Background: Histone deacetylase Hdac1 and Hdac2 regulate gene expression and protein function by removing acetyl groups on lysines. Hdac1 and Hdac2 influence many biological processes, from chromatin organization and cell differentiation, to cell cycle and inflammation. As we reported before, intestinal epithelial cell (IEC) Hdac1 and Hdac2 are essential regulators of intestinal homeostasis related to differentiation and inflammatory responses, among others. However, the specific Hdac1 and Hdac2 downstream targets still remain to be determined.

Aims: To better understand the molecular mechanisms driven by Hdac1 and Hdac2 in IEC, we need a full picture of the proteomic IEC-specific changes occurring upon Hdac1 and/or Hdac2 deletion or HDAC pharmacological inhibition.

Methods: Floxed mice for both Hdac1 and Hdac2 alleles were crossed with villin-Cre mice, to insure IEC-specific deletion. 3-month-old mutated mice were used for the experiments. For pharmacological inhibition, the HDAC class I inhibitor CI994 was injected intraperitoneally in 2-month-old Balb/c mice, once a day for 5 days. Jejunal epithelial cells were isolated by the EDTA method. Cell lysates from control IEC, from Hdac1, Hdac2 and Hdac1/2 deleted IEC and from CI994 treated IEC were labeled with different isobaric chemical tags (Tandem Mass Tag reagents). Labeled cell lysates were mixed, digested with trypsin and used for liquid chromatography-tandem mass spectrometry and data analysis. Bioinformatic pathway analysis was achieved with the DAVID 2.0 software for gene ontology biological processes, for proteins showing two-fold increases or decreases.

Results: More than 3000 proteins were detected by mass spectrometry analysis for each sample. Differential protein expression was observed in Hdac1/2-depleted (800), Hdac1-depleted (150) or Hdac2-depleted (200), and CI994-treated IEC (150). Translation and chromatin assembly were among the top biological processes respectively up- and down-regulated in Hdac1/2-depleted IEC, with decreased expression of goblet and Paneth cell proteins (Zg16, Muc2, Lyz1) and increased enterocyte proteins (Sis, Alpi). Interestingly, while the top negative biological process for both Hdac1- and Hdac2-depleted IEC was “antigen processing and presentation of peptide antigen”, the immune response pathway and the protein kinase/intracellular signaling cascade pathway were increased respectively in Hdac1 and Hdac2 knockout IEC. Surprisingly, CI994-treated IEC displayed “homeostatic process”, as the top increased biological process, and chromatin assembly, as the top decreased biological process.

Conclusions: Targeting HDAC, genetically or pharmacologically, changes IEC behavior. Hdac1 and Hdac2 regulate similar as well as distinct protein expression programs, thereby indicating specific molecular functions in IEC.
Funding Agencies: CCC, CIHR