# Interplay of Altered Signaling Pathways Induces Colon Cancer Formation

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Colorectal cancer is the second leading cause of cancer-related deaths in the United States. Benign adenomas originate from the epithelial cells of the colon and progress to malignancies through the accumulation of genetic and epigenetic changes. Studies in cell lines have determined that some genetic changes individually alter intracellular signaling pathways that can contribute to cell proliferation, survival, and genetic instability. However, the interplay of multiple genetically altered signaling pathways is a more important factor driving tumor initiation and progression *in vivo*, yet these interactions remain ill-defined. Indeed, novel therapeutic targets can be uncovered by understanding these mechanisms. In a recent paper published in *Oncogene*, senior author Dr. William Grady of the Clinical Research Division reports that alterations of two key oncogenic signaling pathways mutated in human colon cancers cooperate to induce colon cancer in an *in vivo* mouse model.

Colorectal cancers are classified into three subsets based on their type of genomic instability. About 15% of all colon cancers are of the microsatellite instability (MSI) subtype, which results from defects in DNA mismatch repair machinery causing the expansion or contraction of DNA repeats throughout the genome. A new *in vivo* mouse model created in the Grady laboratory recapitulates central molecular features found in the MSI class of colorectal cancers. According to Dr. Grady, "this model has the potential for use as a preclinical model for colorectal cancer for testing novel therapeutic agents."

Several oncogenic signaling pathways are disrupted in colorectal cancers through genetic alterations. In most cases of colon cancer, tumor formation is initiated by mutations that activate the Wnt signaling pathway. Additional genetic changes then promote tumor cell growth and survival by activating the mitogen activated protein kinase (MAPK) pathway and/or the phosphoinositide-3-kinase (PI3K)-AKT pathway. The MSI type of colorectal cancer is more dependent on the PI3K-AKT pathway, as evidenced by its susceptibility to PI3K inhibitors. Phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) is a negative regulator of the PI3K-AKT pathway, and loss of the *PTEN* gene occurs in 20-40% colorectal cancers, with a high frequency in the MSI class of colorectal cancers. Also co-occurring with high frequency in the MSI subset of colorectal cancers is the inactivation if the TGF-β signaling pathway, which acts as a tumor suppressor in the colon.

Inactivating mutations of the TGF-β receptor 2 gene, *TGFBR2*, block the TGF-β signaling pathway in colon cancer cells.

Postdoctoral fellow Dr. Ming Yu and colleagues addressed whether disruption of TGF-β signaling and loss of *PTEN* specifically in the epithelial cells of the intestine cooperated to induce intestinal cancer formation in mice. Yu *et al*. found that genetic deletions of either *TGFBR2* or *PTEN* individually had little to no effect on colon tumor formation in mice. However, 37 out of 43 mice (86%) developed colon tumors and had significantly shortened median survival ($P < 0.0001$) with deletions of both *TGFBR2* and *PTEN*. The tumors were histologically characterized as primary adenocarcinomas, and the majority had a mucinous histology, which means they secrete mucin proteins (see figure). These lesions occurred in the small intestine, cecum, colon, and cecal junctions. Furthermore, 8% of the mice had metastasis of the tumors to surrounding tissues, including the pancreas, peritoneal cavity, and liver. These results showed that inhibition of TGF-β signaling and loss of *PTEN* cooperated to induce intestinal cancer in mice.

To understand the mechanism of tumor formation in these double mutant mice, the activation of various signaling pathways was examined. *PTEN* deletion activated AKT in the normal tissues of mice as expected, but the tumors of *PTEN* and *TGFBR2*knockout mice had less activated AKT compared to the adjacent normal tissue. This suggests that lower PI3K activity may favor cancer initiation. *PTEN* is thought to have other functions in the cell, which could also contribute to tumor formation. Surprisingly, the Wnt pathway was not activated in the tumors of these mice, suggesting tumor initiation occurred independent of this pathway. Some colon cancers do not have Wnt pathway mutations, so this mouse model may shed light on the mechanisms involved in tumor initiation in those colon cancers that arise through Wnt-independent pathways. The researchers did find activation of the MAPK pathway without finding additional mutations, accompanied by increased proliferation and decreased apoptosis in the tumor cells. No additional gross chromosomal gains or losses were found in these tumors that would indicate additional genetic events that contributed to tumor formation.

Normal TGF-β signaling induces the expression of cyclin dependent kinase (CDK) inhibitors $p15I^{NK4B}$, $p21^{CIP1}$, and $p27^{KIP1}$, which negatively regulate the cell cycle and proliferation. Expression of all three CDK inhibitors was decreased in tumor versus normal tissue. *PTEN* and *TGFBR2* re-expression in cells synergized to increased activity of the p21 promoter, proving direct regulation of these inhibitors by the two pathways. "These findings raise the possibility that agents that target the cyclin–CDK enzyme complexes, which are currently in early phase clinical studies, may be particularly effective in colorectal cancers that carry *TGFBR2* mutations and lack *PTEN* expression," according to Dr. Grady.

Importantly, the majority of the double mutant mice displayed only one or two intestinal tumors, suggesting that additional cooperating events have occurred in the tumor tissue and are involved in initiating tumor formation. Future studies will address if additional mechanisms are involved in promoting tumor formation in this mouse model.

Yu M, Trobridge P, Wang Y, Kanngurn S, Morris SM, Knoblaugh S, Grady WM. 2013. Inactivation of TGF-β signaling and loss of PTEN cooperate to induce colon cancer in vivo. *Oncogene* Epub ahead of print, doi: 10.1038/onc.2013.102.

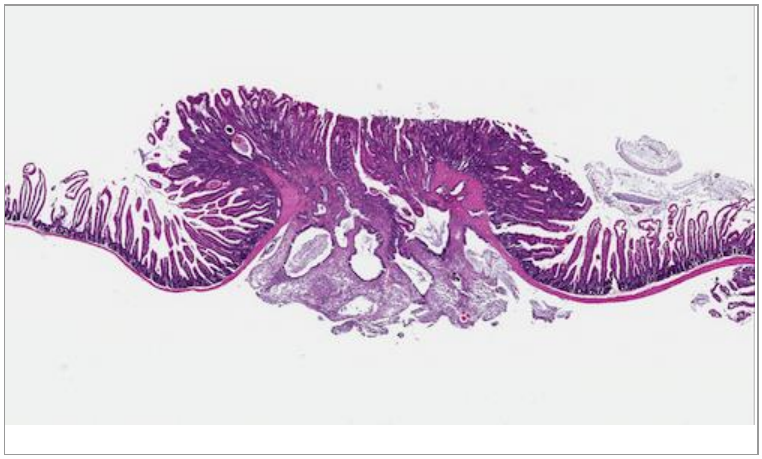

*Image provided by Ming Yu and William Grady.*

An H&E stained section of an intestinal adenocarcinoma (center mass) from a mouse deficient for TGF-beta receptor 2 and PTEN. Note the malignant cells penetrating into the serous membrane surrounding the intestine and tumor-specific mucin production (20x magnification).