# Wnt Down, Tumors Wind Up?

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**In mouse intestinal tumors induced by the inhibition of APC, the restoration of APC function causes complete tumor regression with normal differentiation and return of stem cell function irrespective of whether tumors also carried mutations in Kras and p53. These findings by Dow et al. validate the Wnt pathway as an exquisite target for intervention.**

Effective cancer treatments require targets that upon inactivation cause tumor cell death, senescence, or that force cells to differentiate. The dependence of tumors on such targets, which are usually instrumental in conferring aberrant signaling, has been coined "oncogene addiction," implying that tumor cell growth will be preferentially impaired upon inhibition of such targets. Addiction to the inactivation of tumor suppressor genes is less well documented as restoration of function is more difficult to assess. For Myc-driven lymphomas lacking functional p53, restoration of p53 results in tumor cell apoptosis, yet mutations in p53 or other components in the pathway occur easily (Martins et al., 2006). In comparison, in a Kras-driven model of non-small-cell lung cancer, restoration of p53 had very little effect (Junttila et al., 2010). In this issue of *Cell*, Dow et al. (2015) address the dependence of mouse intestinal cancer on the loss of the tumor suppression by APC.

Different tumor types often show an idiosyncratic mutation pattern with a preponderance of mutations in a distinct gene or pathway, indicative of different wiring. For instance, pancreatic cancers almost invariably carry mutations in K-RAS, whereas small-cell lung cancer shows loss-of-function of the retinoblastoma protein. Similarly, colon carcinomas predominantly bear inactivating mutations in APC (a negative regulator of beta-catenin in the Wnt pathway). One has to assume that these predominant lesions are a requirement for the development of that specific tumor. However, the question is whether these tumors also require these lesions for their maintenance, a critical issue for designing targeted interventions. The notion that a range of additional, often quite diverse, driver mutations are found in these tumors further complicates matters as these additional lesions could abrogate the dependency on the initial defect. The question of addiction of a progressed tumor can best be answered by genetic experiments in which the physiological level of the supposed critical driver lesion is restored.

Dow et al. use a conditional and inducible short hairpin RNA (shRNA) approach to control the level of APC in a small subset of intestinal stem cells in the mouse (Figure 1). Reducing APC to very low levels results in tumors along the intestine including colon. Once tumors are detectable by endoscopy, they restore normal levels of APC by switching off the shRNA. This results in tumor regression. Tumor cells undergo differentiation toward the cell types specified by normal intestinal differentiation. Remarkably, this also occurs in tumors that, besides expressing reduced APC levels, lack p53 and bear a dominant active Kras. This illustrates that loss of APC remains critical for intestinal tumor maintenance even if potent additional oncogenic lesions have been acquired that are known to cause tumor progression in this system.

This implies that cellular processes unleashed by APC loss are appealing targets for intervention. The observation made in a small fraction of patients that beta-catenin mutations are found rather than loss-of-function lesions in APC suggests that beta-catenin or the complexes it forms with other transcription regulators could be suitable targets for drug intervention. Indeed, tankyrase inhibitors, which impair beta-catenin function in APC mutant cells by stabilizing Axin2 (Huang et al., 2009), have shown to impede tumor development in pre-clinical models (Waaler et al., 2012) and are currently being tested in clinical trials.

A number of observations made in this study are noteworthy. First of all, the authors do not see any non-responding tumors or eventual relapses in their model, even if APC is restored in the mutant Kras, p53 setting. A case in point is the Myc transcription factor. Myc is a critical driver in many tumors, and abrogation of its transcription activating capacity results invariably in tumor regression with no sign of escapees (Soucek et al., 2008, 2013). In the case of APC, this lack of escape might be due to the fact that APC constitutes a critical node of the Wnt and other signaling pathways and escape by mutations in APC would require almost complete suppression of its activity, at least in this tumor type. In the study by Dow et al., the required loss-of-function of both alleles might be unlikely to occur in view of the relatively modest tumor mass inherent to mouse models. In the same vein, activation of beta-catenin requires specific mutations, also making the probability low and given its downstream position in the signaling pathway alternative lesions might not be at hand. It would be of interest to see if one can force escapees in this system, for instance, by mutagenesis, and what their nature would be. Furthermore, the fact that APC controls the degradation of multiple factors regulating cell proliferation might add to the strong selection for APC deletions and therefore its critical role in colorectal cancer. The Hippo pathway, another signaling cascade highly relevant in cancer (Harvey et al., 2013), is also controlled by the APC

destruction complex and contributes to the oncogenic capacity of APC loss (Azzolin et al., 2014).

