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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEWS

SLC26A3 (DRA, the Congenital Chloride Diarrhea Gene): A Novel Therapeutic Target for Diarrheal Diseases

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SUMMARY

The DRA (SLC26A3) gene encodes a key intestinal chloride transporter. This review highlights that DRA may serve as a novel therapeutic target for diarrheal diseases, with dual benefits in diarrheal phenotype and gut inflammation in pathogen infection or IBD.

Diarrhea associated with enteric infections, gut inflammation, and genetic defects poses a major health burden and results in significant morbidity and mortality. Impaired fluid and electrolyte absorption or secretion in the intestine are the hallmark of diarrhea. Electroneutral NaCl absorption in the mammalian GI tract involves the coupling of Na^+/H^+ and Cl^-/HCO_3^- exchangers. SLC26A3 (Down Regulated in Adenoma, DRA) is the major anion exchanger involved in luminal Cl^- absorption and HCO_3^- secretion. Mutations in the SLC26A3 gene cause a severe disease called congenital chloride diarrhea (CLD). Multiple studies have shown that DRA function or expression is downregulated in infectious diarrheal disorders caused by EPEC, *C rodentium*, *Salmonella, Clostridioides difficile* and *Cryptosporidium parvum infection.* In addition, DRA levels are severely depleted in colonic mucosa of IBD patients and in mouse models of IBD (eg, DSS, TNBS, adoptive T-cell transfer, anti-CD-40, and IL-10 KO colitis). In addition, genetic defects exhibiting diarrhea including microvillus inclusion disease (MVID), keratin-8 depletion, knock-out mouse models of transcriptional factors (eg, CDX-2 and HNF1α/1β) also exhibit severe down regulation of DRA. Also, recent studies have shown that DRA is not only critical for chloride absorption but also plays a key role in maintaining gut epithelial barrier integrity, microbiome composition, and has now emerged as an IBD susceptibility gene. In this review, we provide strong evidence that DRA may serve as a novel therapeutic target with dual benefits in not only correcting diarrheal phenotype but also improving gut barrier integrity and inflammation in pathogen infection or IBD. *(Cell Mol Gastroenterol Hepatol 2025;19:101452; https://doi.org/10.1016/j.jcmgh.2024.101452)*

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Diarrhea associated with enteric infections, gut inflammation, and genetic defects poses a major health burden and results in significant morbidity and mortality. Impaired fluid and electrolyte absorption and/or secretion in the intestine are the hallmark of diarrhea. Electroneutral NaCl absorption in the mammalian gastrointestinal tract involves the coupling of Na^+/H^+ and Cl^-/HCO_3^- exchangers. SLC26A3 (down regulated in adenoma, DRA) is the major anion exchanger involved in luminal Cl^- absorption and HCO_3^- secretion. Mutations in the SLC26A3 gene cause a severe disease called congenital chloride diarrhea (CLD). Multiple studies have shown that DRA function and/or expression is down-regulated in infectious diarrheal disorders caused by *Enteropathogenic Escherichia coli* (EPEC), *Citrobacter rodentium* (CR), *Salmonella, Clostridioides difficile,* and *Cryptosporidium parvum* (C parvum) infection. In addition, DRA levels are severely

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Abbreviations used in this paper: A/E, attaching and effacing; AAD, antibiotic-associated diarrhea; Aqp8, aquaporin 8; ATRA, all trans retinoic acid; C parvum, *Cryptosporidium parvum*; cAMP, cyclic adenosine monophosphate; CD, Crohn's disease; CD4, cluster of differentiation 40; CDI, *Clostridioides difficile* infection; CFTR, cystic fibrosis transmembrane; ChIP, chromatin immunoprecipitation; CLD, congenital chloride diarrhea; CR, *Citrobacter rodentium*; DRA, down regulated in adenoma; DSS, dextran sodium sulfate; EDMs, enteroid derived monolayers; EMSA, electrophoretic mobility shift assay; EPEC, *Enteropathogenic Escherichia coli*; FMT, fecal microbiota transplant; Foxp3, forkhead/winged helix transcription factor; GAS, gamma activated sites; GR, glucocorticoid receptor; GWAS, genome wide association studies; HNF, hepatocyte nuclear factor; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IEC, intestinal epithelial cell; IFN, interferon; IL10, interlukin 10; KO, knock out; LA, *Lactobacillus acidophilus*; LPA, lysophosphotidic acid; miRNA, micro RNA; mRNA, messenger-RNA; MVID, microvillus inclusion disease; NACl, sodium chloride; NF, necrosis factor; NHE3, sodium hydrogen exchanger 3; NPY, neuropeptidase Y; PAT1, putative anion transporter 1; S1P, sphingosine 1 phosphate; SAR, structural activity relationship; SGLT1, sodium dependent glucose transporter 1; SLC, solute carrier; SNP, single nucleotide polymorphism; STAS, sulfate transporters and anti-sigma factors; TcdA, toxin A; TcdB, toxin B; TFs, transcription factors; Th1, type 1 T helper; Th2, type2 T helper cells; TNBS, 2,4,6-trinitrobenzenesulphonic acid; TNF, tumor necrosis factor; TTSS, type III secretion system; UC, ulcerative colitis; YY1, ying yang 1 factor; ZO-1, zonula occludens 1.

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depleted in colonic mucosa of IBD patients and in mouse models of IBD, eg, dextran sodium sulfate (DSS), 2,4,6-trinitrobenzenesulphonic acid (TNBS), adoptive T-cell transfer, anti-cluster of differentiation 40 (CD-40), and interleukin (IL) 10 knockout (KO) colitis. In addition, genetic defects exhibiting diarrhea including microvillus inclusion disease, keratin-8 depletion, KO mouse models of transcriptional factors, eg, CDX-2 and hepatocyte nuclear factor (HNF)1α/1β, also exhibit severe down-regulation of DRA. Also, recent studies have shown that DRA is not only critical for chloride absorption but also plays a key role in maintaining gut epithelial barrier integrity and microbiome composition and has now emerged as an IBD susceptibility gene. In this review, we provide strong evidence that DRA may serve as a novel therapeutic target with dual benefits in not only correcting diarrheal phenotype but also improving gut barrier integrity and inflammation in pathogen infection or IBD.

