ABSTRACT

Mutations affecting phosphorylation sites in the β-catenin gene have been implicated in the development of human and rodent hepatocellular carcinomas (HCCs). To further investigate the involvement of this gene in hepatocarcinogenesis, we used several transgenic mouse models of hepatic tumors induced by overexpression of c-myc in the liver either alone or in combination with transforming growth factor (TGF)-α or TGF-β1. Activation of β-catenin, as judged by the presence of mutations and/or nuclear translocation of the protein, was most frequent in liver tumors from c-myc (4/17; 23.5%) and c-myc/TGF-β1 (6/18; 33.3%) transgenic mice. However, it was very rare in faster growing and histologically more aggressive HCCs developed in c-myc/TGF-α mice (1/20; 5%). Administration of diethylnitrosamine, phenobarbital, or 2-amino-3,8-diethylimidazo[4,5-f]quinoxaline did not significantly affect the occurrence of β-catenin mutations. Notably, nuclear accumulation of β-catenin was observed only in adenomas and highly differentiated carcinomas with eosinophilic phenotype. Furthermore, preneoplastic lesions with eosinophilic phenotype frequently displayed focal nuclear positivity, colocalized with areas of high proliferation. In contrast, basophilic and clear-cell foci, as well as pseudoglandular and poorly differentiated HCCs, exhibited a normal or reduced membranous immunoreactivity for β-catenin. These studies suggest that nuclear translocation of β-catenin and activation of Wingless/Wnt signaling may represent an early event in liver carcinogenesis, providing a growth advantage in a subset of hepatic tumors with a more differentiated phenotype.

INTRODUCTION

HCC is the major malignant tumor in human liver and one of the most common visceral cancers worldwide. Primary HCC represents 0.5–2% of all of the cancers in most Western countries, but its incidence is extremely high in certain endemic areas of Southeast Asia and Southern Africa. Although major environmental risk factors have been identified (chronic infections with hepatitis-B virus and hepatitis-C virus, exposure to aflatoxin B1, alcohol consumption, estrogenic steroids, rare genetic disorders, etc.), the molecular mechanisms of hepatic tumorigenesis remain poorly understood (1–4). Genetic alterations of neoplastic development in c-myc/TGF-β1 mice (37, 38). Moreover, coexpression of c-myc and TGF-α transgenes in the liver has been shown to induce a tremendous enhancement of neoplastic development in c-myc/TGF-α mouse liver when compared with both parental lines (36). On the other hand, malignant conversion is prevented by hepatocyte growth factor in c-myc/hepatocyte growth factor double transgenic mice (41). To further our understanding of molecular mechanisms of c-myc-associated hepatocarcinogenesis, we examined the involvement of the Wnt pathway in liver tumor development in these models. For this purpose, we determined the β-catenin mutation frequency and intracellular localization of β-catenin protein in hepatocellular neoplasms formed in different transgenic mouse lines overexpressing c-myc in the liver, either alone or in combination with TGF-α and β1. In particular, we were interested in determining whether the β-catenin activation is transgene-phenotype-, or tumor grade-specific. Furthermore, we investigated whether chemical treatments could increase the β-catenin mutation rate, as described previously (16, 17, 18) in different models. In addition, we compared the protein expression and intracellular localization of β-catenin in cell lines derived from c-myc/TGF-α and c-myc tumors.

MATERIALS AND METHODS

Transgenic Mice and Chemical Treatments. Generation of the Alb/c-myc (c-myc) single-transgenic mice and Alb/c-myc/MT/TGF-α (c-myc/
TGF-α) and Alb/c-myc/Alb/TGF-β1 (c-myc/TGF-β1) double-transgenic mice has been described previously (36–38). DEN (10 μg/g) was administered to eight c-myc/TGF-β1 transgenic mice at 15 days of age (36). Phenobarbital was administered to eight c-myc/TGF-α transgenic mice in food at a concentration of 0.05% (42). Twenty bitransgenic Alb/c-myc/Alb/α-lac Z mice (39) were maintained on an American Institute of Nutrition-76-based diet containing 0.06% (w/w) MeIQx starting from weaning age (39).

