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Excess β-catenin promotes accumulation of transcriptionally active p53

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β-catenin is a multifunctional protein, acting both as a structural component of the cell adhesion machinery and as a transducer of extracellular signals. Deregulated β-catenin protein expression, due to mutations in the β-catenin gene itself or in its upstream regulator, the adenomatous polyposis coli (APC) gene, is prevalent in colorectal cancer and in several other tumor types, and attests to the potential oncogenic activity of this protein. Increased expression of β-catenin is an early event in colorectal carcinogenesis, and is usually followed by a later mutational inactivation of the p53 tumor suppressor. To examine whether these two key steps in carcinogenesis are interrelated, we studied the effect of excess β-catenin on p53. We report here that overexpression of β-catenin results in accumulation of p53, apparently through interference with its proteolytic degradation. This effect involves both Mdm2-dependent and -independent p53 degradation pathways, and is accompanied by augmented transcriptional activity of p53 in the affected cells. Increased p53 activity may provide a safeguard against oncogenic deregulation of β-catenin, and thus impose a pressure for mutational inactivation of p53 during the later stages of tumor progression.

Keywords: carcinognesis/β-catenin/colorectal cancer/Mdm2/p53

Introduction

β-catenin is a multifunctional protein that engages in diverse associations with a variety of partners (for recent reviews on β-catenin see Ben-Ze'ev and Geiger, 1998; Bullions and Levine, 1998; Cox and Peifer, 1998; Willert and Nusse, 1998). On the one hand, it is a major component of the cellular adhesion machinery. It binds to the intracellular domain of cadherins, transmembrane proteins that mediate calcium dependent cell–cell adhesion, and through its association with α-catenin it links cadherins to the actin cytoskeleton (Kemler, 1993; Ben-Ze'ev and Geiger, 1998). On the other hand, β-catenin is a pivotal component of the Wnt/Wingless (Wg) signaling pathway, which plays a key role in an array of developmental processes (Cox and Peifer, 1998; Willert and Nusse, 1998). The cellular levels of the β-catenin protein, as well as its subcellular localization, are very tightly regulated. When not associated with cell–cell junctions, β-catenin is incorporated into a large complex that also includes the protein encoded by the *APC* tumor suppressor gene, the GSK-3β serine/threonine protein kinase, and the axin/conductin protein (Behrens *et al*., 1998; Hart *et al*., 1998; Ikeda *et al*., 1998). Formation of this complex results in subsequent proteolytic degradation of β-catenin by the ubiquitin–proteasome system (Aberle *et al*., 1997; Salomon *et al*., 1997). Activation of the Wnt/Wg pathway upon binding of the Wnt ligand blocks this degradation, giving rise to an increased pool of free β-catenin, which translocates into the cell nucleus. Nuclear β-catenin may associate with members of the TCF/LEF family of transcription factors and stimulate transcription of a variety of target genes—mostly still unknown—which presumably orchestrate the phenotypic manifestations of the activated Wnt/Wg pathway (Behrens *et al*., 1996; Molenaar *et al*., 1996; Riese *et al*., 1997; van de Wetering *et al*., 1997).

Recent studies have highlighted a striking association between β-catenin deregulation and the emergence of various types of cancer (reviewed in Clevers and van de Wetering, 1997; Gumbiner, 1997; Peifer, 1997; Ben-Ze'ev and Geiger, 1998; Bullions and Levine, 1998). In particular, aberrant accumulation of non-membrane-associated β-catenin has been extensively documented in colorectal cancer. Colorectal cancer evolves through a series of sequential genetic alterations, each of which presumably confers upon the affected cells a more malignant phenotype and decreases their dependence on environmental cues (reviewed in Kinzler and Vogelstein, 1996). One of the most common of these alterations is the mutational inactivation of the *APC* gene, usually resulting in the synthesis of a truncated APC protein (Polakis, 1997). This defective APC protein is unable to promote the proteolytic degradation of β-catenin, leading to its accumulation and nuclear translocation within the cancer cell (Munemitsu *et al*., 1995). In some other cases of colorectal cancer, a similar outcome is achieved through mutation of the β-catenin gene itself (Ilyas *et al*., 1997; Morin *et al*., 1997). In these cases, the mutations target specific serine residues that are normally phosphorylated by GSK-3β and whose phosphorylation is essential for subsequent ubiquitinylation and degradation of β-catenin. The consequence of either APC inactivation or β-catenin mutation is similar: a failure to degrade β-catenin properly, its accumulation and nuclear translocation leading to a constitutive activation of β-catenin responsive genes (Korinek *et al*., 1997). These genes presumably contribute to the aberrant, cancer-prone behavior of the affected cells. Compelling support for this notion was recently provided through the finding that the c-*myc*

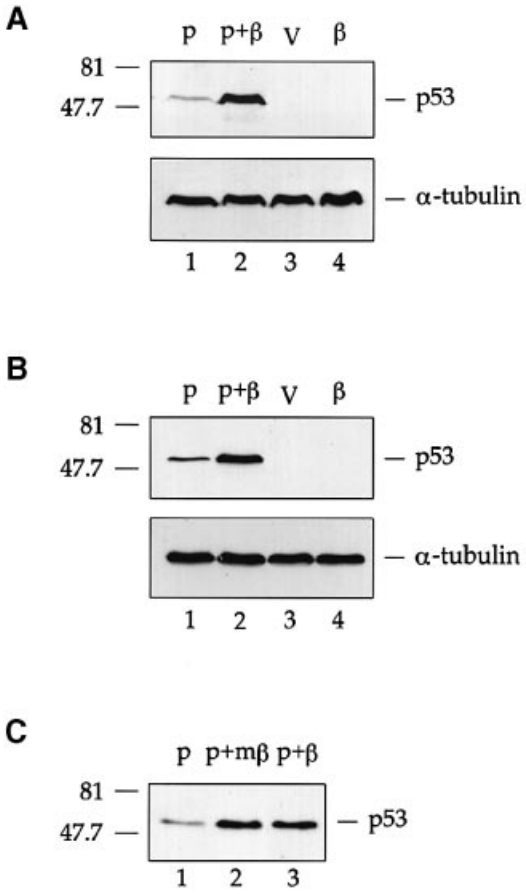

Fig. 1. β-catenin increases the steady-state levels of p53. Human H1299 cells (**A**) or mouse 10(1) cells (**B** and **C**) were transfected with 100 ng of a plasmid encoding human wt p53, either alone (p, lane 1), or together with 5 μg of either wt β-catenin (p+β) or the Ser33Tyr mutant β-catenin (C, p+mβ). As negative controls, cells were transfected with either pCDNA3 empty vector DNA (V) or β-catenin alone (β). In each case, total amounts of transfected DNA were brought up to 5.1 μg with pCDNA3. Cells were harvested 26 h after transfection, and subjected to Western blot analysis with a mixture of the p53-specific antibodies DO-1 and PAb1801. The positions of p53 and of molecular size markers (in kDa) are indicated. Equal sample loading was confirmed by reprobing the same blots with antibodies against α-tubulin.

and cyclin D1 genes, well known positive regulators of cell proliferation, are direct targets for activation by β-catenin (He *et al*., 1998; Shtutman *et al*., 1999; Tetsu and McCormick, 1999). Of note, histological examination of material from familial adenomatous polyposis (FAP) patients, who carry APC germ line mutations, revealed increased levels of both nuclear and cytoplasmic β-catenin in the colorectal epithelium, confirming that aberrant accumulation of β-catenin is one of the earliest events in colorectal carcinogenesis (Inomata *et al*., 1996; Sparks *et al*., 1998).

Oncogenic deregulation of β-catenin is not limited to colorectal cancer. Similar observations were made in a number of other cancers, including melanoma (Rubinfeld *et al*., 1997) and hepatocellular carcinoma (Coste *et al*., 1998; Miyoshi *et al*., 1998). Thus, aberrant accumulation of this protein appears to be an important step in the tumorigenic conversion of many epithelial cell types.

