Gene 536 (2014) 326–331

Contents lists available at ScienceDirect

Gene

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

Arsenic programmes cellular genomic-immunity through miR-2909 RNomics

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ARTICLE INFO

Article history:
Accepted 2 December 2013
Available online 18 December 2013

Keywords:
miR-2909
Gene regulation
APOBEC3G
IFNβ
Viral-RNA sensing

ABSTRACT

It is widely recognized that human cells are equipped with innate antiviral-RNA armour involving the production of type I interferons and APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G) gene-product. Although arsenic has been shown to have paradoxical effect on one arm of this armour involving APOBEC3G, the exact molecular mechanism of its action in this regard is far from clear. The present study, addressed to explore as to how arsenic programmes this innate antiviral-RNA cellular-sensing pathway, clearly revealed that arsenic programmes this innate cellular antiviral genomic response through its inherent capacity to initiate cellular miR-2909 RNomics pathway, involving not only the modulation of APOBEC3G gene but also KLF4 (Kruppel-like factor 4) dependent regulation of gene coding for IKBKε (Inhibitor of nuclear factor kappa-B kinase subunit epsilon) which in turn modulates RIG-I (retinoic acid-inducible gene 1) pathway responsible for the production of IFNβ (interferon beta) through restriction of CYLD (Cylindromatosis) deubiqutinating activity. This restricted inhibitory enzyme activity of CYLD upon NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) also ensures sustained expression of miR-2909. Our results for the first time show that cellular miR-2909 RNomics may constitute an innate genomic armour to promote as well as restrict retroviral infection.

1. Introduction

Extensive studies have shown that host microRNAs (miRNAs) regulate fundamental genetic programmes responsible for providing cells with sensing mechanism against invading RNA viruses (tenOever, 2013). This cellular sensing mechanism has been widely recognized to be provided by both the RIG-I/MAVS pathway involving type-1 interferons (Kato et al., 2005; Rehwinkel et al., 2010) and the induction of APOBEC3G gene product that lethally hyper mutates viral RNA (Russell et al., 2009). In this context, it is interesting to note that the tumour suppressor 'CYLD', a deubiqutinating enzyme has not only been shown to inhibit the IRF3 (Interferon regulatory factor 3) signalling pathway and type-1 interferon production triggered by RIG-I (Friedman et al., 2008) but its expression was also found to be down-regulated upon viral infection. Interestingly, ability of CYLD to deubiquitinate and consequently down-regulate RIG-I is restricted by its own phosphorylation via IKBKε (Zhang et al., 2008). Recently arsenic trioxide, used to treat acute promyelocytic leukaemia, was shown to counteract the APOBEC3G mediated restriction of retroviral (HIV-1, in particular) infection in dendritic cells but not in the more permissive $CD4^+$ T cells (Chou et al., 2005; Stalder et al., 2010). Although CYLD is documented to inhibit NFkB binding activity (Kovalenko et al., 2003), arsenic is known to promote such an activity in order to regulate the expression of its various target proto-oncogenes (Liao et al., 2004). A recent study from our lab suggests a novel epigenetic mechanism of oncogenesis involving arsenic trioxide dependent miR-2909 RNomics (Sharma et al., 2013). Also, it was shown that chronic exposure to low dose arsenic significantly altered the gene and protein expression of many regulators of innate immunity and led to a compromised response to a subsequent immune challenge (Kozul et al., 2009).

Keeping in view all the above mentioned features of arsenic biology, the present study was addressed to understand the detailed mechanism through which arsenic could programme the genomic immunity involving the interplay between arsenic-induced cellular miR-2909 expression and the genes involved in the regulatory pathway responsible for the generation of type I interferons and APOBEC3G expression.

2. Materials & methods

2.1. Bioinformatics analysis

Promoter sequences for relevant genes were obtained from Mammalian Promoter sequence database (http://rulai.cshl.edu/CSHLmpd2/). Putative binding sites for various transcription factors were retrieved from JASPAR database (http://jaspar.cgb.ki.se/) at a default threshold

Abbreviations: miRNA, MicroRNA; PBMCs, Peripheral blood mononuclear cells; CYLD, Cylindromatosis; KLF4, Kruppel-like factor4; NFκB, Nuclear factor kappa B; FCS, Foetal Calf Serum; As, Arsenic; IFNβ, Interferon beta; APOBEC3G, Apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G; IKBKε, Inhibitor of nuclear factor kappa-B kinase subunit epsilon; RIG1, Retinoic acid-inducible gene 1; IRF3, Interferon regulatory factor 3.

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http://dx.doi.org/10.1016/j.gene.2013.12.004

score of 85.0. The presence of putative target site of miR-2909 on 3′UTR of KLF-4 gene was explored bioinformatically using RNA Hybrid tool (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/).

2.2. Cellular model employed

Human peripheral blood mononuclear cells (PBMCs) were obtained from 25 normal healthy volunteers (with their prior informed consent), who were fasting for 12 h or had abstained from any medication for 2 weeks before blood donation and subsequently these cells were cultured in vitro as per the standard procedure reported by us earlier (Sharma et al., 2013).

2.3. Cell transfection studies

miR-2909 knock-down PBMCs were generated by transfecting mercury LNA™ miR-2909 inhibitor (EXIQON) using lipofectamine transfection reagent (Invitrogen) and subsequently these PBMCs were exposed to medium enriched with or without sodium arsenite (2 μM) and incubated for 36 h at 37 °C in 5% CO_2 atmosphere. KLF4 expression plasmid (Addgene plasmid 17967) (Lowry et al., 2008) was transfected into PBMCs using escort transfection reagent (Sigma) and incubated for 48 h at 37 °C in 5% CO_2 atmosphere. At the end of incubation period, the cells from each well were processed for RNA and protein isolation.

