Activation of the canonical Wnt/β-catenin pathway confers growth advantages in c-Myc/E2F1 transgenic mouse model of liver cancer

Diego F. Calvisi†, Elizabeth A. Conner †, Sara Ladu, Eric R. Lemmer, Valentina M. Factor, Snorri S. Thorgeirsson *

Laboratory of Experimental Carcinogenesis, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Building 37, Room 4146A, 37 Convent Drive MSC 4262, Bethesda, MD 20892-4262, USA

Background/Aims: Previously, we showed that activation of the β-catenin/Wnt pathway is a dominant event during c-Myc/E2F1 hepatocarcinogenesis. Majority of c-Myc/E2F1 HCCs displayed nuclear accumulation of β-catenin in the absence of β-catenin mutations, suggesting that alterations in other members of the Wnt pathway might be responsible for nuclear localization of β-catenin. Here, we investigated the mechanisms responsible for nuclear translocation of wild-type β-catenin and addressed the potential contribution of the Wnt pathway in c-Myc/E2F1 hepatocarcinogenesis.

Methods: Status of the members of the Wnt pathway was determined through microsatellite and Western blot analysis.

Results: Majority of c-Myc/E2F1 HCCs exhibited multiple abnormalities in the Wnt pathway regardless of the presence of β-catenin mutations. The observed abnormalities included overexpression of Wnt-1, Frizzled 1 and 2 receptors, Dishevelled-1, downregulation of Secreted frizzled-related protein-1, GSK-3β inactivation, microsatellite instability at the Axin locus as well as induction of β-catenin target genes, such as glutamine synthetase, glutamate transporter-1, and Wisp-1. HCCs with β-catenin activation displayed significantly higher proliferation rate and larger tumor size when compared with β-catenin negative tumors.

Conclusions: The data demonstrate that multiple abnormalities in the members of the Wnt pathway lead to nuclear accumulation of β-catenin and suggest that activation of Wnt pathway provides proliferative advantages in c-Myc/E2F1-driven hepatocarcinogenesis.

Published by Elsevier B.V. on behalf of European Association for the Study of the Liver.

Keywords: C-Myc; E2F1; β-Catenin; Hepatic tumors; Transgenic mouse models

1. Introduction

C-Myc and E2F1 are implicated in the development of a wide variety of both experimentally and naturally occurring tumors, including hepatocellular carcinoma (HCC) [1–7].
p53-dependent and -independent mechanisms [12–17]. Thus, it is not surprising that these two genes which share common functional properties may also regulate each other [18–20]. Indeed, it has been shown that there is a requirement for distinct E2F members to mediate Myc-induced proliferation versus apoptosis [19]. Moreover, a recent report suggests that survival of c-Myc-over-expressing cells may depend on E2F1 activity [21].

Activation of other oncogenes can also modify c-Myc-mediated apoptosis. In an inducible c-MycER expression system, Wnt/β-catenin signaling suppressed apoptosis by inhibiting c-Myc-induced release of cytochrome c and caspase activation [22], and potentiated c-Myc-induced oncogenic transformation. Accordingly, in c-Myc driven experimental hepatocarcinogenesis and in human HCC, activation of β-catenin has been frequently reported [23–25]. β-Catenin, a key player in the Wnt cascade, is involved in two major functions: homotypic cell–cell interaction by its association with E-cadherin and transmission of the proliferative/survival signal of the Wingless/Wnt pathway [26]. In the canonical Wnt pathway, Wnt-1 ligand binds transmembrane Frizzled (FRZ) receptors, resulting in activation of the downstream effector Dishevelled (Dvl). Transactivation of Dvl prevents phosphorylation and degradation of β-catenin by inhibition of glycogen synthase-3β (GSK-3β). The elevated free β-catenin in the cytoplasm binds to members of the Tcf/LEF family, translocates to the nucleus and activates the transcription of a variety of genes involved in control of cell proliferation or apoptosis [26]. In the absence of Wnt, the active GSK-3β protein, complexed with the Adenomatous polyposis coli (APC), Axin and Conductin (Axin2), phosphorylates specific serine/threonine residues near β-catenin’s N-terminal region [27–30]. Once phosphorylated, β-catenin binds the F box protein β-TrCP, targeting it for ubiquitination and degradation by the proteasome system [30].