The p53 tumor suppressor protein is among the most frequent targets for inactivation in cancer (for recent reviews on p53 see Hansen and Oren, 1997; Levine, 1997; Agarwal *et al*., 1998; Almog and Rotter, 1998; Giaccia and Kastan, 1998; Prives, 1998). The presence of a functional wild type (wt) p53 protein safeguards against the emergence of cells with deregulated growth properties, which are otherwise likely to give rise to cancer. The p53 protein serves to maintain cellular genomic stability, by eliminating cells with damaged genomes from the proliferating pool, as well as by facilitating the repair of such damage. The underlying mechanism involves the activation of p53 in response to detection of genomic damage, leading to accumulation of biochemically active p53 within the nucleus. Key to this process is the substantial increase in the intracellular stability of p53, usually a very short-lived protein under non-stressed conditions. Normally, p53 is maintained at very low steady-state levels owing to continuous degradation through the ubiquitin–proteasome pathway (Maki *et al*., 1996). This degradation is greatly dependent on the Mdm2 oncoprotein (Bottger *et al*., 1997; Haupt *et al*., 1997; Kubbutat *et al*., 1997), probably acting on p53 as an E3 ubiquitin protein ligase (Honda *et al*., 1997).

An increase in p53 activity can give rise to a variety of biological consequences, most notably arrest of the cell cycle in G_1 and/or G_2 and induction of apoptotic cell death. In addition to overt damage to the cellular genome, activation of p53 can also occur in response to the presence of deregulated oncogenes, most clearly exemplified by the cellular *ras* and c-*myc* and the adenoviral E1A (Serrano *et al*., 1997; de Stanchina *et al*., 1998; Zindy *et al*., 1998). Conceivably, activation of p53 under these circumstances serves to prevent cells carrying such deregulated oncogenes from giving rise to full-blown cancer. This provides at least part of the explanation for p53's tumor suppressor activity.

Like the APC/β-catenin pathway, p53 is also a very common target for genetic alterations in colorectal cancer (Kinzler and Vogelstein, 1996). Elimination of p53's tumor suppressor capacity appears to be a prerequisite for the conversion of a transformed colorectal cell into a malignant tumor. Interestingly, p53 mutations occur relatively late during the course of colorectal carcinogenesis, suggesting that the inhibitory effects of p53 may become rate limiting only as a consequence of earlier events in this cancer progression sequence.

To examine the possibility of a causal relationship between the early aberrant activation of β-catenin-dependent signaling and the later inactivation of p53 during carcinogenesis, we investigated the effects of deregulated β-catenin on p53 levels and transcriptional activity. We report that forced overexpression of β-catenin, believed to mimic the oncogenic activation of this protein, induces accumulation of transcriptionally active p53. Thus, aberrant accumulation of non-membrane-associated β-catenin, as occurs in certain types of tumors, might eventually trigger an inhibitory p53 response, providing a safeguard against neoplastic conversion. This may exert a strong selective pressure for the subsequent mutational inactivation of p53, resulting in a further increase in tumor malignancy.

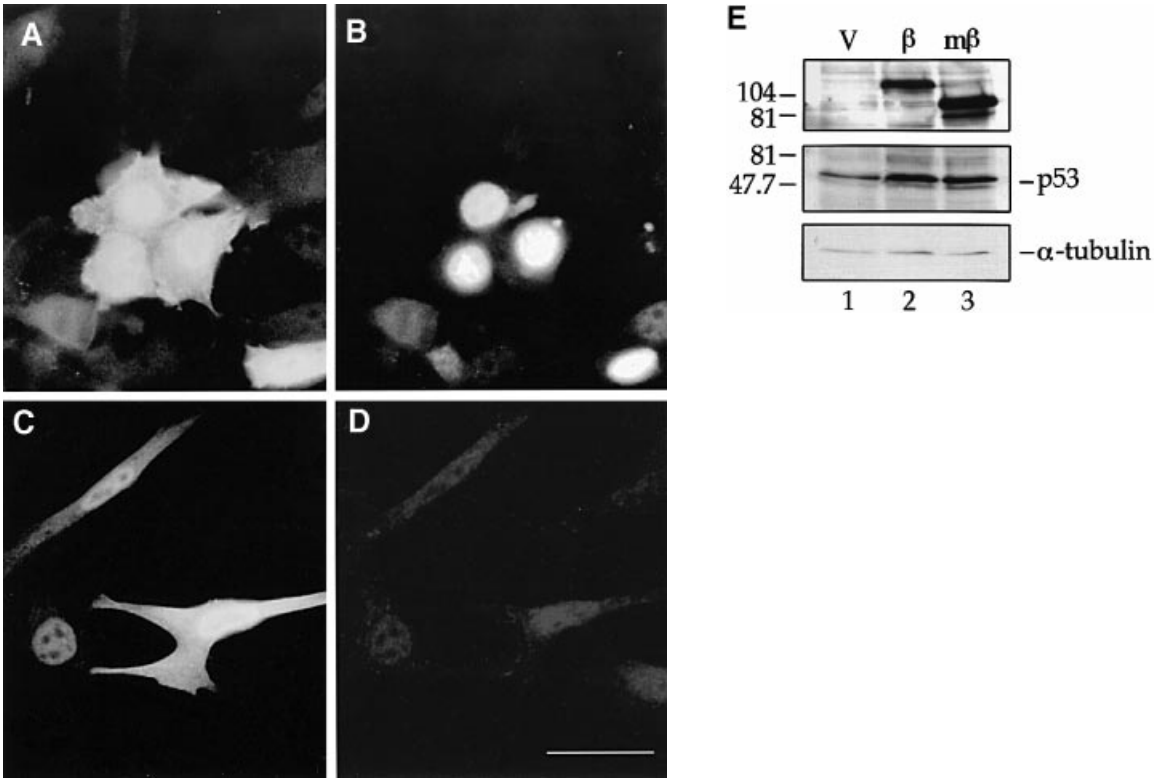

Fig. 2. Overexpression of β-catenin increases nuclear accumulation of endogenous p53. NIH 3T3 cells were transfected with 5 μg of plasmid encoding either HA-tagged human β-catenin (**A** and **B**) or HA-tagged plakoglobin (**C** and **D**). After 36 h, cells were processed for double immunostaining with polyclonal anti-HA antibodies (A and C) and the monoclonal p53-specific antibody PAb248 (B and D). The secondary antibodies were labeled with FITC (A and C) or Cy3 (B and D). (**E**) Mouse embryo fibroblasts were infected with recombinant retroviruses encoding either HA-tagged GFP–β-catenin (β), HA-tagged β-catenin Ser33Tyr (mβ) or puromycin resistance only (V). After 48 h, cells were harvested and cell extracts were subjected to sequential Western blot analysis with antibodies directed against the HA tag (Y11, Santa Cruz; upper panel), p53 (a mixture of PAb248 and PAb421, middle panel) or α-tubulin, serving as a control for equal loading. Positions of molecular size markers (in kDa) are indicated on the left.

Results

β-catenin overexpression increases steady-state p53 levels

Changes in cellular p53 activity, particularly in response to genotoxic or oncogenic stress, are often reflected in altered p53 protein levels. To investigate a possible cross-talk between β-catenin and p53, we assessed the effect of β-catenin overexpression on steady-state p53 levels. p53-null human lung adenocarcinoma H1299 cells (Mitsudomi *et al.*, 1992) were transiently transfected with a human p53 expression plasmid, either alone or together with a β-catenin expression plasmid. p53 levels were determined by Western blot analysis 26 h later. As can be seen in Figure 1A, co-transfection with β-catenin caused a substantial increase in the amount of p53 (lane 2), as compared with cells co-transfected with an empty vector control (lane 1). Similar results were obtained with the p53-null mouse embryo fibroblast cell line 10(1) (Harvey and Levine, 1991; Figure 1B). Essentially identical results were obtained when mouse, rather than human, p53 was used (data not shown). Of note, an increase in steady-state p53 levels was also observed with the tumor-derived β-catenin mutant Ser33Tyr, carrying a serine to tyrosine substitution at position 33 (Figure 1C, lane 2); this mutant, isolated from a colorectal cancer cell line (Morin *et al.*, 1997), lacks a critical GSK-3β phosphoacceptor site and is therefore not efficiently targeted for ubiquitination and degradation.

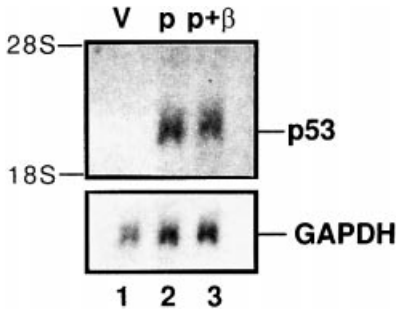

Fig. 3. p53 mRNA levels are not modulated by β-catenin. H1299 cells were transfected with 500 ng of human p53 expression plasmid, either alone (p, lane 2) or together with 10 μg of β-catenin expression plasmid (p+β, lane 3). As negative control, parallel cultures were transfected with pCDNA3 empty vector DNA (V, lane 1). Total RNA was extracted from each culture 26 h after transfection, and 10 μg of each RNA was subjected to Northern blot analysis with a human p53 cDNA probe. The same blot was subsequently reprobed for GAPDH, to normalize for loading variations.

