Vitamin D Receptor Regulates Intestinal Barriers in Colitis

Yong-guo Zhang\textsuperscript{1}, Shaoping Wu\textsuperscript{1}, Rong Lu\textsuperscript{1}, David Zhou\textsuperscript{2}, Jingsong Zhou\textsuperscript{3}, Elaine Petrof\textsuperscript{4}, Erika Claud\textsuperscript{5} and Jun Sun\textsuperscript{1}.

\textsuperscript{1}Biochemistry, Rush University, Chicago, Illinois; \textsuperscript{2}Pathology, University of Rochester; \textsuperscript{3}Physiology, Kansas City University of Medicine and Bioscience; \textsuperscript{4}Medicine, Queen’s University, and \textsuperscript{5}Pediatrics, University of Chicago.

**Background:** The breakdown of intestinal barrier is a common manifestation of gastroenterological disorders including inflammatory bowel diseases. Furthermore, dysfunctions of the intestinal barrier contribute to the development of other diseases such as septic shock, alcoholic liver disease, and type I diabetes. Claudin-2 is a tight junction protein that mediates paracellular water transport in leaky epithelia. Elevated Claudin-2 and increased permeability are reported in patients with inflammatory bowel diseases. Recent evidence suggests that vitamin D and vitamin D receptor (VDR) may regulate functions of tight junction proteins. However, it is unknown how Claudin-2 is regulated by VDR signaling in normal and inflamed intestine.

**Methods:** Using whole body VDR\textsuperscript{-/-} mice, intestinal epithelial VDR conditional knockout (VDR\textsuperscript{ΔIEC}) mice, human samples, and cultured human intestinal epithelial cells (IEC), we performed a series of molecular and biochemical experiments \textit{in vivo} and \textit{in vitro}.

**Results:** Using loss- and gain- of function assays, we provide evidence that the \textit{CLDN2} gene is a direct target of the transcription factor VDR that mediates up-regulation of Claudin-2 in response to treatment of cells with vitamin D\textsubscript{3}. VDR-inducing Claudin-2 promoter activity required the Cdx binding site on the promoter of the \textit{CLDN2} gene. We further identify a functional vitamin D response element in the Claudin-2 promoter. \textit{In vivo}, VDR deletion in intestinal epithelial cells (IEC) led to decreased Claudin-2 at both mRNA and protein levels. Functionally, we found a robust increase of Claudin-2 in inflammatory IECs which lacked VDR regulation and allowed the inflammatory cytokines to take over. Furthermore, in inflamed intestine of ulcerative colitis patients, VDR expression is low and Claudin-2 is enhanced.

**Conclusion:** A lack of intestinal VDR regulation leads to dysfunction of Claudin-2 in inflammatory responses. This study reveals a complex role and novel mechanism for intestinal VDR by regulation of epithelial barriers and inflammation.