Previously, we demonstrated that transgenic overexpression of either c-Myc or E2F1 in the liver was sufficient to induce tumor growth, albeit with different latencies [6,7]. Although the driving force of carcinogenesis appeared to be the mitogenic properties of either oncprotein, there were distinct differences by which these two transcription factors control liver growth [6,7]. Overexpression of E2F1 led to the early establishment of preneoplastic diploid cell population at preneoplastic stage, whereas c-Myc promoted age-related polyplodylation, delaying tumor development [31]. In both transgenic models, there was a reciprocal induction of the other transcription factor, supporting the hypothesis that c-Myc and E2F1 modulate each other’s activity in vivo. Accordingly, it has been recently shown that down-regulation of c-Myc by antisense oligonucleotides inhibits the expression of E2F1 and in vitro growth of HepG2 and Morris 5123 cell lines [32]. To better understand the functional interaction between c-Myc and E2F transcription factors and its significance to liver oncogenesis, we have generated c-Myc/E2F1 double transgenic mice by crossing homozygous c-Myc and E2F1 transgenic mice [31]. We determined previously that neoplastic development in c-Myc/E2F1 double transgenic mice was frequently accompanied by activation of β-catenin, even in the absence of β-catenin activating mutations [33]. Therefore, in this study we specifically addressed [1] the molecular mechanisms responsible for nuclear accumulation of wild-type β-catenin in c-Myc/E2F1 HCCs and [2] the potential contribution of Wnt/β-catenin pathway in c-Myc/E2F1-driven hepatocarcinogenesis. We show here that hepatic co-expression of c-Myc and E2F1 collaborates with the activation of Wnt signaling leading to accelerated and more aggressive hepatocarcinogenesis.

2. Materials and methods

2.1. Transgenic mice

Generation of the Alb/c-Myc (c-Myc) and Alb/E2F1 (E2F1) transgenic mice has been previously described [6,7]. Double transgenic mice were generated by crossing homozygous c-Myc [166.8] with homozygous E2F1 (line [8]) mice. Animal study protocols were conducted according to the National Institutes of Health guidelines for animal care.

2.2. Tissue specimens, histopathological analysis and DNA extraction

Mice were sacrificed by cervical dislocation. Body weights were recorded and livers harvested, weighed, and examined for macroscopic tumors, which were recorded and measured. Slices of liver 4.5 mm in thickness were taken from each lobe and from tumors ≥ 3 mm in size. They were dissected and divided in two parts: half was stored at −80°C, half was fixed in 10% formalin and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin (H&E). Histopathological diagnoses were performed by two distinct investigators (D.F. Calvisi and E.R. Lemmer) based upon criteria previously described [34]. High molecular weight DNA was isolated from frozen mouse liver and tumor tissues as reported [35].

2.3. Western blot analysis

Liver protein lysates were processed as previously described [24]. Mouse monoclonal anti-β-catenin, anti-Cyclin D1, anti-GSK-3β, anti-glutamine synthetase (Transduction Laboratories, Lexington, KY), anti-β Tubulin, anti-Del-1 (Santa Cruz Biotechnology, Santa Cruz, CA), goat polyclonal anti-Conductin, anti-Axin, anti-FRZ 1 and 2 (sc-7429), and anti-Wnt-1 (Santa Cruz Biotechnology), rabbit polyclonal anti-APC (sc-896), anti-SFRP1 (sc-9169) (Santa Cruz Biotechnology), anti-c-Myc (Upstate, Lake Placid, NY), and anti-GLT-1 (Alpha Diagnostic, San Antonio, TX) antibodies were used in a 1:500 dilution. Phosphorylation status of β-catenin and GSK-3β proteins was evaluated by using the rabbit anti-phospho-β-catenin Ser33/37/Thr41 and anti-phospho-GSK-3β/ Ser21/9 polyclonal antibodies in a 1:250 dilution (Cell Signaling Technology, Inc., Beverly, MA). Nuclear extracts from wild-type livers and c-Myc/E2F1 HCCs were prepared by using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce Biotechnology, Inc., Rockford, IL) following the manufacturer’s protocol. Nuclear accumulation of β-catenin was determined in nuclear extracts by using the mouse monoclonal anti-β-catenin antibody (Transduction Laboratories) in 1:300 dilution. Activation of β-catenin was defined when HCCs displayed nuclear accumulation of β-catenin by Western blot analysis.
2.4. Microsatellite analysis

