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Paneth cells: the hub for sensing and regulating intestinal flora

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The complex interplay between symbiotic bacteria and host immunity plays a key role in shaping intestinal homeostasis and maintaining host health. Paneth cells, as one of the major producers of antimicrobial peptides in the intestine under steady-state conditions, play a vital role in regulating intestinal flora. Many studies on inflammatory bowel disease (IBD)-associated genes have put Paneth cells at the center of IBD pathogenesis. In this perspective, we focus on mechanistic studies of different cellular processes in Paneth cells that are regulated by various IBD-associated susceptibility genes, and we discuss the hypothesis that Paneth cells function as the central hub for sensing and regulating intestinal flora in the maintenance of intestinal homeostasis.

inflammatory bowel disease, Paneth cell, commensal bacteria, intestinal homeostasis

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INTRODUCTION

Trillions of bacteria reside in the human intestinal lumen. These bacteria are usually termed as the intestinal flora or microbiota. The microbiota plays important roles in host health by assisting nutrient digestion and absorption, educating the immune system, regulating metabolism, and fending off intestinal pathogens. Microbial imbalance, known as dysbiosis, has been linked to the development of a wide range of disorders, including metabolic syndromes, autism spectrum disorders, inflammatory bowel disease (IBD), and so on. Therefore, it is important to understand how the intestinal microbiota is established after birth and dynamically regulated throughout life. The intestinal microbiota and mucosal immunity constantly interact and reciprocally shape each other to achieve a steady state which is referred to as intestinal homeostasis. IBD, one of the clinical manifestations of disrupted intestinal homeostasis, has been widely studied to understand the important players in maintaining intestinal homeostasis.

IBD OVERVIEW

IBD includes two main forms: Crohn's disease (CD) and ulcerative colitis (UC). IBD is generally thought to arise from inappropriate and sustained responses of host immunity to intestinal microbiota. IBD is a complex disease influenced by both environmental and genetic factors. Genome-wide association studies (GWAS) have identified over 100 distinct loci that confer risk of or protection against developing IBD (Anderson et al., 2011; Franke et al., 2010; Huttenhower et al., 2014; Liu et al., 2015). Mechanistic studies on these associated genes have revealed complex interplay between intestinal microbiota and mucosal immunity. When the interplay goes awry, the host is at risk of intestinal infection or inflammation. For example, a delicate balance between inflammatory Th17 cells and regulatory Treg cells is critical for intestinal homeostasis. Interestingly, among the commensal bacteria, *segmented filamentous bacteria* (SFB) induce Th17 cell differentiation

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while *Clostridia* induce the differentiation of colonic Treg cells (Atarashi et al., 2011; Ivanov et al., 2009). Dysbiosis in intestinal flora may tilt the balance between intestinal Th17 and Treg cells, and conversely, the imbalance of intestinal Th17 and Treg cells may set the stage for intestinal dysbiosis.

RECIPROCAL INTERACTION BETWEEN PANETH CELLS AND MICROBES

Strikingly, a number of studies on IBD-associated genes have since converged on Paneth cells. Paneth cells, a group of secretory cells located at the bottom of small intestinal crypts, secrete a range of anti-microbial peptides (AMPs) into the intestinal lumen (Bevins and Salzman, 2011; Clevers and Bevins, 2013). This perspective focuses on the crosstalk between Paneth cells and intestinal micriobiota and implication in IBD. More comprehensive views on Paneth cells are covered in several review articles (Bevins and Salzman, 2011; Clevers and Bevins, 2013). In-depth mechanistic studies also reveal a complex interplay between Paneth cells and intestinal flora, in which Paneth cells play a critical role in sensing and regulating intestinal flora (Furusawa et al., 2014; Lupp et al., 2012). The AMPs secreted by Paneth cells include cryptdin, Reg3γ, lysozyme and so on (Bevins and Salzman, 2011; Clevers and Bevins, 2013). Cryptdin suppresses pathogenic microbes and neutralizes a range of bacterial toxins (Kudryashova et al., 2014). Reg3γ specifically kills Gram-positive bacteria, suggesting that each AMP has its own antimicrobial spectrum. AMPs from Paneth cells are essential for controlling intestinal *Listeria monocytogenes* infection (Kaser et al., 2008; Kobayashi et al., 2005). Depleting Paneth cells in mice results in a compromised intestinal barrier and enhanced translocation and dissemination of pathogens (Vaishnava et al., 2008).

