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Research article

Astrocytic expression of the RNA regulator HuR accentuates spinal cord injury in the acute phase

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HIGHLIGHTS

- Transgenic HuR in astrocytes (Tg-HuR) translocates to the cytoplasm in spinal cord injury (SCI).
- Tg-HuR mice have decreased neuronal survival in acute SCI.
- Tg-HuR mice have increased astrocyte activation and vascular permeability in acute SCI.

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ABSTRACT

We recently showed that the RNA regulator, HuR, is translocated to the cytoplasm in astrocytes in the acute phase of spinal cord injury (SCI), consistent with its activation. HuR positively modulates expression of many pro-inflammatory factors, including IL-1β, TNF-α, and MMP-12, which are present at high levels in the early phase of SCI and exacerbate tissue damage. Knockdown of HuR in astrocytes blunts expression of these factors in an *in vitro* stretch injury model of CNS trauma. In this report, we further investigate the impact of HuR in early SCI using a mouse model in which human HuR is transgenically expressed in astrocytes. At 24 h following a mid-thoracic contusion injury, transgenic HuR translocated to the cytoplasm of astrocytes, similar to endogenous HuR, and consistent with its activation. Compared to littermate controls, the transgenic mice showed a global increase in astrocyte activation at the level of injury and a concomitant increase in vascular permeability. There was a significant decrease in neuronal survival at this time interval, but no differences in white matter sparing. Long term behavioral assessments showed no difference in motor recovery. In summary, transgenic expression of HuR in astrocytes accentuated neuronal injury and other secondary features of SCI including increased vascular permeability and astrocyte activation. These findings underscore HuR as a potential therapeutic target in early SCI.

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1. Introduction

Traumatic spinal cord injury (SCI) is a severe clinical condition with an annual incidence of 20–40 cases per million in developed countries [33]. The immediate sequelae of SCI, including ischemia with infarction, edema and hemorrhage, and loss of autoregulation, lead to biochemical and pathological changes in the cord that contribute to secondary injury and clinical worsening [13,33]. A rapid inflammatory response is elicited by glial cells (astrocytes and microglia) in the vicinity of injury, characterized by the release of inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, matrix metalloproteases (e.g. 2,3, 9 and 12), nitric oxide and other free radicals [8,10]. These mediators recruit and activate additional glia and other immune cells, leading to an accumulation of toxic substances and an accentuation of vascular permeability, ischemia and edema [3,10,14,16,26,27].

HuR is an RNA regulator that binds to adenine- and uridine-rich elements (ARE) in non-coding regions of the mRNAs of many

Abbreviations: BMS, Basso Mouse Scale; GFAP, glial fibrillary acidic protein; RBP, RNA binding protein; SCI, spinal cord injury; Tg, transgenic; WT, wild type littermate controls.

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pro-inflammatory mediators that are upregulated in early SCI. While predominantly located in the nucleus, HuR translocates to the cytoplasm when activated, and transports the bound mRNA to polysomes to promote translation and RNA stabilization [1,4]. We recently showed that HuR translocates to the cytoplasm in astrocytes during the early phase of SCI. Using an *in vitro* injury model, we showed that HuR plays a positive regulatory role in astrocytic expression of inflammatory mediators detected in early SCI, including IL1-β, TNF-α and MMP-12 [19]. Given the association of these inflammatory mediators to secondary injury following SCI, we hypothesized that overexpression of HuR in astrocytes would further exacerbate the neuropathological and clinical features of SCI.

2. Materials and methods

2.1. Spinal cord contusion injury in mice

All animal procedures were reviewed and approved by the UAB Institutional Animal Care and Use Committee in compliance with the National Research Council Guide for the Care and Use of Laboratory Animals. The generation of HuR transgenic (HuR-Tg) mice is described elsewhere [35]. Eight week old, female HuR-Tg or littermate controls (WT) were administered a bilateral contusion injury at thoracic level T10 as previously described [19]. Sham control animals received laminectomies only. Sterile Ringers solution (1CC) was applied twice daily for a week as post-operative fluid replacement. Pain management consisted of subcutaneous injection of buprenorphine (0.05 mg/kg) prior to surgery and twice daily for 3 days following surgery. Bladders were manually expressed after surgery and twice daily until euthanasia.

