Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(10): 262
https://doi.org/10.31083/j.fbl2810262

Review

HuR and Its Interactions with Noncoding RNAs in Gut Epithelium Homeostasis and Diseases

Shweta Sharma[1], Lan Xiao[1], Jian-Ying Wang[1,2,3,*]

[1]Cell Biology Group, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[2]Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[3]Baltimore Veterans Affairs Medical Center, Baltimore, MD 21201, USA
*Correspondence: jywang@som.umaryland.edu (Jian-Ying Wang)

Academic Editor: Christian Barbato
Submitted: 20 July 2023 Revised: 10 October 2023 Accepted: 17 October 2023 Published: 25 October 2023

Abstract

The mammalian intestinal epithelium is a rapidly self-renewing tissue in the body and its homeostasis is tightly controlled by numerous factors at multiple levels. The RNA-binding protein HuR (human antigen R) is intimately involved in many aspects of gut mucosal pathobiology and plays an important role in maintaining integrity of the intestinal epithelium by regulating stability and translation of target mRNAs. Nonetheless, deregulation of HuR expression and altered binding affinity of HuR for target transcripts occur commonly in various gut mucosal disorders. In this review, we highlight the essential role of HuR in the intestinal epithelium homeostasis and discuss recent results that interactions between HuR and noncoding RNAs (ncRNAs), including circular RNAs, long ncRNAs, small vault RNAs, and microRNAs, influence gut mucosal regeneration and regulate barrier function in various pathophysiological conditions. These exciting discoveries advance our knowledge of HuR biological function in the gut mucosa and also create a fundamental basis for developing novel therapies to protect intestinal epithelial integrity in critically ill patients.

Keywords: RNA-binding proteins; noncoding RNAs; intestinal epithelium; gut barrier dysfunction; autophagy; inflammatory bowel diseases; cancers

1. Introduction

The mammalian intestinal epithelium is a single layer of columnar cells that function as a dynamic biophysical barrier to separate mucosal tissues from luminal bacteria and noxious substances, in addition to its crucial role in food digestion and absorption [1–3]. The intestinal epithelium undergoes a rapid and continual self-renewal process throughout the entire life, and the epithelium homeostasis depends on intestinal epithelial cells (IECs) to quickly alter gene expression to regulate proliferation, apoptosis, migration, differentiation, and cell-to-cell interaction in response to pathophysiological stresses. Intestinal stem cells and amplified progenitor cells divide continuously in the crypts and drive renewal process, while the newly divided cells differentiate into various mature cell types when they migrate up along the crypt-villus axis. Apoptosis takes place in both the crypt area, where it maintains the balance in cell number between survival cells and newly divided cells, and the luminal surface of the intestine, where apoptosis causes a loss of differentiated cells [4,5]. The intestinal mucosa also displays a spectrum of responses after acute injury and can repair itself quickly to restore epithelial integrity. Nonetheless, disrupted homeostasis of the intestinal epithelium and severe mucosal wounds/erosions occur commonly in various pathologies, resulting in abnormalities of the epithelial structure and the translocation of luminal harmful substances and bacteria to the bloodstream [6–8].

The gene expression programs that govern homeostasis of the intestinal epithelium are tightly regulated at the posttranscriptional level, although transcriptional events also play an important role in this process. In particular, regulation of mRNA stability and translation is essential for control of intestinal mucosal defense, continual renewal, repair after acute injury, and gut barrier function [9,10]. The stability and/or translational control of labile mRNAs require the association of specific mRNA sequences (*cis*-elements) with *trans*-acting factors such as RNA-binding proteins (RBPs) and noncoding RNAs (ncRNAs), specifically microRNAs (miRNAs). RBPs and miRNAs directly bind to *cis*-elements that are frequently located at the 3′-untranslated region (3′-UTR) of target mRNAs and this interaction alters rates of mRNA turnover and translation [11–13]. Long ncRNAs (lncRNAs), circular RNAs (circRNAs), and small vault RNAs (vtRNAs) also regulate gene expression at the posttranscriptional level via distinct mechanisms, including interactions with RBPs and miRNAs. Emerging evidence suggests that homeostasis of the gut epithelium is tightly regulated by RBPs and ncRNAs acting in concert to regulate gene expression synergistically or antagonistically, whereas deregulation of RBPs and ncRNAs is involved in pathogenesis of various human gut mucosal diseases.

HuR (human antigen R) is a ubiquitous RBP and contains two RNA recognition motifs (RRMs) at its N-terminus

with high affinity for uridine-rich and adenine uridine-rich elements located in the 3′-UTRs of target mRNAs, while the third RRM located at the C-terminus recognizes the poly(A) tail [14,15]. HuR is primarily distributed in the nucleus in unstimulated cells, but it can rapidly translocate to the cytoplasm when exposed to various pathophysiological stresses [14–16]. HuR participates in different physiological and pathological processes, including proliferation, apoptosis, migration, tissue injury/repair, inflammation, and angiogenesis [4,17–20] and its dysfunction is linked to several gut mucosal disorders such as inflammatory bowel disease (IBD) and malignancies [21–24]. In this review, we highlight the important roles of HuR in the intestinal epithelial homeostasis and pathologies and further discuss in some detail the mechanisms by which HuR regulates the stability and translation of target mRNAs through interaction with ncRNAs.

2. HuR in the Intestinal Epithelium Homeostasis

2.1 HuR Promotes Growth of Small Intestinal Mucosa

The intestinal epithelium renews itself every 5–7 days in humans and 4–5 days in mice and this rapid process is tightly controlled by numerous factors at multiple levels. As with other tissues in the body, the intestinal epithelial renewal is uniquely regulated by growth hormones, cortisol, insulin, and thyroxin that modulate metabolism in all tissues in the body. However, the intestinal mucosa responds to a host of events triggered by the presence of luminal food that is one of the strongest stimulants of mucosal growth. Fasting in mice or food starvation in patients with critical disorders inhibits growth of the intestinal mucosa, leading to mucosal atrophy [9]. In cultured IECs, HuR silencing by transfection with specific small interference RNA (siRNA) targeting HuR (siHuR) inhibits cell proliferation and causes G1 phase growth arrest by controlling the expression of several genes associated with cell division and differentiation [25–29].

To examine the functional role of HuR in the intestinal epithelium *in vivo*, we used a Cre-LoxP system to generate a mouse bearing with intestinal epithelium tissue-specific HuR deletion (IE-HuR$^{-/-}$) [4]. Although there are no significant differences in gut gross morphology, body weight, and general activity between IE-HuR$^{-/-}$ mice and littermate mice, HuR-deficient mice exhibit disrupted growth of small intestinal mucosa. There is a remarkable reduction in the levels of DNA synthesis and the lengths of crypts and villi in IE-HuR$^{-/-}$ mice compared with littermate mice. The HuR-deficient small intestinal epithelium also loses the ability of regenerative potential of crypt progenitors, since S-phase descendants in villous regions reduces markedly in IE-HuR$^{-/-}$ mice relative to littermate mice after exposure to irradiation. In an *ex vivo* model, growth rates of primarily cultured intestinal organoids derived from the small intestinal crypts of IE-HuR$^{-/-}$ mice decrease significantly compared with those observed in the organoids from control littermate mice [5]. On the other hand, HuR deletion in IECs fails to alter renewal of the colonic epithelium, although mucosal HuR levels are also undetectable in the colon of IE-HuR$^{-/-}$ mice. These findings strongly suggest that HuR is a biological enhancer of the small intestinal mucosal growth, and its activation is absolutely required for maintaining the intestinal epithelium homeostasis.

Although the exact mechanism underlying function of HuR in the regulation of intestinal epithelial renewal remains largely unknown, it has been shown that HuR promotes growth of the small intestinal mucosa at least partially by activating the Wnt signaling pathway [4,29]. Targeted deletion of HuR in the intestinal epithelium decreases levels of the mRNA encoding low-density lipoprotein (LDL)-receptor-related protein 6 (LRP6) that functions as a Wnt-coreceptor and is critical for constant gut mucosal growth. In addition, HuR deletion increases expression levels of p65 and Smad7 in the intestinal mucosa without effect on the levels of Frizzled-7 protein. Further experiments show that HuR physically interacts with the *Lrp6* mRNA via its 3′-UTR rather than 5′-UTR and coding region (CR), and this association enhances LRP6 expression by increasing stability and translation of the *Lrp6* transcript [4]. In support of these findings, another study reveals that HuR also directly associates with the mRNA encoding transcriptional factor c-Myc, a strong stimulator of intestinal mucosal growth, and this interaction increases c-Myc translation but does not affect *c-Myc* mRNA stability [30]. Unlike HuR binding to the *p53* and *nucleophosmin* (*Npm*) mRNAs [26], HuR association with the *c-Myc* mRNA requires HuR phosphorylation induced by checkpoint kinase 2 (ChK2). In addition, NPM serves as a nuclear interactor of Rac1 that is essential for intestinal mucosal renewal and defense [7]. HuR increases NPM abundance in the intestinal epithelium, thus promoting the nuclear accumulation of Rac1 and epithelial renewal, whereas HuR deletion in IECs decreases the levels of NPM and slows down movement of Rac1 to the nucleus, thus contributing to the inhibition of small intestinal mucosal growth in IE-HuR$^{-/-}$ mice [20].

