REVIEW

Novel roles of Na^+,K^+-ATPase ligands in regulating cytokines mRNA stability by HuR signalosome and the underlying pathophysiologic relevance

Yin Wu[1,2], Cao Dan[1], Bian Jinjun[3], Feng Su[1], Chen Wei[1], Zhou Ri[1], Ma Lin[1], Hua Zi-chun[1]

[1]*The State Key Lab of Pharmaceutical Biotechnology, College of life Sciences, Nanjing University, Nanjing, China, 210016*
[2]*The State Key Lab of Natural Medicine, China Pharmaceutical University, Nanjing, China,210025*
[3]*Department of Anesthesiology and Intensive Care Unit, Changhai Hospital, Affiliated Hospital of the Second Military Medical University, Shanghai, China, 200433*

Correspondence: Yin Wu
E-mail:wyin2003@163.com
Received:January 24, 2015
Published: September 22, 2015

Na^+,K^+-ATPase ligands, also called as cardiotonic steroids, are hormone-like immunoregulators, because they are frequently detected in patients with inflammatory-related diseases, moreover, they regulate multiple aspects of immune responses. One of prominent roles of Na^+,K^+-ATPase ligands in regulating immunity is their abilities of modulating cytokines expression. These bioactive chemicals can either upregulate or downregulate IL-1β, TNF-α, IL6, or iNOS expressions in different model system, however, all of those studies pointed to transcriptional upregulation. In our recent studies, we provided evidences that Na^+,K^+-ATPase ligands are capable of regulating cytokines mRNA stability by integrating multiple posttranscriptional mechanisms, including human antigen R (HuR) translocation, generation of miR181s, and formation of stress granules. These mechanisms do not function alone, but act in a synergistic or an antagonistic manner to fine-tune the cytokines expression, HuR nuclear export, however, forms signalosome and plays a core role among these processes. By taking advantage of HuR signalosome, Na^+,K^+-ATPase ligands stabilized cyclooxygenase-2 mRNA in lung epithelial cells and induced acute lung injury. In monocytes, ouabain-induced nuclear export of HuR competed with miR181s on the shared target of TNF-α, also triggered stress granules formation which recruited TNF-α mRNA into it for protection, thereby stabilizing TNF-α mRNA and reversing sepsis-induced immunoparalysis both in vitro and in vivo. Besides its effect in immune-related diseases, HuR also regulates a variety of pro-oncogenes and anti-oncogenes expressions in cancer cells, which determines the cancer cells sensitivity towards Na^+,K^+-ATPase ligands or other chemotherapeutic drugs. In sum, HuR emerges as a very important signaling molecule in Na^+,K^+-ATPase ligands-mediated cytokines production. These results shed new light on the pathophysiologic function as well as pharmacological activities of Na^+,K^+-ATPase ligands, also highlight a fundamental role of Na^+,K^+-ATPase in regulating cytokine mRNA reprogramming and metabolism. Identification of the components of HuR signalosome will offer more novel targets and biomarkers for disease therapy.

Keywords: Na^+,K^+-ATPase ligands; human antigen R; posttranscriptional mechanism; mRNA stability; acute lung injury; sepsis-induced immunoparalysis; cancer cells sensitivity

To cite this article: Yin Wu, *et al*. Novel roles of Na^+,K^+-ATPase ligands in regulating cytokines mRNA stability by HuR signalosome and the underlying pathophysiologic relevance. RNA Dis 2015; 2: e546. doi: 10.14800/rd.546.

Na^+,K^+-ATPase ligands are novel hormone-like immunomodulators

Na^+,K^+-ATPase ligands consist of a family of chemicals known to have potent therapeutic value in the therapy of cardiovascular diseases. They are not only plant-derived, but also produced from amphibian toad or the adrenal cortex and hypothalamus in human beings; ouabain is such a good case in point. In 1991, Hamlyn and colleagues purified endogenous ouabain (EO) from human plasma [1], which boosts the investigations for identifying the novel function of Na^+,K^+-ATPase ligands in human physiology or diseases. So far, the mainstream is that EO is elevated in patients with hypertension[2] or congestive heart failure [3], and is critically involved in cardiac remodeling [4]. However, increasing evidences demonstrate that EO can also act as a hormone-like immunomodulator that regulates immune responses[5,6], for instance, ouabain inhibited lymphocyte proliferation, exacerbated activation-induced cell death (AICD), modulated monocytes, and had remarkable anti-inflammatory effects on various inflammatory animal models. In fact, ouabain resembles glucocorticoids in many aspects of immune regulation, probably because both of them share similar core steroid structure and can be released from the adrenal under the stimulus of ACTH[7]. However, the detailed immunomodulatory mechanisms for Na^+,K^+-ATPase ligands are far from being understood.

