

Annual Progress Report

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Carcinoid tumors of the small intestine resist conventional chemotherapy. Knowing the underlying genetic changes will inform efforts to develop new treatments, but to date specific genes are not implicated in the disease and molecular pathways of intestinal carcinoid tumor pathogenesis are largely unknown. We therefore sought to investigate the genetic alterations underlying ileal carcinoid tumorigenesis. We used high-resolution arrays of single nucleotide polymorphisms (SNPs) to identify regions of allelic imbalance in 24 primary and metastatic small bowel carcinoid tumors derived from 18 patients; results were compared against matched germline (non-tumor) DNA. Regions of gain or loss comprising whole chromosome arms or large chromosomal regions constituted the most common class of anomalies. Loss of all or most of chromosome 18 was the commonest finding, evident in 13 of the 24 specimens; heterozygosity was also lost on chromosomes 9p and 16q in fewer samples. The amplitude of observed gains was modest in comparison to those reported in some other tumor types. A novel and unexpected result of these studies was that ileal carcinoid tumors seem to belong to two distinct groups: those with simultaneous gain of chromosomes 4, 5, 7 and 14, and those with modest allelic imbalance. One focal region of recurrent gain on chromosome 14q mapped to the locus of the gene encoding the anti-apoptotic protein DAD1 (Defender Against Death 1), and antibody staining confirmed DAD1 protein expression in tumor tissue. These studies represent the deepest genomic analysis conducted to date on human intestinal carcinoid tumors and the results provides a basis to investigate putative oncogenes and tumor suppressor genes in this disease. The results are scheduled to be published as follows:

Kulke MH, Freed E, Chiang D, Philips J, Zahrieh D, Glickman JN, Shivdasani RA. High-resolution analysis of genetic alterations in small bowel carcinoid tumors reveals areas of recurrent amplification and loss. *Genes Chrom Cancer* 2008; In Press.

Normal “neuroendocrine” cells derive from pluripotent gut epithelial progenitors but the cell of origin of intestinal carcinoid tumors is unclear and much remains to be learned about lineage hierarchies among gastrointestinal secretory cell lineages. Definition of these hierarchies will identify molecular pathways that function to generate or maintain particular neuroendocrine cell lineages; such pathways may be targeted for rational therapy of carcinoid tumors. Homeodomain transcription factors often function in differentiation of specific cell types. For example, the Nkx6 subfamily controls differentiation in the central nervous system and endocrine pancreas. We isolated a new member of this subfamily, *Nkx6.3*, and observed that *Nkx6.3* transcripts are restricted to the epithelium of the most distal stomach region, the antrum and pylorus, where they localize in mature endocrine cells at the base of gastric gland units. We used targeted gene disruption to generate mice lacking Nkx6.3 expression. These mice develop and grow normally, with a grossly intact digestive tract, but show markedly reduced *gastrin* mRNA, many fewer gastrin-producing (G) cells in the stomach antrum, hypogastrinemia, and increased stomach luminal pH. There is also a corresponding increase in *somatostatin* mRNA levels and antral somatostatin-producing (D) cells. Levels of other transcription factors required for gastric endocrine cell differentiation, Pdx1, Pax6 and Ngn3, are normal. These studies implicate Nkx6.3 as a novel and selective regulator of G- and D-

cell lineages, which are believed to derive from a common progenitor, and they set the stage for further characterization of enteroendocrine cell differentiation pathways. The results were published in the following paper:

Choi MY, Romer AI, Wang Y, Wu MP, Ito S, Leiter AB, **Shivdasani RA**. Requirement of the tissue-restricted homeodomain transcription factor Nkx6.3 in differentiation of gastrin-producing G-cells in the stomach antrum. *Mol Cell Biol* 2008; 28:3208-3218.