Fall 2023 OVPR Seed Grant Program

Title: Development of neutral ceramidase (nCDase) inhibitors for colorectal cancer.

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OVERVIEW / ABSTRACT

Colorectal cancer (CRC) is the second-leading cause of cancer-related deaths in the U.S., with increasing incidence of aggressive disease in younger individuals. An attractive drug target for CRC is the human protein neutral ceramidase (nCDase), a membrane bound enzyme primarily expressed in the intestine which hydrolyzes and reduces the extracellular pro-apoptotic lipid ceramide to sphingosine. nCDase knock out (KO) mice show increased colonic ceramide levels and a 93% decrease in adenocarcinoma formation in carcinogen-induced colon cancer as compared with wild type mice. Thus, nCDase inhibition has emerged as an important mechanism for drug design. Further, sphingomyelin- and ceramide–rich diets have also been shown to increase colonic ceramide levels and reduce colon adenocarcinomas in mouse models and in human epigenetic studies. Thus, both nCDase inhibition and increased dietary ceramides have established anti-CRC activity, but the combination of these approaches and their mechanisms of action remain unexplored. ***Our hypothesis is that combining a potent inhibitor of ceramide metabolism in the gastrointestinal tract with a ceramide-rich diet will exhibit a synergistic reduction in colon cancer incidence and recurrence.***

In previous studies, members of our team working with multiple collaborators have solved the crystal structure of human nCDase and identified the structural basis for ceramide recognition in a deep active site,[1] expressed recombinant nCDase and developed multiple biochemical and cell-based assays optimal for drug development,[2] and carried out a high-throughput screen of ~670K compounds.[3] Importantly, several promising small molecule series were identified and promising initial structure activity relationships (SAR) were established as recently reported in *Bioorg Chem* **2023**, *139*, 106747 (Haley, Rizzo, Tonge, and coworkers).[4] In this work, a comprehensive molecular modeling study centered around three of the most promising hits (SB-17, SB-26, SB-37) was performed which established putative binding geometries with nCDase that can be leveraged in the design of improved compounds. Here, in this application, we propose to build on the results of our recent publications by utilizing powerful new computational refinement tools developed in the Rizzo lab to optimize and refine these initial hits. The most promising analogs from modeling will be chemically synthesized in the Tonge lab, and the inhibitory activity, as well as *in vitro* metabolic stability and *in vivo* mouse pharmacokinetics, will be performed in the Haley lab. Our objective is to generate more potent compounds and establish additional metabolic and pharmacokinetic data to support a multi-PI U01 application to the National Cancer Institute at NIH. The project is arranged as two Specific Aims:

Aim #1: Computer-based optimization starting from previously identified nCDase inhibitors followed by chemical synthesis of the most promising analogs.

Aim #2: Inhibition, stability, and pharmacokinetic measurements of oral nCDase inhibitors in mouse intestine, colon, liver and plasma.