefore, our results suggest that the molecule may contribute to spindle function at a late stage of mitosis in HCC cells.

AF is a protein secreted from human pituitary gland [52]. This is a potent inhibitor of intestinal hypersecretion and inflammation induced by cholera toxin [53]. Interestingly, the amino acid sequence of AF is virtually identical to that of human S5a, a ubiquitin conjugate binding subunit of 26S protease, although the two mRNAs differ in their 5’ untranslated region [52,54]. Our reverse transcription (RT)-PCR analysis using specific primer pairs for both cDNAs revealed that only a transcript representing AF could be detected in liver tissues from HCC patients (not shown). However, their structural identity suggests that the two gene products could share common functions. Ubiquitin-mediated proteolysis is involved in many cellular processes including cell cycle
progression and transcriptional regulation [55,56]. A homologue of this gene has also been identified as the p44 subunit of the basal transcription factor IIH (TFIIH) [57]. Furthermore, S5a can interact with Id1, a negative regulator for the basic helix-loop-helix transcription factors, and reverse Id1-mediated repression [58]. These facts suggest that the AF/S5a protein could be a potential regulator of transcription factors important for HCC progression. Our results demonstrated the overexpression of components of the ubiquitin-proteasome system, including AF/S5a and Cdc34, in HCCs. This could support the previous report that protein ubiquitination is activated in HCC cells [59]. These findings suggest that comprehensive remodeling of the gene regulation system at the posttranslational level may be involved in HCC progression.

The DAD1 gene was identified originally as a defender against apoptotic death gene in a mutant hamster cell line, with no sequence similarity to any other known cell death suppressor genes [60]. DAD1 mRNA is downregulated in human prostatic tumor cells when they undergo apoptosis by staurosporine, a potent inhibitor of protein kinases [61]. This gene encodes ubiquitously expressed hydrophobic rough ER protein, which consists of 113 amino acids and is a subunit of the mammalian oligosaccharyltransferase (OST) catalyzing N-linked glycosylation of many nascent polypeptides in the ER [62]. Inhibition of this enzyme by tunicamycin can induce apoptosis in human promyelocytic HL-60 cells [63]. Therefore, high expression of DAD1 in HCC cells can activate OST and block apoptosis, thereby enhancing tumor cell survival.

It has been proposed that aberrations of the anti-oncogenes, including p53 and Rb, are common events in advanced liver malignancies [17,64,65]. On the other hand, though there are exceptions in animal systems, the extensive contribution of major oncogenes, including the ras family, has not yet been established in human HCCs [66,67]. Using the DD method, we have identified several genes overexpressed in HCC cells. Functional analysis of these genes could provide further insight into understanding liver carcinogenesis. Furthermore, these genes should be used as tumor markers for diagnosis or indices for the prognosis of HCC patients.

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References

[13] S. Takada, K. Koike, Activated N-ras gene was found in...


E. Schwob, T. Bohm, M.D. Mendenhall, K. Nasmyth, The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *Saccharomyces* Cell 79 (1994) 233–244.