Diarrhea is a leading cause of significant morbidity and mortality worldwide, resulting in ~1.3 million deaths annually.[1] It is particularly more devastating for children, with nearly 1.7 billion cases of childhood diarrhea reported globally each year, contributing to 1 in 9 child deaths worldwide.[2] In addition, diarrhea is a common symptom of chronic conditions such as IBD and genetic intestinal disorders, further increasing its prevalence. Despite being a natural defense mechanism, diarrhea can lead to severe dehydration and death due to fluid loss.[3]

Diarrheal diseases vary in nature, ranging from acute to chronic and stemming from multiple causes. In developing countries, poor hygiene contributes to high prevalence of acute diarrhea caused by infectious agents including bacteria, viruses, and parasites.[4] Conversely, in developed nations like the United States, diarrhea is more often linked to inflammatory disorders such as IBD,[5,6] as well as in irritable bowel syndrome (IBS),[7] side effects of medications,[8] and chronic congenital conditions.[9]

Key intestinal mechanisms, which are known to underlie diarrheal diseases include inhibition of fluid absorption, increase in secretion, or both.[6,10] In this regard, intestinal ion transporters are central mediators regulating fluid and electrolyte homeostasis in the intestine.[11] Specifically, the electroneutral sodium chloride absorption in the mammalian intestine is primarily mediated by 2 key ion transporters, comprising the chloride bicarbonate exchanger SLC26A3 (commonly known as DRA) and sodium hydrogen exchanger SLC9A3 (NHE3).[2,12] However, electrogenic chloride secretion is mediated by the cystic fibrosis transmembrane (CFTR)[13] channel. In most diarrheal disorders the dysregulated function and/or expression of these ion transporters are central in mediating increased passage of watery stools. Previously, efforts have been made to develop therapies to manage diarrhea by targeting ion transporters in the SLC superfamily including CFTR and NHE3.[14–17] However, recent studies from our group and others in multiple models of pathogen infection,[18–22] inflammatory insults,[23–28] and genetic mutations[29–34] (summarized in Table 1 and Figure 1) have now established that DRA is one of the most important players in the development of diarrhea and is, therefore, a novel therapeutic target for diarrheal diseases. Further, the emerging role of DRA as an IBD susceptibility gene is reviewed with recent developments linking loss of DRA protein to compromised intestinal epithelial integrity, microbial dysbiosis, and increased susceptibility to colitis.[35,36] Finally, the current perspectives of research and future directions, focusing on strategies to up-regulate DRA as a potential therapy in not only alleviating diarrhea but gut inflammation, are also discussed.

DRA: A Member of the SLC26 Gene Family

The SLC26 family, part of the SLC superfamily, comprises 11 members facilitating transepithelial anion exchange including sulfate, chloride, bicarbonate, iodide, formate, oxalate, SCN, OH^- and NO_3^-.[62] Although most SLC26 transporters are broadly expressed in the body, some have more specific distribution.[63] Notably, SLC26A3 and SLC26A6 are the key players in intestinal anion transport.

SLC26A3 or DRA was initially identified as a tumor suppressor gene.[64] Later, its significance as an important Cl^-/HCO^-_3 exchanger was recognized, when the specific gene mutations of DRA were linked with congenital chloride diarrhea or CLD.[65–67] A crucial role of DRA as a major transporter involved in Cl^- absorption was further confirmed by vesicle transport studies in SLC26A3 deficient mice exhibiting a significant reduction in apical membrane chloride/base exchange activity. SLC26A3 KO mice also exhibited high chloride content diarrhea, volume depletion, and stunted growth similar to CLD patients.[68] Apart from its usual monovalent anion substrates Cl^- and HCO^-_3, studies have also demonstrated the transport of SO_4^{2-} and oxalate by DRA; however, these are transported at a much lower rate.[69] Conversely, unlike SLC26A3, the deficiency of putative anion transporter 1 (PAT1) or SLC26A6 (a small intestinal anion exchanger) does not give rise to diarrhea.[70–72]

Membrane Topology

Structurally, DRA is a glycoprotein consisting of 764 amino acids with a molecular mass ranging approximately from 85-116 kDa.[73,74] The protein contains around 12 transmembrane α-helices with both -COOH and -NH2 termini located intracellularly. The C-terminal harbors the sulfate transporter and anti-sigma factor (STAS) domain, crucial for the transporter activity, and a PDZ domain, facilitating protein-protein interactions. The STAS domain mutations lead to the loss of ion transporter function,[75] whereas the PDZ domain is more important for the formation of protein-protein complexes with other transporter proteins such as CFTR and NHE3.[74]

Expression Profile

Expression of SLC26A3 protein is highest on the apical membrane of the intestinal columnar epithelial cells. Also, along the crypt-villus axis, the expression is greatest on the villus tip as opposed to the crypt base.[74] Along the

Table 1. Agents That Down-regulate or Up-regulate DRA Expression/Function

DRA down-regulation in models of infectious diarrhea		
Models of infectious diarrhea	Effect on DRA expression/function	Citation
Enteropathogenic E coli	⇓ Function and surface levels	19
Citrobacter rodentium	⇓ mRNA and protein	18, 37
C difficile	⇓ Protein	20, 38
Salmonella	⇓ mRNA and protein	39, 40
Cryptosporidium parvum	⇓ mRNA, protein and function	21
Genetic models of diarrhea associated with marked DRA repression		
Genetic defects/mutations/KO mouse models/TFs/miRNA	Effect on DRA expression/function	Citation
MVID (Myo5b KO/mutations)	⇓ mRNA, protein and function	32–34
HNF1 α, HNF1β, HNF4 α KO	⇓ mRNA and protein	41–43
CDX-2 KO	⇓ mRNA and protein	29
SATB2 KO	⇓ mRNA	44
Keratin-8 KO	⇓ mRNA and protein	30
STAT1	⇓ mRNA	45
NF-κB	⇓ mRNA and protein	46
miR-494	⇓ Protein	47
IBD, mouse models of IBD and cytokines	Effect on DRA expression/function	Citation
Ulcerative colitis	⇓ mRNA and protein	48, 49
Crohn's disease	⇓ mRNA and protein	6, 50
DSS colitis	⇓ mRNA and protein	23, 25
Anti-CD-40 colitis	⇓ mRNA and protein	26
Adoptive T-cell transfer colitis	⇓ mRNA and protein	24
$TNF^{+\Delta ARE}$ mice	⇓ mRNA and protein	27
IL10 KO spontaneous colitis	⇓ mRNA	28
TNBS colitis	⇓ mRNA and protein	51
HLA-B27/β2m (rat)	⇓ mRNA	31
Interferon gamma	⇓ mRNA, protein and function	31, 45, 46
Tumor necrosis factor	⇓ mRNA and protein	46
Interleukin 1 beta	⇓ mRNA	31
Agents that up-regulate DRA expression/function (anti-diarrheal)		
Agents	Effect on DRA expression/function	Citation
All-trans retinoic acid	⇑ mRNA and protein	52, 53
Neuropeptide Y (NPY)	⇑ Function and surface levels	26, 54
Vasoactive intestinal peptide	⇑ mRNA and protein	55
Lysophosphatidic acid	⇑ mRNA, protein and function	56
Sphingosine-1 phosphate	⇑ mRNA, protein and function	57
Butyrate	⇑ mRNA	42
Dexamethasone	⇑ mRNA and protein	58
Lactobacillus spp.	⇑ mRNA, protein and function	23, 59
Bifidobacterium spp.	⇑ mRNA, protein and function	60
Akkermansia muciniphila	⇑ mRNA	61