2.2 HuR Regulates Apoptosis in the Intestinal Epithelium

HuR functions as an anti-apoptotic factor in the intestinal mucosa and protects IECs against apoptosis by altering expression levels of several apoptosis-associated genes at the posttranscription level [31]. The products of these genes regulated by HuR in IECs include X chromosome-linked inhibitor of apoptosis protein (XIAP), transcription factor JunD, mitogen-activated protein kinase kinase-1 (MEK-1) and activating transcription factor-2 (ATF-2). HuR directly binds to the mRNAs encoding XIAP, ATF-2, JunD, and MEK-1 proteins and enhances their expression, thus protecting cells against apoptosis. HuR stabilizes the *Xiap* mRNA by interacting with both its 3′-UTR and CR and increases expression of XIAP, desensitizing IECs to apop-

totic cell death in response to stress [32]. On the other hand, HuR only binds to 3′-UTRs of the *Atf-2* and *JunD* mRNAs and this association primarily increases the stability of *Atf-2* and *JunD* mRNAs with only minor influence on their translation [33,34]. Interaction of HuR with the *mek-1* transcript increases both stability and translation of *mek-1* mRNA [25]. Interestingly, all associations of HuR with the *Xiap*, *Atf-2*, *JunD*, and *mek-1* mRNAs are tightly controlled by cellular polyamines that act as biological regulators of intestinal epithelial regeneration and adaptation. Mechanistically, polyamines regulate HuR binding affinity for given mRNAs by altering Chk2-mediated HuR phosphorylation [20] and polyamines also affect the subcellular distribution of HuR via adenosine monophosphate (AMP)-activated protein kinase-regulated phosphorylation and acetylation of importin α1 [35].

2.3 HuR is Essential for Intestinal Mucosal Repair after Acute Injury

The integrity of the intestinal mucosa is quickly restored through a series of coordinated processes after acute injury [36]. One primary way by which the mucosa repairs itself rapidly and prevents further tissue damage is early epithelial restitution, characterized by stages of cell spreading and migration to quickly cover the wound area and restore the epithelial integrity [37]. HuR plays an essential role in stimulating early epithelial restitution after acute injury [17,38–40]. In an *in vitro* model of epithelial restitution, HuR silencing by transfection with siHuR inhibits early rapid epithelial restitution after wounding. In an injury model in mice, exposure to mesenteric ischemia/reperfusion (I/R) leads to small mucosal injury and erosions, but IE-HuR$^{-/-}$ mice exhibit a remarkable delay in the mucosal repair after acute injury. The intestinal mucosa is almost completely restored 6 h after I/R in littermate mice, but this rapid epithelial repair is inhibited by HuR deletion, since mucosal surface in IE-HuR$^{-/-}$ mice remains discontinuous and shows sloughed cells and debris at the same time after I/R. HuR deletion in IECs also enhances colitis induced by 3% of dextran sulfate sodium (DSS) in drinking water. Administration of DSS for 5 days causes acute colitis in both littermate and IE-HuR$^{-/-}$ mice, but histological injury scores in the colonic mucosa of IE-HuR$^{-/-}$ mice are ~two-fold that of littermate mice. Moreover, HuR deletion delays recovery of DSS-induced colitis, since the degree of granulocyte infiltration and injury/erosions in the colonic mucosa are much high in IE-HuR$^{-/-}$ mice relative to littermate mice when examined at the same time after DSS-induced injury.

HuR stimulates rapid healing of the intestinal mucosa after acute injury by enhancing expression of multiple signaling proteins such as stromal interaction molecule 1 (Stim1) [28], cell division control protein 42 (Cdc42) [17], 14-3-3ζ [39], caveolin-1 [38,41], and vitamin D receptor (VDR) [40] via direct interactions with their mRNAs. For example, Stim1 acts as a Ca^{2+} sensor within stores and is important for the activation of store-operated Ca^{2+} entry that stimulates IEC migration over the wounded area after injury. HuR binds to and stabilizes the *stim1* mRNA via its 3′-UTR, thus increasing Stim1 levels and promoting early epithelial restitution by inducing Ca^{2+} influx [28]. Cdc42 is a member of the Rho family of small GTPases and is critical for actin organization that is essential for cell migration after wounding. The *Cdc42* mRNA is a novel target of HuR, and interaction of HuR with the *Cdc42* mRNA promotes Cdc42 translation without effect on its total mRNA level. HuR also modulates subcellular localization of Rac 1, another member of the Rho family. The levels of cytoplasmic Rac1 in the intestinal mucosa increases in IE-HuR$^{-/-}$ mice, although HuR deletion fails to alter total Rac1 abundance [17].

2.4 HuR Promotes Gut Barrier Function

The intestinal epithelium lines the luminal surface of the mucosa and acts as a physical and biochemical barrier that directly interfaces with a wide array of luminal toxic substances, pathogens, and microbiota. The structure of this specialized barrier in the intestinal epithelium is comprised of various intercellular junction proteins, including tight junctions (TJs) and adherens junctions (AJs). TJs are the most apical element of the junctional complex and form a selective permeable barrier that blocks even small molecules from leaking between epithelial cells [1]. Immediately below TJs are cadherin-rich AJs, which provide strong cell-to-cell interaction and integrate different signals to modulate epithelial paracellular permeability. Since TJ and AJ complexes are highly dynamic, maintenance of the levels of cellular TJ and AJ proteins is crucial for the integrity of the barrier function in stressful environments.

An increasing body of evidence indicate that HuR enhances expression of TJs and AJs posttranscriptionally and its activation is essential for structure and effectiveness of the epithelial barrier. HuR induces the stability and translation of mRNAs encoding TJ proteins including occludin, claudin-1, claudin-3, and JAM-1, and AJ protein E-cadherin, mostly via direct interaction with their 3′-UTRs, and thus promotes the intestinal epithelial barrier function [42–45]. In an *in vitro* gut permeability model using differentiated IECs, HuR deletion by transfection with siHuR results in the epithelial barrier dysfunction, as shown by a decrease in transepithelial electrical resistance and an increase in paracellular influx of FITC-dextran. In mice exposed to septic stress, inhibiting the HuR binding affinity for its target mRNAs by decreasing Chk2-dependent HuR phosphorylation via polyamine depletion decreases the levels of TJs and AJs and leads to gut barrier dysfunction [42]. Although IE-HuR$^{-/-}$ mice do not exhibit significant change in the structure and function of gut barrier without any pathological stress, the barrier function in the HuR-deficient epithelium is more susceptible to stress, since increased gut per-

meability is much high in IE HuR$^{-/-}$ mice compared to littermates after administration of 3% DSS in drinking water or exposure to mesenteric I/R [17]. Moreover, recovery rates of the gut barrier function after DSS or mesenteric I/R are slower in IE-HuR$^{-/-}$ mice relative to littermate controls.

Chk2-dependent HuR phosphorylation is necessary for HuR association with *occludin* mRNA, and activation of Chk2 activity enhances formation of the HuR/*occludin* mRNA complex and promotes occludin translation [42]. HuR stabilizes *claudin-1* transcript by directly interacting with its 3′-UTR and/or preventing the binding of small vtRNA vtRNA2-1 to the *claudin-1* mRNA [43,45]. Moreover, HuR also induces the expression of cell cycle-related genes, such as cyclin D1 and c-Myc [29,46,47] and this stimulatory effect of HuR on intestinal epithelial renewal also contributes to HuR-mediated enhancement of the gut barrier function.

2.5 HuR Regulates Paneth Cell Function and Autophagy

Paneth cells are specialized IECs that are located at the bottom of the crypts in the small intestinal mucosa. Paneth cells secrete various antimicrobial peptides/proteins (AMPs) that are necessary for protecting the intestinal epithelium from enteric pathogenic invasion [48,49]. HuR enhances the production and release of AMPs via control of Paneth cell function, thus promoting defense of the intestinal epithelium [5]. Target deletion of HuR in IECs leads to defects in Paneth cells, since intestinal tissues from IE-HuR$^{-/-}$ mice exhibit a decrease in the numbers of Paneth cells and fewer lysozyme granules per cell, compared with those observed in littermate mice. Consistently, intestinal organoids generated from IE-HuR$^{-/-}$ mice have fewer lysozyme-positive cells. Human intestinal mucosa from patients with IBD also displays decreased levels of HuR, along with a reduction in the number of Paneth cells [5,50,51]. Mechanistically, HuR deletion reduces cell surface trafficking of Toll-like receptor 2 (TLR2) and impairs TLR2 membrane localization by inhibiting the production of the endoplasmic reticulum chaperone canopy3 (CNPY3). HuR directly binds to the *Cnpy3* mRNA via its CR but not 3′-UTR, and this interaction increases CNPY3 expression by stabilizing *Cnpy3* mRNA and stimulating its translation. These results indicate that the *Cnpy3* mRNA is a target of HuR and that HuR enhances epithelial host defense by increasing Paneth cell function via TLR2 as a result of HuR-mediated CNPY3 expression.

Autophagy is a process by which unwanted materials and pathogens in the cytoplasm are targeted to the lysosome for degradation. There are more than 30 members of autophagy-related genes (ATGs) identified in mammalian tissues. Autophagy activation is critical for the recognition and degradation of intracellular pathogens, which acts as an innate barrier of bacterial infection, whereas deregulation of autophagy compromises intestinal epithelial host defense and tissue homeostasis. Furthermore, the role of autophagy in preserving gut health is exemplified by the fact that certain bacteria can manipulate the autophagic process to promote their survival and colonization in the gut. Defects in autophagy-mediated clearance of intracellular bacteria may contribute to the chronic inflammation observed in IBD patients [52,53], since genetic variations in the *Atg16l1* gene induces the risk of developing Crohn's disease [54].

