

# Regulation of intestinal homeostasis and tumorigenesis by the transcriptional coregulator RIP140

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Colon cancer frequently results from mutations that constitutively activate the Wnt signaling pathway, a major target being the tumor suppressor gene adenomatous polyposis coli (APC). We recently identified the transcription factor RIP140 as a new inducer of APC gene transcription that inhibits colon cancer cell growth and impedes the Wnt signaling pathway by reducing  $\beta$ -catenin activation.

The canonical Wnt/ $\beta$ -catenin signaling pathway is generally considered to be essential for gut homeostasis, especially for self-renewal of intestinal stem cells.  $\beta$ -catenin is an essential cytoplasmic signal transducer of this canonical Wnt pathway. In the absence of pathway stimulation by Wnt ligands,  $\beta$ -catenin is phosphorylated and targeted for degradation by the proteasome. The degradation complex responsible for  $\beta$ -catenin destabilization contains the tumor suppressor gene products axin and adenomatous polyposis coli (APC) as well as glycogen synthase kinase 3 $\beta$  and casein kinase I (CKI).<sup>1</sup> In response to Wnt ligands the activity of the destruction complex is inhibited, therefore  $\beta$ -catenin is no longer degraded but translocates to the nucleus where it interacts with TCF/LEF transcription factors to induce transcription of Wnt target genes.

Canonical Wnt activation is considered the critical initiating event toward tumor development. Colon cancers often result from mutations that constitutively activate the Wnt signaling pathway, and the major target gene *APC* is mutated in up to 80% of colorectal carcinoma (CRC) tumors.<sup>2</sup> Moreover, specific deletion of the *APC* gene in LGR5<sup>+</sup> stem cells triggers the

formation of numerous adenomas in mouse small and large intestines.<sup>3</sup>

We recently showed for the first time that the transcriptional corepressor receptor interacting protein of 140 kDa (RIP140),<sup>4</sup> also known as nuclear receptor-interacting protein 1 (NRIP1), plays a major role in normal and tumoral development of the intestinal epithelium. Using loss of function and gain of function mouse models, we demonstrated that RIP140 is able to negatively regulate the Wnt/ $\beta$ -catenin pathway and to control homeostasis and tumorigenesis of gut mucosa.

First, we observed that RIP140 inhibits cell proliferation and apoptosis in the murine intestinal epithelium, this effect being associated with a decrease in intestinal cell renewal after whole body irradiation. In addition, we showed that RIP140 exerts a negative control on Wnt/ $\beta$ -catenin signaling, which is an essential pathway for the proliferation of stem and progenitor cells in the intestinal epithelium. In intestinal epithelial cells overexpressing RIP140 we observed a reduction in staining for active nuclear unphosphorylated  $\beta$ -catenin (the hallmark of activation of this signaling pathway), associated with a decrease in several  $\beta$ -catenin target genes. In line with this observation, we noticed that

the number of Paneth cells per crypt decreased in the intestinal epithelium of mice overexpressing RIP140, corroborating negative regulation of the Wnt signaling pathway by RIP140.

This effect of RIP140 involves positive transcriptional regulation of the tumor suppressor gene *APC*, a member of the  $\beta$ -catenin degradation complex. Regulation of *APC* gene expression was observed in both mouse intestinal epithelium and in human colon cancer cells, in which we demonstrated recruitment of RIP140 on the proximal region of the *APC* promoter.

We also showed that overexpression of RIP140 in HCT116 colorectal cancer cells disrupted their cell cycle distribution, with an increase in the proportion of cells in G1 phase and a decrease in the number of cells in S phase. Moreover, subcutaneous xenografts of these cells in athymic mice exhibited a significant decrease in tumor cell growth compared to xenografts of control HCT116 cells.

Finally, using tumor biopsies from patients with colon cancer we demonstrated that RIP140 expression is lower in tumor biopsies than in adjacent healthy tissue, with a significant decrease in expression at both the mRNA and protein

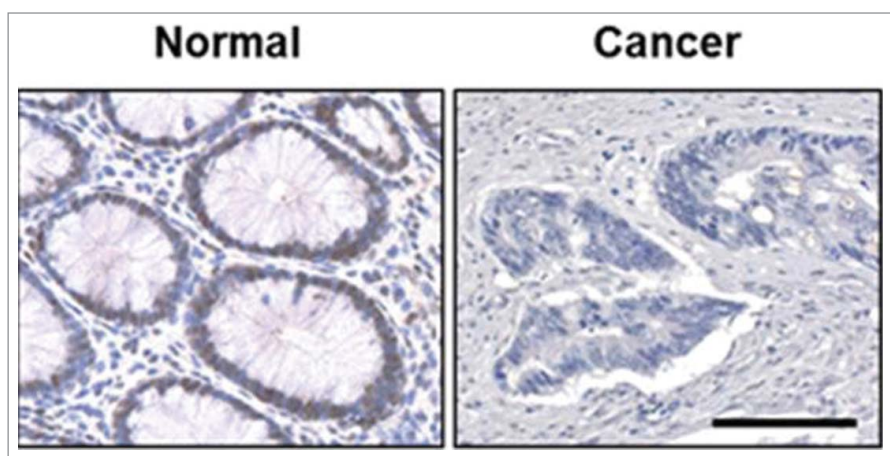
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**Figure 1.** RIP140 expression in human colon cancers. RIP140 expression was measured by immunohistochemical analysis using an anti-RIP140 antibody on a tissue microarray containing normal tissues and adenocarcinoma samples. A significant decrease in the number of cells positive for nuclear RIP140 in colon adenocarcinomas compared to normal tissues was observed in 60% of the adenocarcinomas (scale bar: 100  $\mu\text{m}$ , original magnification: 20 $\times$ ).

levels (Fig. 1). Using a cohort of approximately 400 colon cancer biopsies we confirmed a significant correlation of RIP140 expression with APC expression and an inverse correlation with expression of target genes of the Wnt signaling pathway. Most interestingly, we noticed that RIP140 expression (assessed at the mRNA or protein level), correlated with patient survival. Indeed, patients with tumors that exhibited high levels of RIP140 expression had a better prognosis in term of progression-free and overall survival than patients whose tumors had low levels of RIP140.

Several issues arise from this work and additional studies are needed to resolve

the complex roles of RIP140 in colorectal carcinogenesis. Our data clearly indicate that the effects of RIP140 on  $\beta$ -catenin activation rely on the regulation of APC expression in human colon cancer cells. However, other nuclear signaling pathways targeted by RIP140 such as those involving nuclear receptors<sup>5</sup> might mediate some of the effects that we observed. Indeed, several nuclear receptors exhibit strongly deregulated expression in intestinal epithelium and have been shown to play an important physiopathologic role in the intestine.<sup>6</sup>

Moreover, several negative transcriptional responses to activation of the

Wnt/APC/ $\beta$ -catenin pathway have been reported<sup>7</sup> and an attractive hypothesis to explain the decreased expression of the *RIP140* gene in colon cancer is negative regulation of *RIP140* expression by the Wnt/APC/ $\beta$ -catenin pathway itself.

Finally, our study demonstrated a link between RIP140 expression and patient survival and further work is now required to define the clinical relevance of RIP140 as a prognostic marker compared to previously reported gene signatures.<sup>8</sup>

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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