length of the intestine, higher amounts are detected in the duodenum and colon with less in jejunum and ileum.[76] In addition, it is also expressed in the male reproductive tract and pancreas. In this regard, DRA appears to be more important in the intestine and plays critical roles in its homeostasis, and the severe consequences of DRA

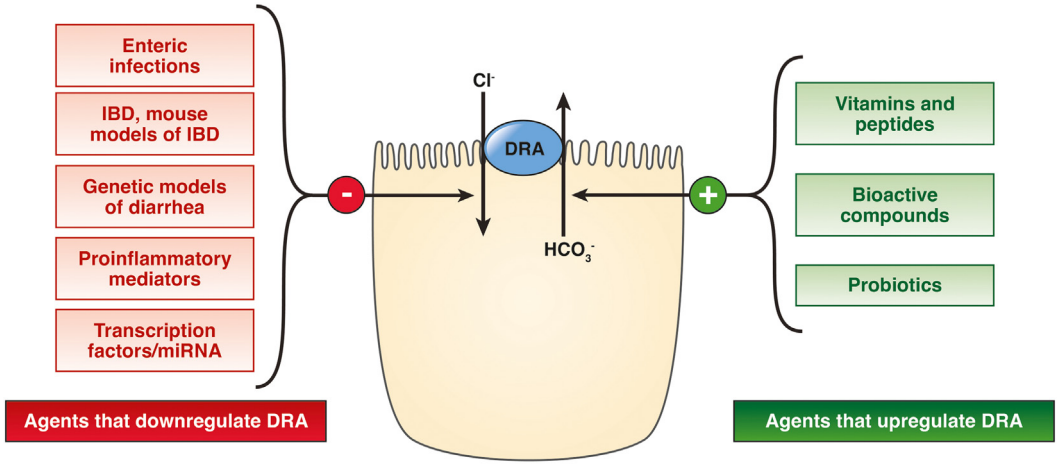

Figure 1. Regulation of DRA expression or function with potential implications in diarrheal diseases. Down-regulation of DRA function or expression has been implicated in various diarrheal disorders, including enteric infections (EPEC, *Salmonella, C difficile, C parvum, C rodentium*); IBD (UC and CD); mouse models of IBD (DSS, TNBS, adoptive T-cell transfer, anti-CD-40, and IL10 KO colitis); genetic models of diarrhea: CLD, MVID, and keratin 8 KO; proinflammatory mediators: (IFNγ, TNFα, NF-κB, IL1β, H_2O_2, NO, ROS); loss of specific transcription factors (HNFs, CDX2, SATB2), and miRNAs (miR 494). Up-regulation of DRA function and expression by various agents may have antidiarrheal implications such as by vitamins and peptides (ATRA, GLP1, VIP, NPY); bioactive compounds (dexamethasone, LPA, S1P, butyrate); and probiotics (*Lactobacilli* and *Bifidobacteria*). Therefore, targeting DRA represents a potentially novel strategy for the development of therapeutic interventions to manage diarrheal disorders.

deficiency in diarrheal diseases as well as gut inflammation are summarized below.

DRA Down-regulation in Diarrhea Associated With Enteric Infections

Diarrhea caused by infection with enteropathogens is a major cause of morbidity and mortality worldwide.[77] In majority of bacterial infection-induced diarrhea, normal absorptive function is diminished and/or secretory processes are enhanced that ultimately lead to the rapid loss of fluid and electrolytes.[78] Here we discuss the important pathogenic microorganisms including enteropathogenic *Escherichia coli, Clostridioides difficile, Salmonella,* and *C parvum,* which produce severe diarrhea associated with significant down-regulation of DRA expression.

Enteropathogenic E coli Infection

EPEC is a foodborne human enteric pathogen known to cause infantile diarrhea.[79] Upon infection, EPEC induces specific histopathological changes in intestinal enterocytes known as attaching and effacing (A/E) lesions.[80] EPEC produces its effects via a type III secretion system (TTSS), and its main effectors are Tir, espF, espG, espH, escN, espA, espB or espD, and Map, which play crucial roles in EPEC pathogenesis.[80] However, the ion transport basis of EPEC-induced diarrhea has only recently been delineated by our group.[19,22,78] Notably, a marked decrease in function and expression of DRA in response to infection with EPEC was shown.[19] EPEC infection also decreased DRA levels on the apical surface of enterocytes, causing internalization of DRA to the cytoplasm via clathrin independent processes.[22] The effects of EPEC infection on DRA activity were mediated via effector proteins EspG1 and EspG2 in decreasing apical membrane DRA level via disrupting host microtubule network by degrading cytoskeletal protein, tubulin.[19] Similarly, studies with the murine counterpart of EPEC, CR, showed that CR infection in FVB/N mice resulted in fatal diarrhea due to extensive loss of electrolytes.[37] This diarrhea was associated with almost complete loss of colonic DRA expression.[81] Interestingly, decreased levels of expression of DRA and NHE3 in response to CR infection were counteracted by the probiotic *Lactobacillus acidophillus*, confirming the importance of these proteins.[18]

Salmonella Infection

Salmonella infection spreads through contaminated food and water, often leading to diarrhea, and is a major global health concern. Marchelletta et al[39] demonstrated a marked loss of DRA surface expression in colonic epithelial cells in *Salmonella*-infected mice, indicating the critical role of reduced DRA expression in pathogen-induced diarrhea. Recently, Quach et al[40] further demonstrated a marked decrease in DRA expression in *Salmonella*-infected enteroid-derived monolayers (EDMs) with a concomitant inhibition in the Notch signaling pathway, suggesting that *Salmonella* infection may hinder fluid absorption both by inhibiting DRA expression and by inhibiting Notch signaling, promoting a shift from absorptive to secretory cell lineage.