HCCs from c-Myc/E2F1 mice were screened for microsatellite alterations at the APC (D18Mit27, D18Mit15), Axin (D17Mit55, D17Mit100), Conductin (D11Mit11, D11Mit61) and GSK-3β (D3S1303, D3S3576) loci. The PCR primer pairs used were purchased from Research Genetics (Huntsville, AL) and the PCR reactions were performed according to the supplier’s protocol. Loss of heterozygosity (LOH) was recorded when a 50% or greater reduction in electrophoretic band intensity was detected. Microsatellite instability (MSI) was identified by the presence of band shifts or novel bands in the PCR product.

2.5. PCNA and apoptotic indices, tumor size

PCNA-labeling index was determined by counting PCNA-positive cells after staining with the mouse monoclonal anti-PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1:1000 dilutions according to the manufacturer’s recommendation and counterstaining with hematoxylin. Apoptotic index was scored by counting the apoptotic figures after staining with the ApoTag peroxidase in situ detection kit (Serologicals Corporation, Norcross, GA) following the manufacturer’s protocol. At least 2000 nuclei were counted per animal. The indices were represented as a percentage (mean ± SE) of the total cells counted. Tumor size was measured in mm² on H&E sections obtained from the central part of HCCs using the ImageJ software.

2.6. Statistical analysis

Data were evaluated by Student’s t-test to identify significant differences. Differences were considered significant if P values were less than 0.05.

3. Results

3.1. Development of preneoplastic and neoplastic lesions in transgenic mice

Previously, we have demonstrated that co-expression of c-Myc and E2F1 transgenes driven by the albumin/enhancer promoter accelerates HCC development in double transgenic mice when compared to both parental lines [6,33]. Almost all c-Myc/E2F1 as well as the majority of E2F1 transgenic mice developed diffuse liver dysplasia by 3 months of age, while dysplasia was delayed in c-Myc transgenic mice (Fig. 1). Similarly, preneoplastic foci and adenomas appeared more rapidly in both c-Myc/E2F1 and E2F1 mice. However, only double transgenic mice (22%, 2/9) developed HCCs by 6 months of age when no HCC was found in the single transgenic counterparts. All c-Myc/E2F1 mice (30/30) harbored HCCs by 9 months whereas only 23% (7/30) of c-Myc mice and 60% (18/30) of E2F1 mice developed HCC by 12 months. The histopathology of HCC derived from c-Myc/E2F1 mice resembled those in c-Myc mice, but the changes were more severe and tumors arose faster than in the c-Myc or E2F1 single transgenic mice. While tumors from the single transgenic lines were mostly well differentiated, HCCs from c-Myc/E2F1 mice were predominantly moderately well differentiated with trabecular, solid and trabecular/pseudoglandular pattern (Fig. 1). Thus, the kinetics of HCC development in c-Myc/E2F1 double transgenic mice suggests that E2F1 contributes to the early stages of disease, whereas overexpression of c-Myc is critical for its progression.

3.2. Multiple abnormalities of the GSK-3β complex promote β-catenin stabilization

In a previous study we found that activation of β-catenin, as judged by the presence of mutations and/or nuclear translocation of the protein, was a predominant feature of c-Myc/E2F1 hepatocarcinogenesis, whereas it was less frequent in c-Myc and rare in E2F1 HCCs [33]. Here, we extended the collection of examined tumors and focused specifically on the status of β-catenin in c-Myc/E2F1 HCCs by Western blot analysis. The majority (13/24, 54.2%) of c-Myc/E2F1 HCCs displayed nuclear accumulation of
**Table 1**

Summary of β-catenin activation in HCC developed in c-Myc, E2F1 and c-Myc/E2F1 Transgenic line of HCC

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>Number of HCC</th>
<th>β-Catenin nuclear accumulation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With β-catenin mutation</td>
<td>Without β-catenin mutation</td>
</tr>
<tr>
<td>c-Myc</td>
<td>30</td>
<td>4 (13.3%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>E2F1</td>
<td>30</td>
<td>1 (3.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>c-Myc/E2F1</td>
<td>30</td>
<td>11 (36.7%)^c,d</td>
<td>13 (54.2%)</td>
</tr>
</tbody>
</table>

Statistical significance as determined by two-tail Student’s t-test.
^a P=0.002 versus E2F1.
^b P=0.0002 versus c-Myc.
^c P=0.04 versus c-Myc.
^d P=0.0009 versus E2F1.
^e P=2.7×10^−13 versus E2F1.