Overexpressed β-catenin can upregulate not only co-transfected p53, but also the endogenous protein. Transient transfection of NIH 3T3 cells with β-catenin resulted in a marked nuclear accumulation of the endogenous wt p53 within cells overexpressing the transfected β-catenin, but not those failing to express it (Figure 2B; staining of transfected β-catenin, identified through its HA tag, is shown in Figure 2A). Of note, a similar nuclear accumulation of p53 did not occur when NIH 3T3 cells

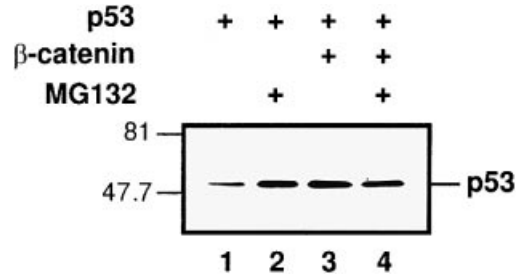

Fig. 4. Induction of p53 accumulation by MG132 and β-catenin. H1299 cells were transiently transfected with a human p53 expression plasmid (100 ng/6 cm dish), with or without the addition of 5 μg β-catenin expression plasmid. Twenty-three hours after transfection the proteasomal inhibitor MG132 was added, where indicated, to a final concentration of 25 μM. After an additional 3 h, cells were harvested and processed as described in Figure 1.

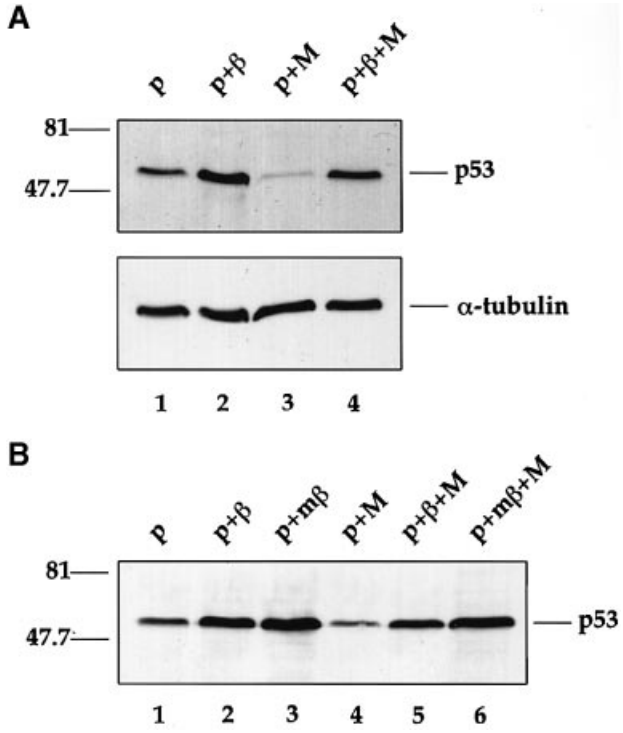

Fig. 5. Effect of β-catenin on Mdm2-mediated p53 degradation. (**A**) H1299 cells were transfected with 100 ng of p53 expression plasmid (p). Where indicated, expression plasmids for β-catenin (β, 5 μg) and murine wt Mdm2 (M, 100 ng) were also included. Cells were processed as in Figure 1. (**B**) p53-null mouse 10(1) cells were transfected with 100 ng of p53 expression plasmid (p), together with the indicated combinations of plasmids encoding wt β-catenin (β; 5 μg/dish), Ser33Tyr mutant β-catenin (mβ; 5 μg/dish), and mouse Mdm2 (M; 200 ng/dish). Cells were processed as in Figure 1.

were transfected with plakoglobin (Figure 2C and D), despite the fact that this protein, also known as γ-catenin, is a close relative of β-catenin that shares with it many structural and biochemical properties (Ben-Ze'ev and Geiger, 1998; Simcha *et al*., 1998). Likewise, infection of primary mouse embryo fibroblasts with recombinant retroviruses encoding either wt β-catenin fused to green fluorescent protein (GFP; Figure 2E, lane 2) or the β-catenin mutant Ser33Tyr (mβ, lane 3) led to a several-fold elevation in endogenous p53 relative to cells infected with a control retrovirus (V, lane 1).

Together, these data demonstrate that overexpression of

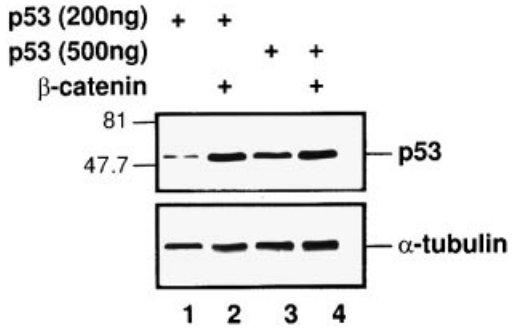

Fig. 6. β-catenin induces p53 accumulation in the absence of Mdm2. Immortalized fibroblasts derived from $p53^{-/-}$;$mdm2^{-/-}$ double knock-out mouse embryos (McMasters *et al.*, 1996; 5×10^5 cells/6 cm dish) were transfected with the indicated amounts of DNA encoding wt human p53, either alone or together with 5 μg β-catenin expression plasmid. Total amounts of DNA were kept constant with pCDNA3. Cells were harvested 26 h after transfection, and processed as in Figure 1.

β-catenin can lead to a substantial increase in the amount of nuclear p53 within cells.

β-catenin regulates p53 protein but not p53 mRNA

To investigate the molecular mechanism responsible for the accumulation of p53 protein in the presence of excess β-catenin, we tested whether β-catenin could increase the intracellular levels of p53 mRNA. As shown in Figure 3, excess β-catenin had no detectable effect on p53 mRNA levels in transiently transfected H1299 cells under conditions that cause a strong elevation of p53 protein levels. Hence, the effect of β-catenin is likely to be translational or post-translational.

Intracellular p53 concentrations are often regulated at the level of protein stability (Hansen and Oren, 1997; Levine, 1997; Kubbutat and Vousden, 1998), involving tightly controlled degradation via the ubiquitin–proteasome pathway (Bottger *et al*., 1997; Haupt *et al*., 1997; Kubbutat *et al*., 1997). To assess whether the effect of β-catenin is mediated through interference with the proteasomal degradation of p53, transiently transfected H1299 cells were incubated with the proteasomal inhibitor MG132 (Palombella *et al*., 1994). As expected, MG132 caused p53 accumulation in cells transfected with p53 alone (Figure 4, lanes 1 and 2). MG132, however, did not elicit a further increase in p53 beyond the levels induced by excess β-catenin (Figure 4, lanes 3 and 4). This suggests that β-catenin and MG132 mediate p53 accumulation through a similar mechanism, namely by inhibiting the proteasomal degradation of p53.