Animal housing and care were in accordance with NIH guidelines. Neoplastic and corresponding surrounding liver tissues were obtained from male mice at different ages (12–20 months). Histopathological diagnoses were based upon criteria described by Frith et al. (43).

### DNA and RNA Extraction
DNAs were extracted from frozen liver tissue or paraffin-embedded liver sections, as reported (44, 45). Total RNAs were isolated with guanidinium thiocyanate, followed by centrifugation in cesium chloride solution. Poly(A) RNAs were selected by oligo(deoxy-thymidine)-cellulose chromatography.

### Genomic PCR and RT-PCR
PCR amplifications were performed on a 9600 Perkin-Elmer DNA cycler (Perkin-Elmer Corp., Norwalk, CT) using genomic DNA from 71 samples with a pair of primers (13), PCAT-1F, 5′-TACAGGTAGCATTTTCAGTTCAC-3′ (sense) and PCAT-2R, 5′-TAGCTTCCAAACACAAATGC-3′ (antisense), which produced an amplicon of 296 bp encompassing the putative GSK-3β phosphorylation sites in β-catenin exon 2 (corresponding to human exon 3). The PCR was carried out in a reaction volume of 50 μl consisting of 1.0 μM each primer, 0.2 mM each deoxynucleotide triphosphate, 10 μl of 0.05% (42). Twenty bitransgenic Alb/c-myc/Alb/TGF-β1 mice at different ages (12–20 months). Histopathological diagnoses were based upon criteria described by Frith et al. (43).

### DNA Sequencing
DNA Sequencing. PCR and RT-PCR products were electrophoresed, excised, and purified from 2% TAE-agarose gel using the QIAEX II gel extraction kit (Qiagen, Inc., Valencia, CA) and sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) following the supplied original method. Sequencing was performed in both strands on an ABI 377 sequencer, using primers reported above, and analyzed with Sequencer 4.0.2 and BLAST software. All of the ambiguous and mutated samples (50 ng) were sequenced again using the same primers. A third round of sequencing has been conducted with RT-PCR primers described elsewhere (17) when the results remained doubtful.

### Immunohistochemistry
Immunohistochemical staining was performed on 10% formalin-fixed, paraffin-embedded sections as described previously (14). The mouse monoclonal anti-β-catenin antibody (Transduction Laboratories, Lexington, KY) and the goat anti-axin and anti-conductin polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were used at 1:100 dilution. The immunoreactivity was visualized with the Vectastain Elite ABC Kit (Vector Laboratories) and 3,3′-diaminobenzidine (Dako Corporation, Carpinteria, CA) as the chromogen. Slides were counterstained with Mayer’s hematoxylin. For immunofluorescence, after overnight incubation with the primary antibody, all of the slides were incubated 1 h with fluorescein isothiocyanate (FITC) antibody (Dako Corporation; diluted 1:50) and then counterstained with propidium iodide.

For PCNA immunostaining, deparaffinized sections were incubated in 0.01 M periodate in PBS for 10 min and 0.1 M sodium borohydride in PBS for 10 min to inhibit the endogenous peroxidase activity. Immunolabeling was performed using the PCNA mouse monoclonal antibody (Dako Corporation; dilution 1:100 in PBS). Antibody binding was visualized as reported above. As negative controls, the sections were immunostained omitting the primary antibody.