HuR up-regulates autophagy activity by affecting ATG expression. HuR silencing represses formation of autophagosome that relies on products of the *Atg* genes and is critical for activation of autophagy. HuR directly interacts with the mRNAs encoding ATG16, ATG12, and ATG5 primarily through their 3′-UTRs and increases their stability and translation, thus inducing levels of cellular ATG proteins. Consistently, expression levels of HuR are positively correlated with the abundances of cellular ATG5 and ATG12 in cancer cells, while levels of both HuR and ATG16L1 are reduced in human intestinal mucosa obtained from patients with IBD. Targeted HuR deletion in mice lowers the levels of ATG16L1 in the intestinal mucosa, but it does not alter mucosal abundance of ATG5 [55]. Furthermore, the levels of mucosal microtubule-associated protein light chain 3 (LC3)-I and LC3-II also decrease in the small intestine of IE-HuR$^{-/-}$ mice compared with littermates. In contrast, HuR deletion does not alter transcription of the *Atg* genes, since the mucosal levels of all *Atg16l1*, *Atg5*, and *Atg7* mRNAs in IE-HuR$^{-/-}$ mice are indistinguishable from those observed in littermate mice.

Taken together, these exciting findings collected from cultured IECs, intestinal organoids, mice with ablated HuR, and human tissue samples strongly suggest a novel working model by which HuR is required for maintaining the intestinal epithelium homeostasis in various pathophysiological conditions (Fig. 1). According to this model, HuR activation enhances the intestinal epithelial integrity by stimulating mucosal growth, protecting IECs against apoptosis, promoting early epithelial restitution after wounding, increasing the barrier function, and elevating Paneth cell function and autophagy. In contrast, disrupted HuR activity impairs the processes of mucosal regeneration and adaptation, thus compromising the intestinal epithelial homeostasis and contributing to mucosal pathologies such as massive injury/erosions, delayed healing, gut barrier dysfunction, and inflammation.

3. HuR in Gut Mucosal Diseases

3.1 HuR in IBD

Several studies show that altered levels of tissue HuR in the intestinal mucosa are implicated in pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), two types of IBD in [45,55,56]. We examined the levels of HuR and Paneth cells in human tissue samples of intestinal mucosa collected from ileal and colonic mucosal tissues in IBD patients [57]. HuR is distributed in both the cytoplasm

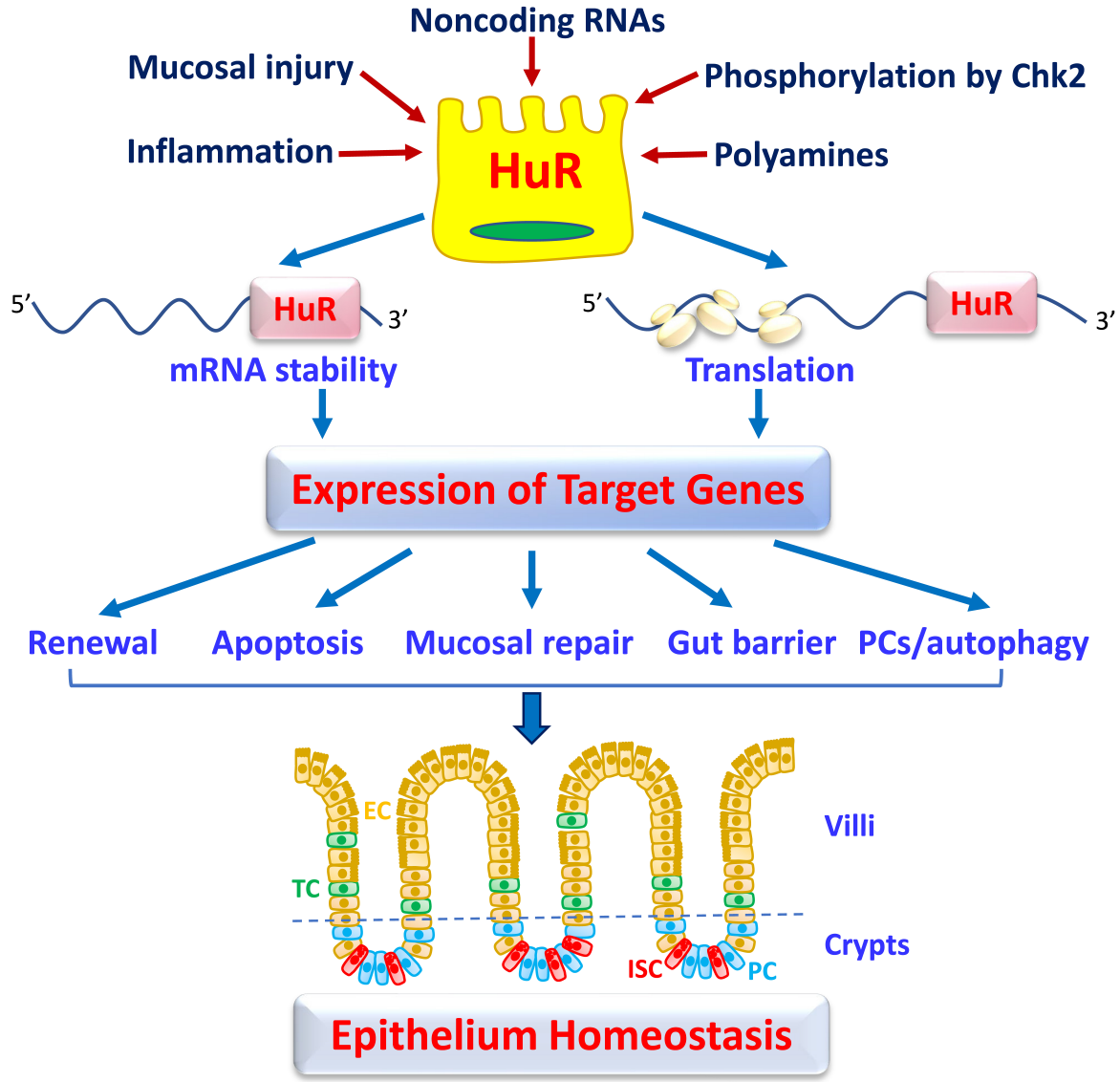

Fig. 1. Essential role of HuR in maintaining the intestinal epithelium homeostasis. HuR stimulates mucosal growth, IEC survival, epithelial repair after injury, gut barrier function, Paneth cell (PC) activity, and autophagy by altering expression levels of its target genes at the posttranscriptional level. HuR binds to and induces stability and translation of mRNAs that encode proteins involved in regulation of migration, proliferation, apoptosis, differentiation, and cell-to-cell interaction. HuR function is tightly controlled by multiple factors, whereas deregulation of HuR expression, its subcellular distribution, and binding affinity for target transcripts disrupts integrity of the intestinal epithelium, thus contributing to pathogenesis of different gut mucosal disorders. HuR, human antigen R; miRNAs, microRNAs; TC, tuft cells; EC, enterocytes; ISC, intestinal stem cells.

and nucleus in normal small intestinal mucosa from control individuals. However, the intestinal mucosa with injury/erosions and inflammation obtained from CD patients exhibits a remarkable reduction in the levels of HuR protein, specifically in the cytoplasm, when compared with those from control individuals. Similarly, levels of HuR in the colonic mucosa also decrease dramatically in UC patients relative to control individuals. Interestingly, the decreased levels of small intestinal mucosal HuR are associated with defective Paneth cells in patients with CD, since lysozyme-positive cells are undetectable in the ileal mucosal tissue samples taken from CD patients. The reduced HuR levels and defective Paneth cells in patients with CD or UC are also associated with an inhibition of mucosal renewal and an inhibition of TJ expression. The levels of several proteins, including Bcl-2, nucleotide-binding oligomerization domain 2 (NOD2), and vitamin D receptor (VDR), also decrease in the intestinal mucosa of patients with IBD [40,57–60]; all mRNA encoding these proteins are targets of HuR. These findings suggest that reduced HuR and subsequent defects in Paneth cell function are involved in the pathogenesis of human IBD.

3.2 HuR in Cancers

Altered HuR expression and deregulation of its binding affinity occur commonly in gastrointestinal cancers, specifically colorectal cancer, but the exact roles of HuR in carcinogenesis are cell type-dependent manner [19,61–63]. For instance, HuR promotes cell proliferation and inhibits apoptosis in gastric cancer cells [19,64], while HuR enhances angiogenesis and induces metastasis in colorectal cancer cells [62], although the association of induced amounts of HuR with cancer aggressive behavior is similar in both cancers. Moreover, HuR up-regulates expression levels of cyclin D1, Bcl-2, c-Myc, cyclooxygenase 2 (COX-2) and increases the activity of Wnt/β-catenin signaling, mTOR, MAPK, and NF-κB pathways [64–67]; all these factors and signaling pathways are involved in pathogenesis of cancers. HuR is also implicated in chemoresistance in colorectal cancers by altering apoptosis and drug efflux pumps that transport cytotoxic agents out of cells [63]. In esophageal cancer, HuR not only enhances the expression of survivin and counters the expression of tumor suppressor esophageal cancer-related gene 2 [64,68] but also impacts on the radiosensitivity of esophageal cancer by elevating the levels of the epithelial-to-mesenchymal transition-related protein Snail [69]. In addition, HuR acts as a prognostic marker since induced expression of HuR is associated with poor overall survival rates in patients with gastric or colorectal cancer. These results provide evidence showing that HuR plays an essential role in pathogenesis of gut cancers and is a potential target for therapeutic interventions.

