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Correspondence re: T. Zhang *et al.*, Evidence That APC Regulates Survivin Expression: A Possible Mechanism Contributing to the Stem Cell Origin of Colon Cancer. Cancer Res., *61:* 8664–8667, 2001.

Letter

Survivin is highly expressed in the majority of colorectal cancers (1). The possible involvement of survivin (a structurally unique member of the inhibitor of apoptosis family proteins that is potentially involved also in the control of cell division) in the pathogenesis of colorectal cancer has been also supported by Zhang *et al.* (2) in a recent issue of *Cancer Research*. The authors demonstrated a down-regulation of survivin mRNA and protein expression in the HT-29 colorectal cancer cell line by the *wild-type adenomatous polyposis coli (wt-APC)* gene, which plays a pivotal role in the modulation of the T-cell factor/β-catenin signaling pathway through β-catenin degradation. Moreover, Zhang *et al.* (2) found survivin preferentially expressed in the lower section of the normal human colonic crypts, confirming an inverse pattern with wt-APC expression. Again, at preclinical level, in the HCT-116 colorectal cancer cell line, it has been shown recently by Kim *et al.* (3) that a deregulation of the T-cell factor/β-catenin signaling pathway leads to an increased expression of survivin, which, in turn, results in the disruption of the balance between cell proliferation/differentiation and apoptosis. All together these findings would suggest a link between survivin expression and APC mutation and/or β-catenin accumulation.

We investigated survivin expression by immunohistochemistry (4) in a series of 71 colon cancers from patients submitted to radical surgery at the National Cancer Institute of Milan, Italy, between 1998 and 2000 to confirm the observed preclinical data also at a clinical level. Microsatellite instability was present in 10 of the 71 colon cancers, and APC exon 15 mutations were detected in 46 of the 61 microsatellite instability (MSI) negative cases and in 3 of the 10 MSI positive cancers. β-Catenin was overexpressed both at cytoplasmic and nuclear level in all of the MSI-negative tumors without APC mutation, in agreement with the occurrence of alterations in the APC/β-catenin pathway as the first hit of carcinogenesis in MSI-negative colon cancers, whereas in all of the MSI-positive specimens, as in healthy mucosa, β-catenin expression was limited to the plasma membrane in keeping with the hypothesis of an alternative pathway of colonic tumorigenesis (5).

Survivin appears to be expressed in the majority of colon cancers, regardless of APC status and microsatellite instability/β-catenin accumulation (Table 1). Similarly, apoptotic and proliferation indices did not seem to be significantly different in MSI-positive and MSI-negative tumors. However, survivin expression appears to be strongly and directly associated with cell proliferation in MSI-positive (Spearman's regression coefficient, $r_s = 0.9$; $P = 0.0003$) but not in MSI-negative/β-catenin accumulating cancers ($r_s = 0.04$). Such a finding could indirectly support the hypothesis that in MSI-negative colon cancers with altered APC/β-catenin pathway survivin may contribute to neoplastic transformation by increasing cell resistance to apoptosis, whereas in MSI-positive colon cancers a prevalent role of survivin to enhance tumor cell proliferation might be suggested. The association between survivin expression and cell proliferation or apoptosis in colon cancer has been reported previously by other authors (6) but never investigated as a function of dysregulation in the APC/β-catenin/T-cell factor pathway. Our findings on clinical tumors are in part consistent with observations derived from elegant studies on colon carcinoma cell lines (2, 3), and the identification of survivin as a possible downstream target of the APC/β-catenin/T-cell factor pathway might open perspectives for the use of approaches contrasting survivin expression in preventive and therapeutic interventions (6).

