Molecular Targets for Calcium and Vitamin D

Leonard H. Augenlicht, Ph.D.
Montefiore Medical Center
Albert Einstein College of Medicine and Cancer Center

Two approaches have identified potential targets involved in the reprogramming of colonic epithelial cells by vitamin D. First, we have used a western-style diet formulated to mimic the levels of nutrients in the human diet that are identified risk factors for colon cancer: higher fat and lower levels of calcium, vitamin D, choline, methionine, and fiber. This diet increased tumor development modestly in $Apc1638^{+/-}$ mice, a model in which β-catenin-Tcf signaling is elevated in the tumors, and more dramatically in the $Muc2^{-/-}$ mouse, in which tumor formation does not involve increased β-catenin-Tcf signaling. Moreover, this diet, fed to wild-type C57Bl6 mice over two-thirds of their lifespan (i.e., 2 years), resulted in significant colon tumor formation, providing a new model of sporadic colon cancer. In each of these three models, adjusting calcium and vitamin D to higher levels was effective in reversing the tumor formation induced by the diet, even though fat was left high and donors to the single carbon pool remained low. Gene expression profiling of the flat colonic mucosa in each model identified genes and pathways that track calcium and vitamin D levels in the diet and, hence, also track probability of tumor formation. These data sets reveal genes and pathways that commonly respond to calcium and vitamin D in the three models, as well as those that are distinct to each model.

A second approach follows up our observations that in colon carcinoma cell lines, both the short-chain fatty acid butyrate as well as vitamin D, induced a pause in transcription of both the *c-myc* and the *cyclin D1* genes downstream of the site of their transcriptional initiation. This raised the question of how often this mechanism is recruited to other loci that are repressed by either butyrate or vitamin D in induction of cell maturation. To address this, we designed and fabricated a novel chip to interrogate the transcription of the 5' and the 3' ends of every gene in the RefSeq database. Replicate experiments on both butyrate and vitamin D identified sets of genes that respond to each nutrient for which expression of the 3' end of the transcript is repressed, but for which the 5' end is increased. A second-generation chip has been designed that tiles through each of these candidate genes, as well as a set of control genes, at a resolution of 5–20 nucleotides. This will be used to confirm the transcriptional attenuation of these genes in response to butyrate and vitamin D, determine the boundaries of the site of attenuation in each case, and investigate the cis and trans acting regulatory elements that impose the transcriptional attenuation.