4. HuR Interactions with NcRNAs

Most (>98%) of the mammalian genome is transcribed into huge amounts of ncRNAs, while protein-coding RNAs (mRNAs) only account for a minority (<2%) of the transcriptional products [70–72]. Emerged evidence indicates that interactions of HuR with ncRNAs, including lncRNAs, circRNAs, small vault RNAs, and miRNAs, play an important role in HuR-modulated intestinal epithelial homeostasis. Through the complex molecular associations of HuR with ncRNAs, HuR alters expression levels of target genes and enables IECs to adapt and respond to different stresses, thus maintaining integrity of the intestinal epithelium [23,28,73,74]. On the other hand, disrupted interactions between HuR and ncRNAs compromise the epithelium homeostasis and have a great impact on various gut mucosal disorders.

4.1 HuR Interaction with lncRNAs

LncRNAs can be transcribed from antisense, intergenic, or promoter-proximal regions but they have little potential to encode functional proteins. LncRNAs are poorly conserved in general and they modulate gene expression via a broad range of posttranscriptional and/or transcriptional mechanisms. LncRNAs can function as decoys, scaffolds, or signals and also act through *cis*- and *trans*-regulatory factors, genomic targeting, and antisense molecules. Enriched in the intestinal epithelium, several lncRNAs, including *SPRY4-IT1*, *H19*, *TUG1*, and *GMDS-AS1*, control mucosal renewal, adaptation, and repair after acute injury predominantly by interacting with HuR [23,73–75].

H19 is a 2.3-kb lncRNA transcribed from the *H19/Igf2* gene cluster and it is involved in regulating the gut epithelial barrier function [23]. The levels of tissue *H19* increase dramatically in inflamed colonic mucosa and this induction in the level of tissue *H19* damages small intestinal mucosa in patients with sepsis and IBD. Induced *H19* affects intestinal epithelial renewal, wound healing, and gut barrier function. Ectopically expressed *H19* suppresses expression of TJ ZO-1 (Zonula occludens-1) and AJ E-cadherin by reducing their mRNA stability and translation via miR-675, leading to gut barrier dysfunction. HuR directly binds to *H19*, prevents miR-675 processing from *H19*, thus promoting ZO-1 and E-cadherin expression and rescuing the barrier function. In contrast, target deletion of HuR in IECs increases miR-675 abundance in the intestinal mucosa and delays recovery of the gut barrier function in mice exposed to septic or mesenteric I/R stress [17,23]. These results demonstrate the importance of HuR interaction with *H19* in regulation of the gut epithelial barrier function.

LncRNA *SPRY4-IT1* is derived from the *SPRY4* gene, and it is shown to regulate proliferation and apoptosis in cancer cells. The expression of *SPRY4-IT1* is upregulated in patients with gastric cancer or colorectal cancer, which is correlated with cancer metastasis. *SPRY4-IT1* also plays a critical role in maintaining intestinal epithelium homeostasis by affecting gut barrier function through control of TJ expression. HuR directly binds to *SPRY4-IT1* and enhances binding of *SPRY4-IT1* to the mRNAs encoding TJs including occludin, claudin-1, claudin-3, and JAM-1 (junctional adhesion molecule 1), thereby promoting *SPRY4-IT1*-mediated stimulation of TJ translation [73]. HuR silencing decreases *SPRY4-IT1* association with these TJ mRNAs and causes dysfunction of the epithelial barrier. These results indicate that *SPRY4-IT1* and HuR stimulate TJ expression and promote the epithelial barrier function synergistically.

TUG1 is a 7.1-kb lncRNA that is distributed across multiple tissues including the intestinal epithelium. *TUG1* regulates expression of several target genes through distinct mechanisms such as chromatin remodeling, miRNA sequestration, and interactions with RBPs. Aberrant *TUG1* expression is commonly observed in the intestinal mucosa of patients with IBD and colorectal cancers. HuR is able to interact with *TUG1*, and this association affects HuR function in various pathologies [74]. Ectopically expressed *TUG1* attenuates mucosal inflammatory progression of DSS-induced colitis in mice partially by altering c-Myc expression via control of the balance between HuR and miR-29b-3p. The mucosal tissue levels of *TUG1* are

decreased by high glucose and high fat, which promotes apoptosis and release of pro-inflammatory cytokines in the intestinal epithelium. In contrast, *TUG1* overexpression reverses these effects and protects IECs against the damage induced by high glucose and high fat.

LncRNA *GMDS-AS1* is expressed in the intestinal mucosa and its overexpression plays an important role in the pathogenesis of colorectal carcinoma. Recently, *GMDS-AS1* has been shown to physically interact HuR, and this interaction prevents HuR from being degraded by ubiquitination or the proteasome [75]. HuR association with *GMDS-AS1* also stabilizes the *Stat3* mRNA and results in the activation of *GMDS-AS1*-induced STAT3 signaling. Ectopically overexpressed HuR restores proliferation of colorectal cancer cells in the absence of cellular *GMDS-AS1*.

4.2 HuR Interaction with circRNAs

CircRNAs are a class of diverse endogenous ncRNAs in mammalian tissues and play critical roles in intestinal epithelium homeostasis and diseases [10,76]. *CircRNAs* are produced via back-splicing reactions and have a covalently closed structure, preventing degradation and increasing their half-life. *CircRNAs* function as transcriptional regulators, miRNA sponges, protein decoys, scaffolds, and recruiters, and they also interact with RBPs such as HuR [77,78].

CircPABPN1 is transcribed from the *PABPN1* gene and initially identified to modulate PABPN1 expression by altering HuR interaction with the *PABPN1* mRNA in HeLa cells [79]. C*ircPABPN1* also regulates intestinal epithelial autophagy by affecting ATG16L1 expression through interaction with HuR [78]. *CircPABPN1* directly binds to HuR in IECs, and ectopically overexpressed *circPABPN1* specifically decreases association of HuR with the *Atg16l1* mRNA and represses the expression of ATG16L1 without effect on the expression levels of ATG5 or HuR. Furthermore, elevating the levels of cellular HuR rescues expression of ATG16L1 in cells overexpressing *circPABPN1*. Conversely, *circPABPN1* overexpression and HuR silencing repress ATG16L1 expression synergistically. *CircPABPN1* inhibits ATG16L1 translation primarily by preventing HuR binding to the *Atg16l1* transcript, since the *Atg16l1* mRNA does not have potential binding sites for *circPABPN1*. Importantly, human intestinal mucosal tissues from IBD patients display induced levels of *circPABPN1* and reduced HuR abundances, along with a decrease in the level of ATG16L1 and autophagy inactivation. Since HuR targets multiple mRNAs in IECs, it is possible that *circPABPN1* also regulates other transcripts via its association with HuR to modulate the intestinal epithelial autophagy and homeostasis.

CircRHOBTB3 plays a tumor suppressive role in the aggressiveness of colorectal cancer by regulating HuR. *CircRHOBTB3* physically interacts with HuR and promotes degradation of HuR through ubiquitination [62]. *CircRHOBTB3* also functions as a miRNA sponge in gastric cancer, although target genes by *circRHOBTB3* remain largely unknown. It has been reported that *circ0104103* inhibits cell growth of colorectal cancer and decreases metastasis at least partially by interacting with HuR [80]. In addition, expression level of *circAGO2* is upregulated in patients with gastric and colon cancers, which is associated with poor clinical outcomes. *CircAGO2* interacts with and induces HuR translocation from nucleus to the cytoplasm, thus altering expression of HuR target genes and enhancing aggressiveness of cancer [19]. On the other hand, decreasing the interaction between *circAGO2* and HuR reduces tumorigenesis and cancer cell aggressiveness.

4.3 HuR Interaction with Small vtRNAs

vtRNAs are small ncRNAs synthesized by RNA polymerase III and they are associated with giant cytoplasmic ribonucleoprotein (RNP) particles termed vaults. Humans express four vtRNA paralogs *vtRNA1-1*, *vtRNA1-2*, *vtRNA1-3*, and *vtRNA2-1*, while mice only produce one vtRNA [81]. vtRNAs have important roles outside of vault RNPs and have been linked to many cellular processes, including mRNA splicing, nuclear transport, drug resistance, synaptogenesis, lysosome function, apoptosis, and tumorigenesis. The free *vtRNA1-1* regulates selective autophagy by directly interacting with the RBP p62 [82]. We have recently found that the expression patterns of vtRNAs in the intestinal mucosa change significantly in response to stress and that intestinal mucosa from patients with IBD exhibits increased levels of vtRNAs [43]. Further studies show that *vtRNA2-1* inhibits expression of TJs occludin, claudin 1, and AJ E-cadherin posttranscriptionally and leads to gut barrier dysfunction. *vtRNA2-1* represses the translation of TJs occludin and claudin 1 by disrupting interaction of the respective mRNAs with HuR. *vtRNA2-1* associates with HuR and abolishes HuR binding to the mRNAs encoding occludin and claudin 1, thus decreasing their translation. These results indicate that induced *vtRNA2-1* damages function of the intestinal epithelial barrier by inhibiting HuR-mediated translation of occludin and claudin 1.