Na^+,K^+-ATPase ligands regulate cytokines expression in a variety of pathological settings

Cytokines expression profile plays critical roles in the progression of pathological diseases. One of prominent roles for ouabain in regulating immune responses is its ability of modulating cytokines expression. Ouabain was found to increase IL-1β, IL-6, and TNF-α expressions in human peripheral blood mononuclear cells (PMBC) [8], enhance the production of inflammatory cytokines in patients who are diagnosed with rheumatoid arthritis[9], and stimulate the expressions of VCAM-1 and iNOS in endothelial cells [10]. Mesenchymal cells produced abundant GM-CSF in response to ouabain treatment, and this effect is dependent on Ca^{2+} increase [11]. Besides, ouabain has an additive effect on GM-CSF production induced by TNF-α [12]. Notably, most of these studies considered that ouabain regulated cytokines expression at transcriptional level. On the contrary, we found in recent studies that ouabain had a novel role in regulating cytokines expression at posttranscriptional level. Moreover, ouabain integrates multiple posttranscriptional mechanisms, including microRNAs, RNA binding proteins, stress granules, and/or long non-coding RNAs with a purpose of delicately controlling cytokines expression, which plays pivotal roles in regulating inflammatory process of acute lung injury [13], sepsis-induced immunoparalysis [14], and cancer cells apoptosis. The following is the detailed review of the projects we are currently undertaking.

Na^+,K^+-ATPase ligands regulates cyclooxygenase-2 mRNA stability in acute lung injury-HuR is involved[13]

Acute lung injury is a frequently-occurring but life-threatening pulmonary disease in clinic. Recently, increasing evidence demonstrates that Na^+, K^+-ATPase impairment in alveolar epithelial cells is greatly involved in the pathogenesis of acute lung injury (ALI) [15]. For example, hypoxia trigged the endocytosis and ubiquitin-mediated degradation of alveolar epithelial Na^+, K^+-ATPase, and as a result, lung edema clearance is greatly impaired [16].

Na^+, K^+-ATPase is a ubiquitously expressed membrane ion transporter which maintains the ion and electrolyte homeostasis across membrane by consuming ATP supply. In light of this, it is not surprising that Na^+, K^+-ATPase impairment in lung epithelial cells inevitably leads to inefficient water absorption and lung edema clearance in ALI. However, it is noteworthy to mention that ALI is a well-characterized inflammatory disorder, whether Na^+, K^+-ATPase impairment also makes a contribution to the inflammation of ALI deserves investigation. To solve this problem, an acute lung injury model with the impaired Na^+, K^+-ATPase activity in lung is absolutely needed, however, because Na^+, K^+-ATPase is fundamental in physiology, knockout of Na^+, K^+-ATPase gene is expected to cause animal death, we thus adopted an alternative strategy by using Na^+, K^+-ATPase inhibitor ouabain. As a result, mice inhaled with aerosolized ouabain developed acute inflammatory response in lung. However, this pathological process was largely alleviated by rofecoxib, suggesting the effect of ouabain is dependent on COX-2[13]. In addition to ouabain, similar results were also obtained by other Na^+,K^+-ATPase ligands. Meanwhile, it seemed that both transcriptional and posttranscriptional layers of regulation were involved in COX-2 upregulation by Na^+,K^+-ATPase ligands. Promoter analysis revealed that NF-IL6/c/EBP and CRE-binding sites are required for the increased COX-2 gene transcription by ouabain.

Besides transcription, ouabain also persistently increased COX-2 mRNA as well as protein expressions over 16 h, suggesting additional mechanisms might be involved, because COX-2 is a cytokine with short half-life, after transcription occurs, COX-2 mRNA will be rapidly degraded as a host defense mechanism to eliminate its damaging effect on tissues or organs. As a result, the COX-2 transcripts were largely prevented from decay by ouabain after they were transcribed. Bioinformatics analysis reveals that multiple

"AUUUA" element ("AU"-rich elements, ARE) are present in the 3'-untranslated region (UTR) of COX-2 transcripts. These AREs can be recognized and bound by a variety of ARE binding proteins (ARE-BPs) that either increase or decrease the half-life of the gene [17]. The well-recognized ARE-BPs include human antigen R (HuR), AUF1, TTP, KSRP, and so on [18]. Interestingly, ARE-BPs can associate with each other and form a complex on the ARE motif, thereby delicately controlling the mRNA stability or translation efficacy of the target gene[18]. We coined a phrase for this way of regulation as ARE-BP signalosome.

