mediating tumor suppression. It has been well documented that genotoxic stress, including telomere dysfunction, triggers apoptosis in some cell types, whereas in others senescence is the primary response. While such choices are not well understood, it is comforting that, in B cells, which prefer apoptosis, senescence can act as a robust backup system. Finally, the possibility that oncogene- and telomere-induced senescence may act as reinforcing, two-tiered defense systems, especially in human cancers, needs to be considered.

REFERENCES

Ben-Porath, I., and Weinberg, R.A. (2004). J. Clin. Invest. *113*, 8–13.

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E. (1998). Science *279*, 349–352.

Campisi, J. (2005). Cell *120*, 1–10.

Cech, T.R. (2004). Cell *116*, 273–279.

Collado, M., and Serrano, M. (2006). Nat. Rev. Cancer *6*, 472–476.

Cosme-Blanco, W., Shen, M.F., Lazar, A.J.F., Pathak, S., Lozano, G., Multani, A.S., and Chang, S. (2007). EMBO R. Published online March 30, 2007. 10.1038/sj.embor.7400937.

d'Adda di Fagagna, F., Teo, S.H., and Jackson, S.P. (2004). Genes Dev. *18*, 1781–1799.

Feldser, D.M., and Greider, C.W. (2007). Cancer Cell, this issue.

Hayflick, L. (1965). Exp. Cell Res. *37*, 614–636.

Herbig, U., and Sedivy, J.M. (2006). Mech. Ageing Dev. *127*, 16–24.

Shay, J.W., and Wright, W.E. (2005). Carcinogenesis *26*, 867–874.

von Zglinicki, T., Saretzki, G., Ladhoff, J., d'Adda di Fagagna, F., and Jackson, S.P. (2005). Mech. Ageing Dev. *126*, 111–117.

Role of *c-Myc* in *Apc* Mutant Intestinal Phenotype: Case Closed or Time for a New Beginning?

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DOI 10.1016/j.ccr.2007.04.015

Inactivation of the *adenomatous polyposis coli* (*APC*) tumor suppressor gene occurs in most colorectal cancers. The proto-oncogene *c-MYC* was one of the first genes linked to *APC* inactivation, but the in vivo significance of *c-MYC*'s enhanced expression in intestinal cells with *APC* defects has been uncertain. Sansom et al. recently reported that targeted inactivation of c-Myc in murine intestinal epithelium potently inhibited phenotypical and transcriptional changes seen in *Apc*-deficient intestinal epithelium. While these findings are very interesting, some questions remain about the assignment of *c-Myc* as the pre-eminent β-catenin-regulated gene in intestinal epithelium.

The small intestine's epithelial lining is characterized by invagination of the so-called crypts into surrounding tissue and by the presence of finger-like protrusions (i.e., villi) extending into the lumen (Figure 1A). In the lower region of each crypt, there may be four to six stem cells (Marshman et al., 2002). Following their proliferative expansion in the crypt, progenitors differentiate into enterocytes, goblet cells, and enteroendocrine cells as they migrate upward along the crypt-villus axis to populate the villus surface, or into Paneth cells as they migrate down to the base of the crypt. Enterocytes undergo apoptosis and/or are shed from the mucosal surface only days after their birth.

The β-catenin protein has previously been established to have a central role in regulating proliferation, differentiation, and migration in intestinal epithelium (Batlle et al., 2002; Ireland et al., 2004; van de Wetering et al., 2002). Most of the cell's pool of β-catenin is tethered to E-cadherin as an adherens junction component to mediate intercellular adhesion. A less abundant "free" pool of β-catenin can serve as a transcriptional coactivator upon its translocation to the nucleus and binding to DNA-binding proteins of the T cell factor (TCF) family (Figures 1B and 1C). In most cells, the free pool of β-catenin is tightly regulated by a destruction complex composed of the adenomatous polyposis coli (APC) and Axin tumor suppres-