Clostridioides difficile Infection

Another important pathogen, *Clostridioides difficile,* is known to cause diarrhea via secretion of toxin A (TcdA) and toxin B (TcdB) and the binary toxin.[82] *Clostridioides difficile* infection (CDI) is a primary cause of antibiotic-associated diarrhea and the most common cause of hospital acquired infection in the United States.[83] The diarrhea associated with CDI ranges from self-limiting nature

to severe in extreme cases. Studies by us and others have shown that both *C difficile* toxins, TcdA and TcdB, treatment caused a marked decrease in DRA protein expression in both intestinal epithelial cells (IECs) and toxigenic mouse model with no change in its mRNA levels,[20,38] suggesting a post-transcriptional mechanism regulating DRA expression. In addition, at higher doses, TcdB was also capable of reducing NHE3 function and translocation to the subapical endomembrane compartment.[84] Furthermore, a transcriptome analysis study performed in *C difficile* toxins TcdA and TcdB treated mice showed that DRA/SLC26A3 was the second-most down-regulated gene in the mouse colon, followed by other solute and water transporters Aqp8, Clca1, Slc20a1, Slc26a2, Nhe3 (Slc9a3), and Sglt1 (Slc5a1). In addition, the study has also shown that both the TcdA and TcdB treatment significantly decreased the DRA and SGLT1 expression, SGLT1 function, and CFTR dependent chloride secretion and increased barrier permeability in the mouse colon.[85] Consistent with these mouse studies, patients with recurrent CDI also exhibited diminished expression of DRA protein on the apical surface of the colonic epithelium, corroborating the aforementioned findings in animal and cellular models.[20] Thus, DRA down-regulation appears to be one of the key contributors in CDI associated diarrhea.

C parvum Infection

C parvum is a protozoan parasite that causes diarrhea and is a leading cause of waterborne diseases.[86,87] To understand the mechanisms underlying diarrhea in cryptosporidiosis, we recently demonstrated that *C parvum* infection significantly inhibited the DRA expression in in vitro IECs and in vivo mouse models.[21] Interestingly, *C parvum* infection also decreased the DRA function in Caco-2 cells.[21] It is also noteworthy to mention that *Cryptosporidium* infection also down-regulated the expression of several important tight (occludin and zonula occludens 1 [ZO-1]) and adherens junction proteins (E-cadherin) in the ileum, which could also contribute to diarrhea.[88] However, detailed future studies are warranted to delineate the precise mechanisms underlying DRA repression by *C parvum* infection.

DRA Down-regulation in Diarrhea Associated with Genetic Defects/KO Mouse Models

DRA expression has also been shown to be down-regulated in mouse models of multiple genetic diseases or KO mouse models of certain key transcription factors that exhibit diarrheal phenotype. For example, mutations in DRA gene have been shown to be responsible for CLD. Also, diarrhea associated with mutations in the key actin motor gene Myo5b responsible for microvillus inclusion disease involved a decrease in DRA expression. In addition, the KO mice for several essential transcription factors, eg, HNFs, CDX2, and cytoskeletal protein Keratin 8, also exhibited diarrheal phenotype. However, the ion transport basis of diarrhea in these models has only recently been delineated and included a marked down-regulation of DRA expression. These studies, presented below, further support the notion that DRA is a novel therapeutic target for diarrheal diseases.

Congenital Chloride Diarrhea

CLD is a rare genetic recessive disorder caused by mutations in the DRA gene, characterized by extensive loss of chloride in acidic stools accompanied by metabolic alkalosis, which requires life-long replacement therapy to account for the water and electrolyte deficit.[68,89–91] Similar to the CLD phenotype, DRA KO mice also develop severe diarrhea, metabolic alkalosis, and serum electrolyte imbalance. Higher incidences of CLD are found in countries including Finland, Poland, Kuwait, and Saudi Arabia.[92] The cause of CLD is a genetic mutation at chromosome 7, identified by genetic and physical mapping, giving rise to a defective DRA protein.[93] The founder mutation of CLD, caused by a three-base deletion at nucleotides 951-953, results in the loss of the amino acid valine at position 317 (V317del) in the protein. This results in the loss of both chloride and sulfate exchange capability of the transporter.[90] Almost all mutations characterized in the high incidence populations, including the founder mutation V317del, are at the same locus in all cases of CLD.[65] Four mutations in CLD are associated with STAS domain and cause misfolding and mis-trafficking of the transporter leading to the loss of function.[75] Interestingly, a recent study showed that subjects with CLD were predisposed to IBD.[94]

Microvillus Inclusion Disease

Microvillus inclusion disease (MVID) is one of the most common congenital secretory diarrheal diseases, which is characterized by histologic abnormalities such as intestinal villus atrophy, intracytoplasmic inclusions of enterocytes, and brush border defects.[95] Although the reason for disease development is not well-understood, in most cases it arises because of a loss of function mutation in the actin motor gene Myo5b.[96] MVID causes death of newborns and children and requires continuous support of parenteral nutrition due to the presence of severe diarrhea.[97]

In epithelial cells, Myo5b is known to direct apical polarity.[96] Before the identification of the mutations in Myo5b, the abnormalities in NaCl absorption in diarrhea in MVID patients were well-documented.[95] Recent studies showed a marked decrease in the expression of DRA and NHE3 in mouse models of MVID, in the human MVID enterocytes, as well as in cellular models recapitulating the disease phenotype.[33,34] However, the secretory effects of CFTR gene were unaffected[33] in these patients, indicating the involvement of dysfunctional NaCl absorption in MVID diarrhea.[32]

Knockout Models of Transcription Factors

HNFs play a crucial role in regulating the expression of genes involved in diverse physiological processes including cell differentiation, cell fate, barrier function, transport, and

metabolism.[98] A previous study by D'Angelo et al[41] showed that the expression of ileal and colonic DRA was substantially reduced in mice deficient in either intestinal HNF-1α or HNF-1β, and knocking down both isoforms of HNF1, HNF1$\alpha^{-/-}$/HNF1$\beta\Delta$, in the intestine completely abolished expression of DRA. These mice also exhibited severe diarrheal phenotype. In addition, our earlier studies showed a marked decrease in the basal DRA promoter activity by deleting the HNF-4α binding site.[42] In addition, using knockdown or overexpression of HNFs in Caco-2 cells, our group further showed direct binding to promoter and regulation of DRA expression by HNFs in IECs.[43]