In the study, by constructing a series of deletion variants of COX-2 3'-UTR, we finally identified that the sequence of Site I ARE largely contributes to COX-2 mRNA stabilization in the presence of ouabain treatment. More intriguingly, ouabain and other Na^+,K^+-ATPase ligands can trigger HuR translocation from nucleus to cytoplasm, which subsequently binds with the sequence of Site I ARE and increases the mRNA stability of COX-2 [13].

This is the first report involving HuR as an important RNA binding protein in Na^+,K^+-ATPase ligands-mediated cytokines expression at posttranscriptional level.

Na^+,K^+-ATPase ligands regulates tumor necrosis factor α mRNA stability in sepsis-induced immunoparalysis

HuR competes with miR181 and forms stress granules to stabilize TNF-α mRNA [14]

Besides COX-2, lots of other inflammation-related genes contain AREs in their 3'-UTRs that can be recognized by HuR, such as interleukin 6 (IL6) and various chemokines. The aforementioned study raises a possibility that Na^+,K^+-ATPase ligands may regulate the expressions of a repertoire of these AREs containing messages as a general mechanism. If this is the case, what is the underlying mechanisms and biological significance?

A previous study attracted our attention. In that case, ouabain increased TNF-α expression in mononuclear cells isolated from patients with rheumatoid arthritis [9]. To confirm it, the effects of ouabain and LPS on TNF-α mRNA and protein expressions in PBMC and THP1 cell lines were examined. Interestingly, both LPS and ouabain were able to increase TNF-α mRNA expression in first 2 hours, however, the TNF-α mRNA decay in ouabain-treated was significantly slowed down as compared to that occurred in LPS-treated cells. In PBMC, ouabain treatment even led to persistent TNF-α cytokine expression over a 24-h period [14]. These results invited us to speculate that ouabain may also regulate TNF-α expression at posttranslational level.

Similar as that of COX-2, the 3'-UTR of TNF-α transcript also contains multiple AREs. By constructing deletion mutants of TNF-α 3'-UTR, we identified the minimal cis-regulatory element (T55) required for ouabain's effect. Notably, unlike that in COX-2 3'-UTR in which the AREs are separately distributed, seven "AUUUA" motif sequences exactly clustered and overlapped within the T55 region of TNF-α 3'-UTR. Ouabain again triggered HuR nuclear export in human monocytes, which stabilized TNF-α mRNA. However, LPS failed to induce HuR export. Given this result, it seems understandable that TNF-α mRNA decay was delayed in ouabain-treated cells, but accelerated in LPS-treated cells.

Unexpectedly, T55 region was also found to be the minimal cis-regulatory element required for LPS-mediated TNF-α destabilization. Targetscan software predicted that a highly conserved miR181-binding site (UGAAUGU) is present within two adjacent "AUUUA" motif sequences. The microRNA181 family consists of four members: miR181a, miR181b, miR181c, and miR181d. The "mimics" of miR181a/b/c/d greatly reduced the luciferase activity of TNF-α 3'-UTR reporter, TNF-α mRNA and protein expressions. Moreover, LPS triggered miR181c/d transcription through Egr-1 expression. All of these data suggest both mRNA stabilizing and destabilizing cis-elements co-exist in T55, meanwhile, miR181 is a newly identified negative regulator of TNF-α expression.

More interestingly, ouabain can also trigger Egr-1-dependent miR181 production as that of LPS, which is conflicting with the fact that ouabain stabilized TNF-α mRNA by triggering HuR translocation. To explain this inconsistency, we presume that HuR complex may spatially mask the binding sites for miR181s in the 3'-UTR of TNF-α. This presumption is indeed reasonable, because only one miR181 binding site is present in the minimal TNF-α 3'-UTR; in contrast, seven "AUUUA" motifs are located in the immediate vicinity of the miR181 binding site. To confirm this hypothesis, we transfected cells with HuR expression plasmid, miR181s, and T55 reporter, the results clearly demonstrated that there is a competition between miR181s and HuR on regulating TNF-α mRNA stability. Similar result was also obtained in RNA immunoprecipitation experiment. After ouabain treatment, the association between HuR and TNF-α mRNA was greatly enhanced. In another experiment, we also found that in cells after ouabain treatment, many small cytoplasmic foci occurred, RNA-FISH experiment revealed that TNF-α mRNAs co-localized with these small cytoplasmic organelles which were finally identified as stress granules. Therefore, in order to stabilize TNF-α mRNA, ouabain integrates multiple mechanisms; one is to prevent TNF-α mRNA from