β-catenin partially inhibits the Mdm2-mediated proteolysis of p53

The Mdm2 oncoprotein is a key regulator of p53 turnover, targeting p53 for rapid proteasomal degradation (Bottger *et al*., 1997; Haupt *et al*., 1997; Kubbutat *et al*., 1997). The *mdm2* gene is a target for transcriptional activation by p53, thereby establishing a negative feedback loop that keeps p53 in check (reviewed in Lozano and Montes de Oca Luna, 1998). To determine whether β-catenin elevates steady-state p53 levels by interfering with Mdm2-driven p53 proteolysis, H1299 cells were transfected with plasmids encoding p53 and various combinations of Mdm2

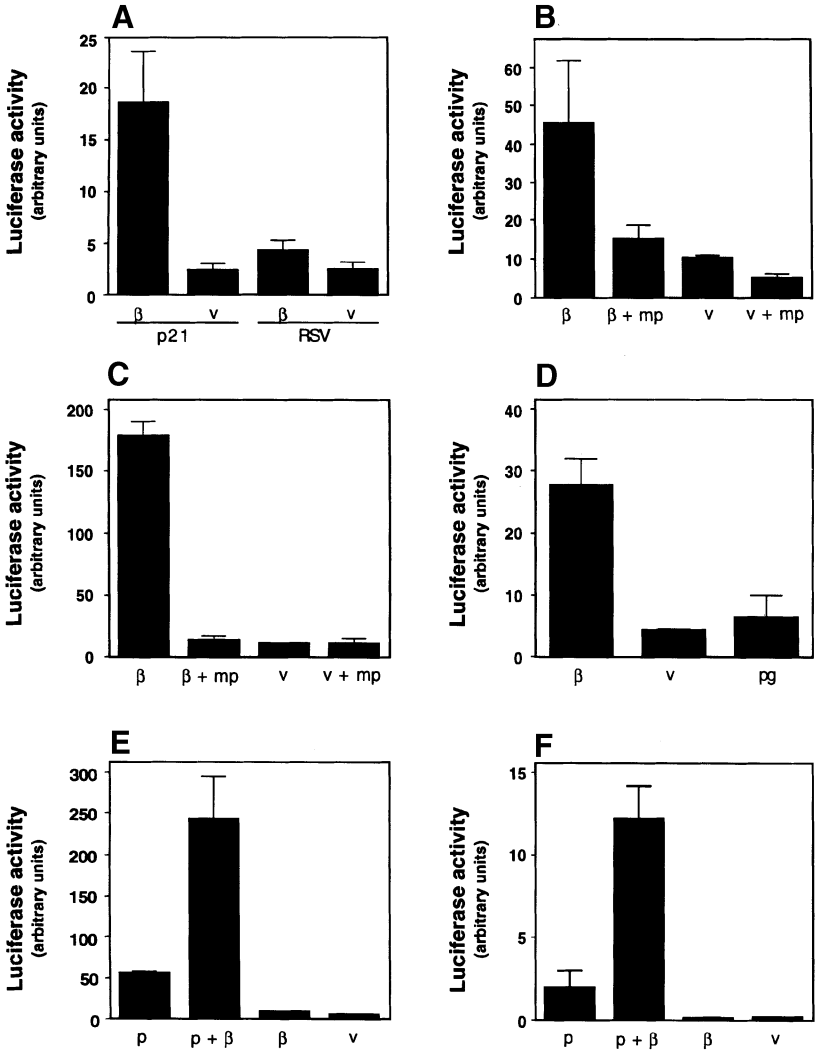

Fig. 7. β-catenin enhances the transcriptional activity of p53. (**A**) NIH 3T3 cells were transfected with reporter plasmids expressing the firefly luciferase gene under transcriptional control of either the $p21^{Waf1}$ promoter or the RSV long terminal repeat (40 ng per 6 cm dish), together with 2 μg of β-catenin expression plasmid DNA (β) or pCDNA3 empty vector (V). Luciferase activity in cell extracts was assayed 48 h post-transfection. Transfections were carried out in triplicate; the standard error is indicated. (**B**) NIH 3T3 cells were transfected with 40 ng of $p21^{Waf1}$ promoter-driven luciferase plasmid as in (A), but including also 200 ng of DNA encoding the negative *trans*-dominant mutant p53Phe132 (mp) where indicated. (**C**) NIH 3T3 cells were transfected and processed as in (B), except that the luciferase reporter was driven by the rat cyclin G promoter (40 ng/dish). (**D**) NIH 3T3 cells were transfected with 40 ng of $p21^{Waf1}$ promoter-driven luciferase plasmid, together with 2 μg of either β-catenin expression plasmid (β), plakoglobin expression plasmid (pg) or empty pCDNA3 vector (V). (**E**) p53-null 10(1) fibroblasts were transfected with 100 ng/6 cm dish of $p21^{Waf1}$ promoter-driven luciferase plasmid, together with 2.5 ng of human wt p53 expression plasmid (p), 1 μg DNA of β-catenin expression plasmid (β) or 1 μg empty pCDNA3 vector (V). Luciferase activity was assayed 48 h post-transfection. Other details as in (A). (**F**) 10(1) cells were transfected and processed as in (E), except that the luciferase reporter was driven by the rat cyclin G promoter.

and β-catenin. p53 levels were strongly reduced in the presence of Mdm2 (Figure 5A, compare lanes 1 and 3), and augmented by β-catenin (lane 2). Of note, overexpression of β-catenin resulted in significant protection of p53 against downregulation by Mdm2 (Figure 5A, lane 4). Similarly, the decrease in p53 levels conferred by Mdm2 in 10(1) cells was prevented by co-transfection with either wt β-catenin (Figure 5B, lane 5) or the β-catenin Ser33Tyr mutant (Figure 5B, lane 6). These findings argue strongly that β-catenin overexpression can interfere with Mdm2-mediated p53 degradation.

Inhibition of Mdm2-mediated proteolysis, however, does not account in full for the effect of excess β-catenin on p53 protein levels. This was revealed by using embryonic fibroblasts derived from $p53^{-/-}$;$mdm2^{-/-}$ double knock-out mice (McMasters *et al.*, 1996). As shown in Figure 6, β-catenin upregulated p53 even in the complete absence of Mdm2 (compare lanes 1 and 2); the effect of β-catenin was more pronounced with lower concentrations of p53 plasmid (Figure 6, compare lanes 1 and 2 with 3 and 4).

Taken together, these findings imply that β-catenin confers the elevation in p53 through a combination of at least two mechanisms, one involving protection against Mdm2-mediated proteolysis and the other presumably operating through an alternative, currently undefined pathway that may also regulate p53 degradation. The ability to simulate the full effect of β-catenin by treating the cells with MG132 suggests that the second mechanism is also dependent on the proteasomal machinery.

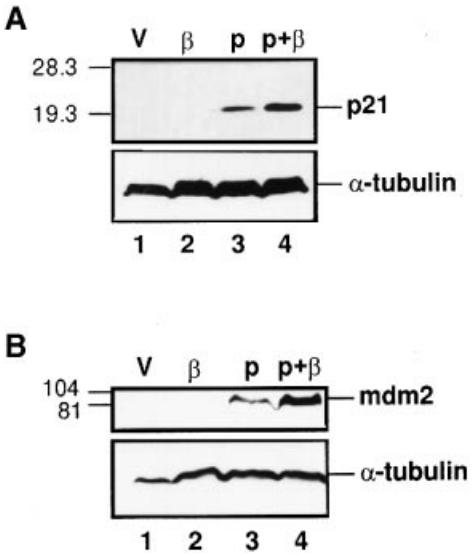

Fig. 8. β-catenin enhances the induction of p21^{Waf1} and Mdm2 proteins by p53. H1299 cells were transfected with the indicated combinations of plasmids encoding wt human p53 (p, 500 ng/10 cm dish) or β-catenin (β, 10 μg/dish) or pCDNA3 vector control (V). Protein levels of p21^{Waf1} (**A**) and human Mdm2 (**B**) were determined by Western blot analysis. The analyses shown in (A) and (B) were performed using 15% and 7.5% polyacrylamide gels, respectively. Equal loading was confirmed by reprobing for α-tubulin.

The p53 protein induced by β-catenin is transcriptionally active

To assess whether the p53 accumulated in the presence of excess β-catenin is transcriptionally active, we determined the effect of β-catenin overexpression on the ability of p53 to stimulate transcription from p53-responsive promoters. Two cell types were used for this purpose: NIH 3T3 cells, in which the transcriptional activity of the endogenous wt p53 was studied, and p53-null 10(1) cells in which the effect of β-catenin on co-transfected p53 was followed.

NIH 3T3 fibroblasts were transiently transfected with a luciferase reporter gene driven by the p53-responsive p21^{Waf1} promoter (El Deiry *et al*., 1993). As shown in Figure 7A, the activity of the p21^{Waf1} promoter was stimulated 7-fold by co-transfection with β-catenin (β), relative to the empty vector control (V). The p53-irrelevant RSV promoter was only slightly stimulated under the same conditions. The effect of β-catenin on the activation of the p21^{Waf1} promoter was p53 dependent, being markedly attenuated by the p53Phe132 p53 mutant (mp, Figure 7B), a negative *trans*-dominant inhibitor of wt p53 function (Shaulian *et al*., 1992). Even stronger inhibition by p53Phe132 was evident when employing another p53-responsive promoter, that of the cyclin G gene (Okamoto and Beach, 1994; Zauberman *et al*., 1995) (Figure 7C). The accumulation of transcriptionally active p53 in NIH 3T3 cells was specific for β-catenin and was not elicited by plakoglobin (pg, Figure 7D).