2.4. Gene expression analysis

The isolated RNA from each culture-well was subjected to expression analysis of genes coding for miR-2909, KLF4, APOBEC3G, IFNβ and IKBKε using gene-specific primers and Real-Time PCR method. U6 and β actin were used as invariant controls for the expression analysis of miR-2909 and other genes respectively. The isolated proteins from each culture well were subjected to Western-blotting followed by immuno-detection using specific antibodies against APOBEC3G, KLF4, CYLD and β actin (used as an invariant control). The densitometry scanning of bands was done using scion image analysis software.

2.5. Promoter activity

In another set of experiments, KLF4 transcription factor binding site on IKBKε gene promoter was amplified by PCR and subsequently cloned into pBlueTOPO reporter vector (Invitrogen). This vector was transfected into PBMCs and then these cells were exposed to medium enriched with or without sodium arsenite (2 μM) and incubated for 48 h at 37 °C under 5% CO_2 atmosphere. At the end of this incubation period, the cells from each well were processed for the β-galactosidase activity by assay kit (Invitrogen).

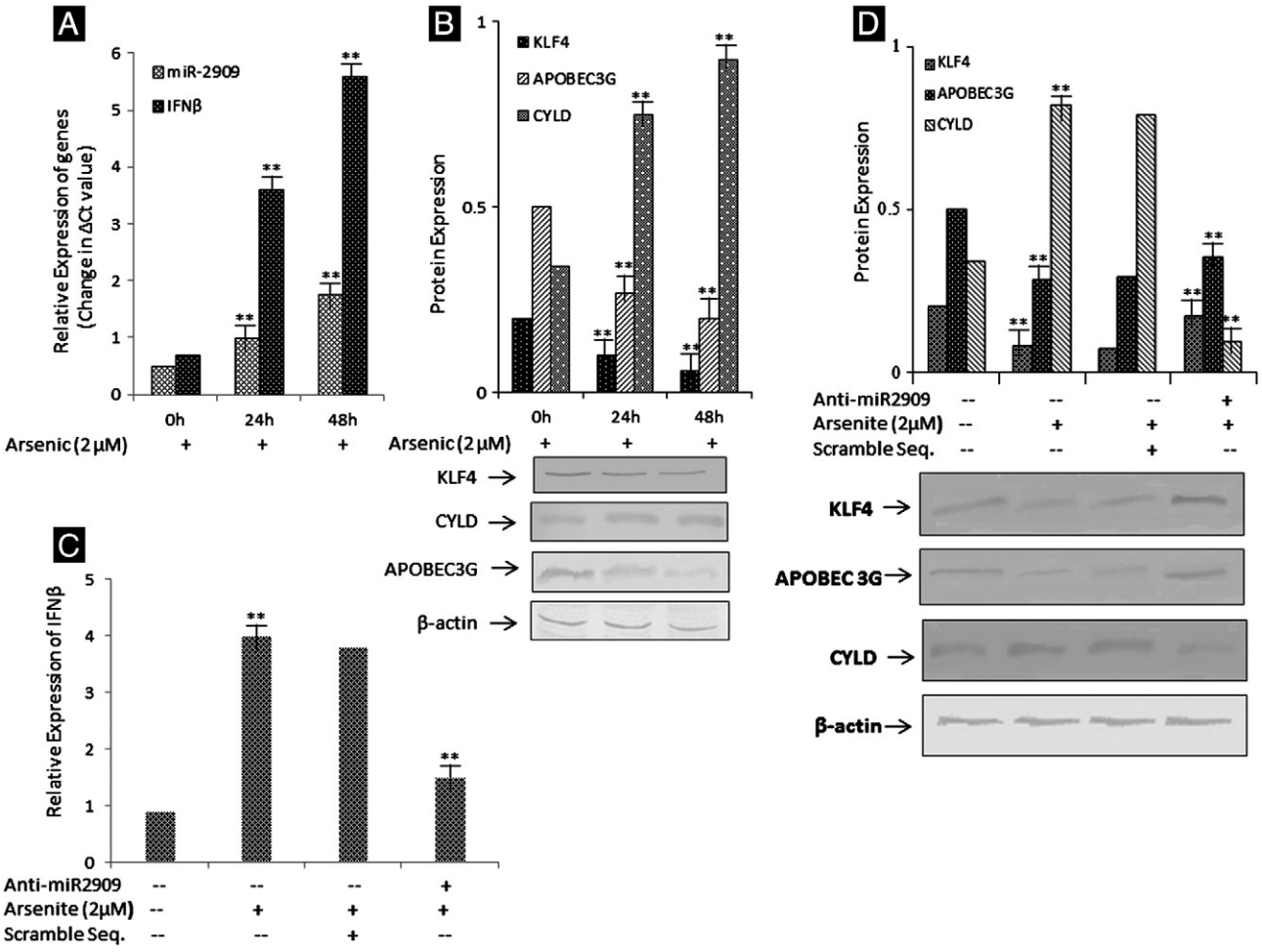

Fig. 1. Time dependent expression of genes coding for miR-2909 and IFNβ (A), CYLD and APOBEC3G and KLF4 (B) within human PBMCs exposed to sodium arsenite (2 μM) for 48 h in vitro culture. '0' hour in each group represents the basal expression level of indicated genes of untreated cells. Expression of genes coding for IFNβ (C), APOBEC3G, CYLD and KLF4 (D) at the translational level within human PBMCs transfected with either antigo-miR-2909 or its scrambled sequence and subsequently exposed to sodium arsenite (2 μM) in vitro culture for 36 h. Each bar represents mean ± SD of experiments done in triplicate. Statistical significance is shown as $^{**}p < 0.01$.