nuclease-mediated degradation by triggering HuR nuclear export and recruiting them onto the 3'-UTR of TNF-α mRNA; the second is to compete the shared target of TNF-α mRNA with miR181s, thereby antagonizing the TNF-α mRNA destabilizing effect of miR181s; the third is to provoke stress granules formation, which recruits TNF-α mRNA into it in case of being degraded by miR181s. Obviously, HuR integrates different posttranscriptional mechanisms and plays a core role in these processes; for these characteristics, we assigned it a new name, HuR signalosome.

Ouabain reverses sepsis-induced immunoparalysis by reprogramming T_H1 cytokines expression

While we appreciated the role of HuR signalosome in ouabain-mediated TNF-α mRNA stabilization, more important questions emerge. Why TNF-α mRNA stability is needed to be fine-tuned by ouabain? Why the 3'-UTR of TNF-α harbors two functionally distinct cis elements in regulating TNF-α mRNA stability, and why LPS failed to trigger HuR export? To answer these questions, we examined the effect of ouabain on sepsis-induced immunoparalysis, because TNF-α production is critically involved in this pathological disorder; on the other hand, an elevated level of EO was detected in patients with severe sepsis, but the clinical significance remains unknown.

Sepsis is a life-threatening disease and normally appreciated as a systematic inflammatory response, however, clinical therapies that are designed to specifically deactivate inflammatory cytokines such as TNF-α and IL-1β antagonists have failed, or, in some cases, have worsen the survival[19]. Recently, increasing evidence demonstrated that a compensatory anti-inflammatory response to counter-regulate the immune response in sepsis can result in a phase of immunosuppression, which has been convincingly established as a major pathogenic mechanism in sepsis[20,21]. Sepsis-induced immunosuppression, or called as immunoparalysis, is characterized by impaired TNF-α production, immune effector cell apoptosis, shift from T_H1 to a T_H2 immune phenotype[20], upregulation of T_{reg} cells, and a decrease in human leukocyte antigen-DR (HLA-DR) expression in monocytes[22,23]. Unfortunately, the effective approach to reverse sepsis-induced immunoparalysis is currently not available in clinic. GM-CSF and IFN-γ were shown to partially reverse immunoparalysis, but the results are only limited to experimental study or small-scale clinical trials [24,25].

In the study, we established a clinically relevant "two-hit" model of sepsis that can mimic the pathological setting of sepsis-induced immunoparalysis, consisting of cecal ligation and puncture (CLP) followed by the induction of Salmonella typhimurium (S.tm) infection. In this model, we found ouabain significantly improved animal survival when administered at a low dose of 0.1 mg/kg at 54 and 78 h post-CLP. But surprisingly, when ouabain was administered before S.tm infection, at 6 and 30 h after CLP, increased mortality was observed. This result suggests that ouabain exerts protective effect only at the stage of immunoparalysis, but not the stage of hyperinflammation, as such, immune status monitoring is necessary for the proper use of ouabain in sepsis therapy[14].

To understand why ouabain was able to reverse immunoparalysis, we found the effect of ouabain can be largely blocked by infliximab, a TNF-α monoclonal antibody, suggesting TNF-α is required for ouabain to have effect. This result, indeed, not only explained the necessity of increasing TNF-α mRNA stability by ouabain, but also highlighted the importance of TNF-α in pathology of immunoparalysis.

Based on different immune characteristic, sepsis can be divided into two closely linked immune phases, that is, an initial phase of hyperinflammation followed by a subsequent phase of immunosuppression[22]. TNF-α has different roles in these two phases. At the stage of hyperinflammation, TNF-α is massively produced as a major pro-inflammatory cytokine to combat bacterial infections; however, the overproduction of TNF-α is detrimental to tissue and organ that may lead to multiple organs dysfunction. To circumvent this damaging effect, host immunity initiates an anti-inflammatory response as a defense mechanism to protect itself, which includes the production of IL10, TGF-β, and others. Among them, IL10 was found to decrease TNF-α mRNA stability by suppressing p38 MAPK [26]. In this study, miR181s production activated by LPS or ouabain can also be considered as a host defense mechanism against endotoxin injury, because it can potently promote TNF-α mRNA degradation. The results from clinical samples also confirmed that miR181b/c/d was dramatically upregulated in patients with severe sepsis, and accordingly, the TNF-α mRNA levels were downregulated.

