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Review

Posttranscriptional control of intestinal epithelium homeostasis by RNA-binding protein HuR

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Abstract: The mammalian intestinal epithelium is a rapidly self-renewing tissue in the body and directly interfaces with a wide array of luminal noxious contents and microorganisms. Homeostasis of the intestinal epithelium is preserved through well-controlled mechanisms including posttranscriptional regulation. RNA-binding protein (RBP) HuR regulates the stability and translation of target mRNAs and is intimately involved in many aspects of gut mucosal pathophysiology. Here we highlight the biological roles of HuR in maintaining the integrity of the intestinal epithelium, with particular focus on the emerging evidence of HuR in the regulation of intestinal epithelial renewal, mucosal repair, defense, and gut permeability. We also further analyze the mechanisms through which HuR and its interactions with other RBPs and noncoding RNAs (ncRNAs) such as microRNAs and long ncRNAs modulate the intestinal epithelial homeostasis. With rapidly advancing knowledge of RBPs and ncRNAs, there is growing recognition that posttranscriptional control of the intestinal epithelium homeostasis might be promising therapeutic targets in our efforts to protect the integrity of the intestinal epithelium under critical pathological conditions.

Key words: RNA-binding proteins; noncoding RNAs; intestinal epithelial renewal; mucosal repair; gut permeability; posttranscriptional regulation

RNA结合蛋白HuR对肠道上皮稳态的转录后调控

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摘 要：哺乳动物的肠上皮组织具备快速和强大的自我更新能力，以适应与肠内容物中众多有害物质和微生物的直接接触。基因的转录后调控是肠上皮组织维持稳态的重要机制之一。RNA结合蛋白HuR在细胞中的主要功能是调节靶向基因转录本的稳定性和翻译，密切参与肠道黏膜病理生理调节。本综述将重点介绍HuR在维持肠上皮完整性方面的生物学作用，尤其是HuR在调节肠上皮细胞更新、肠道黏膜修复以及肠壁通透性方面的最新发现和研究进展；并深入分析HuR与其它RNA结合蛋白以及非编码RNA (包含微小RNA和长链非编码RNA)之间的相互作用在肠上皮稳态调节中的共同作用。随着RNA结合蛋白和非编码RNA研究领域的不断深入，肠上皮组织稳态的转录后调控将为如何在特定病理条件下保护肠上皮的治疗提供新的线索。

关键词：RNA结合蛋白；非编码RNA；肠上皮更新；黏膜修复；转录后调控
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1 Introduction

The mammalian intestinal epithelium undergoes a continual renewal process, defined as active proliferation of intestinal stem cells localized near the base of the crypts, progression of these cells up the crypt-villus axis with cessation of proliferation and subsequent differentiation and apoptosis [1]. Differentiated intestinal epithelial cells (IECs) in the mucosa primarily include absorptive enterocytes, mucus-secreting goblet cells, innate immune Paneth cells, hormone-secreting enteroendocrine cells, and tuft cells. With distinct functions in food digestion and host defense, these IECs actively communicate with each other and function cooperatively [2]. Homeostasis of the intestinal epithelium is essential for normal mucosal function, which is tightly regulated by numerous factors. In response to stress, rapid changes in gene expression patterns in IECs control cell division, migration, differentiation, apoptosis, and cell-to-cell interaction, thereby preserving the intestinal epithelial homeostasis and integrity. Although the gene expression programs that govern the intestinal epithelium homeostasis are strongly regulated at the transcription level, posttranscriptional events, particularly altered mRNA stability and translation in response to stressful environments, play an essential role in maintaining the intestinal epithelium integrity. Changes in mRNA stability and translation are controlled by two major types of trans-acting factors: RNA-binding proteins (RBPs) and noncoding RNAs (ncRNAs) such as microRNAs (miRNAs) [3–5]. RBPs and miRNAs directly interact with cis-elements on the mRNAs, frequently present at the 3'-untranslated regions (3'-UTRs), and modulate the stability and translation of target transcripts negatively or positively.