### RESULTS

#### β-Catenin Mutations
The sequence of β-catenin exon 2, including the GSK-3β phosphorylation consensus motif in control DNA samples, was identical to the GenBank sequence for mouse β-catenin cDNA. Only 4 of 91 tumors in c-myc, c-myc/TGF-α, and c-myc/TGF-β1 transgenic mice revealed point mutations involving the putative phosphorylated codons (Table 1). All of the β-catenin mutations, revealed by PCR analysis of genomic DNA and sequencing, occurred in spontaneous well-differentiated HCCs. Three of four mutations were found within the codon 45 and led to a single base transition TCC to TTC (Ser 45→Phe). This mutation has been observed in human HCC, melanoma, thyroid anaplastic carcinoma, colorectal cancer, and murine hepatoblastoma (13, 19, 20, 23, 24).

The fourth mutation was the commonly occurring transversion TCT to TAT (Ser 33→Tyr) in codon 33, found in both humans and rodents (13–18). No mutations were detected in DEN and phenobarbital-treated mice.

#### β-Catenin Immunohistochemistry
Because signal transduction via β-catenin involves its posttranscriptional stabilization and translocation into the nucleus (26–29), we next performed immunohistochemistry and determined the subcellular localization of β-catenin protein in the same neoplasms and adjacent nontumoral parenchyma. Nontumoral tissue, including dysplastic hepatocytes characteristic for c-myc-overexpressing livers, showed an apparently normal β-catenin

---

Table 1 Summary of β-catenin activation in hepatic tumors developed in different transgenic mouse lines

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>Genetic background</th>
<th>Chemical treatments</th>
<th>β-catenin mutations</th>
<th>Mutated codon</th>
<th>Nuclear expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc/c-myc/Alb</td>
<td>CBA/J × C57BL/6J</td>
<td>No</td>
<td>2/17 (11.7%)</td>
<td>TCC→TTC</td>
<td>4/17 (23.5%)</td>
</tr>
<tr>
<td>c-myc/AlaZ</td>
<td>[CBA/J × C57BL/6J]×[BALB/c × DBA/2]</td>
<td>MelQx</td>
<td>0/20</td>
<td>TCC→TAT</td>
<td>Not determined</td>
</tr>
<tr>
<td>c-myc/TGF-β1</td>
<td>CBA/J × C57BL/6J</td>
<td>No</td>
<td>1/18 (5.5%)</td>
<td>TCC→TTC</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>c-myc/TGF-α</td>
<td>[CBA/J × C57BL/6J]×CD1</td>
<td>DEN</td>
<td>0/8</td>
<td>TCC→TTC</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>1/20 (5%)</td>
<td>TCC→TTC</td>
<td>0/8</td>
</tr>
</tbody>
</table>

---

Downloaded from cancerres.aacrjournals.org on November 9, 2017. © 2001 American Association for Cancer Research.
expression pattern (Fig. 1A). In all of the cases, β-catenin was localized predominantly at the cell membranes and occasionally in the cytoplasm, consistent with previous reports (13, 14, 48). Three of four mutations involving both codons 45 and 33 led to a nuclear accumulation of β-catenin. Strong nuclear and cytoplasmic expression was observed either focally or uniformly throughout the tumor (Fig. 1, B and C). One of the mutations affecting codon 45, found in c-myc/TGF-α HCC, was associated only with a strong albeit very heterogeneous cytoplasmic staining (Fig. 1D).

The inappropriate subcellular distribution of β-catenin was also detected in nine tumors (six well-differentiated HCCs and three HCAs) lacking exon 2 mutations and appeared to be more frequent in liver neoplasms developed in c-myc/TGF-β1 mice (Table 1; Fig. 1F). In tumors with wild-type β-catenin exon 2, only a small fraction of cells, typically 5–10%, showed a strong nuclear and/or cytoplasmic staining, indicating stabilization of the β-catenin protein. The β-catenin nuclei-positive neoplastic cells formed focal areas within HCCs and HCAs that otherwise showed a predominantly normal membranous pattern of staining (Fig. 1B).