Reciprocally, intestinal microbes regulate synthesis and secretion of AMPs. AMPs in Paneth cells are stored in dense core vesicles (DCVs) before being secreted into the intestinal lumen. Studies in the 1970s showed that DCVs in Paneth cells from germ-free mice are smaller in size and greater in number (Satoh, 1988). Microbial colonization greatly stimulates DCV secretion in germ-free mice (Satoh, 1988). A study by Ayabe shows that secretion in Paneth cells depends on the presence of bacteria or their ligands (Ayabe et al., 2000). Aside from AMP secretion, the production of Reg3γ critically depends on the presence of commensal bacteria. In fact, Reg3γ is one of the most upregulated genes in the intestine when germ-free mice are colonized with commensal bacteria (Cash et al., 2006). Both events, bacteria-stimulated secretion and Reg3γ induction, depend on the expression of toll-like receptors (TLRs) and their downstream adaptor protein Myd88 in Paneth cells. The precise control of AMP synthesis or secretion depends on host factors, and faulty regulation leads to increased risk of intestinal inflammation, exemplified by point mutations in two IBD-associated genes, *TCF4* (transcription factor 4) and *KCNN4* (potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4) (Simms et al., 2010; Wehkamp et al., 2007). *TCF4* encodes a transcription factor that regulates the transcription of AMPs in Paneth cells, while *KCNN4* encodes a calcium-activated potassium channel involved in DCV exocytosis.

THE CELLULAR PROCESSES MODULATED BY COMMENSAL BACTERIA IN PANETH CELLS

Two different pathways are utilized in Paneth cells for sensing microbes: TLR-Myd88 and nucleotide-binding oligomerization domain-containing protein 2 (Nod2). Activation of the TLR-Myd88 pathway is required for upregulation of Reg3γ upon microbial colonization and DCV exocytosis. However, the role of Nod2 in Paneth cells has been more elusive. Nod2 is a cytosolic bacterial sensor that induces cytokine and antimicrobial peptide gene expression in response to the bacterial peptidoglycan muramyl dipeptide (MDP). Mutations in the gene encoding Nod2 are most commonly associated with increased risk of CD (Hugot et al., 2001; Ogura et al., 2001). The three major *NOD2* mutations that are associated with CD abolish or reduce the ability to sense bacterial products (Billmann-Born et al., 2011; Girardin et al., 2003; Inohara et al., 2003; Li et al., 2004; Netea et al., 2005), suggesting that loss of function of Nod2 contributes to the pathogenesis of CD. Our understanding of the function of Nod2 in mucosal immunity is largely based on studies in $Nod2^{-/-}$ mice. Kobayashi observed a marked reduction in production of a few members of the cryptdin family in $Nod2^{-/-}$ Paneth cells; however, later studies suggest that genetic background may have affected the original observation (Kobayashi et al., 2005; Shanahan et al., 2014). $Nod2^{-/-}$ Paneth cells display less antimicrobial activity *in vitro* in response to MDP. $Nod2^{-/-}$ mice display altered composition of commensal bacteria, and increased susceptibility to enteric infection and piroxicam-induced ileitis (Kobayashi et al., 2005; Petnicki-Ocwieja et al., 2009; Ramanan et al., 2014). Despite such progress, the cellular and molecular mechanisms underlying the intestinal abnormalities in $Nod2^{-/-}$ mice remain to be further investigated. A recent study has shown that sensing of microbes by Nod2 directs lysozyme sorting in Paneth cells (Zhang et al., 2015). In Paneth cells of germ-free mice or $Nod2^{-/-}$ mice, newly synthesized lysozyme is targeted to lysosomes for degradation.