Many RBPs have housekeeping functions and interact with various cellular transcripts, but several RBPs associate with specific subsets of mRNAs and control gene expression levels in response to physiological stress or pathologies. HuR (also named as ELAV1) is among the most prominent sequence-specific translation and turnover regulatory RBPs. HuR has two N-terminal RNA-recognition motifs through which it binds with high affinity and specificity to AU-rich elements located in the 3'-UTRs of labile mRNAs, and this interaction commonly enhances the stability and translation of target transcripts [2, 6, 7]. HuR also affects gene regulatory programs by interplaying with different ncRNAs, including miRNAs, long ncRNAs (lncRNAs), and circular RNAs [8–12]. In the intestinal epithelium, HuR distributes predominantly in the nucleus of non-stimulating cells, but it can be rapidly translocated to the cytoplasm where HuR interacts directly with target mRNAs in response to different stresses, thus altering the gene expression levels. Recently, HuR has been emerging as a master biological regulator of the intestinal epithelium homeostasis by actively participating in the control of intestinal epithelial renewal, adaptation, defense, repair, and gut barrier function [7–9, 13, 14]. In this review, we highlight the key functions of HuR in the intestinal epithelium and further discuss the mechanism by which HuR regulates gene expressions at the posttranscriptional level by interacting with other RBPs and ncRNAs.

2 HuR plays an essential role in renewal of the intestinal epithelium

The intestinal epithelium has the most rapid turnover rate in the body, and it renews itself every 4–5 days in mice and 5–7 days in humans. This rapid epithelial renewal is tightly regulated by numerous intracellular and extracellular factors through distinct mechanisms [15, 16]. Like other tissues in the body, the intestinal mucosal growth is uniquely modulated by several factors such as growth hormones, insulin, cortisol, and thyroxin that alter metabolism in all tissues in the body. On the other hand, the intestinal epithelium responds to a host of events triggered by the presence of food and microbiota within the gastrointestinal (GI) tract. Food in the intestinal lumen is one of the strongest stimulants of mucosal growth by inducing the release of several gut hormones that only target mucosal tissues of the GI tract [15, 17]. Fasting in mice or food starvation in patients with critical disorders inhibits renewal of the intestinal epithelium and causes mucosal atrophy [17]. Intestinal epithelial renewal and repair of damaged mucosa in the GI tract also require the supply of polyamines (spermidine, spermine and their precursor putrescine) to the dividing cells in the crypts, whereas polyamine depletion represses epithelial regeneration and delays mucosal repair after injury [15].

To investigate the *in vivo* function of HuR in the intestinal epithelium, we generated mice bearing intestinal epithelium tissue-specific HuR deletion (IE-$HuR^{-/-}$) by crossing $HuR^{flox/flox}$ mice with mice carrying villin-Cre [18]. Although there are no significant differences in body weight, GI gross morphology, reproduc-

tion ability, and general appearances between IE-HuR$^{-/-}$ mice and control littermate mice, targeted deletion of HuR in IECs inhibits mucosal growth in the small intestine, as evidenced by remarkable decreases in the levels of DNA synthesis and the lengths of villi and crypts in IE-HuR$^{-/-}$ mice. The levels of cell proliferation marker proteins such as PCNA and Ki67 are also decreased in the small intestinal mucosa of IE-HuR$^{-/-}$ mice. Moreover, the loss of HuR in IECs inhibits the regenerative potential of crypt progenitors, since S-phase descendants in villous regions decrease significantly in IE-HuR$^{-/-}$ mice compared with those observed in control littermates after exposure to radiation. Consistently, growth rates of primarily cultured intestinal organoids isolated from IE-HuR$^{-/-}$ mice decrease dramatically relative to those observed in the organoids generated from control littermate mice [19]. The structures of intestinal organoids isolated from control littermates exhibit multiple differentiated epithelial cells and buds 10 days after primary culture, but HuR-deficient intestinal organoids from IE-HuR$^{-/-}$ mice are small and display decreased buds. DNA synthesis also decreases in HuR-deficient intestinal organoids compared with the organoids from control littermate mice, as measured by BrdU incorporation assay. In cultured IECs, HuR silencing by transfection with small interfering RNA targeting HuR (siHuR) also inhibits cell proliferation *in vitro*. In a separate study, conditional deletion of HuR in IECs also represses intestinal tumorigenesis in mice [18]. These findings obtained from *in vivo*, *ex vivo* and *in vitro* systems strongly support the essential role of HuR in the intestinal mucosal renewal that is absolutely required for the intestinal epithelium homeostasis.

HuR promotes renewal of the intestinal mucosa primarily by activating the Wnt signaling pathway [10, 18, 20]. The HuR-deficient intestinal epithelium is associated with a significant decrease in the levels of Lrp6 mRNA and LDL-receptor-related protein 6 (LRP6) that functions as a Wnt-coreceptor. HuR deletion fails to alter the levels of Frizzled 7 in the intestinal mucosa, but it increases expression levels of mucosal p65 and Smad7. Mechanistically, HuR directly binds to the Lrp6 mRNA via its 3'-UTR rather than 5'-UTR and coding region (CR), and this interaction increases LRP6 expression by stabilizing the Lrp6 mRNA and enhancing its translation [10]. In another study, we further found that HuR also directly interacts with the mRNA encoding transcriptional factor c-Myc and enhances its translation without effect on *c-Myc* mRNA stability [20]. Unlike HuR association with the *Npm* and *p53* mRNAs [21], HuR binding to the *c-Myc* mRNA is highly dependent on HuR phosphorylation mediated by checkpoint kinase 2 (ChK2).

