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Research Article

Numb modulates the paracellular permeability of intestinal epithelial cells through regulating apical junctional complex assembly and myosin light chain phosphorylation ☆

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ABSTRACT

Numb is highly expressed throughout the crypt-villus axis of intestinal mucosa and functions as cell fate determinant and integrator of cell-to-cell adhesion. Increased paracellular permeability of intestinal epithelial cells is associated with the epithelial barrier dysfunction of inflammatory bowel diseases (IBDs). The apical junctional complex (AJC) assembly and myosin light chain (MLC) phosphorylation regulate adherens junctions (AJ) and tight junctions (TJ). We determined whether and how Numb modulate the paracellular permeability of intestinal epithelial cells. Caco-2 intestinal epithelial cells and their Numb-interfered counterparts were used in the study for physiological, morphological and biological analyses. Numb, expressed in intestinal epithelial cells and located at the plasma membrane of Caco-2 cells in a basolateral to apical distribution, increased in the intestinal epithelial cells with the formation of the intestinal epithelial barrier. Numb expression decreased and accumulated in the cytoplasm of intestinal epithelial cells in a DSS-induced colitis mouse model. Numb co-localized with E-cadherin, ZO-1 and Par3 at the plasma membrane and interacted with E-cadherin and Par3. Knockdown of Numb in Caco-2 cells altered the F-actin structure during the Ca^{2+} switch assay, enhanced TNFα-/INF-γ-induced intestinal epithelial barrier dysfunction and TJ destruction, and increased the Claudin-2 protein level. Immunofluorescence experiments revealed that NMIIA and F-actin co-localized at the cell surface of Caco-2 cells. Numb knockdown in Caco-2 cells increased F-actin contraction and the abundance of phosphorylated MLC. Numb modulated the intestinal epithelial barrier in a Notch signaling-independent manner. These findings suggest that Numb modulates the paracellular permeability by affecting AJC assembly and MLC phosphorylation.

Abbreviations: AJC, apical junction complex; MLC, myosin light chain; IBD, inflammatory bowel diseases; DSS, dextran sulfate sodium; TEER, trans-epithelial electric resistance; AJ, adherens junction; TJ, tight junction; NMIIA, non-muscle myosin II A.

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Introduction

Numb, originally identified in Drosophila, is a membrane-associated protein that asymmetrically segregates in dividing cells and is believed to determine distinct cell fates by interacting with and inhibiting Notch activity [1]. Previously we reported that Numb modulated intestinal epithelial cells toward goblet cell phenotype by inhibiting the Notch signaling pathway [2].

Numb is evolutionarily conserved, and the mammalian homolog encodes four alternatively spliced transcripts, generating four different proteins. Numb contains multiple protein–protein interaction regions, including an amino-terminal phosphotyrosine-binding (PTB) domain, a carboxyl-terminal proline rich region (PRR), and Eps15 homology (EH) regions (DPF and NPF) [3]. The different isoforms result from presence or absence of exon 3 (found within the PTB domain) and/or exon 9 (found within the PRR domain) [4]. The key factors that crosstalk with Numb within the microenvironment to trigger cell fate specification are unknown. Nevertheless, a number of pathways that control stem/progenitor cell proliferation and differentiation are known to interact with Numb [5]. For instance, the involvement of Numb in Notch or Hedgehog activation, combined with loss of p53 function, promotes stem cell maintenance and expansion [6–8]. Numb displays a complex pattern of functions, probably due to the diverse isoforms encoded by the two homolog (Numb and Numb-like) and the heterogeneity of interacting proteins. The modular structure of Numb makes it an adapter protein that interacts with several molecules, thereby regulating its multiple cell functions [9].