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Table 1 *Biological features associated with proliferation and apoptosis in colon cancers characterized for dysregulation in the APC/β-catenin/T-cell factor pathway*

		Survivin expression[a]		Apoptotic index[b]	Proliferation index[c]
	Total cases	Median value (range)	% of positive cases	median value (range)	median value (range)
β-Catenin negative[d] (microsatellite instability positive)	10	70 (0–100)	80%	0.6 (0–1.2)	72.5 (50–100)
β-Catenin positive (microsatellite instability negative)	61	50 (0–90)	74%	0.4 (0–18.7)	85.0 (50–100)

[a] Percentage of tumor cells with cytoplasmic immunostaining. Cut-off value for positive cases = 25% of positive cells.
[b] Percentage of tumor cells with cytoplasmic immunostaining for M30.
[c] Percentage of tumor cells with nuclear immunostaining for MIB-1.
[d] β-Catenin protein expression was evaluated on formalin-fixed, paraffin-embedded samples by immunohistochemistry (mouse monoclonal anti-β-catenin antibody; Transduction Laboratories, Lexington, KY) for the presence of nuclear, cytoplasmic, and membranous accumulation in both tumor and normal surrounding tissues.

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Reply

On the basis of studies using carcinoma cell lines, we (1) and others (2) reported that survivin expression is down-regulated by wild-type adenomatous polyposis coli (APC) via the inhibition by APC of β-catenin/T-cell factor (TCF) -4 signaling. We hypothesized that up-regulation of survivin expression occurs when *APC* is mutant and that this mechanism helps promote colon cancer development (Scheme 1, see next page). The objective of the study by Daidone *et al.* (3) was to confirm these preclinical reports using clinical data, which hitherto had not been done, by determining whether survivin expression is associated with *APC* mutation or with up-regulation of β-catenin/TCF-4 signaling in colon cancers. To this end, they investigated survivin protein expression by immunohistochemistry in colon carcinomas from an unselected series of surgical patients ($n = 71$). They correlated survivin expression with *APC* mutation, β-catenin immunostaining patterns, and microsatellite instability (MSI) status.

On the basis of detection of *APC* mutations, they state that "survivin appears to be expressed in the majority of colon cancers regardless of APC status. . . ". However, they do not provide the data on *APC* mutation status with regard to survivin immunostaining status. They found mutations in exon 15 of *APC* in 49 (69%) of their total of 71 cases and survivin positivity in 54 (76%) of these 71 cases. The question is: what proportion of cases with and without an *APC* mutation have induction of survivin expression? Without this information, it would seem difficult to reach their stated conclusion. Moreover, it would also be useful to know whether these cases had homozygous or heterozygous *APC* mutations because cases with a heterozygous *APC* mutation would be predicted to have less (or no) activation of β-catenin/TCF-4 signaling compared to those cases with homozygous mutations in *APC*.

In their study, detection of nuclear and cytoplasmic (*versus* membraneous) immunostaining for β-catenin was used to classify colon cancers as having up-regulated (dysregulated) β-catenin/TCF-4 signaling. Their data showed that survivin is frequently overexpressed regardless of the β-catenin immunostaining pattern. Detecting survivin overexpression in tumors with nuclear β-catenin staining is consistent with our hypothesis (1) that mutant *APC*, via up-regulated β-catenin/TCF-4 signaling, leads to increased survivin expression that contributes to colon tumorigenesis by inhibiting apoptosis and promoting cell proliferation. Unexpectedly, they also found survivin overexpression in colon cancers that lack nuclear β-catenin staining, which raises our *first* question: why does survivin overexpression occur in cases where β-catenin/TCF-4 signaling does not appear to be up-regulated?

Lack of nuclear β-catenin staining in their series (10 of 71 cases) strongly correlated (100%) with MSI positivity. This included 3 MSI+ cases with mutant *APC* that had negative nuclear β-catenin immunostaining. Their results are consistent with a prior study showing that nuclear β-catenin staining is infrequent (13%) in sporadic colon cancers that are MSI+ (4). However, the frequency of nuclear β-catenin staining is somewhat higher (34%) in MSI+ colon cancers from hereditary nonpolyposis colorectal cancer patients (4), suggesting that nuclear β-catenin positivity can and does occur in some MSI+ colon cancers. The finding of Daidone *et al.* (3) that 30% of MSI+ cases have *APC* mutations is consistent with reports that truncation-causing *APC* mutations occur in ~50% of MSI+ colon cancers (5). Although not tested in their series, activating mutations in β-catenin are also frequent (up to 40% or more) in MSI+ colon cancers, and these mutations appear to have the same tumorigenic effect as *APC* mutations: they both activate the TCF-4 transcription factor (6–8). Indeed, immunohistochemical analysis of MSI+ tumors with β-catenin mutations shows that they all have nuclear β-catenin immunostaining (8).