HuR also regulates IEC apoptosis to maintain stable numbers of enterocytes in the intestinal epithelium by altering expression of apoptosis-related genes such as X chromosome-linked inhibitor of apoptosis protein (XIAP), mitogen-activated protein kinase kinase-1 (MEK-1), JunD, and activating transcription factor-2 (ATF-2) [22–25]. XIAP is a member of the inhibitor of apoptosis protein (IAP) family, which promotes cell survival by protecting cells from caspase-mediated apoptosis. HuR associates with both the 3'-UTR and CR of the *Xiap* mRNA, stabilizes it, and increases its expression, thus desensitizing IECs to apoptotic cell death under stressful environments [26]. HuR also has a strong binding affinity for the mRNAs encoding ATF-2, JunD, and MEK-1. Interactions of HuR with the *Atf-2* and *JunD* mRNA primarily increase their stability with minor effect on ATF-2 and JunD translation [25]. On the other hand, association of HuR with the *mek-1* mRNA increases both stability and translation of *mek-1* transcript [22]. Interestingly, all interactions of HuR with the *Xiap*, *Atf-2, JunD*, and *mek-1* mRNAs are highly regulated by cellular polyamines that function as biological regulators of gut mucosal growth and adaptation. Polyamines also modulate the subcellular localization of HuR through AMP-activated protein kinase (AMPK)-regulated phosphorylation and acetylation of importin α1 [27].

Taken together, the findings obtained from mice with ablated HuR, primary intestinal organoids, and cultured IECs suggest a model by which HuR plays an essential role in the control of intestinal epithelial renewal and adaptation under pathophysiological conditions (Fig. 1). Based on this model, elevating HuR activity enhances intestinal mucosal growth by activating the Wnt signaling pathway through up-regulation of LRP6 and c-Myc expression, whereas inhibition of HuR phosphorylation or targeted deletion of *HuR* gene in IECs inhibits renewal of the intestinal mucosa and represses adaptation as a result of inactivated Wnt signals. HuR also protects IECs against apoptosis by targeting mRNAs encoding anti-apoptotic proteins such as XIAP, ATF-2, JunD, and MEK-1, which also contributes to maintaining the intestinal epithelium homeostasis.

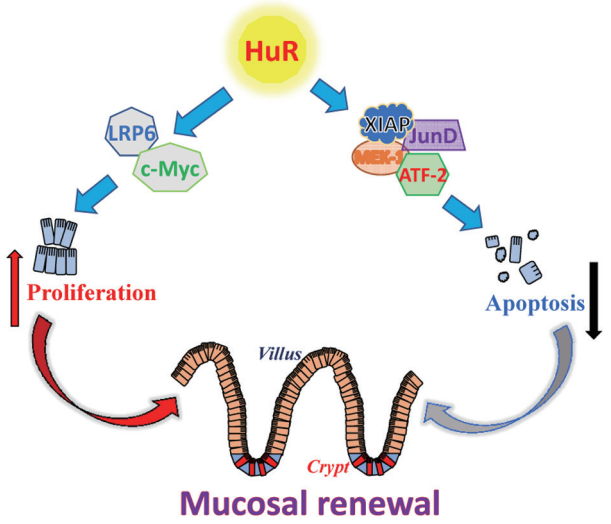

Fig. 1. HuR enhances proliferation and protects intestinal epithelial cells (IECs) against apoptosis in the intestine. HuR directly binds to mRNAs encoding proteins associated with proliferation (Wnt signals) and anti-apoptosis, and induces expression levels of these target genes by increasing their mRNA stability and translation. XIAP: X chromosome-linked inhibitor of apoptosis protein; MEK-1: mitogen-activated protein kinase kinase-1; ATF-2: activating transcription factor-2; LRP6: LDL-receptor-related protein 6.