β-catenin in the absence of β-catenin mutations (Table 1, Figs. 2A and 3), in agreement with our previous data [33]. Western blot analyses showed that β-catenin was expressed at significantly higher levels in all HCCs with nuclear accumulation of β-catenin (Fig. 2A). Furthermore, the levels of phosphorylated β-catenin targeted for degradation was lower in tumors with nuclear localization of β-catenin (Fig. 2A). Although the latter finding argues against the possibility that inefficient proteasome degradation might be responsible for β-catenin nuclear accumulation, the involvement of the proteasome system requires further investigation.

To address whether alterations in other members of the Wnt pathway are responsible for nuclear accumulation of β-catenin, we next examined the status of the GSK-3β complex members, including APC, Axin, Conductin and GSK-3β using the same collection of c-Myc/E2F1 HCCs (Figs. 2B and 3). Western blot analysis for APC revealed the absence of this protein only in one of 30 c-Myc/E2F1 HCCs with β-catenin nuclear translocation (Figs. 2B and 3). Subsequent microsatellite analysis of the APC locus in this tumor showed the presence of a homozygous deletion. Axin was upregulated in all c-Myc/E2F1 HCCs regardless of β-catenin cellular localization, but higher levels of Axin protein were detected in HCCs with membranous β-catenin (Figs. 2B and 3). In contrast, 22/30 (73.3%) HCCs with nuclear accumulation of β-catenin displayed microsatellite instability (MSI) at the Axin locus, whereas HCCs with membranous β-catenin displayed an intact Axin locus (Fig. 4). To better define the region affected by MSI at the chromosome 17, a broader segment of the chromosome was scanned by D17Mit44, D17Mit78, D17Mit113 and D17Mit145 microsatellite repeats. No further genetic changes were observed, indicating that MSI was limited to the Axin locus. Although the relevance of this finding is not clear at present, it is possible that MSI at the Axin locus might contribute to nuclear accumulation of β-catenin. This hypothesis is supported by the findings of high incidence of MSI at the Axin locus in c-Myc HCCs, which also showed frequent nuclear translocation of wild-type β-catenin (Table 1), but not in E2F1 HCCs (data not shown). Furthermore, HCCs with β-catenin nuclear positivity displayed a marked upregulation of conductin, whereas low or undetectable protein levels were observed in tumors with membranous localization of β-catenin (Figs. 2B and 3). This finding is in accordance with Axin2 (human homolog of conductin) over-expression in colon and liver tumors harboring β-catenin defects [37–39]. Conductin is a feedback inhibitor of the Wnt signaling pathway, whose negative regulatory role is neutralized in human tumors by
alternative overriding pathways [37–39]. No loss of heterozygosity (LOH) was detected at the conductin locus. Further, GSK-3β protein was downregulated in 3/30 (10%) HCCs showing nuclear localization of β-catenin (Fig. 3). More importantly, phosphorylation of GSK-3β, a marker of GSK-3β inactivation, was markedly increased in c-Myc/E2F1 HCCs with nuclear translocation of β-catenin when compared with HCCs with membranous immunolabeling for β-catenin (Figs. 2B and 3). No loss of heterozygosity was detected at the GSK-3β locus. Taken together, these data show that β-catenin escapes GSK-3β complex inactivation in the majority of c-Myc/E2F1 HCCs.