The transcriptional competence of the p53 that accumulated in the presence of β-catenin was further validated with p53-null 10(1) fibroblasts. While a limiting amount of transfected p53 (p, Figure 7E) stimulated the p21^{Waf1} promoter several-fold over the vector control (V), the stimulation became much more pronounced when β-catenin was also included (p+β). Similar results were obtained with the cyclin G promoter (Figure 7F).

It was important to confirm that the enhanced transcriptional activity of p53 operates not only on artificial promoter constructs, but also on authentic p53-responsive chromosomal genes. To that end, intracellular levels of proteins encoded by representative p53 target genes were monitored in transfected H1299 cells. Transfection with β-catenin alone did not affect the very low levels of endogenous p21^{Waf1} protein (β, Figure 8A). While transfection with p53 (p) increased the expression of p21^{Waf1}, co-transfection with β-catenin (p+β) had a significantly stronger stimulatory effect. A similar induction was also observed with Mdm2, another protein encoded by a physiologically relevant p53 target gene in H1299 cells (Figure 8B), as well as in 10(1) cells (data not shown). Hence, excess β-catenin can trigger accumulation of transcriptionally active p53 and stimulate the expression of p53-regulated genes.

p53 levels are elevated in cells overexpressing the Dishevelled gene product

Nuclear translocation of β-catenin with consequent transcriptional activation can be induced by triggering of the Wnt/Wg signaling pathway (Cox and Peifer, 1998; Willert and Nusse, 1998). The product of the Dishevelled (*Dsh*) gene is a component of the Wnt/Wg pathway, whose activation prevents β-catenin degradation and promotes the nuclear accumulation of β-catenin. To find out whether aberrant activation of the Wnt/Wg pathway can also give rise to p53 accumulation, NIH 3T3 cells were transiently transfected with DNA encoding the *Xenopus laevis* Dishevelled protein (XDsh; Sokol 1996). As expected, cells overexpressing Dishevelled exhibited strong nuclear β-catenin staining (Figure 9C and D). Importantly, many of the Dishevelled overexpressors also displayed nuclear accumulation of p53 (Figure 9A and B), whereas overexpression of GFP did not have a similar effect on p53 levels (Figure 9E and F). Hence, p53 accumulation can also be triggered when β-catenin is activated through excess signaling by the Wnt/Wg pathway. It should be noted, however, that while the vast majority of Dishevelled-overexpressing cells exhibited nuclear accumulation of β-catenin, a clear increase in nuclear p53 was seen in only about half of these cells (data not shown). This suggests that additional factors may modulate the ability of excess Wnt/Wg signaling to induce p53 accumulation in a cell context-dependent manner.

Discussion

The data presented in this study demonstrate that overexpression of β-catenin can promote the accumulation of functionally competent p53. This appears to occur through the ability of excess β-catenin to interfere with the normal proteasomal degradation of p53. Thus, aberrant overexpression of β-catenin may qualify as another stress signal that triggers a cellular p53 response.

What might be the mechanism underlying this effect of β-catenin on p53? In the absence of evidence for a direct physical association between p53 and β-catenin (data not shown), the mechanism is most likely indirect. One possibility is that a rate-limiting component of the

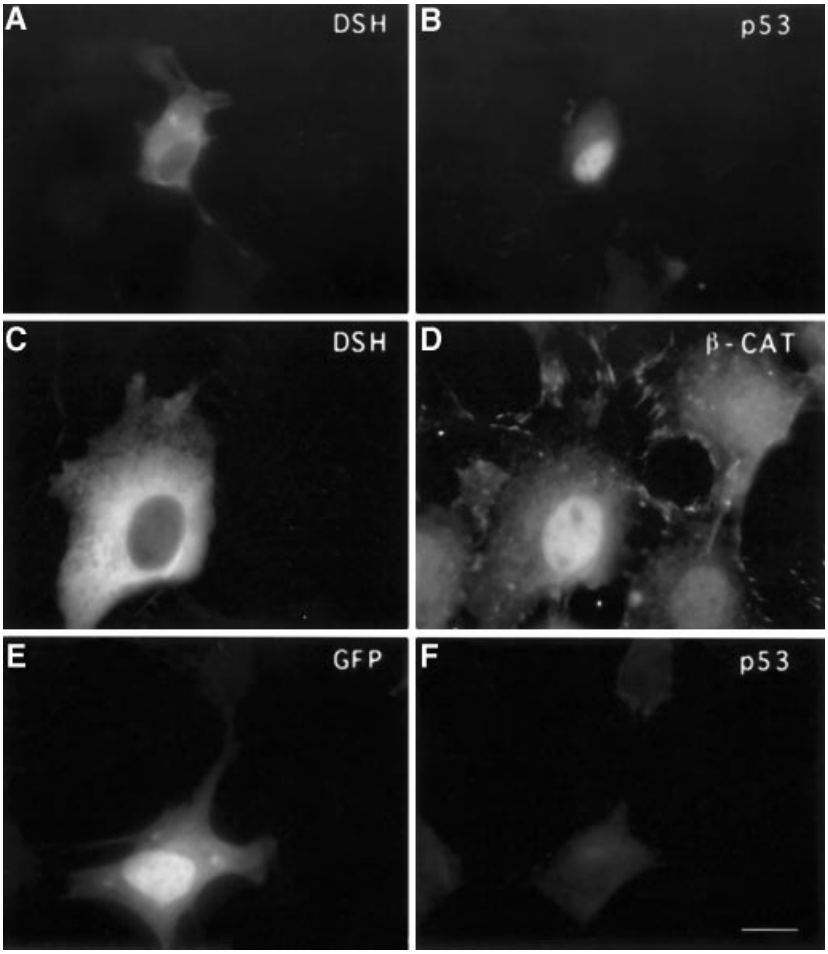

Fig. 9. Overexpression of Dishevelled causes elevation of nuclear p53. NIH 3T3 cells were transiently transfected with *Xenopus laevis* Dishevelled (DSH, A–D) or with GFP (E and F) and double-stained for DSH together with either p53 (**A** and **B**) or β-catenin (**C** and **D**), or for GFP together with p53 (**E** and **F**). The bar represents 10 μm.

ubiquitin–proteasome pathway is shared between p53 and β-catenin, which like p53 is also a target for proteolytic degradation by this pathway (Aberle *et al*., 1997; Salomon *et al*., 1997). The ubiquitination of mammalian β-catenin has recently been shown to be mediated through its interaction with the F-box protein β-TrCP, a homologue of the *Drosophila* Slimb protein (Hart *et al.*, 1999; Latres *et al*., 1999; Winston *et al*., 1999). It will be of interest to examine whether β-TrCP might be such a shared component, particularly in the context of Mdm2-independent stabilization of p53 by excess β-catenin.

Alternatively, β-catenin may reprogram the cell in a way that compromises the normal rapid degradation of p53. The latter may account for the Mdm2-dependent component of the β-catenin effect. Specifically, one of the oncogenic proteins reported to induce p53 accumulation is c-myc (Hermeking and Eick, 1994). It now appears that c-myc exerts this effect by stimulating the synthesis of mRNA encoding the alternative reading frame product (ARF) of the p16/INK4a tumor suppressor gene (Zindy *et al*., 1998). ARF, in turn, binds to both p53 and Mdm2 (Kamijo *et al*., 1998). The consequence of this molecular interaction is interference with the ability of Mdm2 to target p53 for rapid degradation (Pomerantz *et al*., 1998; Zhang *et al*., 1998). Accordingly, induction of ARF expression by excess c-myc can eventually lead to nuclear accumulation of p53 (Zindy *et al*., 1998). Interestingly, the c-*myc* gene has recently been identified as a target for transcriptional activation by the β-catenin–TCF transcription complex (He *et al*., 1998). One can therefore envisage a scenario where deregulated β-catenin, through induction of c-myc expression, triggers an increase in ARF levels with a consequent inhibition of p53 proteolysis. This, however, is most probably not the only explanation for β-catenin-induced p53 accumulation, as such accumulation was also observed in NIH 3T3 cells (Figure 2) that do not express detectable ARF (Kamijo *et al*., 1998; O.Shifman and M.Oren, unpublished observations).

