Neurotoxicology and Teratology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Arsenic exposure during embryonic development alters the expression of the long noncoding RNA growth arrest specific-5 (Gas5) in a sex-dependent manner

Kevin K. Caldwell, Alexander Hafez, Elizabeth Solomon, Matthew Cunningham, Andrea M. Allan*

Department of Neurosciences, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, United States

ARTICLE INFO

Keywords:
Development
Brain
Arsenic
lncRNA
Gas5
Glucocorticoid
Sex differences

ABSTRACT

Our previous studies suggest that prenatal arsenic exposure (50 ppb) modifies epigenetic control of the programming of the glucocorticoid receptor (GR) signaling system in the developing mouse brain. These deficits may lead to long-lasting consequences, including deficits in learning and memory, increased depressive-like behaviors, and an altered set-point of GR feedback throughout life. To understand the arsenic-induced changes within the GR system, we assessed the impact of *in utero* arsenic exposure on the levels of the GR and growth arrest-specific-5 (Gas5), a noncoding RNA, across a key gestational period for GR programming (gestational days, GD 14–18) in mice. Gas5 contains a glucocorticoid response element (GRE)-like sequence that binds the GR, thereby decreasing GR-GRE-dependent gene transcription and potentially altering GR programming. Prenatal arsenic exposure resulted in sex-dependent and age-dependent shifts in the levels of GR and Gas5 expression in fetal telencephalon. Nuclear GR levels were reduced in males, but unchanged in females, at all gestational time points tested. Total cellular Gas5 levels were lower in arsenic-exposed males with no changes seen in arsenic-exposed females at GD16 and 18. An increase in total cellular Gas-5 along with increased nuclear levels in GD14 arsenic-exposed females, suggests a differential regulation of cellular compartmentalization of Gas5. RIP assays revealed reduced Gas5 associated with the GR on GD14 in the nuclear fraction prepared from arsenic-exposed males and females. This decrease in levels of GR-Gas5 binding continued only in the females at GD18. Thus, nuclear GR signaling potential is decreased in prenatal arsenic-exposed males, while it is increased or maintained at levels approaching normal in prenatal arsenic-exposed females. These findings suggest that females, but not males, exposed to arsenic are able to regulate the levels of nuclear free GR by altering Gas5 levels, thereby keeping GR nuclear signaling closer to control (unexposed) levels.

1. Introduction

Arsenic readily crosses the placenta during development (Ahmed et al., 2011; He et al., 2007; Vahter, 2009) and all forms of arsenic, including inorganic and methylated arsenicals, accumulate in the brain (Sanchez et al., 2010). Several epidemiological studies have demonstrated that arsenic exposure is associated with neurological and cognitive deficits (Carroll et al., 2017; Edwards et al., 2014; Gong et al., 2011; Hsieh et al., 2014; Parajuli et al., 2015; Rodrigues et al., 2016; Roy et al., 2011; Yorifuji et al., 2016). Many of the cognitive deficits seen following arsenic exposure may be mediated by the glucocorticoid receptor (GR) and hypothalamic pituitary adrenal (HPA) signaling system (Kino, 2015; Maccari et al., 2014; Tyler and Allan, 2014; Wolf et al., 2016). In our previous work, we observed significant cognitive deficits, which corresponded with alterations in GR signaling, in mice that were exposed to arsenic throughout gestation and during the early postnatal period (Allan et al., 2015; Caldwell et al., 2015; Goggin et al., 2012; Martinez-Finley et al., 2009; Martinez et al., 2008; Tyler and Allan, 2013; Tyler et al., 2015). We have shown that exposure to 50 ppb arsenic, a concentration that is moderately higher than the EPA recommended limit of 10 ppb, affects glucocorticoid signaling including reduced expression of GR in both the embryonic and adult mouse brain (Allan et al., 2015; Caldwell et al., 2015; Goggin et al., 2012), suggesting that arsenic disrupts the fetal programming of the GR system. Aberrant GR programming during development has lasting effects into adulthood, including increased susceptibility to psychiatric disorders (Maccari et al., 2014; Nemoda and Szyf, 2017; Zierold et al., 2004). Most of our published studies using this model of prenatal arsenic exposure have used males. However, we recently reported that sex-specific effects within the glucocorticoid signaling system in fetal placenta