Caudal type homeobox 2 KO mice also exhibit diarrhea, and it was shown that this was associated with a marked decrease in DRA expression.[29] In this study, ectopic overexpression of caudal type homeobox 2 in IECs as well as electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assays further confirmed that regulation of DRA by caudal type homeobox 2 involved direct binding of caudal type homeobox 2 to DRA promoter.[29]

Special AT-rich sequence-binding protein 2 KO mice develop spontaneous colitis and also exhibit diarrheal phenotype, dysbiosis compromised barrier integrity, and down-regulation of DRA expression.[44] Furthermore, in vitro luciferase reporter and ChIP studies showed that special AT-rich sequence-binding protein 2 can function as a transcription factor in direct regulation of Slc26a3 gene expression.[44]

In addition, studies from our group have also shown direct repression of DRA expression by transcription factors (TFs) STAT1[45] and nuclear factor kappa B[46] (Table 1).

Keratin 8 are cytoskeletal intermediate filament proteins within epithelial cells, playing pivotal roles in including cell polarity, migration, signaling, organelle organization, and susceptibility to apoptosis[99] Keratin-8-KO mice are known to develop Th2-type colonic inflammation, epithelial hyperproliferation with diarrheal phenotype.[99] Intriguingly, various mutations in keratin-8 have also been reported in IBD.[100] To elucidate the ion transport basis of diarrhea in K-8-KO mice, expression of various ion transporters was examined. The data showed a significant reduction in DRA expression in both the small intestine and colon, without changing the expression of other key transporters, eg, CFTR, PAT-1, and NHE3.[30] Taken together, the loss of DRA in K-8-KO mice appears to be the main driver of diarrheal phenotype in these mice.

DRA Down-regulation in Diarrhea Associated With IBD

In addition to genetic diarrheal disorders, inflammatory conditions of the intestine also display diarrhea.[6] Particularly, IBD consisting of 2 disease types, ulcerative colitis (UC) and Crohn's disease (CD), are chronic in nature.[6] Almost all IBD patients suffer from diarrhea and metabolic disturbances mainly because of the damage to the intestinal epithelium.[6] Nearly all UC patients and most CD patients commonly have inflammation in the colon, where DRA levels are markedly reduced.[6] Studies have demonstrated that both transcript and protein levels of DRA are severely down-regulated in inflamed colon in the human, and this is also recapitulated in mouse models of colitis.[18,24,89] In addition, in recent years genome wide association studies have identified SLC26A3 as an IBD susceptibility gene in multiple cohorts of UC patients.[101–103]

Malabsorption of NaCl and water is a well-characterized feature of the inflamed intestinal mucosa leading to diarrhea.[104–106] Contributing factors may include inflammation-induced reduction in electrolyte absorption, a compromised barrier function of the epithelium, and gut dysbiosis.[107] Another plausible contributing factor to the pathogenesis of diarrhea in IBD could be the presence of high concentrations of proinflammatory cytokines and chemokines in the lumen that are capable of triggering Cl^- secretion.[108] However, chloride secretion has not been shown to be altered in studies conducted in IBD patients as well as in animal models of IBD.[106,109] Studies from colonic mucosa of UC patients have demonstrated that electrogenic sodium absorption is also reduced in the inflamed intestine.[106,110]

In addition to a decrease in sodium and chloride absorption in UC, studies from Dr Sandle's group have shown that cyclic AMP (cAMP) activated potassium channel activity was also significantly increased in UC.[106,111] The studies suggested that defective sodium chloride absorption, coupled with increased potassium ion permeability, is the main pathophysiological mechanism of diarrhea in UC, rather than enhanced chloride secretion. In addition, the studies indicated that the pattern of BK channel (Big K^+ channels) distribution is altered in UC patients. Although it remains unclear whether this altered distribution directly results in increased luminal (apical) K^+ permeability, if proven to be true, it could explain the increased K^+ secretion in some patients with active UC, which can lead to colonic fecal K^+ losses and hypokalemia.[111] However, future in-depth studies are warranted in an IBD setting to explain the potassium channel interplay alone or in association with NHE3 and DRA in IBD-associated diarrhea. The role of K+ channels in normal pathophysiological situations has been reviewed in detail elsewhere.[111]

The above studies confirm the notion that diarrhea in IBD is mainly due to the anti-absorptive pathway (involving marked down-regulation of DRA/NHE3), rather than increased chloride secretion.[104] These studies further attest to the crucial role played by the DRA in IBD-associated diarrhea and suggest that strategies that can up-regulate DRA could prove to be effective therapies to manage diarrhea. In recent years, several studies in murine inflammatory models have further emphasized the important role of DRA in diarrheal diseases discussed below.

Mouse Models of Colitis

Mouse models of colitis using chemicals, pathogens/toxins, and genetically modified mice have further evolved the current understanding of the critical role of DRA in IBD-associated diarrhea (Table 1) and IBD pathogenesis. We[23] and others[25] have shown that DRA mRNA and protein are significantly reduced in the colon of mice with DSS colitis. In another study where an immune-based model of IBD was used for developing inflammation, ie, adoptive T-cell

transfer colitis, the DRA mRNA and protein in the distal colon were significantly down-regulated.[24] In addition, anti-CD-40–induced colitis animal model driven primarily by innate inflammatory responses, which mirrors the pathophysiology of IBD, including the manifestation of diarrhea, also showed significant repression of DRA expression.[26]

On the other hand, transgenic rodent models including HLA-B27/β2m in rat[31] and IL10 KO mouse studies[28] also showed a very similar phenomenon, where the expression of DRA mRNA was strikingly reduced. In addition, DRA mRNA, protein, and function were also found to be lower in tumor necrosis factor (TNF)α overexpressing mice ($TNF^{+\Delta ARE}$ mice).[27] Table 1 summarizes various animal models of colitis and potential mechanisms involved in DRA down-regulation.

In studies, the NHE3 (Na^+ transporting counterpart of DRA), although reduced in patients with IBD,[112,113] similar trend of reduction is not always observed for mRNA and protein levels in several models of colitis.[24,27]

Although all models of IBD described above show down-regulation of DRA, it should be noted that because mutations in DRA are the critical determinants of diarrhea in CLD and the DRA KO mice exhibit diarrheal phenotype similar to CLD, it is likely that the down-regulation of DRA is the key causative factor in IBD-associated diarrhea.