3 HuR enhances mucosal repair after acute injury

The intestinal mucosa exhibits a spectrum of responses after damage and repairs itself quickly to restore epithelial integrity [28]. The repair process of damaged mucosa consists of: *i*) epithelial restitution, characterized by stages of cell spreading and migration into the wound; *ii*) the replacement of lost cells by cell proliferation; and *iii*) differentiation of epithelial cells into a polarized monolayer. Early rapid epithelial restitution is an initial host response to prevent noxious agents from causing deeper tissue damage and occurs as a consequence of epithelial cell migration, a process independent of cell proliferation [29]. In the acute response to mucosal injury such as after exposure to critical surgical stress or nonselective non-steroidal anti-inflammatory drugs, damaged cells are sloughed, and remaining viable cells adjacent to a wound extend lamellipodia and migrate to cover the wounded area to reestablish epithelial continuity. Stimulation of IEC migration over the wounded area is the result of a series of coordinated cellular and molecular events including dynamic adhesion, spreading, and motility of cells [30]. This complex process is tightly regulated by multiple intracellular and extracellular factors including HuR.

We have reported that HuR promotes early epithelial restitution after acute injury by increasing IEC migration in mice [31]. Both control littermate and IE-HuR$^{-/-}$ mice subjected to mesenteric ischemia/reperfusion (I/R) exhibit signs of significant mucosal injury and erosions in the small intestine. HuR deletion does not enhance the digress of I/R-induced mucosal injury, since the injury scores in the mucosa of IE-HuR$^{-/-}$ mice are similar to those observed in littermates when measured immediately after mesenteric I/R. However, HuR-deficient epithelium displays a significant delay in the process of mucosal repair after acute injury. The mucosa repairs quickly in littermate mice and the epithelial integrity is almost completely restored 6 h after I/R, but this rapid repair is inhibited by HuR deletion. The mucosal surface remains discontinuous, showing sloughed cells and debris in IE-HuR$^{-/-}$ mice at the same time after I/R. In an *in vitro* model that mimics the early cell division-independent stage of epithelial restitution [31], HuR silencing by transfection with siHuR also inhibits early rapid epithelial restitution after wounding. The numbers of cells migrating over the denuded area after wounding decrease significantly in siHuR-transfected cells compared with cells transfected with control siRNA. These results indicate that targeted deletion of HuR in IECs delays mucosal repair, particularly early epithelial restitution after superficial wounding in the small intestine.

Targeted HuR deletion also enhances dextran sulfate sodium (DSS)-induced colitis in mice [31]. After administration of 3% DSS in drinking water for 5 days, both littermates and IE-HuR$^{-/-}$ mice effectively develop acute colitis, as identified by epithelial damage, granulocyte infiltration, and bloody diarrhea. Reducing HuR expression in IECs increases DSS-induced mucosal injury in the colon; histological scores in the colonic mucosa of IE-HuR$^{-/-}$ mice are ~two-fold that of littermate mice when measured immediately after a 5-day DSS treatment. The colonic mucosa repairs quickly after starting of water drinking in control littermate mice, and histological features of the colonic mucosa are restored to near normal from day 5 onward. In contrast, significant granulocyte infiltration and erosions remain in the colonic mucosa of IE-HuR$^{-/-}$ mice at the same time after administration of water, indicating that

HuR deletion not only enhances DSS-induced colitis, but also delays recovery from this challenge [31].

HuR is essential for intestinal mucosal repair after injury by enhancing expression of stromal interaction molecule 1 (Stim1) and cell division control protein 42 (Cdc42) posttranscriptionally. Stim1 functions as a sensor of Ca^{2+} within stores and plays an important role in the activation of store-operated Ca^{2+} entry (SOCE) that stimulates IEC migration over the wounded area after acute injury. HuR directly binds to and stabilizes *stim1* mRNA via its 3'-UTR, thus increasing Stim1 production. Increased Stim1 by HuR after acute injury promotes early epithelial restitution by inducing SOCE [32]. Interestingly, the stability of *stim1* mRNA is negatively regulated by miR-195. Ectopic miR-195 overexpression enhances *stim1* mRNA association with argonaute-containing complexes, and increases the colocalization of tagged *stim1* RNA with processing bodies (P-bodies); but this translocation of *stim1* mRNA to P-bodies is abolished by HuR overexpression. On the other hand, the induced HuR-*stim*1 mRNA association and subsequent increase in the Stim1 levels after mucosal injury are also blocked by increasing miR-195, indicating the role of HuR/miR-195 interaction in regulating Stim1 expression [32].