Second, the study shows that the extent of APC reduction is a critical factor. Only the most active shRNAs elicit tumor development. Still, the tumor latency is significantly longer than observed for genetic deletion of *Apc*. This indicates that even very low levels of this tumor suppressor protein can substantially impair tumor development. It also stresses the requirement for robust shRNA strategies to prove addiction. The authors also make another remarkable observation. Whereas genetic APC ablation in mice results in tumors largely in the small intestine (Boivin et al., 2003)—an observation often used to question the utility of the mouse as a model for colon carcinomas—the shRNA-mediated inhibition of APC also gives rise to many colon tumors, suggesting that the level of this tumor suppressor protein matters and might be a factor in the preponderance of the specific tumor subtype that arises. Obviously such subtleties can also be brought about by specific mutations in APC, and it will be worthwhile to more closely assess the effects of individual mutations in this regard.

Third, the change brought about by restoring normal APC levels in the tumors, even those carrying the Kras and p53 lesions, is remarkable. Cells would stop proliferation and resume their differentiation program, expressing the markers normally found in the intestine. Even more surprising, the tumor cells that are marked by fluorescent protein appear capable of resuming normal crypt stem cell function, giving rise over time to fully fluorescent reporter-labeled normal crypts. Relatively few normal reporter-labeled crypts are found upon regression. Possibly this relates to a relatively small fraction of tumor cells that are capable of resuming a normal stem cell role or to the limited access to the micro-environmental signals required for such a role. Therefore, it would be of interest to follow the remodeling in these tumors toward normal functionality over time with a number of markers that define the different cell types in the colon and that read out MAPK pathway activation.

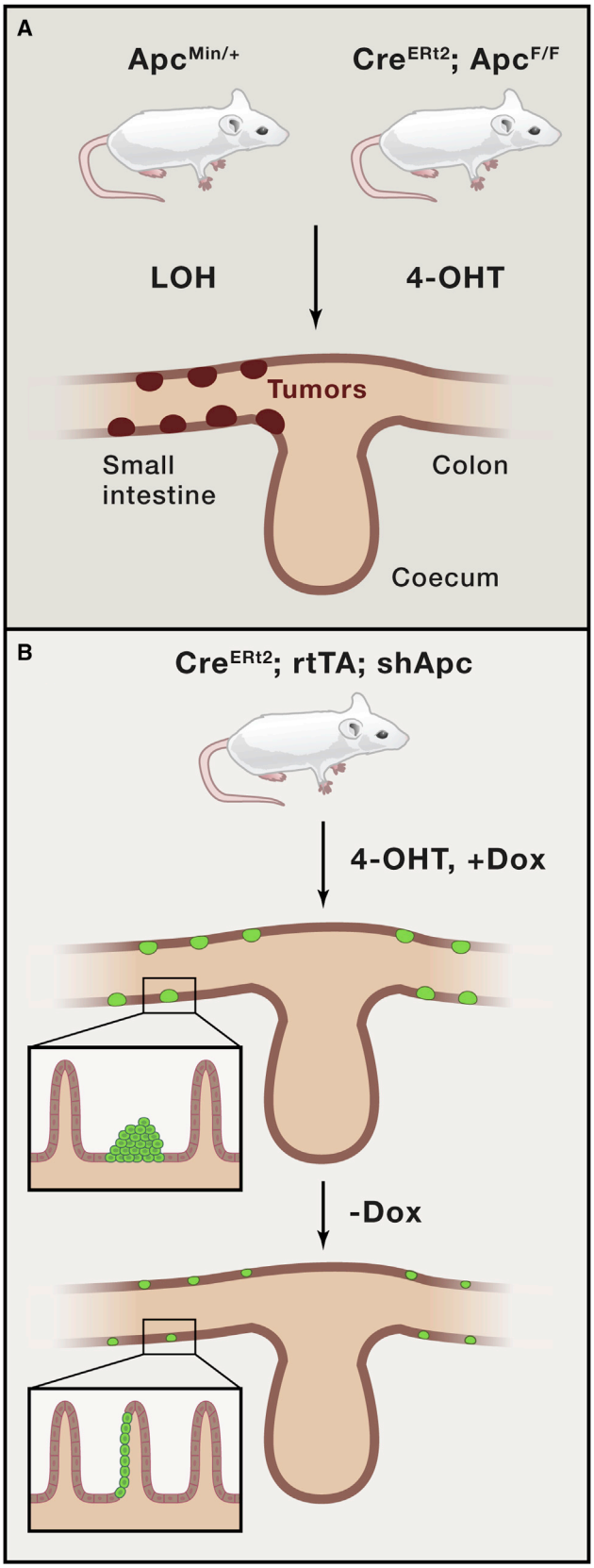


**Figure 1. Addressing the Role of APC in Intestinal Tumor Maintenance**

(A) Genetic inactivation of *Apc* in the mouse intestine, either by LOH in $Apc^{Min/+}$ mice (left) or by temporary Cre expression in intestinal stem cells in conditional $Apc^{F/F}$ mice (right) results in tumors primarily in the small intestine.