3.3. C-Myc/E2F1 HCCs with activated β-catenin express high levels of Wnt-1

Next, we sought to determine the status of Wnt-1, since it promotes β-catenin nuclear accumulation via inhibition of GSK-3β activity [40–42]. Wnt-1 was strongly upregulated in all c-Myc/E2F1 HCCs with β-catenin nuclear accumulation regardless of β-catenin mutations, whereas Wnt-1 levels were comparable in wild-type livers and β-catenin negative HCCs (Figs. 2C and 3). Accordingly, the same lesions displaying high levels of Wnt-1 exhibited elevated expression levels of the Wnt-1-associated receptors, FRZ 1 and 2 (Figs. 2C and 3). Furthermore, only HCCs with membranous localization of β-catenin showed increased levels of the secreted frizzled-related protein 1 (SFRP1)/Sarp-2 (Figs. 2C and 3), known to counteract activation of the FRZ receptors as well as to inhibit nuclear translocation of β-catenin [43]. Similarly, TGF-α and c-Myc/TGF-α HCC with membranous expression of β-catenin were found to over-express SFRP1 [33]. These results indicate that activation of the canonical Wnt-1 signaling with concomitant downregulation of SFRP1 represents a major mechanism leading to nuclear accumulation of wild-type β-catenin in c-Myc/E2F1-driven hepatocarcinogenesis.

3.4. Analysis of β-catenin target genes

Recently, it has been shown that β-catenin promotes inhibition of c-Myc induced apoptosis by induction of
COX-2 and WISP-1 [22]. In addition, mutations of β-catenin gene in the liver resulted in the transactivation of glutamine synthetase and glutamate transporter 1 (GLT-1) [44]. Therefore, we determined whether the same targets were activated by β-catenin in c-Myc/E2F1 liver tumors (Figs. 2D and 3). Upregulation of COX-2 was detected in all c-Myc/E2F1 HCCs regardless of β-catenin subcellular localization (Figs. 2D and 3). In striking contrast, WISP-1 levels were very low in HCCs displaying membranous β-catenin, but elevated in c-Myc/E2F1 HCCs with nuclear accumulation of β-catenin (Figs. 2D and 3). The same pattern of expression was also observed for glutamine synthetase and GLT-1 proteins by Western blot analysis (Figs. 2D and 3).

Furthermore, we investigated the levels of two other canonical β-catenin targets, c-Myc and Cyclin D1. Although these genes are commonly upregulated in HCC, a direct link between activation of β-catenin and induction of c-Myc and Cyclin D1 in the liver is controversial [36,45–47]. We found that c-Myc and Cyclin D1 were induced in all c-Myc/E2F1 HCCs regardless of β-catenin cellular localization (Fig. 2D). Together these data indicate that WISP-1, glutamine synthetase, and GLT-1 are β-catenin target genes in c-Myc/E2F1 HCCs.

3.5. Activation of β-catenin provides proliferative advantages

Since Wnt signaling has been shown to induce oncogenic transformation by both promoting cell proliferation and inhibiting c-Myc-induced apoptosis [22], we compared the growth properties of c-Myc and c-Myc/E2F1 HCCs with and without β-catenin activation (Fig. 5). PCNA labeling indices were significantly higher in both c-Myc/E2F1 and c-Myc HCCs with β-catenin activation when compared with those exhibiting membranous immunolabeling (P < 0.0002 and P < 0.008, respectively). In addition, c-Myc/E2F1 HCCs with nuclear β-catenin displayed a slightly lower rate of apoptosis than HCCs with β-catenin membranous immunostaining (P < 0.05), whereas no overt differences in the apoptotic indices were detected between β-catenin-positive and -negative c-Myc HCCs (P = 0.48). Consequently, the growth rate, estimated by the PCNA/apoptosis ratio, as well as tumor size reached the highest values in c-Myc/E2F1 HCCs with nuclear β-catenin (Fig. 5C, D). Less pronounced, but statistically significant differences were detected between two groups of c-Myc HCCs (Fig. 5). Both c-Myc and c-Myc/E2F1 tumors with deregulated β-catenin displayed a significantly larger tumor size when compared with HCCs with wild-type β-catenin (P < 0.01 and P < 2.56 × 10⁻⁶, respectively). Thus, nuclear translocation of β-catenin provides proliferative advantages during c-Myc and c-Myc/E2F1 hepatocarcinogenesis.