Because the majority of MSI+ colon cancers (50–77% of cases) have mutations in *APC* or β-catenin (8–11), which should up-regulate TCF-4 signaling, it would be predicted that >50% of MSI+ colon cancers should stain for nuclear β-catenin. Hence, the frequency of nuclear immunostaining for β-catenin in MSI+ colon cancers from both hereditary and nonhereditary patients seems low in the current study and in the study by Young *et al.* (4). This raises our *second* question: why do some MSI+ colon cancers harboring *APC* or β-catenin mutations fail to show a nuclear immunostaining pattern for β-catenin, which is an indicator of activation of the β-catenin/TCF-4 signaling pathway?

One possible answer to our *second* question may be that in MSI+ tumors, mutations also occur in the *TCF-4* gene, which encodes the protein that is immediately downstream in the β-catenin signaling pathway. Indeed, frameshift mutations in the *TCF-4* gene frequently occur (30–50%) in MSI+ colon cancers (12–14). *TCF-4* mutations are found in MSI+ tumors with either *APC* or β-catenin mutations, although they are more frequent in tumors with mutant β-catenin. If *TCF-4* becomes mutant in a tumor containing an *APC* or a β-catenin mutation, perhaps this could lead to diminished accumulation (and lack of staining) of β-catenin in the nucleus, either because of reduced capacity of mutant TCF-4 to bind β-catenin or because the mutation affects the half-life of TCF-4, causing decreased intracellular TCF-4 levels. If, as experimental evidence on MSI+ tumors suggests (12), TCF-4 mutation stimulates its own transcriptional activity synergistically with *APC* or β-catenin mutations, up-regulation of this pathway might lead to constitutively elevated intracellular survivin because of an inability to down-modulate survivin expression. While another study indicates that TCF-4 frameshift mutations do not contribute to carcinogenesis of MSI+ colon cancers (13), early evidence (14) suggests that TCF-4 mutations are preferentially selected in tumors with β-catenin mutations because they induce down-regulation of β-catenin/TCF-4 signaling. This might maintain β-catenin/TCF-4 signaling at a specific level according to the "just-right" signaling model (15). We speculate that this mechanism might provide an explanation for our *first* question. That is, TCF-4 signaling might still be activated (by the combined effects of mutations in both β-catenin and TCF-4) even though β-catenin does not show nuclear staining. This could explain how some MSI+ colon tumors can be negative for nuclear β-catenin staining while still being positive for survivin.

Another possibility is that survivin becomes overexpressed in MSI+ tumors due to mechanisms that are independent of β-catenin/

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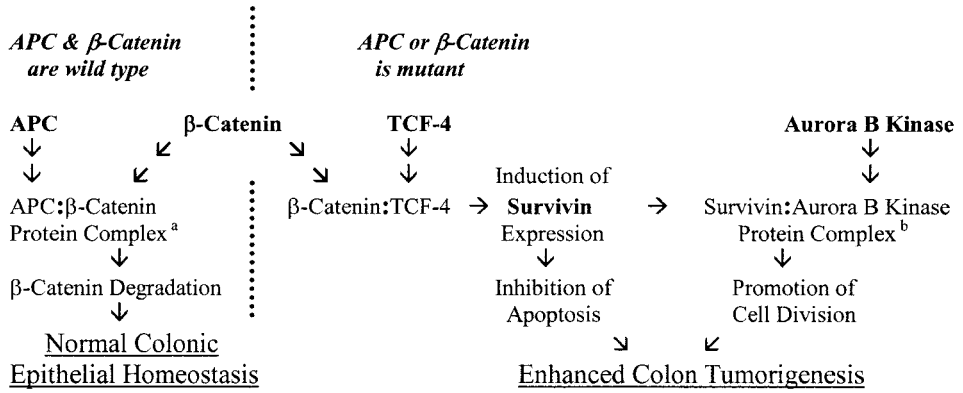