Our findings imply that cells undergoing an aberrant increase in non-membrane-associated β-catenin, resulting for example from mutations in APC or in β-catenin itself, may respond by upregulating p53. This would restrain subsequent proliferation, providing a p53-mediated tumor suppressor function and a pressure for p53 inactivation. A similar sequence of events was proposed to occur in response to the aberrant oncogenic activation of ras (Serrano *et al*., 1997) or c-myc (Hermeking and Eick, 1994; Wagner *et al*., 1994; Zindy *et al*., 1998), both being important contributors to human cancer. Induction of p53 will also provide a safeguard against further accumulation

of genomic instability (Donehower *et al*., 1995; Levine, 1997), which otherwise is likely to accelerate tumor progression (Kinzler and Vogelstein, 1996).

Activation of p53 in cancer cells can promote apoptosis. However, while increased resistance to apoptosis may contribute to intestinal tumor progression (Bedi *et al*., 1995), this is probably not achieved through p53 inactivation (Fazeli *et al*., 1997). Moreover, while APC inactivation and β-catenin accumulation in a mouse intestinal carcinogenesis model correlate with decreased cell proliferation, apoptosis is also reduced in this system (Mahmoud *et al*., 1997). Thus p53 activation, if occurring under such circumstances, is expected to contribute primarily to the observed growth inhibition but not to the induction of apoptosis. In this regard, it is worth noting that β-catenin has been shown to mediate growth arrest in the developing fly wing (Johnston and Edgar, 1998).

It is nevertheless conceivable that under different circumstances the activation of p53 in response to β-catenin overexpression may also promote apoptosis. Interestingly, constitutive activation of Armadillo, the *Drosophila* β-catenin homologue, induces apoptosis in retinal neurons (Ahmed *et al*., 1998). On the other hand, elevated β-catenin correlates with reduced apoptosis in mouse intestinal cells (Mahmoud *et al*., 1997), consistent with the apoptosis induced by APC overexpression in human colorectal cancer cells (Morin *et al*., 1996). Thus, the biological outcome of excess β-catenin can vary greatly with cell type. It is likely that the effect of β-catenin on p53 turnover may also be very different in different cell types.

Our model predicts increased p53 protein levels in early colorectal tumors, subsequent to loss of APC function or acquisition of mutations in β-catenin. Several observations support, at least in part, this conjecture. For example, in cultured colorectal cells progressing *in vitro* from an adenoma to a carcinoma-like state, a concomitant increase in wt p53 is observed (Williams *et al*., 1993). Accumulation of apparently wt p53 has been described in the early stages of some tumors, including colorectal adenoma (Tominaga *et al*., 1993; Bennett, 1995). p53 overexpression, often without overt p53 mutation, also occurs in early adenomas of familial adenomatous polyposis patients carrying APC germline mutations (Levi *et al*., 1996; Kashiwagi *et al*., 1997). The findings described in the present study may provide an explanation for these observations.

It is also of note that reduced E-cadherin expression in breast cancer correlates strongly with accumulation of mutant p53 (Bukholm *et al*., 1997; Charpin *et al*., 1997). This may imply that the decrease in E-cadherin and the consequent release of β-catenin into the cytosol and nucleus may trigger p53 stabilization and provide a selective pressure for loss of wt p53 function.

Interestingly, increased cell–substrate adhesion augments p53 stability in primary keratinocytes, but not in fibroblasts (Nigro *et al*., 1997). Moreover, marked differences in the p53 response exist between normal cells and their immortalized/transformed progeny (Kantak and Kramer, 1998). It is thus possible that the ability of excess β-catenin to upregulate p53 becomes manifest in colorectal epithelial cells only when subsequent genetic alterations, associated with tumor progression, alter the intracellular context in a manner conducive to p53 stabilization. Alternatively, p53 stabilization may take place already at an early stage of colorectal tumor progression, but the consequent increased activity of p53 may become rate limiting only after additional genetic lesions have occurred. Either way, accumulation of active p53 might inhibit further tumor progression, imposing a selective pressure for p53 inactivation. Such a scenario would explain the typically late emergence of p53 mutations in colorectal cancer.

Materials and methods

Cells and transfections

Cells were transfected by the calcium phosphate coprecipitation method, as described earlier (Haupt *et al*., 1997). For luciferase assays and for analysis of p53 protein, cells were plated in 6 cm dishes at a density of 5×10^5 or 2.5×10^5 cells/dish for NIH 3T3 and 10(1) cells, respectively. For analysis of endogenous $p21^{Waf1}$ and Mdm2 proteins, cells were plated at a density of 1×10^6 cells/10 cm dish. Plasmids encoding human wt p53, mutant human p53 and murine Mdm2 were as described (Shaulian *et al*., 1992; Haupt *et al*., 1997). Expression plasmids for mouse wt β-catenin were based on either pCGN (Simcha *et al*., 1998) or pCDNA3 (Invitrogen); essentially identical results were obtained with both types of vectors (data not shown). The total amount of DNA in all parallel transfection mixtures was kept equal by addition of pCDNA3 DNA wherever necessary.

Retroviral infections

Green fluorescent protein was obtained by PCR amplification of the pEGFP-C1 plasmid (Clontech), using the following primers: ACCTTCTAGAATGGTGAGCAAGGGCGAGG and ACCTACTAGTTTACTTGTACAGCTCGTCCATG. After digestion with *Xba*I plus *Spe*I, the PCR product was ligated into pHA β-catenin DNA (Simcha *et al*., 1998) that had been cut with *Xba*I. The *Xba*I site located within the polylinker of pIND (Invitrogen) was abolished by cutting, end-filling and religation, giving rise to pIND-Δ*Xba*. The 5′ UTR of the *tk* gene was obtained by PCR amplification from pCGN DNA (Simcha *et al*., 1998) using the following primers: ACCTCTTAAGATCTTGGTGGCGTGAAACTCC and ACCTGGTACCTCTAGAGGCACGTCATAAGGATAGC.

The PCR product was cleaved with *Bfr*I and *Kpn*I, and subcloned into pIND-Δ*Xba* that had been cut with *Bfr*I plus *Kpn*I, giving rise to plasmid pIND-tkUTR. β-catenin Ser33Tyr and GFP-β-catenin were excised from the corresponding pCGN-based expression plasmids by cleavage with *Xba*I and *Bam*HI, and subcloned into pIND-tkUTR that had been cleaved with *Xba*I and *Bam*HI. This gave rise to plasmids pIND-β-catenin-Ser33Tyr and pIND-GFP-β-catenin, respectively. Each of these two plasmids was then cleaved with *Pme*I and *Xho*I, releasing the fusion protein-encoding inserts. Finally, each insert was ligated into pBabe-Puro retroviral vector DNA that had been cleaved with *Sna*BI and *Sal*I.

High titer retroviral stocks were produced by transfecting each of the retroviral constructs into 293T cells, by the calcium phosphate coprecipitation method, together with ψ^- ecotropic packaging vector pSV-ψ^- E-MLV plasmid providing ecotropic packaging helper function (Muller *et al.*, 1991). Virus-containing culture supernatants were collected 24–72 h post-transfection, at 6 h intervals, and pooled together. Passage 4 mouse embryo fibroblasts (2×10^5/10-cm dish) were infected with filtered supernatants in the presence of polybrene (Sigma). Fresh supernatants were added three times, at 4 h intervals, to achieve a higher overall rate of infection. Cells were then replenished with regular culture medium. At 24 h post-infection, the cultures were placed in medium containing 1% fetal calf serum. After another 24 h, cells were harvested and subjected to Western blot analysis.

Protein and RNA analysis

Steady-state protein levels were monitored as described (Haupt *et al.*, 1997). The following antibodies were used: PAb1801 and DO-1, specific for human p53; C-19 (Santa Cruz Biotechnology), specific for $p21^{Waf1}$; 2A10, specific for Mdm2 (Chen *et al*., 1993). Western blots were developed with the ECL kit (Amersham). RNA was probed by Northern blot analysis as described (Israeli *et al*., 1997).

Luciferase assays

Luciferase assays were performed using a commercial kit (Promega). Luminescence was determined with the aid of a TD-20e luminometer (Turner Design) as described previously (Friedlander *et al*., 1996).

Immunofluorescence

Double immunofluorescence with polyclonal β-catenin antibodies (Sigma), polyclonal anti-HA antibodies and mouse p53-specific monoclonal antibody PAb248 was performed as described previously (Simcha *et al*., 1998).

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References

Aberle,H., Bauer,A., Stappert,J., Kispert,A. and Kemler,R. (1997) β-catenin is a target for the ubiquitin–proteasome pathway. *EMBO J.*, **16**, 3797–3804.