* Corresponding author.
E-mail address: aallan@salud.unm.edu (A.M. Allan).

https://doi.org/10.1016/j.ntt.2017.11.003
Received 25 July 2017; Received in revised form 9 November 2017; Accepted 9 November 2017

and brain (Caldwell et al., 2015). In an effort to identify mechanisms through which arsenic exerts these actions, we conducted a series of studies assessing the effects of arsenic on regulators of GR signaling in the embryonic brain, focusing on noncoding RNAs.

Noncoding RNAs are functional RNA molecules that control gene expression and are not thought to be translated into proteins; however, recent studies suggest that some lncRNAs may be translated into small polypeptides (Cohen, 2014; Makarewich and Olson, 2017). These molecules regulate gene expression at both the transcriptional and post-transcriptional level (Chen and Xue, 2016). Examples of noncoding RNAs include microRNAs, which act as RNA silencers by base-pairing with a complementary sequence on mRNA, small nucleolar RNAs (snoRNAs), which guide chemical modifications of other RNAs, and long noncoding RNAs (lncRNAs), which are distinguished from other noncoding RNAs by having a transcript length > 200 nucleotides (He and Hannon, 2004). While more than one-fifth of the genome has been determined to consist of these new classes of RNA molecules, the physiologic roles of most of them are still unknown (Kapranov et al., 2007). The abundance of these noncoding RNA molecules, combined with data suggesting that certain types of noncoding RNAs are highly conserved throughout species, suggests that they may play important roles as epigenetic gene regulators (Johnsson et al., 2014).

Growth arrest-specific-5 (Gas5) is a lnc RNA which has been implicated in a variety of cellular processes including cell cycle control and growth arrest (Liu et al., 2015; Mourtada-Maarabouni et al., 2008; Schneider et al., 1988) and apoptosis (Hudson et al., 2014; Mourtada-Maarabouni et al., 2008; Mourtada-Maarabouni et al., 2009; Pickard et al., 2013). Gas5 expression has been shown to arrest cells in the G1-S phase of growth, suggesting that it may act as a cell cycle regulator (Guo et al., 2015). Prolonged growth arrest in cells with high levels of Gas5 has been shown to cause a decrease in the transcription of the inhibitor of apoptosis 2 (IAP2) gene, thereby sensitizing cells to apoptosis (Kino et al., 2010). It is important to note that the IAP2 gene contains a glucocorticoid response element (GRE) site within its promoter and its expression is induced by dexamethasone (Webster et al., 2002). Gas5 reduces GR-dependent transcription by forming a double helical, hairpin structure which mimics a GRE site, and which competes with GREs for binding to the DNA binding domain of the GR (Hudson et al., 2014; Kino et al., 2010; Tani et al., 2013). Gas5 also is capable of binding to and blocking the transcriptional activity of the mineralocorticoid, progesterone and androgen, but not estrogen, receptors (Hudson et al., 2014; Kino et al., 2010). In addition to binding steroid hormone receptors, Gas5 is a multiple snoRNA host gene that encodes 9 (10 in humans) C/D box snoRNAs within 11 introns (Smith and Steitz, 1998). Recently, He et al. (He et al., 2015) reported that, in humans, five of these snoRNAs contain sequences that appear to be PIWI-interacting RNAs (piRNAs). Thus, there are several likely sources of transcriptional and translational regulation contained within the Gas5 gene.

Gas5 expression is detected as early as the 8-cell stage of mouse development (Fleming et al., 1997) and continues throughout embryogenesis and fetal development with relatively high expression in the developing brain (Coccia et al., 1992). Fetal mouse Gas5 expression peaks at gestational day (GD) 16 (Coccia et al., 1992). In the adult mouse, Gas5 expression is high in brain and low in liver and spleen (Coccia et al., 1992). Morellini and colleagues (Meier et al., 2010) have shown that Gas5 is expressed in the hippocampus at higher levels than found throughout the rest of the brain (Meier et al., 2010).

