NTRK3 Is a Conditional Tumor Suppressor Inactivated in Colon Cancer

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Colon cancer develops from the accumulation of DNA mutations and epigenetic changes that alter the expression or function of oncogenes and tumor-suppressor genes. A common epigenetic change found in cancer is aberrant DNA methylation, a biochemical change in the DNA of gene promoters that silences the expression of genes. High-throughput assays have uncovered multiple DNA mutations and DNA methylation changes throughout colon cancer genomes. However, not all of the changes are "drivers" that promote tumor formation, many are "passenger" changes that do not affect tumorigeneis. By identifying important epigenetic and genetic drivers, novel targets for therapeutics may be uncovered. A study led by postdoctoral fellow Dr. Yanxin Luo in the laboratory of Dr. William Grady used a genome-wide methylation screen to identify *NTRK3* as a conditional tumor suppressor gene in colon cancer.

NTRK3 is a member of the neurotrophin receptor family and is critical for nervous system development. Previously published studies suggested that NTRK3 is a dependence receptor, which signals in both the ligand-bound ("on") state and ligand-free ("off") state (see figure). When the ligand neurotrophin-3 (NT-3) is present, NTRK3 triggers signals inside the cell via a tyrosine kinase domain to promote cell proliferation and survival. In the absence of NT-3, NTRK3 signals for cell death by activating apoptosis. Therefore, NTRK3 has the potential to be either an oncogene or a tumor suppressor gene depending on the presence of NT-3. In breast and liver cancer, NTRK3 has been identified as an oncogene that promotes tumorigenesis. Conversely, DNA methylation of the*NTRK3* promoter suggests that NTRK3 could function as a tumor suppressor in colon cancer.

The Grady lab found that DNA methylation of the *NTRK3* promoter occurred in tumors isolated from all molecular classes of colon cancer, in 67% colorectal adenocarcinomas and 60% of adenomas, and was not associated with other common DNA mutations. DNA methylation of the promoter silenced both *NTRK3* mRNA and protein expression in colon cancer cell lines and primary tumor samples. In addition, the expression of the ligand NT-3 was significantly lower in both colon cancer cell lines and primary colon cancer tissue compared to normal tissue, and was directly correlated

with NTRK3 expression levels. The NT-3 promoter was also methylated in select colon cancer cell lines and expression could be turned on with drugs that reverse DNA methylation, indicating this was the mechanism for reduced expression in colon cancer.

In the absence of NT-3 expression, reintroducing NTRK3 expression induced cell death through apoptosis in colon cancer cell lines, suggesting that NTRK3 was functioning as a tumor suppressor. Treatment of cells with NT-3 reversed NTRK3-induced cell death, confirming that NTRK3 is a dependence receptor. Re-expression of NTRK3 also suppressed anchorage-independent cell growth of colon cancer cell lines, a hallmark of cancer *in vitro*, and tumor growth *in vivo* in a mouse model of colon cancer. Further supporting the notion that *NTRK3* is a tumor suppressor gene, DNA mutations are present in the *NTRK3* gene in colon, breast, lung, and liver cancer. Luo *et al*. also found a number of mutations in *NTRK3* in primary human colorectal cancers. They expressed a subset of these *NTRK3* mutants (G608S, I695V and L760I) in colon cancer cell lines and found that the*NTRK3* L760I mutant inactivated the ability of NTRK3 to induce apoptosis. The other mutant *NTRK3* genes did not affect NTRK3 function. These results demonstrate that both genetic and epigenetic mechanisms inactivate NTRK3 in colorectal cancer, and also show that some of the *NTRK3* mutations are likely passenger mutations.

According to Dr. Grady, "Our studies show that NT-3 is absent in the colon, which we argue creates selective pressure to inactivate NTRK3 by both genetic and epigenetic mechanisms." These results are similar to another study describing the role of NTRK3 in colon cancer (Genevoi *et al*., 2013). "Our findings suggest that soluble receptors for NT-3 may be an effective anti-cancer treatment. In addition, the high frequency of *NTRK3* methylation in adenoma samples suggests that methylated *NTRK3*might be a useful biomarker for the early detection of colorectal cancer."

Luo Y, Kaz AM, Kanngurn S, Welsch P, Morris SM, Wang J, Lutterbaugh JD, Markowitz SD, Grady WM. 2013. NTRK3 Is a Potential Tumor Suppressor Gene Commonly Inactivated by Epigenetic Mechanisms in Colorectal Cancer. *PLoS Genetics*9:e1003552.

Also see: Genevois AL, Ichim G, Coissieux MM, Lambert MP, Lavial F, Goldschneider D, Jarrosson-Wuilleme L, Lepinasse F, Gouysse G, Herceg Z, Scoazec JY, Tauszig-Delamasure S, Mehlen P. 2013. Dependence receptor TrkC is a putative colon cancer tumor suppressor. *Proc Natl Acad Sci U S A*. 110:3017-22.

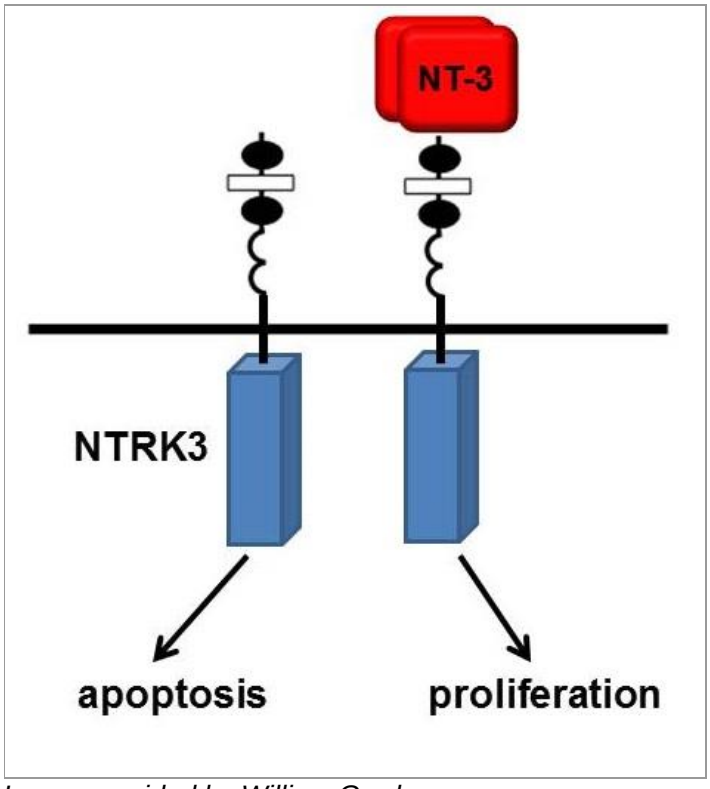

Image provided by William Grady

The biological function of neurotrophin receptor gene NTRK3 depends on whether the ligand NT-3 is present. When NT-3 is present the tyrosine kinase receptor stimulates cell proliferation, while in the absence of ligand the NTRK3 signals for cell death. NTRK3 functions as a tumor suppressor in colon cancer in the absence of NT-3. Decreased NTRK3 expression through DNA methylation or somatic mutations promotes colon tumor formation.