DCV-targeted cargos, like other proteins in constitutive secretory pathways, are synthesized in the endoplasmic re-

ticulum (ER) and processed in the Golgi. It is largely unknown how DCV cargos are selectively sorted and transported to DCVs after exiting the Golgi. Neurons and endocrine cells, such as chromaffin cells in adrenal glands and β-cells in pancreatic islets, employ DCV-mediated exocytosis to release a wide range of cargos in response to different physiological conditions. Two complementary mechanisms have been proposed for DCV cargo sorting: (i) sorting by entry, and (ii) sorting by retention (Kim et al., 2006). Physical aggregation of cargos, either alone or together with chromogranins, is important for their sorting and retention within DCVs (Kim et al., 2006). However, apart from physical aggregation, little is understood about how DCV cargos are retained within DCVs while factors not destined for DCVs are directed to the endosomal/lysosomal route during DCV maturation. An important insight comes from a study in *Caenorhabditis elegans* showing that loss of Rab2a function leads to specific lysosomal degradation of a neuropeptide that is normally located in DCVs (Sumakovic et al., 2009). However, the existence of such a mechanism in mammalian cells has not been demonstrated.

Lysozyme, but not other AMPs, is selectively depleted from DCVs in *Lrrk2*$^{-/-}$ Paneth cells (Zhang et al., 2015). *LRRK2*, originally identified as a gene most mutated in autosomal dominant familial Parkinson's disease (Paisan-Ruiz et al., 2004; Zimprich et al., 2004), encodes a major susceptibility gene for CD (Anderson et al., 2011; Barrett et al., 2008; Franke et al., 2010; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). *LRRK2* encodes a 2,527-amino acid cytosolic protein kinase with several functional domains, including leucine-rich repeats (LRRs), a ROC domain, a COR domain, a Ser/Thr kinase domain and a WD40 repeat domain. LRRK2 is specifically expressed in Paneth cells in the intestinal epithelium and a yeast two-hybrid screen identified Rab2a as an interaction partner of LRRK2. LRRK2 deficiency leads to a failure of Rab2a recruitment on the DCV surface. Lysozyme is mistargeted for lysosomal degradation upon depletion of LRRK2 or Rab2a in Paneth cells. Thus, Paneth cells rely on the LRRK2-Rab2a axis in controlling specific cargo sorting in DCVs.

Furthermore, Nod2-dependent commensal bacteria sensing is required for LRRK2- and Rab2a-mediated lysozyme sorting. In regular specific pathogen-free (SPF) mice, Nod2 localizes to DCVs in Paneth cells. In the absence of commensals, Nod2 no longer localizes to DCVs, and LRRK2 and Rab2a also fail to localize to DCVs. Microbial colonization or MDP alone restores DCV localization of Nod2, recruitment of LRRK2 and Rab2a to DCVs, and lysozyme sorting in Paneth cells. Biochemical studies show that Nod2 physically interacts with LRRK2, suggesting a scenario in which Nod2 recruits LRRK2, and subsequently Rab2a, onto DCVs. Thus, Paneth cells rely on Nod2 to sense intestinal microbes and thereby to direct lysozyme sorting.