Notably, although miR181s-mediated TNF-α degradation is helpful at the stage of hyperinflammation, this effect is obviously deleterious at the stage of immunoparalysis, because the absence of TNF-α increases the risk of secondary infection. In animal experiments, the in vivo delivery of cholesterol miR181s significantly reduced survival and bacterial clearance in the blood and spleen. The antagomir 181 treatment, however, prolonged the survival and improved the bacterial clearance. Therefore, it is necessary to boost TNF-α expression or restore the impaired

TNF-α production at the stage of immunoparalysis. Fortunately, ouabain preferentially increased TNF-α production in monocytes at the stage of immunoparalysis by triggering HuR export and TNF-α mRNA stabilization. In this regard, it seems that the miR181 binding site is intentionally embedded within "AUUUA" motifs in TNF-α 3'-UTR by God, which reserves targets for intervention in sepsis-induced immunoparalysis. As a matter of fact, besides TNF-α, other immunostimulatory cytokines including GM-CSF and IFN-γ all contain classic "AUUUA" motifs within their 3'-UTR that can be recognized by HuR. In the study, ouabain also preferentially stabilized GM-CSF and IFN-γ mRNAs in monocytes isolated from sepsis patients. GM-CSF and IFN-γ have been shown to reverse immunoparalysis by increasing HLA-DR expression in monocytes. Consistently, ouabain increased HLA-DR expression in monocytes from sepsis patients. Thus, via HuR translocation, ouabain may stabilize the mRNAs of many immunostimulatory TH_1 cytokines, including TNF-α, GM-CSF, and IFN-γ, thereby reprogramming cytokine expression in the immunosuppressive state and reversing immunoparalysis.

To the best of our knowledge, this is the first report demonstrating that a small- molecule drug can reverse sepsis-induced immunoparalysis by modulating TNF-α mRNA stability, also, the concept that reprogramming cytokines expression at posttranscriptional level is helpful for reversal of immunoparalysis is proposed for the first time.

Na^+,K^+-ATPase ligands regulate both pro-oncogenes and anti-oncogenes mRNA stability in cancer cells

Another important characteristic of Na^+,K^+-ATPase ligands is their potential anti-cancer activities. Many Na^+,K^+-ATPase ligands can trigger cell death in a variety of cancerous cell lines in vitro, and have anti-cancer effect in vivo[27,28]. Till now, several Na^+,K^+-ATPase ligands have been under phase II trials for cancer therapy in some countries[29]. In addition to directly triggering cancer cell death, Na^+,K^+-ATPase ligands are also found to increase cancer cells apoptosis sensitivity towards chemotherapeutic drugs[29]. For example, Na^+,K^+-ATPase ligands were found to preferentially increase the sensitivity of non-small-cell lung carcinoma (NSCLC) cells towards TRAIL-mediated apoptosis [30].Interestingly, in our previous investigation, suppression of Na^+,K^+-ATPase activity by ouabain enhanced CD95(APO-1)-induced leukemia cell apoptosis, but on the other hand, CD95(APO-1) also triggered the endocytosis and degradation of membrane Na^+,K^+-ATPase in CD95-sensitive leukemia cells to further augment apoptosis [31]. Suppression of Na^+,K^+-ATPase activity by ouabain also aggravated mitochondrial inhibitor-mediated leukemic cell death[32]. Apart from these positive effects, contrasting results for Na^+,K^+-ATPase ligands are also frequently reported. For example, Na^+,K^+-ATPase ligands were found to protect cells from death, especially when they were used at low concentrations, meanwhile, some cancers are refractory to Na^+,K^+-ATPase ligands treatment[29,33].

It is well recognized that the cancer cells destiny as well as their sensitivity towards chemotherapeutic drugs is largely determined by expressions of a panel of pro-oncogenes and oncogenes. If the effects of anti-oncogenes override the effects of pro-oncogenes, the cancer cells are prone to die, or becoming sensitive to chemotherapeutic agents. Notably, lots of pro-oncogenes and anti-oncogenes contain AREs in their 3'-UTR. In view of the fact that Na^+,K^+-ATPase ligands are able to trigger HuR translocation, we set out to determine whether HuR-mediated posttranscriptional mechanisms are also critically involved the effects of Na^+,K^+-ATPase ligands on cancer cells survival or death.