Cdc42 belongs to the Rho family of small GTPases and plays a pivotal role in actin organization, cell migration, and proliferation. We have demonstrated that the *Cdc42* mRNA is a novel target of HuR and that HuR positively regulates Cdc42 expression at the posttranscriptional level [31]. HuR directly interacts with the *Cdc42* mRNA via its 3'-UTR and this association enhances Cdc42 translation without effect on its total mRNA level. HuR also regulates the intestinal epithelium homeostasis by altering subcellular distribution of Rac1, another member of the Rho family. The abundance of cytoplasmic Rac1 in the small intestinal mucosa increases in IE-$HuR^{-/-}$ mice, although HuR deletion does not alter total Rac1 levels [33]. HuR silencing in cultured IECs also increases the cytoplasmic Rac1 levels, without effect on whole-cell Rac1 content. In addition, HuR deficiency also decreases the levels of a molecular chaperone protein nucleophosmin (NPM) in the intestinal epithelium of IE-$HuR^{-/-}$ mice and in cultured IECs. Further study shows that NPM physically interacts with Rac1 and forms NPM/Rac1 complex, whereas NPM silencing decreases NPM/Rac1 association and inhibits nuclear accumulation of Rac1, along with an increase in cytoplasmic abundances of Rac1. In contrast, ectopically expressed NPM enhances Rac1 nuclear translocation and restores Rac1 subcellular localization to near normal in HuR-deficient cells. These results indicate that HuR regulates Rac1 nucleocytoplasmic shuttling in the intestinal epithelium by altering NPM expression [33].

4 HuR promotes gut barrier function and epithelial defense

The intestinal epithelium acts as a dynamic barrier that directly interfaces with a wide array of luminal noxious substances and microbiota [9, 13]. The intestinal epithelial barrier depends on specialized structures named tight junctions (TJs) and adherens junctions (AJs). TJs are located at the apical region of the epithelial lateral membrane and fence the paracellular space in the intestinal epithelium, which is selectively permeable to certain hydrophilic molecules, ions, and nutrients [16, 34]. Immediately below the TJs are the cadherin-rich AJs, which mediate strong cell-to-cell adhesion and integrate distinct cellular signals to regulate epithelial paracellular permeability [35]. TJ and AJ complexes are highly dynamic, and maintenance of the levels of TJ and AJ proteins is essential for the integrity of epithelial barrier function. Several studies from our group and others [36–39] indicate that HuR is crucial for effectiveness of the epithelial barrier function by regulating expression of TJs and AJs at the posttranscriptional level.

HuR was found to bind to several mRNAs encoding TJ proteins including claudin-1, claudin-3, occludin and JAM-1, and AJ protein E-cadherin, mostly via their 3'-UTRs, and thus enhance the stability and translation of these target transcripts [8, 11, 40–43]. In an *in vitro* permeability model using differentiated IECs, HuR knockdown by transfection with siHuR decreases transepithelial electrical resistance and increases paracellular permeability. In mice exposed to septic stress, inhibition of HuR binding affinity for its target mRNAs by decreasing Chk2-dependent HuR phosphorylation is associated with a significant decrease in the levels of TJs and AJs and gut barrier dysfunction [44]. Although IE-$HuR^{-/-}$ mice do not exhibit gut barrier dysfunction without any pathological stress, gut permeability increases remarkably in IE-$HuR^{-/-}$ mice and are more susceptible to stress induced by exposure to DDS and mesenteric I/R [31]. Moreover, recovery rates of the gut

barrier functions after treatment with DSS or mesenteric I/R are much slower in IE-HuR$^{-/-}$ mice compared with control littermate mice.

In addition, HuR also regulates defense of the intestinal epithelium by enhancing the production and release of various antimicrobial peptides/proteins (AMPs) through control of Paneth cell function [27]. Paneth cells are specialized IECs that reside at the bottom of the crypts and produce most AMPs that are crucial for protection of the epithelium from enteric pathogenic invasion [45, 46]. We have recently identified a novel function of HuR in the regulation of Paneth cells in the intestinal epithelium [19]. Intestinal tissues from IE-HuR$^{-/-}$ mice display reduced numbers of Paneth cells as indicated by a decrease in lysozyme-positive cells, and Paneth cells have fewer lysozyme granules per cell, compared with tissues from control littermate mice. Intestinal organoids isolated from IE-HuR$^{-/-}$ mice are smaller and have fewer lysozyme-positive cells. Consistently, intestinal mucosa from patients with inflammatory bowel diseases also exhibits reduced levels of HuR and fewer Paneth cells. Mechanistically, HuR knockout mice inhibits cell surface trafficking of Toll-like receptor 2 (TLR2) and disrupts TLR2 membrane localization by repressing the production of the endoplasmic reticulum chaperone canopy 3 (CNPY3). HuR interacts directly with the *Cnpy3* mRNA coding region and enhances CNPY3 expression by stabilizing *Cnpy3* mRNA and stimulating its translation. These results indicate that HuR regulates Paneth cell function by altering TLR2 localization via posttranscriptional control of CNPY3 abundance [19].