4. Discussion

Current evidence suggests that deregulation of c-Myc and E2F1 expression plays a crucial role in human and rodent hepatocarcinogenesis. In human HCC, c-Myc, E2F1, and E2F1 heterodimeric partner DP1 were found to be frequently over-expressed [48,49]. Accordingly, increase in E2F1/DP1 and E2F2/ DP1 heterodimers as well as transcriptional induction of E2F1 target genes, including c-myc, characterized hepatic malignant transformation in c-Myc and c-Myc/TGF-α transgenic mice [50]. In addition, upregulation of c-Myc and E2F1 occurred only in HCCs from susceptible F344 rats, but not in slowly growing lesions from Wistar and BN rats resistant to hepatocarcinogenesis [51]. Previous reports indicate that the functional relationship between c-Myc and E2F1 is complex and might depend on the biological context or tissue-specific [19–21]. We have shown that in the liver, simultaneous over-expression of c-Myc and E2F1 results in accelerated tumor development as compared to either of the single transgenic lines [33]. In particular, the co-expression of c-Myc and E2F1 enhanced tumor progression, resulting in a shorter latency period and a higher rate of malignant conversion. At the molecular level, c-Myc/E2F1 over-expressing livers showed a significantly higher incidence of Wnt/β-catenin pathway activation when compared with both single transgenic lines. In particular, reactivation of the Wnt cascade as well as alterations in the GSK-3β degradation complex suggests that co-expression of c-Myc and E2F1 favors the selective growth of neoplastic clones with β-catenin activation. Furthermore, activation of Wnt-1 signaling in HCCs with mutated β-catenin implies increased selective pressure for activation of Wnt/β-catenin pathway in c-Myc/E2F1 transgenic mice. In accordance with our results, previous reports have shown that β-catenin mutations in
human liver and colon cancer may occur in the presence of alterations in other members of the Wnt pathway, such as mutations in Axin and Axin 2 genes, as well as promoter methylation of secreted frizzled-related proteins [52–54].

The role of Wnt/β-catenin pathway activation in the liver is still under debate. Accumulating evidence suggests that the Wnt cascade regulates liver homeostasis by stimulating sustained hepatocellular proliferation and survival. Thus, it has been shown that β-catenin promoted both cell proliferation and inhibition of apoptosis in the developing liver [55]. In addition, elevated expression of oncogenic forms of β-catenin induced hepatomegaly and hepatocellular hyperplasia in the mouse liver [45,56]. Importantly, targeted disruption of the APC gene in the mouse liver leads to development of HCC, demonstrating for the first time that activation of the Wnt/β-catenin signaling (without c-Myc expression) is able per se to drive hepatic transformation [47].

Here, we show that activation of Wnt/β-catenin pathway provides proliferative advantages in c-Myc and c-Myc/E2F1 transgenic mouse models of liver cancer. Since the morphological features and the proliferation properties of c-Myc/E2F1 HCCs resembled those of c-Myc tumors, it is tempting to speculate that Wnt signaling activation is a direct consequence of c-Myc overexpression. These data are in disagreement with the findings that co-expression of c-Myc and Wnt-1 promotes the malignant transformation in vitro via inhibition of c-Myc-induced apoptosis [22]. On the other hand, β-catenin activation correlated with increased cell proliferation in HCCs from c-Myc/TGF-α transgenic mice subjected to phenobarbital treatment [57]. Therefore, it seems likely that β-catenin activation results in proliferative and/or anti-apoptotic advantages depending upon the experimental model. c-Myc/E2F1 HCCs with β-catenin activation did not display striking advantages in cell survival over tumors with wild-type β-catenin, indicating that down-regulation of apoptosis in c-Myc and E2F1 HCCs is mainly β-catenin independent.

In summary, we showed that multiple abnormalities in the members of the Wnt pathway are responsible for nuclear accumulation of β-catenin and activation of β-catenin target genes in HCCs from c-Myc/E2F1 double transgenic mice. Activation of β-catenin provides proliferative advantages in the c-Myc/E2F1 mouse model of accelerated hepatocarcinogenesis. This mouse model may represent a prototype system to examine the significance of c-Myc and E2F1 interactions with other oncogenic pathways including Wnt/β-catenin in vivo. Furthermore, the c-Myc/E2F1 mouse model may allow to determine the efficacy of antineoplastic strategies aimed to suppress the Wnt signaling.

Acknowledgements

Dr Calvisi was supported in part by a fellowship from the Fondazione Italiana per la Ricerca sul Cancro (FIRC).

References