Mechanisms of DRA Repression in IBD Models by Cytokines, TFs, and Micro RNAs

Cytokines

The increased cytokines present in the lumen of IBD patients disrupt an array of proteins on epithelial cells. Notably, previous studies have demonstrated how important proinflammatory cytokines such as IL1β, TNFα, and interferon (IFN)γ avidly secreted by immune cells have direct effects on the expression of DRA through transcriptional mechanisms.[31,45,46] Specifically, IFNγ down-regulates DRA expression by binding to its receptor, phosphorylation of JAK and STAT1, resulting in protein dimerization and nuclear translocation of STAT1, which then binds to specific gamma activated site (GAS) region in DRA promoter.[45]

Another important cytokine up-regulated in IBD, TNFα binds to its specific receptor, which activates nuclear factor (NF)-κB by releasing it from I-κB. The released NF-κB directly translocates to the nucleus and binds to DRA promoter to suppress its expression.[46] Specific regulation of DRA levels during inflammatory insults are extensively reviewed elsewhere.[6]

TFs and Micro RNA494

Several TFs have been shown to regulate the expression of SLC26A3 by directly binding to its promoter region. Notably, HNFs (HNF1α, HNF1β, HNF4α) as well as CDX2 have been demonstrated to play a critical role in modulating SLC26A3 expression.[29,37,43] Interestingly, DRA expression along with several of these transcription factors are found to be lower in patients with IBD.[114] Thus, apart from the effect of cytokines, the lack of these regulatory transcription factors could also be responsible for the almost complete loss of DRA expression as opposed to other ion transporters in the same category.

In contrast, the transcription factors such as STAT and NFκB/P65 are known to negatively impact expression of DRA. These transcription factors are predominantly involved in exerting deleterious effects of cytokines on DRA gene transcription. Besides these TFs, post-transcriptional down-regulation of DRA protein expression by micro RNA (miRNA)494 (a miRNA known to be increased in IBD) has also been shown in IECs.[115] The specific cytokines/agents and their action on DRA are listed in Table 1 and Figure 1.

Up-regulation of DRA as a Potential Antidiarrheal Strategy

The mounting evidence now shows that down-regulation of DRA is one of the most critical steps in infectious, inflammatory, as well as other genetic models of diarrhea. Therefore, agents and molecules that can up-regulate the expression and/or function of DRA could serve as therapeutic agents to manage a myriad of diarrheal diseases. Substances that have been shown to increase the expression of DRA and could be potential anti-diarrheal agents are discussed below.

Probiotics and other Biologically Active Dietary Ingredients

Probiotics are live microorganisms that confer health benefits when consumed in adequate amounts.[116] Beneficial probiotic bacteria including *Lactobacillus acidophilus* (LA)[59] and *Bifidobacterium spp*[60] are well-known to decrease the severity of gut inflammation as well as diarrhea in both mice[60,117] and humans.[118] LA and its culture supernatant have been shown to impart antidiarrheal effects by increasing the DRA expression and its apical membrane abundance in the gut epithelium via PI3K-Rac1–dependent mechanisms and also involvement of lipid rafts and NHERF2.[23,59,119] These studies further confirmed that heat stable soluble factor(s) of low molecular mass (3–10 kDa) present in the LA culture supernatant mediated these effects.[119] Another study showed that culture supernatant of *Bifidobacterium spp* or live *Bifidobacterium* increased DRA expression in in vitro and in vivo models. Moreover, this increase in DRA expression in Caco-2 cells appeared to be ERK 1/2 pathway-dependent.[60,120] Another recent study showed that the pasteurized *Akkermansia muciniphila* or its membrane protein Amuc_1100 treatment resulted in significant reductions in both the colonic injury and diarrheal score by increased facilitated water and electrolyte absorption via the up-regulation of DRA, NHE3, and aquaporin (AQ)P4 expression in the mouse model of antibiotic-associated diarrhea (AAD).[61]

On the basis of the above studies, the use of probiotics in diarrheal diseases and the beneficial effects that these microorganisms impart on DRA strengthen their clinical benefit. The antidiarrheal potential of probiotics are extensively discussed elsewhere.[47]

Biologically active dietary ingredients including nutrients and by-products of intestinal microbiota have been shown to mediate beneficial effects in the intestine. In this regard, gut microbial metabolites such as butyrate and other dietary components such as all-trans retinoic acid (ATRA),[52] sphingosine-1 phosphate,[57] and lysophosphatidic acid[56] have been shown to up-regulate DRA function and/or expression, highlighting their potential role in therapeutic modalities via up-regulation of DRA.

ATRA is an active derivative of vitamin A mediating multiple physiological processes of the cell. Previously, our group demonstrated the up-regulation of DRA expression by ATRA via a transcriptional mechanism involving the transcription factor HNF-1β via retinoic acid receptor RAR-β receptor activation.[52] Consistent with this, diarrhea associated with DSS-induced intestinal inflammation was also attenuated when mice were treated with ATRA, highlighting the importance of up-regulation of DRA in alleviating intestinal inflammation as well as diarrhea.[53]

In addition, short chain fatty acid butyrate has also been shown to have direct effects on DRA promoter activity in cell lines via transcription factors HNF4α, ying yang 1 (YY1), and GATA.[42] Interestingly, butyrate has also shown benefit in patients with multiple diarrheal disorders including cholera[121] and CLD[122] where genetic mutations of DRA underlie the pathogenesis of diarrhea.

The metabolic product of dietary sphingolipids, sphingosine-1 phosphate (S1P), a bioactive sphingosine, has emerged as an agent that modulates various cellular processes including epithelial barrier function.[123] S1P receptor modulators dampen the inflammatory response by sequestering lymphocytes in the lymph nodes. In recent studies, several S1P receptor modulators have been developed and have shown a beneficial effect in UC.[124] Interestingly, we have shown S1P to increase DRA function and expression (mRNA and protein) in IECs at the transcriptional level.[57] These effects were specifically mediated by binding of the transcription factor YY1 onto S1P-responsive region in DRA promoter and involving activation of the PI3/Akt pathway.[57] However, more detailed future studies are warranted to evaluate whether the effects of S1P receptor modulators could directly or indirectly (via altered cytokines) affect DRA expression.[124]

Lysophosphatidic acid (LPA), another potent bioactive lipid found in food, was identified to induce DRA expression. Data showed that treatment of IECs in vitro with LPA significantly increased apical expression and its function via membrane trafficking events.[56] Interestingly, LPA was also found to up-regulate DRA through activation of gene transcription via LPA2 receptor and PI3K/Akt signaling pathway.[125] Another group of investigators demonstrated the therapeutic potential of LPA in restoring decreased DRA expression in mouse model of DSS colitis and diarrhea.[25] Taken together, these studies support the potential usage of such dietary ingredients in managing diarrhea during inflammation via stimulating DRA up-regulation.