5 Mechanisms of HuR in intestinal epithelium homeostasis

Given that HuR is involved in many aspects of intestinal mucosal pathophysiology, the mechanisms underlying HuR in controlling mRNA stability and translation have been investigated extensively [8, 9, 11, 43, 47]. An increasing body of evidence also indicates that interactions of HuR with other RBPs and ncRNAs such as lncRNAs play an important role in HuR-modulated intestinal epithelial homeostasis and have a great impact on an important area of various mucosal disorders [8, 24, 43]. Through the complex molecular associations of HuR with other RBPs and lncRNAs, HuR enables IECs in the intestinal mucosa to adapt and respond to different stressful environments and maintains the epithelium homeostasis in different pathological conditions.

5.1 HuR interactions with other RBPs

HuR binds to its target transcripts with high affinity and specificity, but its functions are finely affected by interacting with other RBPs. For an instance, the JunD mRNA is a direct target of both HuR and AUF1 (AU-binding factor 1) [23]. HuR binds to JunD mRNA and stabilizes the transcript as it acts to mRNAs encoding p53, p21, ATF2, NPM, XIAP, and c-Fos [21, 22, 48–51]. However, interaction of AUF1 with JunD mRNA destabilizes JunD transcript and decreases its expression levels in IECs. Therefore, JunD expression levels in IECs are dynamically balanced by competitively binding of JunD mRNA to HuR and AUF1. This competitive binding of JunD mRNA to HuR and AUF1 is implicated in the molecular process by which cellular polyamines control JunD expression and IEC proliferation and apoptosis. Polyamine depletion increases the translocation of HuR to the cytoplasm without any effect on total HuR levels. Increased binding of HuR to JunD mRNA in polyamine-deficient cells inhibits the association of AUF1 with JunD mRNA and increases JunD expression, thus leading to cell growth arrest at G1 phase and resistance to apoptosis. Similarly, competitive interactions between HuR and AUF1 also regulates expression of cyclin D1 and p21 mRNA post-transcriptionally [52].

Like HuR, RBP CUG-binding protein 1 (CUGBP1) is also highly expressed in the intestinal epithelium, but CUGBP1 regulates the stability and translation of target mRNAs negatively [37]. We have reported that HuR and CUGBP1 jointly regulate the translation of TJ occludin and play an important role in the maintenance of TJ barrier integrity and function in the intestine [37]. CUGBP1 and HuR compete for association with the same occludin 3'-UTR element and they modulate occludin translation competitively and in opposite directions. CUGBP1 overexpression reduces the binding of HuR to occludin mRNA, inhibits occludin translation, and impairs the TJ barrier function. In contrast, elevation of cellular HuR represses the binding of CUGBP1 to occludin mRNA, promotes the expression of occludin, thereby improving the epithelial TJ barrier effectiveness. It was further found that repression of occludin translation by CUGBP1 results primarily from the colocalization of CUGBP1 and tagged occludin

mRNA in P-bodies, where mRNAs are believed to be sorted for degradation and/or translational repression. These exciting findings indicate that CUGBP1 represses occludin translation by increasing occludin mRNA recruitment to P-bodies, whereas HuR enhances occludin translation by preventing occludin mRNA translocation to P-bodies via displacement of CUGBP1 [37]. In a separate study, interaction between HuR and CUGBP1 also regulates E-cadherin expression in the intestinal epithelium [53] .

To gain a better understanding of the role of HuR interaction with CUGBP1 in the intestinal epithelium homeostasis, HuR was found to compete with CUGBP1 to modulate the translation of c-Myc that induces mucosal growth and healing after injury [54]. Growth inhibition of the small intestinal mucosa by fasting in mice is associated with increased levels of CUGBP1/c-Myc mRNA complex and a decrease in c-Myc protein [2]. At the molecular level, CUGBP1 binds to the 3'-UTR of c-Myc mRNA and represses c-Myc translation without affecting total c-Myc mRNA levels. HuR also interacts with the same c-Myc 3'-UTR element, and increasing the levels of HuR decreases CUGBP1 binding to c-Myc mRNA. In contrast, increasing the concentrations of CUGBP1 inhibits formation of the HuR/c-Myc mRNA complex. Interestingly, depletion of cellular polyamines also increases CUGBP1 and enhances its association with c-Myc mRNA, thus suppressing c-Myc translation. Moreover, ectopic CUGBP1 overexpression causes G1 phase growth arrest, whereas CUGBP1 silencing promotes cell proliferation. These results indicate that CUGBP1 represses c-Myc translation by decreasing c-Myc mRNA association with HuR and provide new insight into the molecular functions of RBP interaction in the regulation of intestinal mucosal renewal and homeostasis [54].