ER STRESS AND AUTOPHAGY IN PANETH CELLS

Aside from directly controlling AMP synthesis, sorting, and secretion, IBD-associated genes are involved in other cellular processes, such as the unfolded-protein response (UPR) and autophagy, which are also essential for normal function of Paneth cells. Depletion of the UPR transcription factor Xbp1 (X-box binding protein-1) in mouse intestinal epithelium results in exacerbated ER stress and abolition of bactericidal factors in Paneth cells, which causes spontaneous enteritis (Kaser et al., 2008). Defective autophagy in Paneth cells also results in failure of AMP secretion. Impaired Paneth cell function was observed in CD patients who were homozygous for a highly prevalent risk allele of *ATG16L1*, which encodes the autophagy related 16-like 1 protein, and in mice carrying a hypomorphic variant of *Atg16l1* (*Atg16L1*^{HM}) (Cadwell et al., 2008; Cadwell et al., 2010). Persistent norovirus infection abolishes lysozyme secretion in *Atg16L1*^{HM} mice, suggesting that autophagy in Paneth cells is required for stress responses. Indeed, defective autophagy also exacerbates enteritis in mice with Xbp1 deleted in intestinal epithelium (Adolph et al., 2013). Collectively, IBD-associated genes are implicated in a wide range of cellular processes in Paneth cells, highlighting the vital role of Paneth cells in intestinal homeostasis (Figure 1).

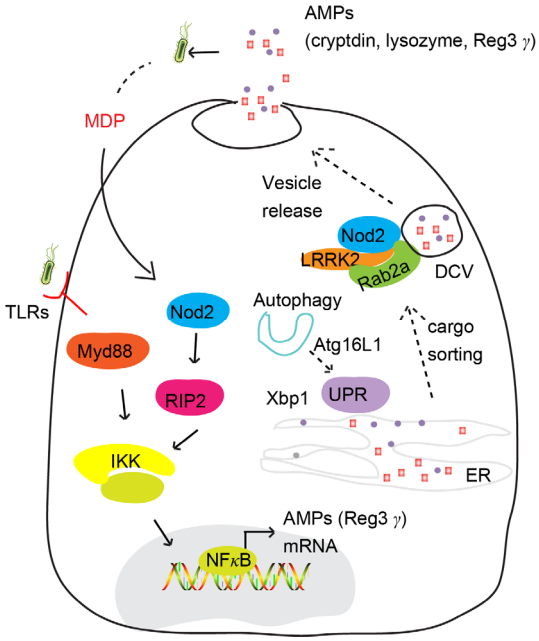

Figure 1 A summary diagram of the cellular processes in Paneth cells which have been implicated in the pathogenesis of IBD. TLRs and Nod2 sense extracellular and intracellular bacterial ligands respectively and activate NF-κB via downstream signaling pathways to induce the transcription of genes encoding AMPs. AMP peptides are synthesized in the endoplasmic reticulum (ER). Xbp1 and Atg16L1, regulators of UPR and autophagy respectively, play an essential role in mitigating ER stress. Nod2, LRRK2 and Rab2a orchestrate the selective sorting of lysozyme in DCVs, before the final exocytosis step once DCVs reach the plasma membrane of Paneth cells. AMPs in the intestinal lumen regulate the composition of the gut microbiota. Solid arrows represent signal transduction pathways, while dashed arrows represent cellular processes.

CONCLUSION

Currently we are still only beginning to understand the complex interplay between Paneth cells and commensal bacteria. Many of the key interactions remain unknown. For example, besides UPR, autophagy and DCV cargo sorting, are there other cellular processes that are critical for Paneth cell function and involved in IBD pathogenesis? Do commensal bacteria regulate cellular processes other than gene transcription and cargo sorting? What is the physiological function of individual AMPs in regulating the microbiota?

In brief, studies on IBD-associated genes have yielded important insights into the role of Paneth cells in maintaining intestinal homeostasis. While secreting large amounts of AMPs to regulate commensals and kill pathogens, Paneth cells are at the same time subject to regulation by the intestinal flora. Failure of Paneth cells to sense microbes or produce AMPs puts the host at risk of intestinal infection and inflammation. We propose that Paneth cells act as a hub for sensing intestinal microbes and regulating the microbiota. Deciphering more of the interplay between commensals and Paneth cells will shed light on the symbiosis between the host and intestinal microbiota.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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