The important pro-absorptive hormone, neuropeptide Y (NPY), has been shown to affect chloride secretion and NaCl absorption to facilitate overall absorption in the intestine. Our group showed that one important pro-absorptive function of NPY included direct effects on apical Cl^-/HCO^-_3 exchange activity.[54] The DRA activity was significantly up-regulated by NPY via the ERK1/2 MAP kinase pathway. Of note, NPY mediated up-regulation of DRA function did not involve alterations in DRA surface levels, but the stimulatory effects of NPY on DRA function were due to increased association of DRA with lipid rafts. In addition, the potential up-regulation of DRA protein levels in Caco2 cells with the important neuropeptide VIP has also been shown.[55] Interestingly, a recent study demonstrated the increased activity/function of DRA via cAMP in human enteroid derived colonic epithelial monolayer and showed it to be dependent on CFTR.[126] Moreover, increased DRA levels were shown to be mediated via trafficking as surface DRA expression increased with cAMP.

Dexamethasone is a broadly active glucocorticoid shown to be promising in managing many inflammatory disorders including IBD.[127] Previous studies had shown that dexamethasone stimulated function and expression of the closely related Na^+ absorbing transporter, NHE3.[128] Consistent with these studies, it was recently shown that dexamethasone also increased DRA mRNA and protein expression via a transcriptional mechanism involving a glucocorticoid receptor (GR) and MKP1 mediated mechanism in IECs.[58] These findings were further validated in an in vivo mouse model.[58] In conclusion, these studies showed how DRA is up-regulated by a variety of agents (Table 1), which may serve as a starting point when developing drug candidates for the management of diarrhea.

Emerging Role of DRA in IBD Pathogenesis

Our current understanding of the role of DRA in an array of intestinal physiology as well as pathologies is rapidly emerging. The DRA KO model serves as a critical tool for precisely delineating the role of DRA down-regulation in various disease states including IBD and pathogen infection. In this section, we highlight the very recent critical reports on DRA supporting the notion that in addition to its role in fluid absorption, DRA has now emerged as a novel IBD susceptibility gene, and has several auxillary roles (Figure 2) which appear to be critical for gut homeostasis.

GWAS Studies

Recent GWAS studies have identified DRA as one of the IBD susceptibility genes where common single nucleotide polymorphisms (SNPs) were observed initially in cohorts of European descent[101] but later in several other cohorts from Japan[129] and Korea,[130] where SLC26A3 or DRA was shown to have significant association with IBD. In a study involving 1384 UC and 3057 control subjects, Asano et al[129] showed that DRA was 1 of the 3 new susceptibility loci for UC in a Japanese population. Furthermore, in an Icelandic UC patient population, damaging variant in SLC26A3 gene received a combined annotation dependent depletion score above 20, demonstrating that this variant was among the top 1% of deleterious variants in the human genome.[103]

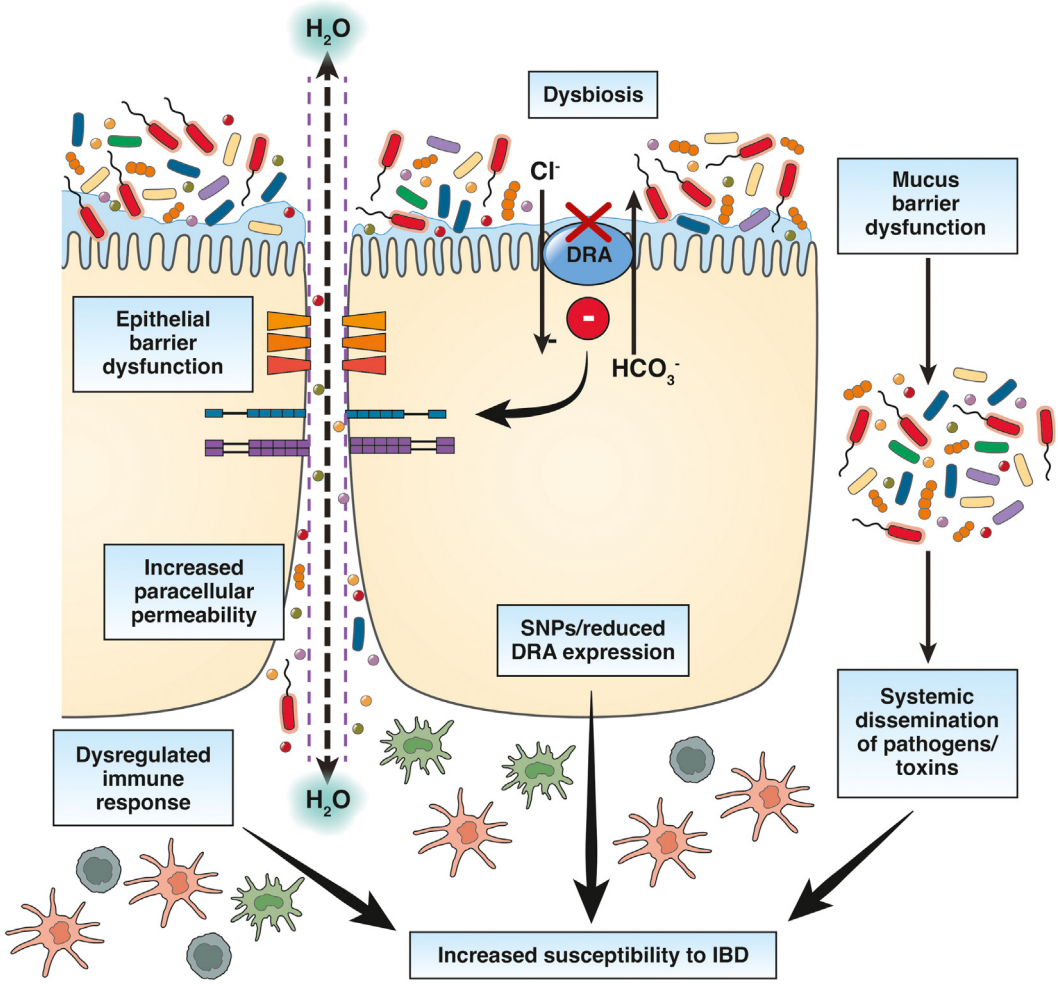

Figure 2. Auxiliary role(s) of DRA in dysbiosis, barrier integrity, and susceptibility to IBD. Single nucleotide polymorphisms (SNPs) or decreased DRA expression and/or function, resulting from inflammation or infection, may contribute to gut microbial dysbiosis; compromised mucus and epithelial barrier function; increased paracellular permeability leading to systemic dissemination of pathogens and toxins; and imbalanced immune response, ultimately increasing susceptibility to IBD.