5.2 Interactions between HuR and lncRNAs

Many regions of the mammalian genome are actively transcribed into lncRNAs that are dynamically expressed in a tissue-specific manner and regulate gene expression at almost every level [55–57]. Several studies conducted in mice and human tissues have revealed the importance of lncRNA in the intestinal epithelium homeostasis and shown that lncRNAs H19 and SPRY4-IT1 perform their regulatory functions through interactions with HuR [7, 13, 58].

5.2.1 HuR/H19 interaction

The lncRNA H19 is transcribed from the evolutionarily conserved imprinted *H19/igf2* gene cluster and regulates the intestinal epithelium homeostasis by altering the gut barrier function [59, 60]. H19 levels are increased in a broad spectrum of pathological conditions such as malignancies and following exposure to various stresses. The levels of tissue H19 increase dramatically in the inflamed human and murine intestinal mucosa, predominantly resulting from an increase in the inflammatory cytokine interleukin-22 [61]. We have demonstrated that increasing the levels of H19 in IECs inhibits expression of TJ ZO-1 and AJ E-cadherin, as well as disrupts the epithelial barrier function [11]. Increased H19 levels decrease production of ZO-1 and E-cadherin by reducing their mRNA stability and translation. Since two highly conserved miRNAs, miR-675-5p and miR-675-3p, are embedded in H19 exon 1, it is possible that the regulatory role of H19 in the epithelial barrier function is mediated by releasing miR-675. Our results further show that ectopic overexpression of H19 increases the levels of both miR-675-5p and miR-675-3p in cultured IECs and that increasing either miR-675-3p or miR-675-5p inhibits expression of ZO-1 and E-cadherin and damages the epithelial barrier function. Interestingly, HuR directly binds to H19, while elevation of cellular HuR levels reduces miR-675 production by preventing the processing of miR-675 from H19, rescues ZO-1 and E-cadherin expression, and abolishes the barrier dysfunction in the epithelium overexpressing H19. In contrast, targeted deletion of *HuR* gene in IE-HuR$^{-/-}$ mice increases miR-675 processing and inhibits the recovery of the epithelial barrier function after exposure to mesenteric I/R. These findings strongly suggest that HuR enhances the barrier function by suppressing the miR-675 release from H19 and represent a conceptual advance by linking HuR/H19 interaction with the control of the barrier function and epithelium homeostasis [11].

5.2.2 HuR/SPRY4-IT1 interaction

SPRY4-IT1 is a 706-bp lncRNA transcribed from the intronic region of the *Spry4* gene and it is the first lncRNA found to regulate gut permeability [8]. SPRY4-IT1 is highly expressed in the intestinal epithelium and distributed in both cytoplasm and nucleus in IECs. Decreased levels of SPRY4-IT1 are commonly observed in the colonic mucosa obtained from patients diagnosed with leaky gut. In cultured IECs, decreasing the levels of SPRY4-IT1 reduces expression of the TJs claudin-1, claudin-3, occludin, and JAM-1, and also results in the

epithelial barrier dysfunction. On the other hand, increasing the levels of SPRY4-IT1 in the intestinal mucosa by recombinant lentiviral SPRY4-IT1 expression vector protects the gut barrier function in mice exposed to septic stress. Mechanistically, SPRY4-IT1 up-regulates the expression of these TJs at the posttranscriptional level by increasing stability and translation of their mRNAs [8].

Since HuR also binds to the 3'-UTRs of mRNAs encoding these TJs and induces their stability and translation, we examined whether SPRY4-IT1 modulates the association of HuR with these TJ mRNAs. Biotinylated SPRY4-IT1 was found to specifically bind to HuR but not to other RBPs such as CUGBP1, TIAR, or AUF1, and these findings are further confirmed by ribonucleoprotein/immunoprecipitation assay using anti-HuR antibody. SPRY4-IT1 fails to directly associate with these TJ proteins. HuR interaction with SPRY4-IT1 does not affect the stability and levels of SPRY4-IT1. However, SPRY4-IT1 silencing represses HuR association with these TJ mRNAs, although it does not alter total HuR levels or its subcellular distribution. In addition, there are several potential SPRY4-IT1-binding sites within the 3'-UTRs of mRNAs encoding claudin-1, claudin-3, occludin, and JAM-1. RNA-RNA pulldown assays show that the levels of these TJ mRNAs in biotinylated SPRY4-IT1 pulldown materials are much higher than those observed in samples pulldown by biotin-labeled Gapdh [8]. HuR silencing only decreases, but not totally prevents, the binding of SPRY4-IT1 to mRNAs encoding these TJs, suggesting that SPRY4-IT1 also directly interacts with these TJ mRNAs, which is independent from its association with HuR. These results indicate that HuR and SPRY4-IT1 simultaneously promote TJ expression and enhance epithelial barrier function synergistically [8].