To address the possibility that a small number of neoplastic cells displaying nuclear positivity would not allow for detection of β-catenin mutation in the predominantly nuclei-negative tumors, the focal areas of nuclear positivity were microdissected from the unstained paraffin-embedded sections and sequenced. Again, no β-catenin exon 2 mutations were observed, suggesting that other mechanisms might be involved in the nuclear translocation of β-catenin protein. Western blot analysis confirmed that β-catenin was expressed at significantly higher levels in all of the tumors displaying nuclear presence of β-catenin, consistent with immunohistochemical data (Fig. 2), whereas no truncated β-catenins were detected.

**Correlation with Tumor Grade.** In our models, c-myc overexpression either alone or in combination with different growth-factors resulted in the development of hepatic neoplasms with different morphology. The c-myc and c-myc/TGF-β1 transgenic mice mostly exhibited preneoplastic and neoplastic lesions with eosinophilic phenotype. HCAs and HCCs were classified as well-differentiated tumors according to the criteria by Frith et al. (43). In contrast, c-myc/TGF-α and c-myc/βlacZ mice showed predominantly more advanced lesions with basophilic-clear cell phenotype and poorly differentiated grade. Chemical treatments with DEN and phenobarbital did not affect the tumor phenotype, whereas MeIQx induced more aggressive and undifferentiated neoplasms.

The pattern of β-catenin staining correlated with histopathological type of liver tumors. Significantly, β-catenin positive nuclei were found only in HCAs and highly differentiated HCCs with mostly eosinophilic cell phenotype. In addition, these types of lesions showed a cytoplasmic accumulation of β-catenin protein compared with the surrounding parenchyma. β-catenin-nuclear positive cells were localized frequently in the periphery of these neoplasms or in the proximity to blood vessels. Notably, no nuclear accumulation of β-catenin was observed in areas of hemorrhages, necrosis, or pseudo-vascular invasion (considered signs of progression to malignancy), although cytoplasmic immunostaining was frequently detected. In contrast, adenocarcinomas (pseudoglandular pattern) and poorly differentiated HCCs with basophilic-clear cell phenotypes showed an apparently normal membranous and rarely a weak cytoplasmic expression of β-catenin.

In the majority of cases, the intensity of the membranous staining was significantly reduced or totally absent compared with the surrounding parenchyma (Fig. 1E). Consistent with this, among the preneoplastic lesions only eosinophilic foci showed the presence of β-catenin-positive nuclei (Fig. 1G), whereas basophilic and clear-cell foci demonstrated normal or reduced membranous immunoreactivity (Table 2).
Additionally, in all of the tumor samples displaying β-catenin nuclear positivity, we examined the status of axin and its homologue, conductin, which form a large complex with APC and β-catenin involved in the β-catenin down-regulation (30, 31). The immunohistochemical staining with axin or conductin antibodies showed similar expression patterns. Both proteins were present in the cytoplasm of tumoral and surrounding hepatocytes. The expression levels of axin and conductin were slightly increased in eosinophilic lesions and decreased in basophilic-clear cell lesions as compared with the surrounding parenchyma (Fig. 3).

### β-Catenin Activation in Different Transgenic Mouse Lines

Aberrant nuclear translocation of β-catenin with or without exon 2 mutations was found most frequently in c-myc/TGF-β1 (8/26) and c-myc (4/17) liver tumors (Table 1). However, β-catenin mutations were absent in histologically more aggressive HCCs developed in c-myc/ΔlacZ transgenic mice treated with MeIQx (Table 1). Similarly, the fast growing tumors from c-myc/TGF-α mice were rarely affected by β-catenin exon 2 mutations. Only 1 of 20 (5%) spontaneous c-myc/TGF-α HCCs displayed β-catenin mutation, and none were found in eight HCCs after promotion with phenobarbital (Table 1). Previously, we have found that hepatocarcinogenesis in c-myc/TGF-α mice is more aggressive and is frequently associated with loss of TGF-β receptor II signaling (49). Notably, tumors developed in c-myc/TGF-α mice have a predominant basophilic-clear cell phenotype, whereas c-myc and c-myc/TGF-β1 mice show a higher frequency of preneoplastic and neoplastic lesions with eosinophilic phenotype. The latter display an intact TGF-β receptor II signaling and undergo more frequently apoptotic cell death (38, 49). Therefore, we examined a possible link between nuclear translocation of β-catenin protein and up-regulation of cell proliferation in hepatic lesions with eosinophilic phenotype. We found that in preneoplastic foci, as well as in focal areas within well-differentiated HCA and HCCs with eosinophilic phenotype, β-catenin nuclei positivity was frequently associated with PCNA-positive cells as compared with the surrounding parenchyma (Fig. 1H). These results suggest that nuclear translocation of β-catenin may provide a selective growth advantage to a subset of liver tumors.