The small intestinal epithelium forms the villi and crypt architecture. Villi are lined by a single-layer of terminally differentiated cells that include enterocytes, goblet cells, and enteroendocrine cells. Crypts have vigorous proliferation potential; the differentiated epithelial cells at the villi tips undergo apoptosis and are replaced every few days by progenitor stem cells that dwell in crypts and move up the villi as they differentiate [10]. Epithelial cells do not move extensively, at least in their final differentiated form. Their proper placement and polarization, in the context of an organ, is regulated by a number of cell-to-cell adhesion structures, including tight junctions (TJs) and adherens junctions (AJs) [11]. TJs are formed by the transmembrane proteins and cytoplasmic proteins. The integral membrane proteins are the claudin family, tight junction-associated MARVEL proteins (TAMPs) composed of occludin, tricellulin and MARVELD3, the immunoglobulin superfamily composed of the JAM family, CAR, and ESAM, Bves and lypolysis-stimulated lipoprotein receptor (LSR). The cytoplasmic proteins divided into two groups. One group consists of the PDZ domain-containing proteins ZO-1, ZO-2, ZO-3, ASIP/Par3, Par6, MAGI-1, MAGI-2, MAGI-3, AF-6/afadin, MUPP1 and PATJ. The other group is comprised of cingulin, 7H6 antigen, symplekin, heterotrimeric G proteins, aPKC, ZONAB, huASH1, Rab-3b, rab-13, PTEN, Pilt, angiomotin/JEAP and protein phosphatase 2A [12–14]. In addition, the Par polarity complex has an important function in the establishment of TJs [15]. AJs, in contrast, are maintained by E-cadherin (also known as Cdh1) and α- and β-catenins [16].

It has been shown that Numb is required for the maintenance of AJs, this seems to be intimately linked to the recycling function of Numb, which localizes to Rab11-recycling endosomes containing internalized E-cadherin and situated near AJs in neural progenitor cells [16]. Numb physically interacts with E-cadherin, Par3 and aPKC in a process regulated by signaling pathways known to induce epithelial–mesenchymal transition (EMT) in ECV304, HeLa, and Vero cells [17]. Indeed, HGF- (or Src-) induced tyrosine phosphorylation of E-cadherin inhibits its interaction with Numb in polarized Madin–Darby canine kidney cells (MDCK cells) [18]. Furthermore, the Numb-associated E3-ligase LNX has been implicated in the removal of claudins from TJs, although it is not known whether Numb is directly involved in this process [19]. It is unclear whether the phenomena derived from either the neural progenitor cells or human endothelial cells, Hela, Vero, MDCK cells is suitable for the intestinal epithelial cells in which Numb is highly expressed [20].

Numb is also expressed in most adult tissues including but not limited to breast, lung, testis, and salivary glands [20–23], suggesting a function for Numb outside of neurogenesis. In the present study, we used the Caco-2 cell line to characterize the role of Numb in modulating the paracellular permeability of intestinal epithelial cells by regulating apical junctional complex assembly and myosin light chain phosphorylation.

Materials and methods

Cell culture

Human colon cancer-derived Caco-2 and T84 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (HyClone, USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, USA), at 37 °C in a humidified incubator with 5% CO_2. Upon reaching 90% confluency, the cells were dissociated with 0.25% trypsin and 0.02% EDTA and split.

Reverse transcription (RT)-PCR

Total mRNA was extracted from Caco-2 and T84 cells using TRIzol reagent (Invitrogen, Carlsbad, CA), and 2 μg of total RNA was used to prepare cDNA in a total volume of 25 μl (PrimeScript® RT-PCR Kit, Takara, Japan). PCR was then performed on 1 μl of cDNA using the following primer pairs: Human Numb forward, 5′-CGATGACCAAACCAGTGACAG-3′ and human Numb reverse, 5′-AGAGGGAGTACGTCTATGACCG-3′; GAPDH forward, 5′-ACCACAGTCCATGCCATCAC-3′ and GAPDH reverse, 5′-TCCACCACCCTGTTGCTGTA-3′.

RNA interference

Numb was targeted for RNAi (shRNA-Numb) as described [2]. Caco-2 cells were seeded on six-well culture plates and grown to 70–80% confluency before transfection. Both pRNAT-U6.1-shRNA-Numb and pRNAT-U6.1-shRNA-Ctr were transfected with Lipofectamine™ 2000. The culture media was replaced with fresh media containing calf serum (150 mL/L) 6 h post-transfection. Forty-eight hours later, transfected cells were selected using G418 (600 μg/mL, Invitrogen). Several individual cell clones grown in the presence of G418 were isolated after 14 days. These cells were cultured and selected using G418 (300 μg/mL) for an additional 10 days to build a stable-transfected cell line.

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