(B) APC suppression by conditional and doxycyclin inducible expression of shRNA's reducing APC in intestinal stem cells results in tumorigenesis in small intestine and colon. Upon doxycylin withdrawal APC returns to physiological levels resulting in sustained tumor regression. Crypt homeostasis is restored by inducing massive differentiation of tumor cells some of which contribute to the normal stem cell compartment even if tumors have acquired additional oncogenic driver mutations such as loss of p53 and/or activated K-ras.

Altogether, this study elegantly shows the power of mouse models to give insight into the role of tumor-causing lesions, their role in tumor maintenance, their dependence, or lack thereof, on other co-occurring mutations, and the consequences of restoring normal function.

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# A Shortcut to Activity-Dependent Transcription

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**Neuronal activity results in the rapid induction of gene transcription through a series of defined molecular events. Madabhushi et al. describe an unexpected role for the cutting of promoter DNA by topoisomerase IIB to facilitate transcription of activity-induced genes.**

Sensory experience induces activity-dependent gene expression in neurons, and this process has been implicated in the function and dysfunction of the nervous system (West and Greenberg, 2011). The ability of a neuron to rapidly induce gene transcription in response to sensory stimuli requires the binding of pre-existing transcription factors such as CREB, SRF, and MEF2 to promoters and enhancers. In response to external stimuli, these factors become modified, typically by phosphorylation or dephosphorylation, leading to enhancer and promoter engagement. This is followed by the release of paused, promoter-bound RNA polymerase II (RNAPII) complexes resulting in productive transcriptional elongation (Figure 1A). Prior to neuronal activity, a time when activity-dependent genes are expressed at low levels, these genes express hallmarks of highly expressed genes (e.g., binding by transcription factors and polymerase, and trimethylation of Histone H3 at lysine 4 at promoters). This suggests that activity-dependent genes are poised for activation but that a switch or set of switches must be flipped in order for transcriptional activation to occur.

In this issue of *Cell*, Madabhushi et al. (2015) propose that some activity-regulated genes are maintained in a state of high torsional stress prior to stimulation such that supercoiling of the DNA keeps RNAPII from extending into gene bodies. The authors provide evidence that upon neuronal depolarization, activation of Topoisomerase IIB (Topo IIB) leads to DNA double-stranded breaks (DSBs) within the promoters, thus allowing the DNA to unwind and RNAPII to productively elongate through gene bodies (Figure 1B).

DSBs have classically been viewed as unwanted DNA damage and have been linked to pathological states including neurological disorders (Madabhushi et al., 2014). However, recent reports have noted that neuronal stimulation leads to the appearance of hallmarks of DSBs in the nucleus of neurons (Suberbielle et al., 2013), including the phosphorylation of serine 139 on the histone variant H2AX (γH2AX), a chromatin mark deposited on adjacent histones by the DNA-damage response pathway immediately after DSBs are detected. This suggested that DSBs might occur as part of the normal cellular response to neuronal activation, but where on the genome these DSBs occur, and what function they play, has remained unclear.

Madabhushi et al. sought to understand the effects of DSBs by inducing them in neurons using a topoisomerase inhibitor drug, etoposide, and investigating the effects on gene expression by RNA-sequencing (RNA-seq) analysis. Etoposide traps type II topoisomerase enzymes (those that make double-stranded cuts in the DNA) in a state where they remain bound to cleaved DNA, and this subsequently can lead to the formation of DSBs. Upon etoposide treatment, the authors observe an increase in transcription at several genes, including *Fos*, *FosB*, and *Npas4*, all of which are known to be rapidly transcribed in response to neuronal activity. Inhibition of the most prevalent type II topoisomerase in neurons (TopIIB) by RNAi knockdown conversely leads to blunted induction of these genes, suggesting that the cutting of DNA by TopIIB is essential for full gene activation.

To further explore if activity-dependent gene induction is linked to DSB formation, the authors examine the distribution of γH2AX in activated neurons by chromatin immunoprecipitation followed