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ORIGINAL RESEARCH

Cell Adhesion Molecule CD166/ALCAM Functions Within the Crypt to Orchestrate Murine Intestinal Stem Cell Homeostasis

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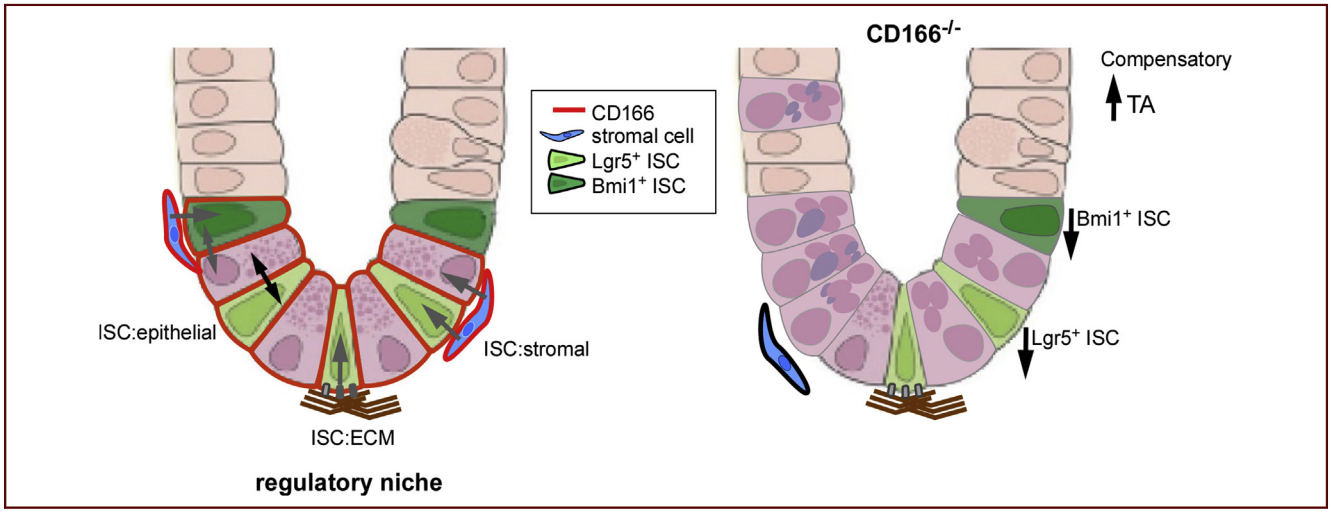

SUMMARY

The cell adhesion molecule and intestinal epithelial crypt–based marker, CD166, functions to maintain the homeostatic niche. Loss of CD166 resulted in decreased active-cycling ISCs and blocked Paneth cell differentiation through a Wnt-deficient signaling environment, highlighting the importance of stem-niche cell interaction in tissue homeostasis.

BACKGROUND & AIMS: Intestinal epithelial homeostasis is maintained by active-cycling and slow-cycling stem cells confined within an instructive crypt-based niche. Exquisite regulating of these stem cell populations along the proliferation-to-differentiation axis maintains a homeostatic balance to prevent hyperproliferation and cancer. Although recent studies focus on how secreted ligands from mesenchymal and epithelial populations regulate intestinal stem cells (ISCs), it remains unclear what role cell adhesion plays in shaping the regulatory niche. Previously we have shown that the cell adhesion molecule and cancer stem cell marker, CD166/ALCAM (activated leukocyte cell adhesion molecule), is highly expressed by both active-cycling Lgr5^{+} ISCs and adjacent Paneth cells within the crypt base, supporting the hypothesis that CD166 functions to mediate ISC maintenance and signal coordination.

METHODS: Here we tested this hypothesis by analyzing a CD166$^{-/-}$ mouse combined with immunohistochemical, flow cytometry, gene expression, and enteroid culture.

RESULTS: We found that animals lacking CD166 expression harbored fewer active-cycling Lgr5^{+} ISCs. Homeostasis was maintained by expansion of the transit-amplifying compartment and not by slow-cycling Bmi1^{+} ISC stimulation. Loss of active-cycling ISCs was coupled with deregulated Paneth cell homeostasis, manifested as increased numbers of immature Paneth progenitors due to decreased terminal differentiation, linked to defective Wnt signaling. CD166$^{-/-}$ Paneth cells expressed reduced Wnt3 ligand expression and depleted nuclear β-catenin.