4.4 HuR Interaction with miRNAs

MiRNAs are small ncRNA with ~22 nucleotides and induce gene silencing by guiding argonaute proteins to bind to 3′-UTR's of target mRNAs in the miRNA-induced silencing complex. Each miRNA is often able to regulate more than one mRNA, while each of mRNAs is commonly targeted by several miRNAs. Emerged evidence indicates that miRNAs regulate distinct cellular functions and play a critical role in the pathogenesis of various disorders including cancers, delayed healing of wounds, inflammation, and heart malfunctions. MiRNAs functionally interact with RBPs such as HuR to jointly modulate shared target mRNAs antagonistically or synergistically. As such, HuR associations with miRNAs are essential for maintaining intestinal epithelium homeostasis.

MiR-195 is a key regulator of intestinal epithelial homeostasis in health and pathologies by targeting different mRNAs encoding proteins involved in proliferation and migration [28,83]. Increased miR-195 also inhibits IGF signaling by decreasing translation of the IGF2-receptor [84] but the levels of cellular miR-195 are reduced by lncRNA *uc.173* [85]. Ectopically overexpressed miR-195 represses early rapid mucosal restitution after acute injury by destabilizing the mRNA encoding Stim1, a protein necessary for stored-operated Ca^{2+} influx [28]. Both HuR and miR-195 target the 3′-UTR of the *Stim1* mRNA and alter decay of *Stim1* mRNA antagonistically, by which they control the levels of cellular Stim1 in response to stress. Interaction between miR-195 and HuR also regulates function of Tuft cells by altering expression of double cortin-like kinase 1 (DCLK1) [86]. Deregulation of Tuft cells by miR-195 and inactivated HuR contributes to disruption of epithelial defense and is involved in different gut mucosal diseases [87,88]. Transgenic overexpression of miR-195 in the intestinal epithelium (miR195-Tg) in mice decreases numbers of Tuft cells and induces vulnerability of the gut barrier when exposed to lipopolysaccharide. Mechanistically, miR-195 directly binds to the *Dclk1* mRNA via its 3′-UTR and represses DCLK1 translation. HuR competes with miR-195 for binding to the *Dclk1* mRNA, thus increasing DCLK1 expression.

MiR-29a/b is highly expressed in the intestinal epithelium and its tissue levels change markedly in mucosal atrophy and gut barrier dysfunction. Ectopic overexpression of miR-29b causes G1 phase growth arrest of IECs, whereas decreasing the levels of tissue miR-29b by treatment with LNA-mediated silencer stimulates renewal of the small intestinal mucosa in mice [89]. MiR-29a/b interacts with the mRNAs encoding cyclin-dependent kinase 2, HMGB1, PTEN, claudin-1, and ZO-1 via their 3′-UTRs and decreases the stability and translation of these mRNAs, but the interactions of miR-29a/b with these transcripts are negatively regulated by HuR [90–94]. HuR interaction with miR-29b also regulates LRP6 expression in IECs by preventing miR-29b binding to the *Lrp6* mRNA [95,96].

MiR-519 is downregulated in gastric and colorectal cancer, while its overexpression inhibits migration, proliferation, and invasion of these cancer cells by suppressing activity of the Wnt/β-catenin signals [97,98]. Interestingly, miR-519 directly binds to the CR of *HuR* mRNA, resulting in the repression of HuR translation. This reduction of HuR abundance by miR-519 is associated with a decrease in expression levels of several HuR target mRNAs and subsequent inhibition of cell proliferation [99]. Furthermore, the levels of miR-22 also decrease in colorectal cancer, while its induction inhibits HuR translation by interacting with 3′-UTR of the *HuR* mRNA, contributing to the suppression of tumor growth [100]. On the other hand, transcription factor Jun enhances HuR expression by inhibiting transcription of the *miR-22* gene via specific AP1-binding sites in its promoter region. MiR-16 is another regulator of HuR in the intestinal epithelium, since increased miR-16 inhibits expression of HuR at translation level, resulting in an inhibition of COX-2 expression in colon cancers [66].

Together, these observations indicate that HuR and ncRNAs jointly regulate the stability and translation of target mRNAs in the intestinal epithelium. Interactions between HuR and ncRNAs, including lncRNAs, circRNAs, small vtRNAs, and miRNAs, alter HuR binding affinity for mRNAs and affect the turnover and translation of HuR target transcripts synergistically or antagonistically, which is essential for maintaining homeostasis of the intestinal epithelium in stressful environments (Fig. 2). In contrast, disruption of HuR association with ncRNAs is widely involved in many aspects of pathogenesis of various gut mucosal disorders. Although the precise mechanisms underlying the regulation of HuR function remain to be fully investigated, its subcellular localization is intimately linked to its effects upon target transcripts. Emerged evidence indicates that most HuR-regulated phenomena in the intestinal mucosa take place in the cytoplasm, while little is known about the roles of nuclear HuR in intestinal epithelium homeostasis and diseases. These findings strongly suggest that HuR and its binding to ncRNAs are novel therapeutic targets for intervention to protect integrity of the intestinal epithelium and gut barrier function in the clinical setting.

5. Conclusions

HuR plays an essential role in maintaining the intestinal epithelium homeostasis by regulating mucosal renewal, protection, repair after acute injury, barrier function, Paneth cells, Tuft cells, and autophagy. HuR enhances stability and translation of target mRNAs by directly interacting with their 3′-UTRs or CRs and it functions as a biological regulator of gut mucosal regeneration and adaptation. Activity of HuR in the intestinal epithelium is tightly regulated by interacting with ncRNAs, including lncRNAs, circRNAs, small vtRNAs, and miRNAs, which alter expression levels of target genes. Competitive binding of HuR and ncRNAs to particular target mRNAs is important for their function in the control of mRNA stability and translation and thus affects epithelium homeostasis. The integrity of the intestinal epithelium depends on a dynamic balance between the actions of HuR and its interactions with ncRNAs in various pathophysiological conditions. On the other hand, altered expression levels of HuR and loss of its binding affinity for given target mRNAs occur commonly in various pathologies and contribute to various gut mucosal disorders such as IBD, delayed healing, and cancers.

Clearly, there is still a big gap in our knowledge regarding HuR function and its regulation by ncRNAs in health and damaged intestinal epithelium. The exact mechanisms underlying control of HuR expression, its subcellular trafficking, and Chk2-dependent HuR phosphorylation remain to be fully investigated at the molecular level. The

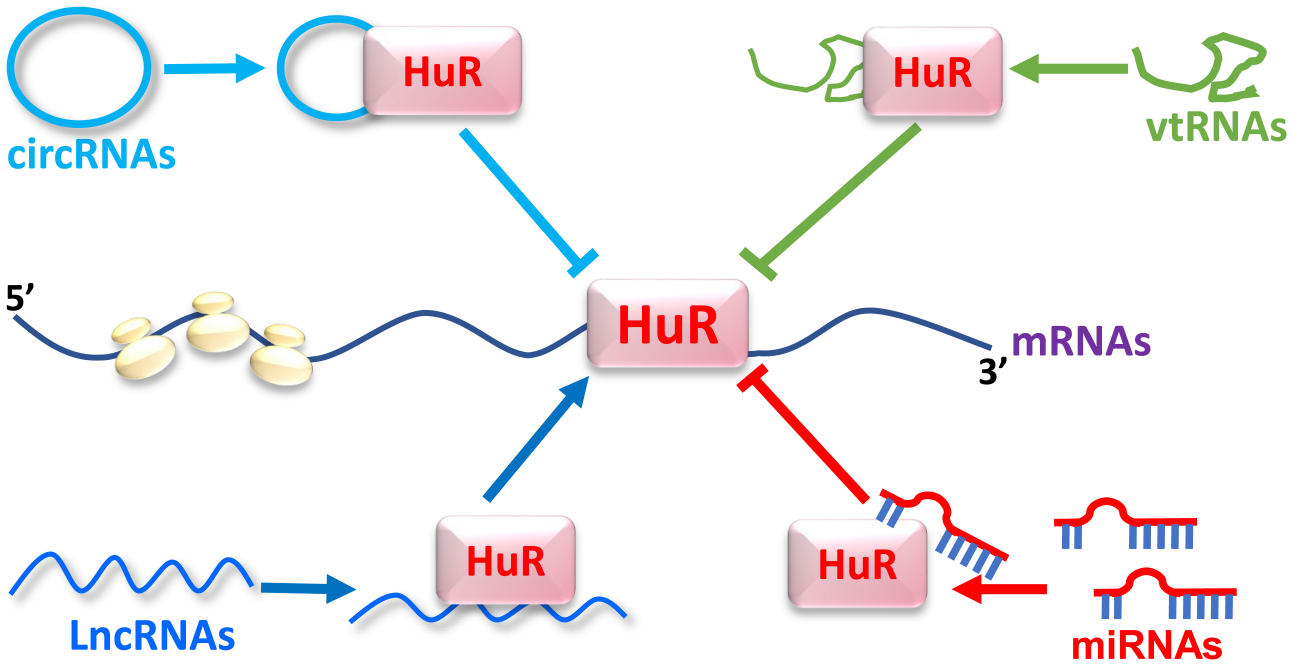

Fig. 2. Regulation of HuR function by ncRNAs in the intestinal epithelium. NcRNAs, including lncRNAs, circRNAs, small Vault RNA (vtRNAs), and miRNAs, are able to directly interact with HuR and alter HuR binding affinity for mRNAs, thus jointly regulate stability and translation of target mRNAs synergistically and antagonistically. Some ncRNAs also regulate HuR function via control of HuR expression levels. On the other hand, interactions between HuR and ncRNAs affect ncRNA binding to given mRNAs and alter the regulatory effects of ncRNAs on target transcripts or genes.

applications of various state-of-the-art new techniques such as tissue-specific genetic loss- or gain-function approach to examine HuR functions *in vivo* and single cell RNA-sequence analysis to define novel targets of HuR in individual cell type are badly needed and will point out new directions. Consistent with this effort, targeted deletion of HuR in IECs is recently shown to specifically alter gene expression profiles in Paneth cells and impairs the integrity of the Paneth cell/intestinal stem cell niche [101]. More studies are also needed to define the mechanisms by which HuR increases the stability and translation of target mRNAs and determine if mutations in specific domains of HuR alter its ability to interact with ncRNAs and affect HuR binding affinity for 3′-UTRs or CRs of target transcripts.