Scheme 1. This scheme is a diagram of the β-catenin/TCF-4/survivin pathway and its possible role in promoting colon tumorigenesis. The left side of the scheme shows the effect of APC on β-catenin when both proteins are wild type. The right side shows the consequences of homozygous mutations in *APC* or an activating mutation in β-catenin. These two conditions, which are depicted on the left and right side of the scheme, portray mechanisms linked to normal colonic epithelial homeostasis and enhanced colon tumorigenesis, respectively. [a]The APC:β-Catenin protein complex also contains the proteins axin and GSK3β. [b]The Survivin:Aurora B Kinase protein complex also contains the INCENP protein.

TCF-4 signaling. For example, survivin expression is negatively regulated by wild-type p53 via a p53 response element in the promoter region of survivin (16) or by chromatin structural modifications that expose the promoter region to p53 binding (17). The presence of mutant p53 is thought to contribute to inhibition of apoptosis through the antiapoptotic effects of survivin (18). However, p53 immunostaining studies indicate that p53 mutation is uncommon (3%) in MSI+ colon cancers (19). Nevertheless, in the occasional MSI+ colon cancer having mutant p53, loss of wild-type p53 might contribute to survivin overexpression. Moreover, there are other sequences in the survivin promoter region for putative transcription factor binding in addition to the ones for p53 and TCF-4, factors that could also regulate survivin expression (20). This suggests that there may be yet other mechanisms that could lead to survivin overexpression.

An additional factor that may confound correlative studies on survivin, such as the one by Daidone *et al.* (3), is that survivin expression may be affected by drug therapy. For example, the chemotherapeutic agent doxorubicin (18) and the nonsteroidal anti-inflammatory drug sulindac (21) have been reported to decrease survivin expression. This confound could potentially lower the proportion of survivin positive cases.

Yet another factor to consider is the method for evaluating survivin expression, which was based on the percentage of cells within a tumor that stain positive for survivin. It would have been informative if they had also provided information on survivin staining patterns compared with β-catenin staining patterns, because nuclear expression of β-catenin is known to be localized predominantly at the invading margin of colorectal cancers, whereas the central bulk of the tumor often lacks nuclear β-catenin staining (22). Thus, it is possible that tumors will only show focal areas that are positive for nuclear β-catenin at the invading margin and that these areas will also display survivin staining.

Despite these caveats, the study by Daidone *et al.* (3) provides valuable clinical data that extends the preclinical studies, because they show that survivin overexpression not only is associated with colon cancers having nuclear β-catenin staining, but also with colon cancers that arise due to an alternative pathway in colon tumorigenesis (MSI+). Additional novel findings by Daidone *et al.* (3) that survivin expression correlates with proliferation also provides important information because it is known that survivin enhances aurora B kinase activity, a protein that catalyzes chromosome segregation and cytokinesis (23, 24). Hence, the intracellular survivin concentration should increase tumor cell proliferation in proportion to the amount of survivin that binds to and activates aurora B kinase, which promotes cell division. Moreover, aurora B kinase is overexpressed in >50% of colorectal carcinomas (25, 26). Thus, in future studies, it will be important to determine whether survivin expression has prognostic significance for MSI+ colon cancers.

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Note Added in Proof

While our manuscript was in press we discovered a recent publication (27) which reports that immunostaining for nuclear β-catenin is rarely observed in colonic adenomas (which are typically homozygous for mutant *APC*) and is detected in fewer than half of colon carcinomas. Thus, results from this study failed to show a direct correlation between mutation of *APC* and elevated β-catenin levels in the nucleus and cytoplasm and the authors (27) concluded that "nuclear translocation of β-catenin may not be an immediate consequence of the loss of APC".

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