Claudin-23 Expression in Intestinal Epithelial Cells Surrounding Mucosal Wounds Enhances Wound Repair *In Vivo*

Kristen M. Lozada-Soto[1,2,3*], Arturo Raya-Sandino[3*], Vicky Garcia-Hernandez[3], Miguel Quiros[3], Charles A. Parkos[3], and Asma Nusrat[3]

[1]Medical Scientist Training Program, University of Michigan Medical School, Ann Arbor, MI
[2]Molecular and Cellular Pathology Graduate Program, Rackham Graduate School, University of Michigan, Ann Arbor, MI
[3]Department of Pathology, University of Michigan Medical School, Ann Arbor, MI
*K.L-S. and A. R.-S. contributed equally to the work presented in this abstract.

Background: Epithelial cells play a crucial role in controlling mucosal barrier function. Mucosal injury resulting in wounds is seen in chronic in inflammatory diseases. In response to damage, epithelial cells migrate as a collective sheet to restore critical barrier function. Claudins in epithelial tight junctions are key regulators of barrier function, and recent studies suggest they contribute to epithelial homeostasis, migration, and proliferation. We observed increased claudin-23 (CLDN23) expression in intestinal epithelial cells (IECs) within crypts surrounding mucosal wounds. Therefore, our studies were aimed at investigating the role of CLDN23 in regulation of intestinal epithelial wound repair.

Methods: *Cldn23*$^{ER\Delta IEC}$ mice were used to silence CLDN23 expression in IECs, with *Cldn23*$^{f/f}$ controls. Human and murine IECs (colonoids) were used to analyze CLDN23 expression post-injury. Human model IEC lines with overexpression (OE) and knockdown (KD) of CLDN23 were generated as previously described.[1] CLDN23 expression in colonic wounds was detected using scRNAseq, RNAScope, and immunofluorescence labeling. *In vitro*, scratch-wounded CLDN23 OE or KD IEC monolayers were monitored for 24h via time-lapse imaging. *In vivo*, punch-biopsy wounding was used to assess repair over 72h using colonoscope videos. To assess the role of CLDN23 in cell migration, colonoid movement was tracked for 48h. Cell directionality and speed were calculated in the DiPer software.[2]

Results: Upon injury, CLDN23 mRNA and protein expression increased in crypts adjacent to the wound at 6 and 12h, suggesting a role for CLDN23 in regulating mucosal wound healing. Mice lacking epithelial CLDN23 expression showed significantly delayed mucosal wound repair (p<0.0001) following biopsy-induced injury in the colon. In keeping with *in vivo* findings, scratch-wounded colonoids from CLDN23 KD mice exhibited delayed wound repair compared with colonoids from floxed mice. Furthermore, in vitro studies using IECs with either CLDN23 OE or KD confirmed functional effect of CLDN23 modulation on epithelial wound repair. Mechanistically, perturbed wound repair observed in the absence of CLDN23 was attributable to a reduced speed of cell migration without affecting cell directionality.

Conclusion: CLDN23 plays a novel role in mucosal wound repair by regulating collective epithelial cell migration. Understanding this mechanism could lead to new therapies for promoting wound repair in chronic inflammatory diseases.

References:

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(2) Gorelik R, Gautreau A. Quantitative and unbiased analysis of directional persistence in cell migration. *Nat Protoc*. 2014;9(8):1931–1943.