

## **Atonal Homolog 1 Mediates the Effect of Gamma Secretase Inhibitors on Intestinal Neoplasms**

**Douglas A. Brown, Avedis Kazanjian, Noah F. Shroyer**

*Department of Gastroenterology, Hepatology, & Nutrition, Cincinnati Children's Hospital Medical Center, University of Cincinnati, OH*

Colon cancer is the second most common cause of death from cancer in the U.S. This places a high value on the investigation of novel drugs that could serve as additional treatment options for this disease. Gamma secretase inhibitors (GSIs) are a class of drugs that have been shown to decrease proliferation in the intestine; therefore, they may be useful in anti-cancer therapy. GSIs function by inhibiting the Notch signaling pathway, which controls cell fate in the intestine by directing progenitors to give rise either to absorptive or secretory cells. Notch suppresses the transcription factor Atonal Homolog 1 (Atoh1), which the Shroyer lab has shown to be a tumor suppressor. Our hypothesis is that GSIs exert their anti-proliferative effect by increasing expression of Atoh1.

We obtained tissue sections from the intestines of mice treated with GSI. These mice were either Atoh1 knockouts or wild types, and some also had the polyp-forming APC<sup>min</sup> genotype. These sections were stained via immunohistochemistry and assessed for proliferation, cellular differentiation, and apoptosis. In addition, we treated several human colon cancer cell lines with GSI and examined mRNA expression with real-time PCR.

GSI decreased proliferation and increased secretory cell counts both in healthy intestinal tissue and in polyps from Atoh1-wild type mice, but not in Atoh1 knockouts. Tissue from Atoh1 knockouts displayed no secretory cell types. We found no GSI effect on apoptosis in either wild type or Atoh1 mutant tissue. rtPCR analysis showed that GSI increased expression of Atoh1 in cell lines in which the Shroyer lab had previously found GSI to cause a reduction in proliferation. Our results indicate that Atoh1 expression is integral to the reduction in proliferation seen in GSI treatment. Future research into Atoh1 as a drug target may lead to new options in colon cancer therapy.

This study was supported in part by NIH grant T35 DK 60444.