In our unpublished observations, ouabain-induced HuR export stabilized death receptor 5 mRNA, which may explain why ouabain was able to increase NSCLC cells apoptotic sensitivity towards TRAIL. Surprisingly, low concentration of ouabain treatment also posttranscriptionally upregulated VEGF expression, which promoted cancer cell growth and increased resistance. More intriguingly, we found in a recent study that under ouabain treatment, HuR formed stress granules and recruited p21 mRNA into them for suppressing its pro-apoptotic effect, the HuR-involved stress granules formation was regulated by general control non-depressible kinase 2 (GCN2). Ouabain enhanced GCN2 protein expression by suppressing its degradation machinery mediated by β-arrestins and NEDD4L, and this effect was relevant to GCN2 phosphorylation at threonine 899, moreover, GCN2 had dual roles in regulating cancer cells sensitivity towards Na^+,K^+-ATPase ligands[34].

Na^+,K^+-ATPase ligands and HuR

In view of the central role of HuR in the effect of ouabain on modulating cytokines expression, we tried to understand the underlying mechanisms. By generating a series of HuR deletion mutants and point mutants, we finally identified that serine 88 and serine 158 are critically involved in HuR translocation under stimulus of ouabain treatment, however, the upstream signaling events that lead to HuR phosphorylation still remain as a mystery.

Concluding remarks

HuR emerges as a very important signaling molecule in Na^+,K^+-ATPase ligands-mediated effects (Figure 1), which opens new avenues for understanding the pathophysiologic

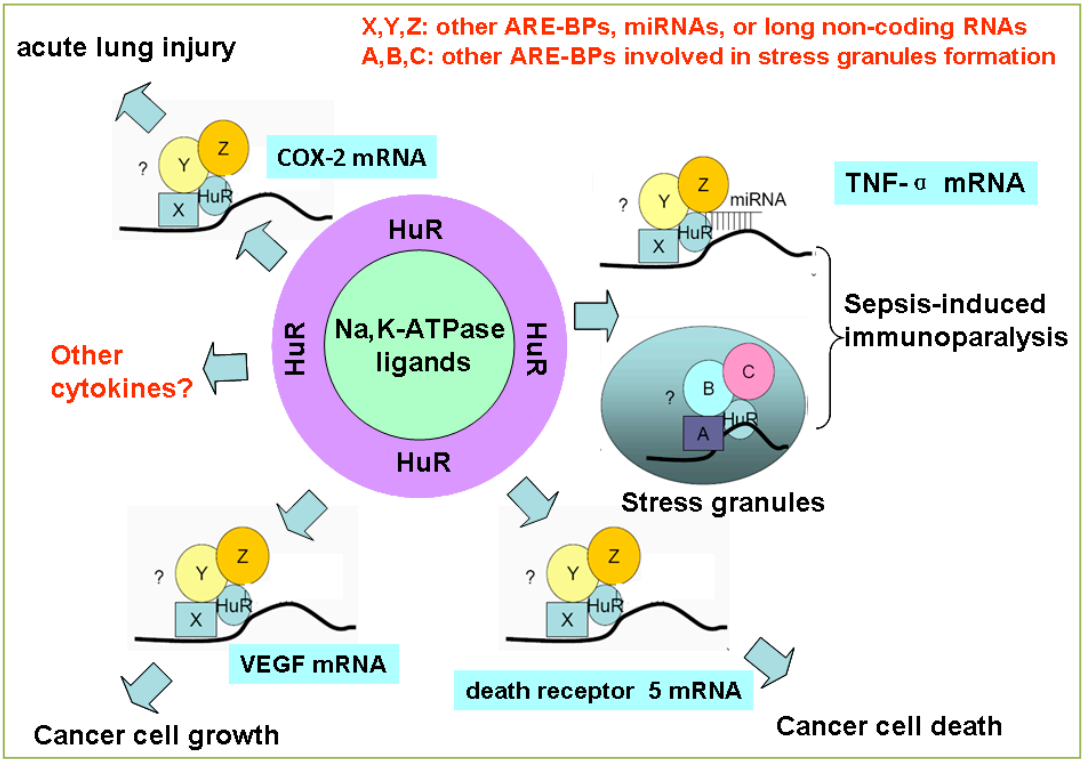

Figure1.Involvement of HuR signalosome in the regulatory effects of Na^+,K^+-ATPase ligands on cytokines expression at posttranscriptional levels.