Agarwal,M.L., Taylor,W.R., Chernov,M.V., Chernova,O.B. and Stark,G.R. (1998) The p53 network. *J. Biol. Chem.*, **273**, 1–4.

Ahmed,Y., Hayashi,S., Levine,A. and Wieschaus,E. (1998) Regulation of armadillo by a *Drosophila* APC inhibits neuronal apoptosis during retinal development. *Cell*, **93**, 1171–1182.

Almog,N. and Rotter,V. (1998) An insight into the life of p53: a protein coping with many functions! *Biochim. Biophys. Acta Rev. Cancer*, **1378**, R43–R54.

Bedi,A., Pasricha,P.J., Akhtar,A.J., Barber,J.P., Bedi,G.C., Giardiello, F.M., Zehnbauer,B.A., Hamilton,S.R. and Jones,R.J. (1995) Inhibition of apoptosis during development of colorectal cancer. *Cancer Res.*, **55**, 1811–1816.

Behrens,J., von Kries,J.P., Kuhl,M., Bruhn,L., Wedlich,D., Grosschedl,R. and Birchmeier,W. (1996) Functional interaction of β-catenin with the transcription factor LEF-1. *Nature*, **382**, 638–642.

Behrens,J., Jerchow,B.A., Wurtele,M., Grimm,J., Asbrand,C., Wirtz,R., Kuhl,M., Wedlich,D. and Birchmeier,W. (1998) Functional interaction of an axin homolog, conductin, with β -catenin, APC and GSK3 β. *Science*, **280**, 596–599.

Ben-Ze'ev,A. and Geiger,B. (1998) Differential molecular interactions of β-catenin and plakoglobin in adhesion, signaling and cancer. *Curr. Opin. Cell Biol.*, **10**, 629–639.

Bennett,W.P. (1995) p53 alterations in progenitor lesions of the bronchus, esophagus, oral cavity and colon. *Cancer Detect. Prev.*, **19**, 503–511.

Bottger,A., Bottger,V., Sparks,A., Liu,W.L., Howard,S.F. and Lane,D.P. (1997) Design of a synthetic Mdm2-binding mini protein that activates the p53 response *in vivo*. *Curr. Biol.*, **7**, 860–869.

Bukholm,I.K., Nesland,J.M., Karesen,R., Jacobsen,U. and Borresen,D.A. (1997) Expression of E-cadherin and its relation to the p53 protein status in human breast carcinomas. *Virchows Arch.*, **431**, 317–321.

Bullions,L.C. and Levine,A.J. (1998) The role of β-catenin in cell adhesion, signal transduction and cancer. *Curr. Opin. Oncol.*, **10**, 81–87.

Charpin,C., Garcia,S., Bouvier,C., Devictor,B., Andrac,L., Choux,R. and Lavaut,M. (1997) E-cadherin quantitative immunocytochemical assays in breast carcinomas. *J. Pathol.*, **181**, 294–300.

Chen,J., Marechal,V. and Levine,A.J. (1993) Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell. Biol.*, **13**, 4107–4114.

Clevers,H. and van de Wetering,M. (1997) TCF/LEF factor earn their wings. *Trends Genet.*, **13**, 485–489.

Coste,A.L. *et al*. (1998) Somatic mutations of the β-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc. Natl Acad. Sci. USA*, **95**, 8847–8851.

Cox,R.T. and Peifer,M. (1998) Wingless signaling: the inconvenient complexities of life. *Curr. Biol.*, **8**, R140–R144.

de Stanchina,E. *et al*. (1998) E1A signaling to p53 involves the p19 (ARF) tumor suppressor. *Genes Dev.*, **12**, 2434–2442.

Donehower,L.A. *et al*. (1995) Deficiency of p53 accelerates mammary tumorigenesis in Wnt-1 transgenic mice and promotes chromosomal instability. *Genes Dev.*, **9**, 882–895.

El-Deiry,W. *et al*. (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825.

Fazeli,A. *et al*. (1997) Effects of p53 mutations on apoptosis in mouse intestinal and human colonic adenomas. *Proc. Natl Acad. Sci. USA*, **94**, 10199–10204.

Friedlander,P., Haupt,Y., Prives,C. and Oren,M. (1996) A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis. *Mol. Cell. Biol.*, **16**, 4961–4971.

Giaccia,A.J. and Kastan,M.B. (1998) The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.*, **12**, 2973–2983.

Gumbiner,B.M. (1997) Carcinogenesis: a balance between β-catenin and APC. *Curr. Biol.*, **7**, R443–R446.

Hansen,R. and Oren,M. (1997) p53; from inductive signal to cellular effect. *Curr. Opin. Genet. Dev.*, **7**, 46–51.

Hart,M.J., de los Santos,R., Albert,I.N., Rubinfeld,B. and Polakis,P. (1998) Downregulation of β-catenin by human Axin and its association with the APC tumor suppressor, β-catenin and GSK3 β. *Curr. Biol.*, **8**, 573–581.

Hart,M. *et al.* (1999) The F-box protein β-TrCP associates with phosphorylated β-catenin and regulates its activity in the cell. *Curr. Biol.*, **9**, 207–210.

Harvey,D.M. and Levine,A.J. (1991) p53 alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts. *Genes Dev.*, **5**, 2375–2385.

Haupt,Y., Maya,R., Kazaz,A. and Oren,M. (1997) Mdm2 promotes the rapid degradation of p53. *Nature*, **387**, 296–299.

He,T.C., Sparks,A.B., Rago,C., Hermeking,H., Zawel,L., da Costa,L.T., Morin,P.J., Vogelstein,B. and Kinzler,K.W. (1998) Identification of c-MYC as a target of the APC pathway. *Science*, **281**, 1509–1512.

Hermeking,H. and Eick,D. (1994) Mediation of c-Myc-induced apoptosis by p53. *Science*, **265**, 2091–2093.

Honda,R., Tanaka,H. and Yasuda,H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.*, **420**, 25–27.

Ikeda,S., Kishida,S., Yamamoto,H., Murai,H., Koyama,S. and Kikuchi,A. (1998) Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3 β and β-catenin and promotes GSK-3 β-dependent phosphorylation of β-catenin. *EMBO J.*, **17**, 1371–1384.

Ilyas,M., Tomlinson,I.P., Rowan,A., Pignatelli,M. and Bodmer,W.F. (1997) β-catenin mutations in cell lines established from human colorectal cancers. *Proc. Natl Acad. Sci. USA*, **94**, 10330–10334.

Inomata,M., Ochiai,A., Akimoto,S., Kitano,S. and Hirohashi,S. (1996) Alteration of β-catenin expression in colonic epithelial cells of familial adenomatous polyposis patients. *Cancer Res.*, **56**, 2213–2217.

Israeli,D., Tessler,E., Haupt,Y., Elkeles,A., Wilder,S., Amson,R., Telerman,A. and Oren,M. (1997) A novel p53-inducible gene, PAG608, encodes a nuclear zinc finger protein whose overexpression promotes apoptosis. *EMBO J.*, **16**, 4384–4392.

Johnston,L.A. and Edgar,B.A. (1998) Wingless and Notch regulate cell-cycle arrest in the developing *Drosophila* wing. *Nature*, **394**, 82–84.

Kamijo,T., Weber,J.D., Zambetti,G., Zindy,F., Roussel,M.F. and Sherr,C.J. (1998) Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc. Natl Acad. Sci. USA*, **95**, 8292–8297.

Kantak,S.S. and Kramer,R.H. (1998) E-cadherin regulates anchorage-independent growth and survival in oral squamous cell carcinoma cells. *J. Biol. Chem.*, **273**, 16953–16961.

Kashiwagi,H., Spigelman,A.D., Talbot,I.C., Debinski,H.S., McKie,A.B., Lemoine,N.R. and Phillips,R.K. (1997) p53 and K-ras status in duodenal adenomas in familial adenomatous polyposis. *Br. J. Surg.*, **84**, 826–829.

Kemler,R. (1993) From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.*, **9**, 317–321.

Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.

Korinek,V., Barker,N., Morin,P.J., van Wichen,D., de Weger,R., Kinzler,K.W., Vogelstein,B. and Clevers,H. (1997) Constitutive transcriptional activation by a β-catenin–Tcf complex in APC–/– colon carcinoma. *Science*, **275**, 1784–1787.