### Liver Cancer Cell Lines

To further investigate downstream pathways involved in cellular proliferation in the absence of β-catenin activation, we performed Western blot analysis and compared the expression levels of two β-catenin target genes (Cyclin D1 and c-jun/AP-1) in six tumor cell lines derived from c-myc or c-myc/TGF-α HCCs and two human colon cancer cell lines (LS 180 and HCT 116) containing mutations in β-catenin (24, 46). None of the liver cancer cell lines showed mutations involving the β-catenin exon 2 or protein accumulation in the nucleus. The levels of β-catenin were clearly lower in mouse cell line compared with that in colon cancer cell lines. However, the expression of both cyclin D1 and c-jun/AP-1 was strongly up-regulated in all of the examined mouse cell lines, particularly in those derived from c-myc/TGF-α tumors (Fig. 4).

---

**Table 2: Correlation of β-catenin expression levels with type of preneoplastic and neoplastic hepatic lesions**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Hepatic lesions</th>
<th>Overexpression</th>
<th>Normal</th>
<th>Down-regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilic</td>
<td>Foci (n = 46)</td>
<td>22 (47.8%)</td>
<td>24 (52.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenomas (n = 25)</td>
<td>15 (60%)</td>
<td>10 (40%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcinomas (n = 18)</td>
<td>11 (61.1%)</td>
<td>7 (38.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foci (n = 27)</td>
<td>8 (29.6%)</td>
<td>19 (70.3%)</td>
<td></td>
</tr>
<tr>
<td>Basophilic-clear cell</td>
<td>Adenomas (n = 28)</td>
<td>8 (28.6%)</td>
<td>20 (71.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcinomas (n = 20)</td>
<td>4 (20%)</td>
<td>16 (80%)</td>
<td></td>
</tr>
</tbody>
</table>

*Eosinophilic foci and neoplastic lesions displayed mainly overexpression of β-catenin, whereas the majority of basophilic-clear cell foci, HCAs, and HCCs showed reduced β-catenin levels.*
Considering that the c-myc was overexpressed in these cell lines, these data suggest that the up-regulation of nuclear β-catenin-specific target genes can occur in the absence of β-catenin activation during liver carcinogenesis.

**DISCUSSION**

Interstitial deletions and missense mutations affecting β-catenin genes have been identified in rat, mouse, and human HCCs with variable frequency. In this study, only 4 of 91 hepatic tumors in c-myc, c-myc/TGF-β1, and c-myc/TGF-α transgenic mice showed the presence of genetic alterations at the β-catenin putative phosphorylation sites, harboring all of the known β-catenin mutations (13, 16, 17). Three tumors had a mutation affecting codon 45 and resulting in TCC to TTC (Ser→Phe) transition. This type has been found in numerous human malignancies and murine hepatoblastoma but not in mouse HCC (13, 19, 20, 23, 24). The mutation in the remaining tumor was located at codon 33 in a specific serine residue corresponding to a known GSK-3β target.