In addition, the influence of microbiota dysbiosis induced by target HuR deletion on regeneration and adaptation of the intestinal mucosa remains largely unknown and will provide useful information about the *in vivo* functions of HuR via interaction with microbiota. It is possible that the presence of certain microbial species can enhance or suppress HuR expression, thus indirectly shaping cellular responses and contributing to gut mucosal-related pathologies. Recently, it has been reported that *C. difficile* infection induces gut mucosal injury and increases gut permeability, whereas treatment with *Lacticaseibacillus casei* strain T21 reduces *C. difficile* toxin effect by altering HuR expression [102]. Finally, experiments using various human mucosal tissue samples from patients with critical illnesses such as IBD, sepsis, leaky gut, and cancers will increase the impact of HuR on pathogenesis of intestinal mucosal disorders and potential clinical application.

Abbreviations

RBPs, RNA-binding proteins; IECs, intestinal epithelial cells; ncRNAs, noncoding RNAs; lncRNAs, long ncRNAs; IBD, inflammatory bowel diseases; RRM, RNA recognition motif; miRNA, microRNA; CRs, coding regions; UTRs, untranslated regions; circRNAs, circular RNAs.

Author Contributions

SS: Conceptualization, data curation, and original draft of manuscript. LX: Conceptualization, data curation, and preparation of Figures. JYW: Conceptualization, data curation; formal analysis and supervision, original draft, review, and editing of manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This work was supported by Merit-Review Award from US Department of Veterans Affairs (to JYW) and by NIH Grants DK-57819, DK-61972, and DK-68491 (to JYW).

Conflict of Interest

The authors declare no conflict of interest. Jian-Ying Wang is a Senior Research Career Scientist, Biomedical Laboratory Research & Development Service, US Department of Veterans Affairs.

References

[1] Turner JR. Intestinal mucosal barrier function in health and disease. Nature Reviews. Immunology. 2009; 9: 799–809.

[2] Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cellular and Molecular Life Sciences. 2013; 70: 631–659.

[3] Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Experimental & Molecular Medicine. 2018; 50: 1–9.

[4] Liu L, Christodoulou-Vafeiadou E, Rao JN, Zou T, Xiao L, Chung HK, *et al*. RNA-binding protein HuR promotes growth of small intestinal mucosa by activating the Wnt signaling pathway. Molecular Biology of the Cell. 2014; 25: 3308–3318.

[5] Xiao L, Li XX, Chung HK, Kalakonda S, Cai JZ, Cao S, *et al*. RNA-Binding Protein HuR Regulates Paneth Cell Function by Altering Membrane Localization of TLR2 via Post-transcriptional Control of CNPY3. Gastroenterology. 2019; 157: 731–743.

[6] Horowitz A, Chanez-Paredes SD, Haest X, Turner JR. Paracellular permeability and tight junction regulation in gut health and disease. Nature Reviews. Gastroenterology & Hepatology. 2023; 20: 417–432.

[7] Martínez-Sánchez LDC, Ngo PA, Pradhan R, Becker LS, Boehringer D, Soteriou D, *et al*. Epithelial RAC1-dependent cytoskeleton dynamics controls cell mechanics, cell shedding and barrier integrity in intestinal inflammation. Gut. 2023; 72: 275–294.

[8] Barral M, Dohan A, Allez M, Boudiaf M, Camus M, Laurent V, *et al*. Gastrointestinal cancers in inflammatory bowel disease: An update with emphasis on imaging findings. Critical Reviews in Oncology & Hematology. 2016; 97: 30–46.

[9] Rao JN, Xiao L, Wang JY. Polyamines in Gut Epithelial Renewal and Barrier Function. Physiology. 2020; 35: 328–337.

[10] Xiao L, Rao JN, Wang JY. RNA-binding proteins and long noncoding RNAs in intestinal epithelial autophagy and barrier function. Tissue Barriers. 2021; 9: 1895648.

[11] Myer VE, Fan XC, Steitz JA. Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. The EMBO Journal. 1997; 16: 2130–2139.

[12] López de Silanes I, Zhan M, Lal A, Yang X, Gorospe M. Identification of a target RNA motif for RNA-binding protein HuR. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 2987–2992.

[13] Scheiba RM, de Opakua AI, Díaz-Quintana A, Cruz-Gallardo I, Martínez-Cruz LA, Martínez-Chantar ML, *et al*. The C-terminal RNA binding motif of HuR is a multi-functional domain leading to HuR oligomerization and binding to U-rich RNA targets. RNA Biology. 2014; 11: 1250–1261.

[14] Fan XC, Steitz JA. HNS, a nuclear-cytoplasmic shuttling sequence in HuR. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95: 15293–15298.

[15] Doller A, Pfeilschifter J, Eberhardt W. Signalling pathways regulating nucleo-cytoplasmic shuttling of the mRNA-binding protein HuR. Cellular Signalling. 2008; 20: 2165–2173.

[16] Grammatikakis I, Abdelmohsen K, Gorospe M. Posttranslational control of HuR function. Wiley Interdisciplinary Reviews. RNA. 2017; 8: e1372.

[17] Liu L, Zhuang R, Xiao L, Chung HK, Luo J, Turner DJ, *et al*. HuR Enhances Early Restitution of the Intestinal Epithelium by Increasing Cdc42 Translation. Molecular and Cellular Biology. 2017; 37: e00574–16.

[18] Chung HK, Wang SR, Xiao L, Rathor N, Turner DJ, Yang P, *et al*. α4 Coordinates Small Intestinal Epithelium Homeostasis by Regulating Stability of HuR. Molecular and Cellular Biology. 2018; 38: e00631–17.

[19] Chen Y, Yang F, Fang E, Xiao W, Mei H, Li H, *et al*. Circular RNA circAGO2 drives cancer progression through facilitating HuR-repressed functions of AGO2-miRNA complexes. Cell Death and Differentiation. 2019; 26: 1346–1364.

[20] Liu L, Xiao L, Chung HK, Kwon MS, Li XX, Wu N, *et al*. RNA-Binding Protein HuR Regulates Rac1 Nucleocytoplasmic Shuttling Through Nucleophosmin in the Intestinal Epithelium. Cellular and Molecular Gastroenterology and Hepatology. 2019; 8: 475–486.

[21] Tili E, Michaille JJ, Piurowski V, Rigot B, Croce CM. MicroRNAs in intestinal barrier function, inflammatory bowel disease and related cancers-their effects and therapeutic potentials. Current Opinion in Pharmacology. 2017; 37: 142–150.

[22] Liu H, Li T, Zhong S, Yu M, Huang W. Intestinal epithelial cells related lncRNA and mRNA expression profiles in dextran sulphate sodium-induced colitis. Journal of Cellular and Molecular Medicine. 2021; 25: 1060–1073.

[23] Zou T, Jaladanki SK, Liu L, Xiao L, Chung HK, Wang JY, *et al*. H19 Long Noncoding RNA Regulates Intestinal Epithelial Barrier Function via MicroRNA 675 by Interacting with RNA-Binding Protein HuR. Molecular and Cellular Biology. 2016; 36: 1332–1341.

[24] Christodoulou-Vafeiadou E, Ioakeimidis F, Andreadou M, Giagkas G, Stamatakis G, Reczko M, *et al*. Divergent Innate and Epithelial Functions of the RNA-Binding Protein HuR in Intestinal Inflammation. Frontiers in Immunology. 2018; 9: 2732.

[25] Wang PY, Rao JN, Zou T, Liu L, Xiao L, Yu TX, *et al*. Post-transcriptional regulation of MEK-1 by polyamines through the RNA-binding protein HuR modulating intestinal epithelial apoptosis. The Biochemical Journal. 2010; 426: 293–306.

[26] Zou T, Mazan-Mamczarz K, Rao JN, Liu L, Marasa BS, Zhang AH, *et al*. Polyamine depletion increases cytoplasmic levels of RNA-binding protein HuR leading to stabilization of nucleophosmin and p53 mRNAs. The Journal of Biological Chemistry. 2006; 281: 19387–19394.

[27] Zou T, Rao JN, Liu L, Xiao L, Yu TX, Jiang P, *et al*. Polyamines regulate the stability of JunD mRNA by modulating the competitive binding of its 3' untranslated region to HuR and AUF1. Molecular and Cellular Biology. 2010; 30: 5021–5032.