Kubbutat,M.H. and Vousden,K.H. (1998) Keeping an old friend under control: regulation of p53 stability. *Mol. Med. Today*, **4**, 250–256.

Kubbutat,M.H., Jones,S.N. and Vousden,K.H. (1997) Regulation of p53 stability by Mdm2. *Nature*, **387**, 299–303.

Latres,E., Chiaur,D.S. and Pagano,M. (1999) The human F box protein β-Trcp associates with the Cul1/Skp1 complex and regulates the stability of β-catenin. *Oncogene*, **18**, 849–854.

Levi,E., Stryker,S.J. and Rao,M.S. (1996) p53 protein overexpression in colorectal tumors from patients with familial adenomatous polyposis: is it an early or late event? *Am. J. Gastroenterol.*, **91**, 11–14.

Levine,A.J. (1997) p53, the cellular gatekeeper for growth and division. *Cell*, **88**, 323–331.

Lozano,G. and Montes de Oca Luna,R. (1998) MDM2 function. *Biochim. Biophys. Acta*, **1377**, M55–M59.

McMasters,K.M., Montes de Oca Luna,R., Pena,J.R. and Lozano,G. (1996) mdm2 deletion does not alter growth characteristics of p53-deficient embryo fibroblasts. *Oncogene*, **13**, 1731–1736.

Mahmoud,N.N., Boolbol,S.K., Bilinski,R.T., Martucci,C., Chadburn,A. and Bertagnolli,M.M. (1997) Apc gene mutation is associated with a dominant-negative effect upon intestinal cell migration. *Cancer Res.*, **57**, 5045–5050.

Maki,C.G., Huibregtse,J.M. and Howley,P.M. (1996) *In vivo* ubiquitination and proteasome-mediated degradation of p53. *Cancer Res.*, **56**, 2649–2654.

Mitsudomi,T. *et al*. (1992) p53 gene mutations in non-small-cell lung cancer cell lines and their correlation with the presence of ras mutations and clinical features. *Oncogene*, **7**, 171–180.

Miyoshi,Y., Iwao,K., Nagasawa,Y., Aihara,T., Sasaki,Y., Imaoka,S., Murata,M., Shimano,T. and Nakamura,Y. (1998) Activation of the β-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res.*, **58**, 2524–2527.

Molenaar,M., van de Wetering,M., Oosterwegel,M., Peterson-Maduro,J., Godsave,S., Korinek,V., Roose,J., Destree,O. and Clevers,H. (1996) XTcf-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos. *Cell*, **86**, 391–399.

Morin,P.J., Vogelstein,B. and Kinzler,K.W. (1996) Apoptosis and APC in colorectal tumorigenesis. *Proc. Natl Acad. Sci. USA*, **93**, 7950–7954.

Morin,P.J., Sparks,A.B., Korinek,V., Barker,N., Clevers,H., Vogelstein,B. and Kinzler,K.W. (1997) Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science*, **275**, 1787–1790.

Muller,A.J., Young,J.C., Pendergast,A.-M., Pondel,M., Landau,N.R., Littman,D.R. and Witte,O.N. (1991) BCR first exon sequences specifically activate the BCR/ABL tyrosine kinase oncogene of Philadelphia chromosome-positive human leukemias. *Mol. Cell. Biol.*, **11**, 1785–1792.

Munemitsu,S., Albert,I., Souza,B., Rubinfeld,B. and Polakis,P. (1995) Regulation of intracellular β-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc. Natl Acad. Sci. USA*, **92**, 3046–3050.

Nigro,J.M., Aldape,K.D., Hess,S.M. and Tlsty,T.D. (1997) Cellular adhesion regulates p53 protein levels in primary human keratinocytes. *Cancer Res.*, **57**, 3635–3639.

Okamoto,K. and Beach,D. (1994) Cyclin G is a transcriptional target of the p53 tumor suppressor protein. *EMBO J.*, **13**, 4816–4822.

Palombella,V.J., Rando,O.J., Goldberg,A.L. and Maniatis,T. (1994) The ubiquitin–proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. *Cell*, **78**, 773–785.

Peifer,M. (1997) β-catenin as oncogene: the smoking gun. *Science*, **275**, 1752–1753.

Polakis,P. (1997) The adenomatous polyposis coli (APC) tumor suppressor. *Biochim. Biophys. Acta*, **1332**, F127–F147.

Pomerantz,J. *et al*. (1998) The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell*, **92**, 713–723.

Prives,C. (1998) Signaling to p53: breaking the MDM2–p53 circuit. *Cell*, **95**, 5–8.

Riese,J., Yu,X., Munnerlyn,A., Eresh,S., Hsu,S.C., Grosschedl,R. and Bienz,M. (1997) LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell*, **88**, 777–787.

Rubinfeld,B., Robbins,P., El-Gamil,M., Albert,I., Porfiri,E. and Polakis,P. (1997) Stabilization of β -catenin by genetic defects in melanoma cell lines. *Science*, **275**, 1790–1792.

Salomon,D., Sacco,P.A., Roy,S.G., Simcha,I., Johnson,K.R., Wheelock,M.J. and Ben-Ze'ev,A. (1997) Regulation of β-catenin levels and localization by overexpression of plakoglobin and inhibition of the ubiquitin-proteasome system. *J. Cell Biol.*, **139**, 1325–1335.

Serrano,M., Lin,A.W., McCurrach,M.E., Beach,D. and Lowe,S.W. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*, **88**, 593–602.

Shaulian,E., Zauberman,A., Ginsberg,D. and Oren,M. (1992) Identification of a minimal transforming domain of p53: negative dominance through abrogation of sequence-specific DNA binding. *Mol. Cell. Biol.*, **12**, 5581–5592.

Shtutman,M., Zhurinsky,J., Simcha,I., Albanese,C., D'Amico,M., Pestell,R. and Ben-Ze'ev,A. (1999) The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. *Proc. Natl. Acad. Sci. USA*, in press.

Simcha,I., Shtutman,M., Salomon,D., Zhurinsky,J., Sadot,E., Geiger,B. and Ben-Ze'ev,A. (1998) Differential nuclear translocation and transactivation potential of β-catenin and plakoglobin. *J. Cell Biol.*, **141**, 1433–1448.

Sokol,S.Y. (1996) Analysis of Dishevelled signalling pathways during Xenopus development. *Curr. Biol*, **6**, 1456–1467.

Sparks,A.B., Morin,P.J., Vogelstein,B. and Kinzler,K.W. (1998) Mutational analysis of the APC/β-catenin/Tcf pathway in colorectal cancer. *Cancer Res.*, **58**, 1130–1134.

Tetsu,O. and McCormick,F. (1999) β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*, **398**, 422–426.

Tominaga,O., Hamelin,R., Trouvat,V., Salmon,R.J., Lesec,G., Thomas,G. and Remvikos,Y. (1993) Frequently elevated content of immunochemically defined wild-type p53 protein in colorectal adenomas. *Oncogene*, **8**, 2653–2658.

van de Wetering,M. *et al*. (1997) Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell*, **88**, 789–799.

Wagner,A.J., Kokontis,J.M. and Hay,N. (1994) Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. *Genes Dev.*, **8**, 2817–2830.

Willert,K. and Nusse,R. (1998) β-catenin: a key mediator of Wnt signaling. *Curr. Opin. Genet. Dev.*, **8**, 95–102.

Williams,A.C., Browne,S.J., Yeudal,W.A., Paterson,I.C., Marshall,C.J., Lane,D.P. and Paraskeva,C. (1993) Molecular events including p53 and k-ras alterations in the *in vitro* progression of a human colorectal adenoma cell line to an adenocarcinoma. *Oncogene*, **8**, 3063–3072.

Winston,J.T., Strack,P., Beer-Romero,P., Chu,C.Y., Elledge,S.J. and Harper,J.W. (1999) The SCF β–TRCP–ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination *in vitro*. *Genes Dev.*, **13**, 270–283.

Zauberman,A., Lupo,A. and Oren,M. (1995) Identification of p53 target genes through immune selection of genomic DNA: the cyclin G gene contains two distinct p53 binding sites. *Oncogene*, **10**, 2361–2366.

Zhang,Y., Xiong,Y. and Yarbrough,W.G. (1998) ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell*, **92**, 725–734.

Zindy,F., Eischen,C.M., Randle,D.H., Kamijo,T., Cleveland,J.L., Sherr,C.J. and Roussel,M.F. (1998) Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev.*, **12**, 2424–2433.

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