β-Catenin mutation frequency found in either c-myc, c-myc/TGF-α, or c-myc/TGF-β1 liver tumors (5–12%) was lower than in HCCs derived in t-type pyruvate kinase/H-ras, t-type pyruvate kinase/c-myc, and woodchuck hepatitis virus/c-myc transgenic mice (40–55%; Ref. 13). Our data are more in accord with the absence of β-catenin mutations in HCCs from SV40 T-antigen transgenic mice (50). The reason for a broad variability in the β-catenin mutation frequency in different transgenic models of liver cancer is unclear. Because all of the transgenic mouse lines were generated in various genetic backgrounds, one possibility is that the deregulation of β-catenin gene might be strain specific. However, it seems more likely that the observed difference indicates that several genetic pathways might support the development of liver cancer in rodents as well as in humans.

Our results are consistent with the observations of Legoix et al. (51), who found a significant correlation between high frequency of β-catenin mutations and a low rate of LOH in human HCCs. Previous work from our laboratory has shown the presence of frequent and nonrandom deletion of chromosomes 1, 4, 6, 7, 9, and 12 during the early stages of c-myc/TGF-α hepatocarcinogenesis (47). As reported for colorectal cancer (52–54), our data might suggest the existence of two major genetic pathways of neoplastic development in the liver. The first was characterized by disruption of Wnt signaling not limited to activating β-catenin mutation and associated with a low rate of LOH. The second, probably the most common, would be characterized by an intact Wnt signaling and presence of a mutator phenotype and chromosomal instability, as observed in the c-myc/TGF-α tumors as well as in human HCCs (10–12, 51).

Immunohistochemical analysis revealed the nuclear accumulation of β-catenin in 12 cases, although only 3 of them had a β-catenin mutation. The absence of mutations in the other nine samples suggests that other mechanisms might be involved in nuclear translocation of the protein and, therefore, in its activation. Numerous data indicate that APC inactivation is uncommon in human and rodent HCCs (55, 56). Recently, Satoh et al. (57) observed rare axin mutations at the β-catenin-binding site in human HCCs that showed nuclear accumulation of the protein in the absence of β-catenin mutations. Genomic mutations affecting GSK-3β, conductin, or other members of the Wnt-signaling pathway might determine β-catenin nuclear translocation.

Nuclei positive for β-catenin protein were detected exclusively in hepatocellular lesions with eosinophilic phenotype. This nuclear translocation of β-catenin was observed in foci as well as in adenomas and carcinomas, suggesting that β-catenin activation is an early event in mouse liver tumor development. We have shown previously (38, 49) that the hepatic lesions with eosinophilic phenotype have intact TGF-β receptor II signaling and demonstrate frequent apoptotic cell death. Although the significance of this relationship is unclear and remains to be investigated, it is tempting to speculate that activation of Wingless/Wnt cascade may give a selective proliferative advantage for preneoplastic and neoplastic cells exposed to TGF-β1. Little is known about molecular mechanisms connecting β-catenin and TGF-β1. Recently, it has been shown that β-catenin and lymphoid enhancer factor-1/Tcf may form a complex with Smad 4 to synergistically induce gene expression during Xenopus embryogenesis (58). Because members of both Wnt and TGF-β1 superfamily are involved in the regulation of cell growth (59–61), a constitutive deregulation of the Wnt/TGF-β signaling may play an important role in liver tumor development. Consistent with this, β-catenin nuclear accumulation was found more frequently in c-myc/TGF-β1 (33.3%) than in c-myc mice (23.5%). Both transgenic lines exhibited the same tumor latency (9–10 months); however, the development of HCC occurred more rapidly in c-myc/TGF-β1 than in c-myc mice. By 13 months of age, 100% of double transgenic mice displayed multifocal neoplasms compared with 60% among c-myc monotransgenics, which developed on average only one to two tumors (38, 40). Taken together with the fact that the presence of β-catenin nuclear positivity was closely associated with areas of high cell proliferation, these data suggest that activation of the Wnt pathway might contribute to the rapid rise in tumor incidence in c-myc/TGF-β1 mice.