[28] Zhuang R, Rao JN, Zou T, Liu L, Xiao L, Cao S, *et al*. miR-195 competes with HuR to modulate stim1 mRNA stability and regulate cell migration. Nucleic Acids Research. 2013; 41: 7905–7919.

[29] Liu L, Ouyang M, Rao JN, Zou T, Xiao L, Chung HK, *et al*.

Competition between RNA-binding proteins CELF1 and HuR modulates MYC translation and intestinal epithelium renewal. Molecular Biology of the Cell. 2015; 26: 1797–1810.
[30] Liu L, Rao JN, Zou T, Xiao L, Wang PY, Turner DJ, *et al*. Polyamines regulate c-Myc translation through Chk2-dependent HuR phosphorylation. Molecular Biology of the Cell. 2009; 20: 4885–4898.
[31] Xiao L, Wang JY. RNA-binding proteins and microRNAs in gastrointestinal epithelial homeostasis and diseases. Current Opinion in Pharmacology. 2014; 19: 46–53.
[32] Zhang X, Zou T, Rao JN, Liu L, Xiao L, Wang PY, *et al*. Stabilization of XIAP mRNA through the RNA binding protein HuR regulated by cellular polyamines. Nucleic Acids Research. 2009; 37: 7623–7637.
[33] Xiao L, Rao JN, Zou T, Liu L, Marasa BS, Chen J, *et al*. Polyamines regulate the stability of activating transcription factor-2 mRNA through RNA-binding protein HuR in intestinal epithelial cells. Molecular Biology of the Cell. 2007; 18: 4579–4590.
[34] Chen J, Xiao L, Rao JN, Zou T, Liu L, Bellavance E, *et al*. JunD represses transcription and translation of the tight junction protein zona occludens-1 modulating intestinal epithelial barrier function. Molecular Biology of the Cell. 2008; 19: 3701–3712.
[35] Zou T, Liu L, Rao JN, Marasa BS, Chen J, Xiao L, *et al*. Polyamines modulate the subcellular localization of RNA-binding protein HuR through AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1. The Biochemical Journal. 2008; 409: 389–398.
[36] Mammen JMV, Matthews JB. Mucosal repair in the gastrointestinal tract. Critical Care Medicine. 2003; 31: S532–S537.
[37] Wang SR, Rathor N, Kwon MS, Xiao L, Chung HK, Turner DJ, *et al*. miR-195 regulates intestinal epithelial restitution after wounding by altering actin-related protein-2 translation. American Journal of Physiology. Cell Physiology. 2022; 322: C712–C722.
[38] Cao S, Xiao L, Wang J, Chen G, Liu Y. The RNA-binding protein HuR regulates intestinal epithelial restitution by modulating Caveolin-1 gene expression. The Biochemical Journal. 2021; 478: 247–260.
[39] Hansraj NZ, Xiao L, Wu J, Chen G, Turner DJ, Wang JY, *et al*. Posttranscriptional regulation of 14-3-3ζ by RNA-binding protein HuR modulating intestinal epithelial restitution after wounding. Physiological Reports. 2016; 4: e12858.
[40] Zhang Y, Cai JZ, Xiao L, Chung HK, Ma XX, Chen LL, *et al*. RNA-binding protein HuR regulates translation of vitamin D receptor modulating rapid epithelial restitution after wounding. American Journal of Physiology. Cell Physiology. 2020; 319: C208–C217.
[41] Vassilieva EV, Gerner-Smidt K, Ivanov AI, Nusrat A. Lipid rafts mediate internalization of beta1-integrin in migrating intestinal epithelial cells. American Journal of Physiology. Gastrointestinal and Liver Physiology. 2008; 295: G965–76.
[42] Yu TX, Wang PY, Rao JN, Zou T, Liu L, Xiao L, *et al*. Chk2-dependent HuR phosphorylation regulates occludin mRNA translation and epithelial barrier function. Nucleic Acids Research. 2011; 39: 8472–8487.
[43] Ma XX, Xiao L, Wen SJ, Yu TX, Sharma S, Chung HK, *et al*. Small noncoding vault RNA2-1 disrupts gut epithelial barrier function via interaction with HuR. EMBO Reports. 2023; 24: e54925.
[44] Sharma A, Bhat AA, Krishnan M, Singh AB, Dhawan P. Trichostatin-A modulates claudin-1 mRNA stability through the modulation of Hu antigen R and tristetraprolin in colon cancer cells. Carcinogenesis. 2013; 34: 2610–2621.
[45] Xu Y, Tian Y, Wang Y, Yang J, Li F, Wan X, *et al*. Human antigen R (HuR) and Cold inducible RNA-binding protein (CIRP) influence intestinal mucosal barrier function in ulcerative colitis by competitive regulation on Claudin1. BioFactors. 2021; 47: 427–443.
[46] Ghosh U, Adhya S. Posttranscriptional regulation of cyclin D1 by ARE-binding proteins AUF1 and HuR in cycling myoblasts. Journal of Biosciences. 2018; 43: 685–691.
[47] Feng Y, Li Y, Zhou D, Li B, Chen G, Li N. Glycyrrhetinic acid reverses antibiotic-induced intestinal epithelial injury through RNA-binding protein human antigen R (HuR). Phytomedicine. 2022; 94: 153836.
[48] Bel S, Pendse M, Wang Y, Li Y, Ruhn KA, Hassell B, *et al*. Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine. Science. 2017; 357: 1047–1052.
[49] Lassen KG, Kuballa P, Conway KL, Patel KK, Becker CE, Peloquin JM, *et al*. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111: 7741–7746.
[50] Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, *et al*. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature. 2008; 456: 259–263.
[51] Adolph TE, Tomczak MF, Niederreiter L, Ko HJ, Böck J, Martinez-Naves E, *et al*. Paneth cells as a site of origin for intestinal inflammation. Nature. 2013; 503: 272–276.
[52] Jin M, Zhang Y. Autophagy and Autoimmune Diseases. Advances in Experimental Medicine and Biology. 2020; 1207: 405–408.
[53] Wittkopf N, Günther C, Martini E, Waldner M, Amann KU, Neurath MF, *et al*. Lack of intestinal epithelial atg7 affects paneth cell granule formation but does not compromise immune homeostasis in the gut. Clinical & Developmental Immunology. 2012; 2012: 278059.
[54] Gao P, Liu H, Huang H, Sun Y, Jia B, Hou B, *et al*. The Crohn Disease-associated ATG16L1^{T300A} polymorphism regulates inflammatory responses by modulating TLR- and NLR-mediated signaling. Autophagy. 2022; 18: 2561–2575.
[55] Li H, Li Q, Sun S, Lei P, Cai X, Shen G. Integrated Bioinformatics Analysis Identifies ELAVL1 and APP as Candidate Crucial Genes for Crohn's Disease. Journal of Immunology Research. 2020; 2020: 3067273.
[56] Lang M, Berry D, Passecker K, Mesteri I, Bhuju S, Ebner F, *et al*. HuR Small-Molecule Inhibitor Elicits Differential Effects in Adenomatosis Polyposis and Colorectal Carcinogenesis. Cancer Research. 2017; 77: 2424–2438.
[57] Thachil E, Hugot JP, Arbeille B, Paris R, Grodet A, Peuchmaur M, *et al*. Abnormal activation of autophagy-induced crinophagy in Paneth cells from patients with Crohn's disease. Gastroenterology. 2012; 142: 1097–1099.e4.
[58] Park SH, Choi HJ, Do KH, Yang H, Kim J, Moon Y. Chronic Nod2 stimulation potentiates activating transcription factor 3 and paradoxical superinduction of epithelial proinflammatory chemokines by mucoactive ribotoxic stressors via RNA-binding protein human antigen R. Toxicological Sciences. 2012; 125: 116–125.
[59] Guo H, Zhang L, Wang Y, He X. Mechanisms of HuR in regulation of epithelial cell apoptosis in rat ulcerative colitis. Cellular Signalling. 2021; 82: 109957.
[60] Gisbert-Ferrándiz L, Salvador P, Ortiz-Masiá D, Macías-Ceja DC, Orden S, Esplugues JV, *et al*. A Single Nucleotide Polymorphism in the Vitamin D Receptor Gene Is Associated With Decreased Levels of the Protein and a Penetrating Pattern in Crohn's Disease. Inflammatory Bowel Diseases. 2018; 24: 1462–1470.
[61] Deng G, Mou T, He J, Chen D, Lv D, Liu H, *et al*. Circular RNA