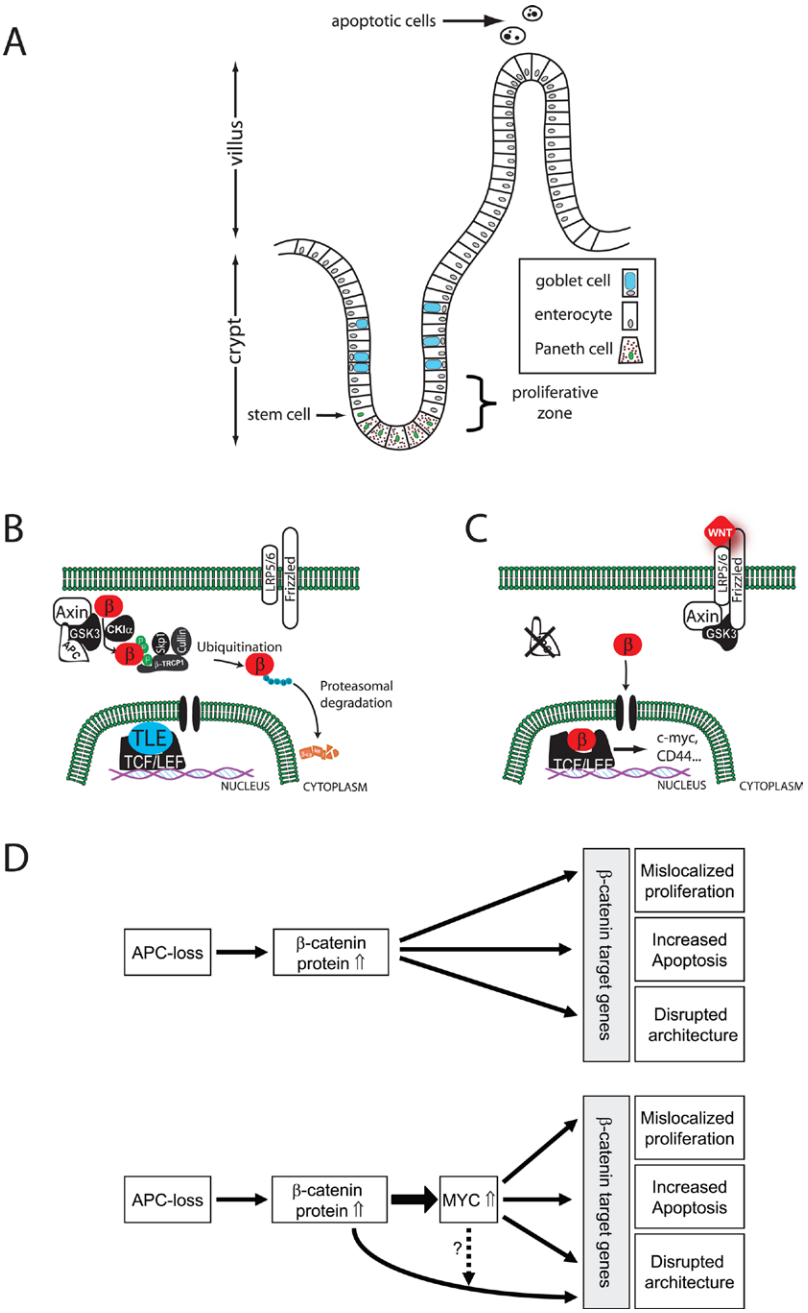

Figure 1. Cell Proliferation and Differentiation in the Small Intestine, β-Catenin-Dependent Wnt Signaling, and the Potential Role of MYC in Regulating β-Catenin-Dependent Transcription in the Intestine

(A) Schematic structure of the architecture of the epithelium in the small intestine. A stem cell (or cells) near the base of the crypt gives rise to a rapidly dividing population that subsequently differentiates into mature enterocytes, goblet cells, and enteroendocrine cells, which migrate up the crypt to populate the villus. After a life span of only a few days, enterocytes undergo apoptosis and are shed into the lumen of the gut. Wnt-β-catenin signaling is involved in the maintenance of a crypt progenitor-like phenotype and differentiation of progenitor cells.

(B) β-catenin (β) is constitutively phosphorylated at several conserved serine/threonine residues in its amino-terminal domain by a destruction complex consisting of the APC, Axin, glycogen synthase kinase 3β (GSK3), and casein kinase Iα (CKIα) proteins. Phosphorylated β-catenin is targeted for ubiquitination and subsequent proteasomal degradation. Transcription of potential β-catenin target genes is repressed due to binding of transcriptional repressors of the groucho/TLE family (TLE) to the DNA-binding proteins of the TCF (or lymphoid enhancer factor [LEF]) family.

(C) Upon binding of Wnt ligands to their cognate receptor, consisting of a protein of the Frizzled protein family and the LDL-lipoprotein receptor-related protein 5/6 (LRP5/6) family, destruction of β-catenin is inhibited. β-catenin translocates to the nucleus, binds to TCF/LEF proteins, recruits coactivators, and activates its transcriptional target genes, such as c-Myc and CD44. Similarly, mutational inactivation of APC or Axin, as well as mutation of conserved serine/threonine residues in β-catenin's N terminus, leads to the accumulation of active β-catenin.