2.2. Immunohistochemistry and western blot

Spinal cord tissue was processed and sectioned into 30 μm serial sections spaced 450 μm apart as previously described [19]. Sections were blocked with 10% goat serum in PBS before incubation with anti-Flag (F4042) antibody (1:100, Sigma, St. Louis, MO) and anti-GFAP (Z0334) antibody (1:500, Dako, Carpinteria, CA) overnight at 4° C. Sections were washed with PBS and incubated with an Alexafluor 555-conjugated secondary antibody (A-21428) for Flag-HuR and an Alexafluor 488-conjugated secondary antibody (A32731) for GFAP at 1:1000 (Thermofisher, Waltham, MA). Slides were treated with Autofluorescence Eliminator Reagent (EMD Millipore, Billerica, MA) before mounting of coverslips using ProLong Diamond Antifade Mountant with DAPI (ThermoFisher, Waltham, MA). Photomicrographs of serial sections spanning the epicenter of injury were taken and relative fluorescence intensity of GFAP immunoreactivity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD). Background was subtracted from the measured integrated density to give relative fluorescence intensity (RFI). RFI was averaged over four random high powered fields for each of four serial sections spanning 1.35 mm across the epicenter of injury. The total RFI for the four serial sections was then calculated.

2.3. Quantification of vascular permeability

Twenty-one hours after injury, 2% Evans Blue in PBS was intravenously injected into mice (8 μL/gram) and allowed to circulate for 3 h. After euthanasia, mice were exsanguinated by intracardiac perfusion of PBS over two minutes and a one cm section of spinal cord centered on the epicenter of injury was carefully removed from the spinal column. After removal of the dura, the spinal cord tissue was frozen at −80° C for 5 min and homogenized. The homogenate was added to a trichloroacetic acid (TCA) solution for a final concentration of 50% TCA and spun down at 1000 g for 30 min. Evans Blue fluorescence in the supernatant was measured at 620 nm excitation/690 nm emission and compared against a standard curve.

2.4. Neuronal counting

Nissl staining for neurons using Cresyl Violet was performed as described previously [11]. Stereo Investigator software (MBF Bioscience, Williston, VT) was used to determine stereological estimates of neuronal counts using the Optical Fractionator probe. The criteria for neuron counting consisted of Cresyl Violet stained cells greater than 10 μm in diameter with distinct cell bodies, and clearly defined nuclei/nucleoli.

2.5. White matter sparing

Serial sections were stained using eriochrome cyanine R to determine white-matter sparing in the injured spinal cord. Spinal cord serial sections were dehydrated through ascending ethanol concentrations (70%–100%), defatted in xylene, and then rehydrated in ethanol solutions in descending concentrations. Sections were stained in a 0.2% eriochrome cyanine R for 10 min and differentiated in 0.5% ammonium hydroxide for 1 min. Tracing of eriochrome cyanine R-stained tissue was performed using Stereo Investigator software (MBF Bioscience, Williston, VT) to determine the areas of the entire cord, gray matter, as well as areas of damaged white matter. Percentage white matter area was calculated against total area of the cord in four serial sections spanning 1.35 mm across the epicenter of injury (based on lowest percent white matter among the entire set of serial sections).

2.6. Basso mouse scale

Functional recovery of spinal cord injured mice was assessed weekly over a four-week period. Briefly, two independent observers naïve to the genotype of the animal scored hind-limb locomotor ability of injured mice in an open-field for four minutes using the Basso Mouse Scale as described previously [5].

2.7. Statistics

Statistical significance was determined by two-tailed Student's *t*-test using Graphpad Prism 7 Software (Graphpad Software, La Jolla, CA).

3. Results

3.1. SCI induces translocation of transgenic HuR to the cytoplasm in astrocytes

We previously developed a transgenic mouse in which Flag-tagged human HuR is expressed in astrocytes under the control of the GFAP promoter [35]. The Flag fusion protein is detected in astrocytes throughout the spinal cord of the HuR transgenic (HuR-Tg) mouse and, as with endogenous HuR, is predominantly nuclear in location. In our previous work, we showed that HuR translocates from the nucleus to the cytoplasm in astrocytes following SCI consistent with its activation [1,4,6,19]. To determine whether transgenically expressed HuR recapitulates this pattern, we subjected HuR-Tg mice to a mid-thoracic contusion SCI and subsequently assessed the intracellular location of Flag-HuR (Fig. 1). In sham-injured mice, Flag-HuR was predominantly nuclear in location. At 24 h post SCI, there was translocation of Flag-HuR to the cytoplasm, as gauged by a strongly merged signal with GFAP, in the epicenter of injury. At levels rostral or caudal to the injury,