Similarly a separate Chinese cohort with 512 UC patients and 658 healthy individuals recognized the association of UC with SLC26A3 (DRA) polymorphism variants to be associated with UC and its expression on colonic tissues.[102] Studies have identified both coding and non-coding variants in the DRA gene, with SNPs being significantly more prevalent in IBD patients.[131] According to Wedenoja et al,[127] these SNPs can affect DRA gene function, protein expression, and membrane localization. Thus, these studies further strengthen the crucial role played by DRA in yet another important chronic diarrheal disorder, IBD.

Another recent study implicating DRA into IBD pathogenesis showed that aluminum exposure was shown to induce cytokine secretion in enteroids from the colon of CD but not healthy patients. It further showed that analysis of genetic polymorphisms and expression of ABCB1 and SLC26A3 transporters revealed that their decreased activity was involved in aluminum-induced gut inflammation, further supporting the role of DRA in IBD pathogenesis.[141]

DRA KO Mouse Model

DRA KO mice exhibit severe diarrhea with high chloride content in the stool, a phenotype similar to CLD. These mice also demonstrate retarded growth and postpartum lethality, increased susceptibility to DSS colitis,[132] and the absence of a firm inner mucus layer.[133] Furthermore, recent studies from our group have shown that loss of DRA causes gut microbial dysbiosis with concomitant reduction in important tight (occludin/ZO-1) and adherens (E-cadherin) junction proteins and increased permeability in mouse colon.[134] Similarly, another study also showed that DRA KO mice displayed dysbiosis with elevated levels of expression of IL22, Reg3β/γ, Relmβ, and other proteins with antimicrobial functions in the slc26a3$^{-/-}$ colon from a juvenile age. The authors speculated that the up-regulation of this panel of antimicrobial proteins may represent a host response aimed at mitigating the inflammatory burden.[133,135]

In addition, further investigations into these mice by our group showed the critical importance of DRA in mucosal

immune homeostasis and its implications in the pathogenesis of gut inflammation. The DRA KO mice exhibited a marked induction of Th2, CD4+ Th2 cells, RORγt+ Th17 and FOXP3+ regulatory T cells in lamina propria of mouse colon. DRA KO colons also showed a marked induction of IL33, where in vivo blocking of IL33 by anti-IL33 antibodies in mice established that T2 immune dysregulation in response to loss of DRA was primarily due to the altered epithelial-immune cell crosstalk via IL33.[136] These recent studies including the GWAS and studies from the DRA KO mice showing compromised barrier integrity, dysbiosis, defective mucus layer, altered immune homeostasis, and increased susceptibility to experimental colitis have now strengthened the notion that DRA deficiency plays a critical role in IBD pathogenesis.

Targeting SLC26A3 for its Roles in Constipation and Nephrolithiasis

Although inhibition of DRA is linked to a decrease in fluid absorption, it is logical that the specific DRA inhibitors could also be used for alleviating constipation. In this regard, a recent study by Lee et al[137] developed a focused library where 4,8-dimethylcoumarin inhibitors were shown to have promising results at low doses in vivo to be potentially beneficial in eliciting DRA inhibition. Along the same lines, in a loperamide-induced constipation model in mice,[138] SLC26A3 inhibitor 7-(2-chloro-phenoxymethyl)-3-phenyl-thiazolo [3,2-a] pyrimidin-5-one (3a) was shown to significantly increase the stool weight, pellet number, and water content. This approach holds promise for the development of targeted therapies to address gastrointestinal disorders associated with altered ion transport.[138] Harnessing the optimal DRA expression also presents a potential therapeutic strategy for other conditions, eg, hyperoxaluria (a major risk factor for calcium oxalate kidney stones) by reducing oxalate absorption. A recent study demonstrated that in a mouse model of oxalate nephropathy produced by a high-oxalate low-calcium diet, the renal injury was largely prevented by DRAinh-A270 (10 mg/kg twice daily). These findings further support a major role of SLC26A3 in intestinal oxalate absorption and suggest the therapeutic utility of SLC26A3 inhibition for the treatment of constipation, hyperoxaluria and prevention of calcium oxalate nephrolithiasis.[139]

Conclusion and Future Perspectives

The studies summarized above attest to the fact that DRA, the major anion exchanger involved in chloride absorption, has now emerged as one of the key players in diarrheal diseases associated with pathogen infection or gut inflammation. Therefore, we speculate that up-regulation of DRA by various agents may be an ideal strategy to manage these diarrheal disorders. It should be cautioned that the use of the compounds (dexamethasone, neuropeptides, probiotics, dietary components) may not solely influence the expression of DRA; thus, specific molecules that can individually target DRA up-regulation may be the key to developing effective therapies. In addition, agents that could inhibit DRA may serve as potential therapy for constipation. There are also several other agents that have been identified to inhibit DRA function.[140] Similar strategies with structure-activity relationship (SAR) studies, where fine-tuning the molecules that up-regulate the DRA activation may be beneficial for developing novel drug candidates for diarrheal disorders. In addition, the validity of many of these agents that have been shown to up-regulate or down-regulate DRA expression in in vitro models using transformed cell lines, eg, Caco2, need to be further tested for their translatability in the human enteroid derived monolayers. Also, there is a need for targeted and untargeted metabolomic analysis in the setting of DRA deficiency or its overexpression to better understand its roles in gut homeostasis, diarrheal diseases, as well as in gut-microbe interactions and susceptibility to gut inflammation and pathogen infection.

Also, on the basis of the recent developments summarized above, it is clear that DRA has auxiliary functions (Figure 2) that are crucial for intestinal epithelial homeostasis, further strengthening the notion that DRA could be a target for not only developing potential drug candidates in managing diarrhea but also in the management of compromised barrier integrity and gut inflammation.

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