CONCLUSIONS: These data support a function for CD166 as an important cell adhesion molecule that shapes the signaling microenvironment by mediating ISC–niche cell interactions. Furthermore, loss of CD166 expression results in decreased ISC and Paneth cell homeostasis and an altered Wnt microenvironment. *(Cell Mol Gastroenterol Hepatol 2017;3:389–409; http://dx.doi.org/10.1016/j.jcmgh.2016.12.010)*

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The continuous renewal and dynamic repair of the intestinal epithelium are fueled by functionally distinct populations of intestinal stem cells (ISCs) located in a confined and instructive microenvironment, the stem cell niche, at the crypt base. Within the niche, cues from adjacent epithelium[1] or underlying stromal cells[2,3] differentially govern these diverse stem and progenitor cells, instructing them to remain quiescent, proliferate, differentiate, or to activate a rapid response to epithelial injury—all to maintain epithelial homeostasis. Within the crypt, ISCs expressing the Wnt target gene Lgr5,[4] defined to be active-cycling,[4–6] are located in close proximity to ISCs that are described to be slow-cycling or quiescent[7] (eg, Bmi1,[8] mTert[9]). The complex cellular relationships between these different classifications of stem cell populations are only beginning to be elucidated in various contexts—homeostasis, after injury, and in disease. Slow-cycling cells, expressing Bmi1, have been shown to give rise to the active-cycling ISC pool.[10,11] Adding to this complexity are the recent findings that injury induces committed progenitors to reacquire stemness and contribute to reestablishing the active-cycling stem cell pool.[12–14] Hence, there are multiple ways to maintain and regenerate the active-cycling stem cell domain,[15] highlighting the need for coordinated proliferative regulation of these cells to establish or reestablish homeostasis. Despite an intense focus on ISC dynamics during the past 10 years,[7,8,11,16] an understanding of how distinct stem cell populations are differentially and coordinately regulated is not clear. Although there are studies describing stem cells as harboring intrinsic regulatory programs,[17] it is suggested that the dominant influence derives from the niche.[18,19]

The ISC niche is composed of both crypt epithelium and surrounding stromal cells.[20,21] The active-cycling Lgr5$^+$ ISCs are interspersed between differentiated Paneth cells that provide additional regulatory factors, including a multitude of Wnt ligands[1,22] to drive both Lgr5$^+$ ISC proliferation and Paneth cell differentiation.[23] Physiologic redundancy within the niche exists, exemplified by studies ablating Wnt3 production from Paneth cells.[2] Loss of this important epithelial-derived signal did not result in loss of the Wnt-dependent Lgr5$^+$ ISC population due to redundant Wnt ligand supply from cells within the crypt-based stroma.[2,3,24] With the influence of both epithelial and stromal factors on proliferation and differentiation within tight quarters, it is likely that an active process exists to differentially regulate the proliferative and quiescence status of ISC populations.

Distinct expression of cell adhesion molecules on cells within the stem cell niche has been reported in numerous organ systems.[25,26] We have previously identified a tightly restricted, high expression domain of the cell adhesion molecule CD166/ALCAM (activated leukocyte cell adhesion molecule) on crypt-based epithelial cells within the niche of the small intestine and colon.[26] Interestingly, CD166 is expressed on both active-cycling Lgr5$^+$ ISCs as well as their Wnt ligand-producing Paneth cell neighbors (Figure 1*A*). This restricted expression domain strongly supports that CD166 plays an important regulatory function in ISC maintenance and proliferative capacity. It is well-accepted that cell adhesion molecules function to bring cells together in physical proximity and impact their shape and polarity.[27] In addition, it is possible that they function to direct the myriad of signals from within the niche to influence ISCs.[27] Notably, adherens junctions represent a critical node of cell-cell contact through coordination of a number of cell signaling networks. Robust evidence exists that many adherens junctional proteins, including the E-cadherin–associated protein β-catenin, can localize to the nucleus and participate in key developmental signaling processes.[28]

CD166 is a transmembrane adhesion protein belonging to the immunoglobulin-like domain superfamily.[29,30] In other organ systems CD166 has a reported myriad of functions. This conserved cell adhesion protein participates in physiologic processes including leukocyte intravasation across the blood-brain barrier, monocyte migration across endothelial junctions, angiogenesis, capillary formation, protection against apoptosis in breast cancer cells, and T-cell activation by both antigen-presenting and tumor cells.[31–37] Furthermore, CD166 has been described as a ligand that functions in heterotypic interactions, binding CD6 on T cells,[38–40] acting in homophilic adhesion complexes between epithelial cells,[41] and as a cell surface marker for a subset of hematopoietic progenitor cells,[42,43] multipotent mesenchymal stem cells,[44,45] and cancer stem cells.[46] Recently, CD166 was reported to be expressed on both hematopoietic stem cells and supportive osteoblasts and to be important in maintaining functional stem cell niche interactions within the bone marrow.[47] Whether this maintenance-associated function is due to the regulation of signaling cues to the stem cell is unclear. Although CD166 currently does not have a defined role in regulating specific cell signaling pathways, it is known that CD166 is a direct target of Wnt5A, a ligand of the non-canonical Wnt signaling pathway.[48] The non-canonical and canonical Wnt signaling pathways function in a regulatory feedback loop to modulate the reciprocal pathway. This intimate relationship between Wnt5A and the canonical Wnt signaling pathway has been demonstrated in many dysregulated and diseased states.[49]

Abbreviations used in this paper: **BrdU, bromodeoxyuridine; CLEM, correlative light and electron microscopy; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; HBSS, Hank's balanced salt solution; IHC, immunohistochemistry; ISC, intestinal stem cell; Lyz, lysozyme; Muc2, mucin 2; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SEM, standard error of the mean; TA, transit-amplifying; TEM, transmission electron microscopy; WT, wild-type.**

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