(D) Postulated models of β-catenin function in *Apc*-deficient intestinal epithelial cells. The upper model suggests that the altered phenotypes seen in *Apc*-deficient intestinal cells are attributable to β-catenin's ability to alter transcription of a variety of downstream target genes. The lower model suggests that β-catenin's effects on cell phenotype are largely due to its ability to activate expression of *c-Myc* and that *c-Myc*'s increased expression is essential in the regulation of downstream β-catenin target genes and the altered cell phenotype of *Apc*-deficient intestinal cells.

sors, GSK3β, and other proteins. This complex promotes phosphorylation of β-catenin at several amino (N)-terminal residues, which leads to ubiquitination followed by proteasomal degradation of phosphorylated β-catenin (Clevers, 2006). The destruction complex is inhibited by binding of certain Wnt family ligands to transmembrane coreceptor complexes consisting of a frizzled family protein and an LRP5/6 protein (Figure 1C). Wnt/β-catenin signaling has an essential role in stem cell regulation in intestinal tissues, as inactivation of the genes for β-catenin or Tcf-4, a major TCF protein in intestinal cells, severely compromises crypt maintenance (Ireland et al., 2004; Korinek et al., 1998). Mutational defects interfering with β-catenin's degradation underlie development of a sizeable fraction of human cancers. *APC* gene defects are found in about 75% of colorectal cancers, while mutations in β-catenin's N terminus are seen in 2%–5% of colorectal cancers. A net consequence of the APC and β-catenin defects is accumulation of free β-catenin and constitutive activation of a β-catenin/TCF transcriptional program. β-catenin dysregulation is found in the earliest stages of colorectal tumorigenesis in man and is inferred to have a key role in initiating development of adenomatous lesions.

The identities of cellular genes regulated by β-catenin/TCF complexes have been of keen interest (Clevers, 2006). Among the first described was the *c-MYC* gene (He et al., 1998). Data from chromatin immunoprecipitation studies indicate that the c-Myc protein can bind to regulatory regions of up to 15% of all genes, leading to activation of certain genes and repression of others. The transcriptional program regulated by c-Myc is context dependent, and the ultimate cellular response to elevated c-Myc levels can range from increased proliferation to apoptosis (Dang et al., 2006). In colorectal cancer cells, c-Myc induction was associated with reduced expression of the cell-cycle inhibitor $p21^{WAF1}$, apparently via c-Myc's function in repression of $p21^{WAF1}$ transcription (van de Wetering et al., 2002). Because this study mainly analyzed c-Myc's role in colorectal cancer cells growth in vitro and there are many other genes whose expression can be induced by β-catenin/TCF, there has been obvious interest in assessing c-Myc's function in *Apc*-defective intestinal cells in vivo.

Studies of c-Myc's function in intestinal epithelium were initially hampered in part by the fact that mice with constitutional defects in *c-Myc* die during embryogenesis. In a recent paper in the journal *Nature*, Sansom and colleagues used a powerful conditional gene inactivation approach to explore *c-Myc*'s role in phenotypes seen in murine intestinal epithelial cells in which *Apc* is inactivated (Sansom et al., 2007). The authors employed a transgenic model in which Cre recombinase expression is induced in small intestinal epithelium by β-naphthoflavone treatment of mice, allowing targeting of floxed alleles in adult intestinal cells (Ireland et al., 2004). Prior work from the group had shown that biallelic inactivation of *Apc* in intestinal epithelium was accompanied by accumulation of transcriptionally active β-catenin in cells, along with profound changes in cell differentiation, proliferation, and survival (Sansom et al., 2004). In contrast to the effects seen upon *Apc* inactivation in intestinal epithelium, where only *c-Myc* was inactivated, no major effects on crypt-villus architecture were seen, and *c-Myc* deletion did not affect apoptotic rates (Muncan et al., 2006). The size of *c-Myc*-deficient crypts was reported to be reduced secondary to a decrease in both cell number and cell size, perhaps due to slower cell-cycle progression and cell division at a smaller cell size. In contrast to what had been suggested by in vitro findings (van de Wetering et al., 2002), no increased expression of $p21^{WAF1}$ was observed in the *c-Myc*-deficient crypts. *c-Myc*-deficient intestinal epithelial cells retained some ability to proliferate, but over time, c-Myc-deficient crypts were replaced via crypt fission events in neighboring crypts where somatic inactivation of *c-Myc* alleles had not been achieved.