The role of Gas5 as a regulator of GR-associated transcription suggests that it may play a significant role in the programming of the HPA axis during development and throughout life. Programming of the HPA axis is believed to be most important during the second trimester (~GD10–19 in the mouse) of fetal development (Huang et al., 2012; Reichardt and Schutz, 1996). Gas5 expression has been shown to be increased during periods of psychogenic stress, suggesting that Gas5 is also a likely regulator of HPA axis programming postnatally (Meier et al., 2010).

The role of lncRNAs in prenatal arsenic-mediated changes in GR programming has not been explored. The goal of the present studies was to assess the impact of prenatal arsenic exposure during fetal brain development on both Gas5 and GR levels to determine if changes in the lncRNA may be responsible for altered GR programming following *in utero* exposure to arsenic.

2. Materials and methods

2.1. Prenatal arsenic exposure model

All procedures involving animals, including the prenatal arsenic exposure model, were approved by the Institutional Animal Care and Use Committee at the University of New Mexico and followed the National Institutes of Health Guide for the care and use of laboratory animals (8th edition, 2011). Male and female C57BL/6J mice were obtained from Jackson Laboratory and were raised in a reverse light/dark cycle with lights on at 20:00 and off at 08:00 daily. Female animals were exposed to arsenic in their drinking water, as previously described (Martinez et al., 2008; Tyler et al., 2014). Prior to mating, female mice were acclimated to arsenic exposure by drinking 50 parts-per-billion (ppb) arsenic (sodium arsenate, Sigma Aldrich) water, prepared using standard tap water. Control mice were provided with tap water, which contained approximately 5 ppb arsenic. Mating occurred over a single 8 h period during the dark cycle. After mating, females were maintained on arsenic water throughout pregnancy. Dams were decapitated, fetuses were removed and the telencephalons were dissected, snap frozen in liquid nitrogen and stored at − 80 °C at GD14, 16 or 18. Mice were euthanized between 0800 and 1000 h.

2.2. Sex determination qPCR analysis

Fetal sex was determined by measuring genomic DNA (gDNA) expression of the Sry gene. Sry gene primers were purchased from Invitrogen (cat: A156612) with the following sequences: forward: GCTGGGATGCAGGTGGAAAA; reverse: CCCTCCGATGAGGCTGATATT (PrimerBank ID: 6766761a1). qPCR was carried out in duplicate using Roche FastStart Universal SYBR Green Master (ROX) (cat: 04913850001, Roche Diagnostics) following the manufacturer's suggested protocol. Sex was determined by comparative analysis of Ct values (Tyler et al., 2017).

2.3. Tissue extraction for protein quantification

Fetal telencephalons were homogenized according to previously established protocols (Buckley and Caldwell, 2003). Individual telencephalons were homogenized in a Kontes RNase-Free Pellet Pestle Grinder (cat: KT749520-0090, VWR) in 100 µL ice cold homogenization buffer (HB) (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 320 mM sucrose, 1:1000 protease inhibitor cocktail (Sigma P8340), 200 µM sodium orthovanadate). Samples were centrifuged at 1000 × g_{max} for 6 min, the supernatant was removed, the pellet was resuspended in 75 µL of HB and spun again at 1000 × g_{max} for 10 min. The supernatant was removed, combined with the first supernatant and frozen at − 80 °C; the preparation was designated the post-nuclear lysate (PNL) and contains cytosolic and membrane proteins. The pellet was resuspended in extraction buffer (EB) (HB containing 75 mM NaCl, 75 mM KCl and 1% v/v Triton X-100). Samples were sonicated (2 × 15 s) in ice then kept in ice for 20 min. Samples were centrifuged at 15,000 × g_{max} for 15 min and the supernatant was collected as the soluble nuclear (nuc) fraction, and frozen at − 80 °C. Protein concentration was determined using the Qubit® Protein Assay Kit following the manual method (cat: Q33212, ThermoFisher Scientific) on a Qubit® 2.0 Fluorometer.