Interestingly, untreated and phenobarbital-treated c-myc/TGF-α transgenic mice developed mostly basophilic-clear cell foci and poorly differentiated HCCs (40, 42) that displayed normal membrane localization of the β-catenin protein. Moreover, the rapid progression from early preneoplastic focal lesions to poorly differentiated HCCs was accompanied by a gradual down-regulation of β-catenin protein associated with the steady loss of the entire chromosome 9 (47) or its band 9E1, where β-catenin is located (62). Similarly, MelQx-fed c-myc transgenic mice developed low-grade HCCs more rapidly and with a higher incidence than untreated c-myc mice (39). Again, no β-catenin mutations were detected in these tumors, suggesting that MelQx-induced mutations were sufficient to induce the neoplastic development. In agreement with these data, we did not find any increase in the rate of β-catenin mutations or nuclear accumulation of β-catenin in DEN-treated c-myc (data not shown) and c-myc/TGF-β1 mice. These observations demonstrate an association be-

---

4 V. M. Factor, unpublished observations.

---

Fig. 4. Western blot analysis of β-catenin, Cyclin D1, and c-jun/AP-1 protein in mouse liver- and human colon cancer-derived cell lines. Lanes 1–5, cells line derived from c-myc/TGF-α HCCs; Lane 6, cell line derived from c-myc HCC; Lanes 7 and 8, HCT 116 and LS 180 human colon cancer cell lines. β-catenin protein levels were higher in colon cancer cell lines with mutated β-catenin gene (Lanes 7 and 8) than in liver cancer cell lines with wild-type β-catenin (Lanes 1–5). Expression levels of Cyclin D1, c-jun, and AP-1, known β-catenin target genes, were elevated in both liver and colon cancer cell lines.
between β-catenin activation, long latency, and a more differentiated histotype of liver tumors.

Nuclear translocation of β-catenin has been shown to result in activation of numerous Wnt target genes (c-myc, Cyclin D1, AP-1/c-jun, Wisp) involved in regulating proliferation and apoptosis (34, 35). To further investigate the status of β-catenin target genes in samples lacking β-catenin activation, we compared expression levels and nuclear immunoreactivity of β-catenin in six c-myc/TGF-α and c-myc tumor cell lines with two human colon cancer cell lines (LS 180 and HCT 116) that contain β-catenin-activating mutations. As expected, β-catenin protein levels were clearly lower in all of the mouse tumor cell lines compared with that in human colon cancer cell lines. However, Cyclin D1 and c-jun/AP-1 protein levels were strongly up-regulated in all of the examined cell lines. These findings demonstrate that the activation of β-catenin target genes in c-myc/TGF-α and c-myc transgenic mice can arise in the absence of β-catenin activation.

The disruption of the pRb/E2F pathway, the loss of TGF-β signaling, and reduction of apoptosis were the major oncogenic events in liver constitutively overexpressing c-myc and TGF-α transgenes (42, 49, 63). Similarly, in SV40 T-antigen transgenic mice, no β-catenin mutations were detected, because T antigen activates cyclin D kinase by sequestration of pRb (49, 64).

Taken together, these data suggest that activation of Wingless/Wnt signaling occurs in only one of several genetic pathways leading to liver cancer.

REFERENCES


62. Guenet, J. L., Simon-Chazottes, D., Ringwald, M., and Kemler, R. The genes for \(\alpha\) and \(\beta\) catenin (Catna1 and Catnb) and plakoglobin (Jup) map to mouse chromosomes 18, 9 and 11, respectively. Mamm. Genome, 6: 363–366, 1995.
Activation of β-Catenin during Hepatocarcinogenesis in Transgenic Mouse Models: Relationship to Phenotype and Tumor Grade

Diego F. Calvisi, Valentina M. Factor, Roberto Loi, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/5/2085

Cited articles
This article cites 61 articles, 27 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/5/2085.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/5/2085.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/61/5/2085.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.