

# HuR: a promising therapeutic target for angiogenesis

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**Abstract:** Multiple angiogenic factors and inhibitors are becoming potential therapeutic targets for ischemia diseases and cancer. Posttranscriptional regulation through the untranslated region of mRNA is emerging as a critical regulating level in nearly all the biological processes. As a kind of RNA binding proteins, HuR plays important role in augmenting the hypoxic or inflammatory signal, stabilizing the resultant angiogenic factors and promoting the proliferation and migration of endothelial cells. These implicate HuR in the proangiogenic factors mediated angiogenesis in the hypoxia and inflammatory. We consider hypotheses that a more effective angiogenesis can be acquired through strengthened and prolonged effects of angiogenic factors, and that progresses in therapeutic angiogenesis might also shed light on the implication of HuR in blocking tumor angiogenesis. These considerations may help us to explain HuR as a promising therapeutic target for angiogenesis related disease. It may be a candidate in hypoxia therapy and cancer management.

**Keywords:** HuR; angiogenesis; hypoxia; inflammatory

Submitted Dec 22, 2013. Accepted for publication Mar 13, 2014.

doi: 10.3978/j.issn.2227-684X.2014.03.02

**View this article at:** <http://dx.doi.org/10.3978/j.issn.2227-684X.2014.03.02>

## Introduction

Ischemia is one of the severest threats to the survival of tissues, which is also a barrier of tumor development. In response to ischemia, tissues or organs have recapitulated many aspects of embryonic circulatory development to enable formation of an accessory vasculature. Multiple angiogenic factors and inhibitors have been implicated in the development of blood vessels, which are becoming potential therapeutic targets for ischemia diseases and cancer. During the past decade, therapeutic angiogenesis clinical trials by direct administration of angiogenic growth factor proteins, injection of non-viral vectors or viral vector constructs carrying angiogenic genes have been used though with compromised outcome (1). The idea that angiogenesis is a complex sequential process, which is orchestrated by a series of molecules, favors the adjuvant therapy or targeting upstream factors which coordinately regulate multiple steps in the angiogenesis. To this end, angiogenic strategies targeting the upstream factors are appealing (2). During the

relatively long process of tumor development, tumor cell and the supporting vasculature has evolved a potent strategy to ensure sufficient angiogenesis, and some of the critical factors detailed studied in cancer research might be qualified to this standard. In view of the potent oncogenic role and the strong ability of HuR in stabilization of multiple angiogenic factors involved in several steps of angiogenesis, we hypothesize that this RNA binding protein may be a candidate in ischemia therapy and cancer management.

## Angiogenesis

Angiogenesis is the formation of neo-vessels from preexisting blood vessels. Angiogenesis in the adult includes at least six sequential steps: (I) detachment of pre-existing pericytes or vascular destabilization; (II) extracellular matrix degradation by endothelial proteases; (III) migration of ECs and/or EPCs; (IV) proliferation of ECs and/or EPCs; (V) tube formation; (VI) reattachment of pericytes or vascular stabilization (3,4). Such a complex process is

an orchestration of endothelial cells, surrounding stromal cells, extracellular matrix and associated factors. Of them, pro-angiogenic factors should play an initial role. To date, dozens of angiogenic factors covering from growth factors, cytokines, interleukins, enzymes, to chemicals, have been identified and characterized, some of which has been used in clinical trials (5). However, these angiogenic factors fails to induce complete angiogenesis when applied individually since nearly all of them just involved in part of the complex process. Strategies coordinately enhance multiple factors should have preferential benefit in angiogenic targeting therapy. Nowadays posttranscriptional regulation through the untranslated region (UTR) of mRNA is emerging as a critical regulating level in nearly all the biological processes. The messenger RNAs of many of these proangiogenic factors are targeted for rapid degradation through AU-rich elements (AREs) located in their 3' UTRs, raising the potential of therapeutic angiogenesis by artificially protecting them from degradation.

### HuR in angiogenesis

HuR, a ubiquitously expressed member of the ELAV family of RNA binding proteins, exhibits specific affinities for ARE-containing RNA sequences in vitro which correlate with their in vivo decay rates, thereby implicating HuR in the proangiogenic factors mediated angiogenesis. Generally speaking, angiogenesis is initiated by hypoxic or inflammatory conditions. Hypoxia and inflammation activate HuR function through modulation of HuR subcellular localization and RNA binding activity (6). Furthermore, another inflammation related cytokine IL-1 could also activate the function of HuR to stabilize a set of target mRNAs for fulfillment of a certain process (7). In turn, activated HuR stabilize TNF $\alpha$  or promote the translation of HIF1 $\alpha$  through the ARE located in the 3' UTR, forming a positive feedback (8-12).