circRHOBTB3 acts as a sponge for miR-654-3p inhibiting gastric cancer growth. Journal of Experimental & Clinical Cancer Research. 2020; 39: 1.
[62] Chen J, Wu Y, Luo X, Jin D, Zhou W, Ju Z, *et al*. Circular RNA circRHOBTB3 represses metastasis by regulating the HuR-mediated mRNA stability of PTBP1 in colorectal cancer. Theranostics. 2021; 11: 7507–7526.
[63] To KKW, Leung WW, Ng SSM. Exploiting a novel miR-519c-HuR-ABCG2 regulatory pathway to overcome chemoresistance in colorectal cancer. Experimental Cell Research. 2015; 338: 222–231.
[64] Donahue JM, Chang ET, Xiao L, Wang PY, Rao JN, Turner DJ, *et al*. The RNA-binding protein HuR stabilizes survivin mRNA in human oesophageal epithelial cells. The Biochemical Journal. 2011; 437: 89–96.
[65] Lin GL, Ting HJ, Tseng TC, Juang V, Lo YL. Modulation of the mRNA-binding protein HuR as a novel reversal mechanism of epirubicin-triggered multidrug resistance in colorectal cancer cells. PLoS ONE. 2017; 12: e0185625.
[66] Xiao H, Ye X, Vishwakarma V, Preet R, Dixon DA. CRC-derived exosomes containing the RNA binding protein HuR promote lung cell proliferation by stabilizing c-Myc mRNA. Cancer Biology & Therapy. 2022; 23: 139–149.
[67] Young LE, Moore AE, Sokol L, Meisner-Kober N, Dixon DA. The mRNA stability factor HuR inhibits microRNA-16 targeting of COX-2. Molecular Cancer Research. 2012; 10: 167–180.
[68] Lucchesi C, Sheikh MS, Huang Y. Negative regulation of RNA-binding protein HuR by tumor-suppressor ECRG2. Oncogene. 2016; 35: 2565–2573.
[69] Hu Y, Li Q, Yi K, Yang C, Lei Q, Wang G, *et al*. HuR Affects the Radiosensitivity of Esophageal Cancer by Regulating the EMT-Related Protein Snail. Frontiers in Oncology. 2022; 12: 883444.
[70] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, *et al*. The transcriptional landscape of the mammalian genome. Science. 2005; 309: 1559–1563.
[71] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009; 136: 629–641.
[72] Shi J, Zhou T, Chen Q. Exploring the expanding universe of small RNAs. Nature Cell Biology. 2022; 24: 415–423.
[73] Xiao L, Rao JN, Cao S, Liu L, Chung HK, Zhang Y, *et al*. Long noncoding RNA SPRY4-IT1 regulates intestinal epithelial barrier function by modulating the expression levels of tight junction proteins. Molecular Biology of the Cell. 2016; 27: 617–626.
[74] Tian Y, Wang Y, Li F, Yang J, Xu Y, Ouyang M. LncRNA TUG1 regulates the balance of HuR and miR-29b-3p and inhibits intestinal epithelial cell apoptosis in a mouse model of ulcerative colitis. Human Cell. 2021; 34: 37–48.
[75] Ye D, Liu H, Zhao G, Chen A, Jiang Y, Hu Y, *et al*. LncGMDS-AS1 promotes the tumorigenesis of colorectal cancer through HuR-STAT3/Wnt axis. Cell Death & Disease. 2023; 14: 165.
[76] Wilusz JE. A 360° view of circular RNAs: From biogenesis to functions. Wiley Interdisciplinary Reviews. RNA. 2018; 9: e1478.
[77] Barrett SP, Salzman J. Circular RNAs: analysis, expression and potential functions. Development. 2016; 143: 1838–1847.
[78] Li XX, Xiao L, Chung HK, Ma XX, Liu X, Song JL, *et al*. Interaction between HuR and *circPABPN1* Modulates Autophagy in the Intestinal Epithelium by Altering ATG16L1 Translation. Molecular and Cellular Biology. 2020; 40: e00492–19.
[79] Abdelmohsen K, Srikantan S, Kuwano Y, Gorospe M. miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 20297–20302.
[80] Tan P, Sun H, Xu M, Liu X, Qin J, Nie J, *et al*. Circular RNA circ0104103 inhibits colorectal cancer progression through interactions with HuR and miR-373-5p. Cancer Science. 2023; 114: 1396–1409.
[81] Hahne JC, Lampis A, Valeri N. Vault RNAs: hidden gems in RNA and protein regulation. Cellular and Molecular Life Sciences. 2021; 78: 1487–1499.
[82] Horos R, Büscher M, Kleinendorst R, Alleaume AM, Tarafder AK, Schwarzl T, *et al*. The Small Non-coding Vault RNA1-1 Acts as a Riboregulator of Autophagy. Cell. 2019; 176: 1054–1067.e12.
[83] Forouzan Jahromi Z, Javeri A, Fakhr Taha M. Tumor suppressive effects of the pleiotropically acting miR-195 in colorectal cancer cells. EXCLI Journal. 2019; 18: 243–252.
[84] Zhang Y, Zhang Y, Xiao L, Yu TX, Li JZ, Rao JN, *et al*. Cooperative Repression of Insulin-Like Growth Factor Type 2 Receptor Translation by MicroRNA 195 and RNA-Binding Protein CUGBP1. Molecular and Cellular Biology. 2017; 37: e00225-17.
[85] Xiao L, Wu J, Wang JY, Chung HK, Kalakonda S, Rao JN, *et al*. Long Noncoding RNA uc.173 Promotes Renewal of the Intestinal Mucosa by Inducing Degradation of MicroRNA 195. Gastroenterology. 2018; 154: 599–611.
[86] Kwon MS, Chung HK, Xiao L, Yu TX, Wang SR, Piao JJ, *et al*. MicroRNA-195 regulates Tuft cell function in the intestinal epithelium by altering translation of DCLK1. American Journal of Physiology. Cell Physiology. 2021; 320: C1042–C1054.
[87] Banerjee A, Herring CA, Chen B, Kim H, Simmons AJ, Southard-Smith AN, *et al*. Succinate Produced by Intestinal Microbes Promotes Specification of Tuft Cells to Suppress Ileal Inflammation. Gastroenterology. 2020; 159: 2101–2115.e5.
[88] van Es JH, Wiebrands K, López-Iglesias C, van de Wetering M, Zeinstra L, van den Born M, *et al*. Enteroendocrine and tuft cells support Lgr5 stem cells on Paneth cell depletion. Proceedings of the National Academy of Sciences of the United States of America. 2019; 116: 26599–26605.
[89] Xiao L, Rao JN, Zou T, Liu L, Cao S, Martindale JL, *et al*. miR-29b represses intestinal mucosal growth by inhibiting translation of cyclin-dependent kinase 2. Molecular Biology of the Cell. 2013; 24: 3038–3046.
[90] Chen X, Li L, Khan MN, Shi L, Wang Z, Zheng F, *et al*. HMGB1 exacerbates experimental mouse colitis by enhancing innate lymphoid cells 3 inflammatory responses via promoted IL-23 production. Innate Immunity. 2016; 22: 696–705.
[91] Palone F, Vitali R, Cucchiara S, Pierdomenico M, Negroni A, Aloi M, *et al*. Role of HMGB1 as a suitable biomarker of subclinical intestinal inflammation and mucosal healing in patients with inflammatory bowel disease. Inflammatory Bowel Diseases. 2014; 20: 1448–1457.
[92] Wang J, Xu J, Fu J, Yuan D, Guo F, Zhou C, *et al*. MiR-29a Regulates Radiosensitivity in Human Intestinal Cells by Targeting PTEN Gene. Radiation Research. 2016; 186: 292–301.
[93] Zhou Q, Costinean S, Croce CM, Brasier AR, Merwat S, Larson SA, *et al*. MicroRNA 29 targets nuclear factor-κB-repressing factor and Claudin 1 to increase intestinal permeability. Gastroenterology. 2015; 148: 158–169.e8.
[94] Zhu H, Xiao X, Shi Y, Wu Y, Huang Y, Li D, *et al*. Inhibition of miRNA-29a regulates intestinal barrier function in diarrhea-predominant irritable bowel syndrome by upregulating ZO-1 and CLDN1. Experimental and Therapeutic Medicine. 2020; 20: 155.
[95] Li Y, Chen G, Wang JY, Zou T, Liu L, Xiao L, *et al*. Post-transcriptional regulation of Wnt co-receptor LRP6 and RNA-binding protein HuR by miR-29b in intestinal epithelial cells. The Biochemical Journal. 2016; 473: 1641–1649.
[96] Tang YJ, Wu W, Chen QQ, Liu SH, Zheng ZY, Cui ZL, *et al*. miR-29b-3p suppresses the malignant biological behaviors of AML cells via inhibiting NF-κB and JAK/STAT signaling pathways by targeting HuR. BMC Cancer. 2022; 22: 909.

[97] Ye X, Lv H. MicroRNA-519d-3p inhibits cell proliferation and migration by targeting TROAP in colorectal cancer. Biomedicine & Pharmacotherapy. 2018; 105: 879–886.
[98] Yue H, Tang B, Zhao Y, Niu Y, Yin P, Yang W, *et al*. MIR-519d suppresses the gastric cancer epithelial-mesenchymal transition via Twist1 and inhibits Wnt/β-catenin signaling pathway. American Journal of Translational Research. 2017; 9: 3654–3664.
[99] Abdelmohsen K, Panda AC, Munk R, Grammatikakis I, Dudekula DB, De S, *et al*. Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1. RNA Biology. 2017; 14: 361–369.
[100] Liu Y, Chen X, Cheng R, Yang F, Yu M, Wang C, *et al*. The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. Molecular Cancer. 2018; 17: 11.
[101] Xiao L, Warner B, Mallard CG, Chung HK, Shetty A, Brantner CA, *et al*. Control of Paneth cell function by HuR regulates gut mucosal growth by altering stem cell activity. Life Science Alliance. 2023; 6: e202302152.
[102] Panpetch W, Phuengmaung P, Cheibchalard T, Somboonna N, Leelahavanichkul A, Tumwasorn S. *Lacticaseibacillus casei* Strain T21 Attenuates *Clostridioides difficile* Infection in a Murine Model Through Reduction of Inflammation and Gut Dysbiosis With Decreased Toxin Lethality and Enhanced Mucin Production. Frontiers in Microbiology. 2021; 12: 745299.