In their most recent studies, Sansom and colleagues found that the increased proliferation and apoptosis seen in *Apc*-deficient crypts was nearly abolished when *c-Myc* was also deleted in parallel, implicating c-Myc function in the proliferative and apoptotic responses of *Apc*-deficient intestinal cells (Sansom et al., 2007). The authors also observed that c-Myc inactivation rescued the altered cell migration and differentiation seen in *Apc*-deficient crypts, resulting in essentially normal crypt-villus architecture. Moreover, though the authors had found that somatic inactivation of *Apc* on its own leads to thousands of small lesions and multiple adenomas in the intestine, *Apc* and *c-Myc* double mutant cells were rapidly lost from the intestine, and no pathology was apparent. Not unexpectedly, *c-Myc* inactivation had no effect on the nuclear accumulation of β-catenin in *Apc*-deficient intestinal cells. Hence, though β-catenin gained access to the nucleus, due to the lack of *c-Myc* function, β-catenin was apparently unable to induce changes in proliferation, differentiation, migration, and apoptosis in the cells. To assess the importance of c-Myc in the global β-catenin/TCF-regulated gene expression, the authors compared gene expression patterns in *Apc*-deficient crypts to those in crypts deficient in both *Apc* and *c-Myc*. The studies revealed that about two-thirds of genes whose expression was activated in intestinal cells upon *Apc* inactivation were significantly less activated when *c-Myc* was also inactivated. The authors conclude the dramatic morphological changes conferred by *Apc* inactivation in murine intestinal epithelium depend entirely on functional c-Myc. By extension, perhaps *c-MYC* function plays a similarly essential role in human colorectal cancer cells with APC or β-catenin defects.

The approach of Sansom et al. is a powerful one, and the authors' data on the effects of combined *c-Myc* and *Apc* inactivation on intestinal crypt morphology, cell proliferation, and apoptosis are clear. However, is it possible that there might be some uncertainty about *c-Myc*'s presumed role as the pre-eminent downstream β-catenin/TCF-regulated gene in *Apc*-defective murine intestinal cells? Some uncertainty might arise from the possibility that *c-Myc* inactivation had powerful and potentially nonspecific effects on intestinal cell phenotype not adequately captured by the assays and endpoints emphasized in the authors' studies. For instance, while the authors found that *c-Myc* inactivation largely reversed the proliferation, migration, differentiation, and apoptosis abnormalities seen in the first 4 days after acute inactivation of *Apc* and *c-Myc*, the doubly mutant intestinal cells were lost when intestinal tissues were studied at 20 days. It is not clear whether the apparent complete loss of intestinal cells lacking *c-Myc* reflects reduced proliferation or increased apoptosis of *c-Myc*-deficient transient amplifying cell population in the crypt or possibly even a failure of *c-Myc*-deficient stem cells. Other issues impacting on interpretation of the results of Sansom et al. are that, besides Wnt signals and β-catenin/TCF complexes, the *c-Myc* gene has various other upstream signaling pathways that regulate its expression, and c-Myc function is a major determinant of biosynthetic capacity in the cell (Dang et al., 2006). Based on these consid-

erations, perhaps the complete loss of *c-Myc* expression and function, rather than the more specific loss of β-catenin-dependent induction of *c-Myc*, underlies the apparent phenotypic rescue of *Apc*-deficient intestinal cells. A more precise approach than the one employed by the authors would be to specifically abrogate the β-catenin/TCF-mediated induction of *c-Myc* transcription, leaving all other aspects of *c-Myc* regulation and function intact. Though substantial conceptual and technical challenges would need to be overcome to achieve this, one wonders whether the results obtained with such an approach might be quite different than those reported by Sansom et al. At this point, a skeptic might wonder whether the studies of Sansom et al. represent the final word on *c-Myc*'s role in the Wnt/β-catenin/TCF pathway or only the basis for a new attack on the question.

REFERENCES

Batlle, E., Henderson, J.T., Beghtel, H., van den Born, M.M., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T., and Clevers, H. (2002). Cell *111*, 251–263.

Clevers, H. (2006). Cell *127*, 469–480.

Dang, C.V., O'Donnell, K.A., Zeller, K.I., Nguyen, T., Osthus, R.C., and Li, F. (2006). Semin. Cancer Biol. *16*, 253–264.

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). Science *281*, 1509–1512.

Ireland, H., Kemp, R., Houghton, C., Howard, L., Clarke, A.R., Sansom, O.J., and Winton, D.J. (2004). Gastroenterology *126*, 1236–1246.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P.J., and Clevers, H. (1998). Nat. Genet. *19*, 379–383.

Marshman, E., Booth, C., and Potten, C.S. (2002). Bioessays *24*, 91–98.

Muncan, V., Sansom, O.J., Tertoolen, L., Phesse, T.J., Begthel, H., Sancho, E., Cole, A.M., Gregorieff, A., de Alboran, I.M., Clevers, H., and Clarke, A.R. (2006). Mol. Cell. Biol. *26*, 8418–8426.

Sansom, O.J., Reed, K.R., Hayes, A.J., Ireland, H., Brinkmann, H., Newton, I.P., Batlle, E., Simon-Assmann, P., Clevers, H., Nathke, I.S., et al. (2004). Genes Dev. *18*, 1385–1390.

Sansom, O.J., Meniel, V.S., Muncan, V., Phesse, T.J., Wilkins, J.A., Reed, K.R., Vass, J.K., Athineos, D., Clevers, H., and Clarke, A.R. (2007). Nature *446*, 676–679.

van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A.P., et al. (2002). Cell *111*, 241–250.