Besides, HuR is found to regulate multiple angiogenic factor mRNAs directly through binding to adenine- and uridine-rich elements within the 3' UTRs. VEGF is a critical mediator of hypoxia-induced angiogenesis in numerous physiological and pathophysiological conditions. Recently, studies on posttranscriptional regulation of VEGF have confirmed that HuR stabilized VEGF mRNA through binding to a 40-bp RNA element under hypoxic condition (13,14). Similar results were observed in gastrocnemius muscle under acute ischemia or hypoxia (15).

In addition to VEGF, the urokinase plasminogen activator

(uPA) system plays an important role in angiogenesis through matrix remodeling, interactions with integrins, cell motility, adhesion and invasion (16). In response to mitogen-activated protein kinase-activated protein kinase 2 activation, HuR accumulated in the cytoplasm and bound with uPA and uPAR, enhancing the role of uPA system (17).

Similar as uPA system, COX2 which encodes the inducible prostaglandin synthase enzyme are essential in angiogenesis through regulating angiogenic factor induction, endothelial cell proliferation, migration, and network assembly (18,19). It is also established that COX2 is essential for mediating the myocardial protective effects of atorvastatin against ischemia-reperfusion injury (20). To date, posttranscriptional regulation of COX2 has been intensely studied. HuR plays a central role in the stabilization of COX2 against multiple conditions (21-24).

MMP-9 is a member of a family of zinc-containing endopeptidases that is involved in degradation of extracellular matrix (ECM) and in vascular remodeling (25). It is an inducible enzyme which is known to be secreted from human endothelial cells and other cell types in response to inflammatory cytokines or angiogenic factors. MMP-9 is involved in mobilizing EPCs and other progenitor cells from the BM niche (26,27), liberating growth factors VEGF from the matrix-bound forms (28). Strikingly, MMP9 is also stabilized by RNA binding protein HuR (29,30).

### HuR in endothelial cell proliferation and migration

As discussed before, angiogenesis is a process associated with endothelial cell migration and proliferation. Molecules promoting endothelial cell migration and proliferation might play a role in angiogenesis. Besides the direct role of HuR on the angiogenic factors, it is reported that HuR can promote cell proliferation, migration.

It has been well established that, in dividing cells, the RNA-binding protein HuR associates with and stabilizes labile mRNAs encoding proliferative proteins, such as mRNAs encoding cyclins A, B1 and E (31). HuR also regulates cyclin mRNA stability during cell proliferation (32), implicating a proliferative role of HuR. Furthermore, Pullmann R Jr *et al.* found that enhanced proliferation of cultured human vascular smooth muscle cells is linked to increased function of RNA-binding protein HuR, implicating a contributing role of HuR in angiogenesis (33).

In terms of migration, which is also related to angiogenesis, Dormov Ralet V *et al.* found that HuR promoted cell migration through stabilizing the beta-actin

mRNA (34). The RNA-binding protein HuR promotes cell migration and cell invasion by stabilizing the beta-actin mRNA in a U-rich-element-dependent manner. Besides, results from Dong *et al.* revealed that Snail, one of the critical regulators in EMT is also stabilized by HuR in the process of ROS induced cell migration (35).

Another evidence suggesting a potential role of HuR in angiogenesis is that inhibition of glycogen synthase kinase-3beta in human endothelial progenitor cells augment the therapeutic angiogenesis (36), while cytoplasmic localization and resultant activation of HuR is one of the main effects of glycogen synthase kinase-3 beta inhibition (37).

In sum, HuR strengthen the hypoxia and inflammatory related angiogenesis at least in three ways: augmenting the hypoxic or inflammatory signal, stabilizing the resultant angiogenic factors, promoting the proliferation and migration of endothelial cells.

### Hypothesis and conclusions

Cytokines, MMPs, growth factors function synergistically in angiogenesis through acting on the sequential steps individually or on the same step corporately. Interestingly, a bundle of angiogenic regulators covering different steps of angiogenesis are regulated by HuR. In other words, HuR is functionally active anti-apoptosis, promoting proliferation, migration and angiogenesis. All of these are required steps for successful formation of functional vasculature and recovery of ischemia, implicating its potential value in therapeutic angiogenesis. It is reasonable to deduce that a more effective angiogenesis can be acquired through strengthened and prolonged effects of angiogenic factors. Future directions should be focused on the ways to strengthen the function of HuR in ischemic disease, such as modulating its expression or the signaling pathways related to the shuttle of HuR. It is also important to note that, progresses in therapeutic angiogenesis might also shed light on the implication of HuR in blocking tumor angiogenesis.

### Acknowledgements

*Funding:* National Natural Science Foundation of China: Post- transcriptional regulation of PDGF $\alpha$  in invasive breast cancer and its role in fibroblast activation (81001181).

*Disclosure:* The authors declare no conflict of interest.

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**Cite this article as:** Dong R, Yang GD, Luo NA, Qu YQ. HuR: a promising therapeutic target for angiogenesis. *Gland Surgery* 2014;3(3):203-206. doi: 10.3978/j.issn.2227-684X.2014.03.02