and pharmacological effects Na^+,K^+-ATPase ligands. While we appreciated the central role of HuR in these processes, the possibilities that other RNA binding proteins involved can not be ignored, because HuR does not function alone, it may associate with other RNA binding proteins such as AUF1, microRNAs, or long non-coding RNAs to form HuR signalosome that controls cytokine expression more specifically and delicately. Whether those new partners could be identified and finally appreciated as novel makers or targets for disease therapy remains an intriguing issue. On the other hand, increasing evidence demonstrates that Na^+,K^+-ATPase ligands-initiated signaling is either Na^+,K^+-ATPase transport activity dependent or independent[6], whether HuR-mediated signaling may crosstalk with the well-established Na^+,K^+-ATPase signaling deserves more experiments to verify.

Finally, although we emphasize here that HuR participates in the regulatory effects of Na^+,K^+-ATPase ligands on immunological disorder and cancer, the story is not yet complete, because Na^+,K^+-ATPase ligands are widely implicated in a variety of biological processes as hormone-like substances. Na^+,K^+-ATPase is more than an ionic pump, that is, besides as a ion pump to exchange Na^+/K^+ across membrane, Na^+,K^+-ATPase also act as a scaffold protein to initiate signaling when they are bound by ouabain or other ligands. Here, we identify another fundamental role of Na^+,K^+-ATPase in regulating mRNA stability and metabolism. Obviously, as knowledge of RNA metabolism increasing, this mechanism, together with others, will make us more aware of the complex role of Na^+,K^+-ATPase in human physiology and pathology.

Acknowledgements

This project was sponsored by Natural Science Fund of China (31071250, 81171843,and 81473293), National Key Basic Research Program from Ministry of Science and Technology of China(2012CB967004, 2014CB744501), open project of National Key Lab of Natural Medicines (SKLNMKF201303), the Fundamental Research Funds for the Central Universities, and Nanjing “321” talents project to Y.W.

Conflict of Interest

The authors declare no conflict of interest on this paper

References

1. Hamlyn JM, Blaustein MP, Bova S, DuCharme DW, Harris DW, Mandel F, *et al*. Identification and characterization of a ouabain-like compound from human plasma. Proc Natl Acad Sci U S A 1991;88:6259-6263.
2. Rossi G, Manunta P, Hamlyn JM, Pavan E, De Toni R, Semplicini

A, *et al*. Immunoreactive endogenous ouabain in primary aldosteronism and essential hypertension: relationship with plasma renin, aldosterone and blood pressure levels. J Hypertens 1995;13:1181-1191.