Taken together, these findings from our group and others suggest a novel model by which HuR regulates the intestinal epithelium homeostasis through association with other RBPs and ncRNAs, particular lncRNAs, under pathophysiological conditions (Fig. 2). According to this model, RBPs AUF1 and CUGBP1, and miRNA miR-195 can compete with HuR to bind to target mRNAs encoding JunD, occludin, Stim1, and c-Myc and modulate the stability and translation of these mRNAs antagonistically. On the other hand, HuR binds to H19 and prevents the processing of miR-675 from H19, thus enhancing the epithelial barrier function. Moreover, HuR interaction with SPRY4-IT1 increases TJ expression and promotes the epithelial barrier function synergistically. The dynamic HuR interactions with other RBPs and lncRNAs are crucial for delicately calculated role of HuR in maintaining the intestinal epithelium homeostasis. These examples illustrate the rich interplay between HuR and other RBPs or ncRNAs whereby HuR alters expression levels of various genes in the intestinal epithelium.

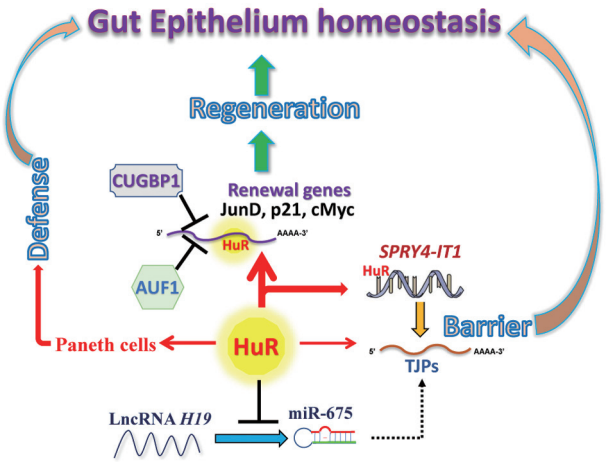

Fig. 2. Interplay between HuR and other RBPs or ncRNAs regulates the intestinal epithelium homeostasis. HuR competes with AUF1, CUGBP1, or miR-195 to jointly modulate stability and translation of target mRNAs. HuR also represses miR-675 processing from H19 and enhances SPRY4-IT1 binding to TJ mRNAs. These interactions of HuR with other posttranscriptional regulators are crucial for maintaining the intestinal epithelium homeostasis.

6 Conclusions and future perspectives

Posttranscriptional regulation of gene expression by HuR in the intestinal mucosa plays an essential role in the epithelium homeostasis by modulating IEC proliferation, migration, apoptosis, cell-to-cell interaction, and innate immunity. HuR increases the stability and translation of target mRNAs by directly interacting with their 3'-UTRs and acts as a biological enhancer of gut epithelial renewal, repair of damaged mucosa, and barrier function. In the intestinal epithelium, functions of HuR are tightly regulated by altering its subcellular localization and phosphorylation and also by modulating HuR interaction with other RBPs and ncRNAs. Competitive binding of HuR and AUF1/CUGBP1 to particular target mRNAs are critical for their stability and translation and affect the epithelium homeostasis remarkably. HuR also interacts with SPRY4-IT1 to

enhance the epithelial barrier function synergistically, and inhibits the processing of miR-675 from H19, in turn preventing the gut barrier dysfunction induced by H19 overexpression. Thus, homeostasis of the intestinal epithelium is dependent on a dynamic balance between the actions of HuR and its interactions with diverse other RBPs and ncRNAs.

There are still many knowledge gaps in our understanding of HuR functions in the intestinal epithelium. The molecular processes controlling the expression levels of HuR, its subcellular distribution, and phosphorylation in response to stressful environments remain largely unknown. Current efforts employ state-of-the-art techniques to define novel targets, binding sequences, and biological functions of HuR to point out new directions. The effect of microbiota dysbiosis induced by HuR knockout on the intestinal epithelium homeostasis remains unknown and will provide novel information on the *in vivo* functions of HuR through interaction with microbiota. Future experiments must also define the mechanism by which HuR enhances the stability and translation of target mRNAs and examine if mutations in specific domains of HuR affect its ability to interact with other RBPs and ncRNAs. Importantly, studies using human mucosal samples from patients with mucosal atrophy, inflammation/erosions, leaky gut, and cancers will improve the impact of HuR on pathogenesis of intestinal mucosal disorders.

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