3. Gottlieb SS, Rogowski AC, Weinberg M, Krichten CM, Hamilton BP, Hamlyn JM. Elevated concentrations of endogenous ouabain in patients with congestive heart failure. Circulation 1992;86:420-425.
4. Nicholls MG, Lewis LK, Yandle TG, Lord G, McKinnon W, Hilton PJ. Ouabain, a circulating hormone secreted by the adrenals, is pivotal in cardiovascular disease. Fact or fantasy? J Hypertens 2009;27:3-8.
5. Rodrigues-Mascarenhas S, Da Silva de Oliveira A, Amoedo ND, Affonso-Mitidieri OR, Rumjanek FD, Rumjanek VM. Modulation of the immune system by ouabain. Ann N Y Acad Sci 2009;1153:153-163.
6. Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets. Pharmacol Rev 2009;61:9-38.
7. Laredo J, Hamilton BP, Hamlyn JM. Ouabain is secreted by bovine adrenocortical cells. Endocrinology 1994;135:794-797.
8. Matsumori A, Ono K, Nishio R, Igata H, Shioi T, Matsui S, *et al*. Modulation of cytokine production and protection against lethal endotoxemia by the cardiac glycoside ouabain. Circulation 1997;96:1501-1506.
9. Foey AD, Crawford A, Hall ND. Modulation of cytokine production by human mononuclear cells following impairment of Na, K-ATPase activity. Biochim Biophys Acta 1997;1355:43-49.
10. Bereta J, Cohen MC, Bereta M. Stimulatory effect of ouabain on VCAM-1 and iNOS expression in murine endothelial cells: involvement of NF-kappa B. FEBS Lett 1995;377:21-25.
11. Yamato K, El-Hajjaoui Z, Kuo JF, Koeffler HP. Granulocyte-macrophage colony-stimulating factor: signals for its mRNA accumulation. Blood 1989;74:1314-1320.
12. Penning LC, Denecker G, Vercammen D, Declercq W, Schipper RG, Vandenabeele P. A role for potassium in TNF-induced apoptosis and gene-induction in human and rodent tumour cell lines. Cytokine 2000;12:747-750.
13. Feng S, Chen W, Cao D, Bian J, Gong FY, Cheng W, *et al*. Involvement of Na(+), K (+)-ATPase and its inhibitors in HuR-mediated cytokine mRNA stabilization in lung epithelial cells. Cell Mol Life Sci 2011;68:109-124.
14. Dan C, Jinjun B, Zi-Chun H, Lin M, Wei C, Xu Z, *et al*. Modulation of TNF-alpha mRNA stability by human antigen R and miR181s in sepsis-induced immunoparalysis. EMBO Mol Med 2014;7:140-157.
15. Vadasz I, Raviv S, Sznajder JI. Alveolar epithelium and Na,K-ATPase in acute lung injury. Intensive Care Med 2007;33:1243-1251.
16. Sznajder JI, Factor P, Ingbar DH. Invited review: lung edema clearance: role of Na(+)-K(+)-ATPase. J Appl Physiol (1985) 2002;93:1860-1866.
17. Ivanov P, Anderson P. Post-transcriptional regulatory networks in immunity. Immunol Rev 2013;253:253-272.
18. Anderson P. Post-transcriptional control of cytokine production. Nat Immunol 2008;9:353-359.
19. Moore TA, Lau HY, Cogen AL, Monteleon CL, Standiford TJ. Anti-tumor necrosis factor-alpha therapy during murine Klebsiella pneumoniae bacteremia: increased mortality in the absence of liver injury. Shock 2003;20:309-315.
20. Muenzer JT, Davis CG, Chang K, Schmidt RE, Dunne WM, Coopersmith CM, *et al*. Characterization and modulation of the immunosuppressive phase of sepsis. Infect Immun 2010;78:1582-1592.
21. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis 2013;13:260-268.
22. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138-150.
23. Oberholzer A, Oberholzer C, Moldawer LL. Sepsis syndromes: understanding the role of innate and acquired immunity. Shock 2001;16:83-96.
24. Meisel C, Schefold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, *et al*. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med 2009;180:640-648.
25. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, *et al*. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med 1997;3:678-681.
26. Rajasingh J, Bord E, Luedemann C, Asai J, Hamada H, Thorne T, *et al*. IL-10-induced TNF-alpha mRNA destabilization is mediated via IL-10 suppression of p38 MAP kinase activation and inhibition of HuR expression. FASEB J 2006;20:2112-2114.
27. Newman RA, Yang P, Pawlus AD, Block KI. Cardiac glycosides as novel cancer therapeutic agents. Mol Interv 2008;8:36-49.
28. Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. Nat Rev Drug Discov 2008;7:926-935.
29. Mijatovic T, Kiss R. Cardiotonic steroids-mediated Na+/K+-ATPase targeting could circumvent various chemoresistance pathways. Planta Med 2013;79:189-198.
30. Frese S, Frese-Schaper M, Andres AC, Miescher D, Zumkehr B, Schmid RA. Cardiac glycosides initiate Apo2L/TRAIL-induced apoptosis in non-small cell lung cancer cells by up-regulation of death receptors 4 and 5. Cancer Res 2006;66:5867-5874.
31. Yin W, Cheng W, Shen W, Shu L, Zhao J, Zhang J, *et al*. Impairment of Na(+),K(+)-ATPase in CD95(APO-1)-induced human T-cell leukemia cell apoptosis mediated by glutathione depletion and generation of hydrogen peroxide. Leukemia 2007;21:1669-1678.
32. Yin W, Li X, Feng S, Cheng W, Tang B, Shi YL, *et al*. Plasma membrane depolarization and Na,K-ATPase impairment induced by mitochondrial toxins augment leukemia cell apoptosis via a novel mitochondrial amplification mechanism. Biochem Pharmacol 2009;78:191-202.
33. Biggar RJ, Wohlfahrt J, Oudin A, Hjuler T, Melbye M. Digoxin use and the risk of breast cancer in women. J Clin Oncol 2011;29:2165-2170.
34. Wei C, Lin M, Jinjun B, Su F, Dan C, Yan C, *et al*. Involvement of general control non-derepressible kinase 2 in cancer cell apoptosis by posttranslational mechanisms. Mol Biol Cell 2015; doi: 10.1091/mbc. E14-10-1438.