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miR-22 contributes to endosulfan-induced endothelial dysfunction by targeting SRF in HUVECs

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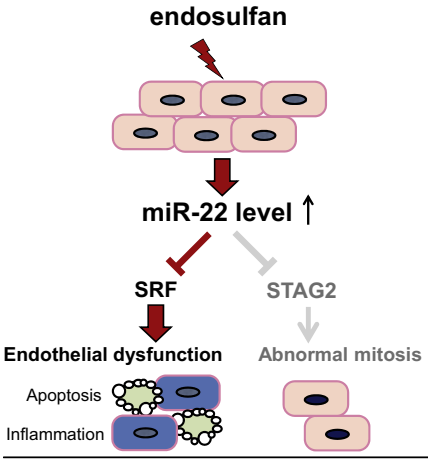
HIGHLIGHTS

- miR-22 was upregulated by endosulfan in HUVECs.
- Anti-miR-22 attenuated endosulfan-induced endothelial dysfunction.
- SRF and STAG2 were novel direct targets of miR-22.
- SRF siRNAs caused apoptosis and inflammation.
- Endosulfan suppressed SRF protein expression.

GRAPHICAL ABSTRACT



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ABSTRACT

microRNAs (miRNAs) function in the posttranscriptional gene regulation, providing new insights into the epigenetic mechanism of toxicity induced by environmental pollutants. miR-22 was discovered to regulate cell proliferation and apoptosis in response to environmental toxicants. We have reported that endosulfan can cause endothelial toxicity in human umbilical vein endothelial cells (HUVECs). In the present study, we investigated the involvement of miR-22 in endosulfan-induced endothelial dysfunction. The expression level of miR-22 was increased in a dose-dependent manner by endosulfan exposure. Overexpression of miR-22 induced apoptosis and inflammation in HUVECs. Anti-miR-22 transfection significantly attenuated the increase in the percentage of apoptotic cells, caspase-3 activity and Interleukin (IL)-6, 8 mRNA levels in endosulfan-exposed HUVECs. Luciferase reporter assay confirmed that SRF and STAG2 were novel direct targets of miR-22. Endosulfan decreased mRNA expression of both SRF and STAG2, but only suppressed protein expression of SRF. Knockdown of SRF via siRNAs resulted in apoptosis and inflammation whereas STAG2 siRNAs only caused abnormal mitosis in

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HUVECs. Taken together, these findings will shed light on the role and mechanism of miR-22 in endosulfan-induced endothelial dysfunction via SRF in HUVECs.

1. Introduction

Endosulfan is one of the representative organochlorine pesticides (OCPs) commonly used in agriculture, which was classified into persistent organic pollutants (POPs) by the Stockholm Convention in 2011 (Weber et al., 2010). With long-term use of the pesticides, endosulfan has a widespread distribution in the environment (Jia et al., 2009). In fact, endosulfan has been detected in human blood, urine and even umbilical cord blood (Damgaard et al., 2006; Shen et al., 2007). Therefore, endosulfan is responsible for adverse effects on human health associated with a variety of human diseases including cardiovascular diseases.

Endothelial dysfunction has been shown to be predictive of adverse cardiovascular events (Tang et al., 2014), characterized by endothelial cell apoptosis and inflammation response. Recently, it is reported that endosulfan exposure impaired vascular tissue in rats and caused mitochondrial damage in endothelial cells of rats due to oxidative stress and inflammation (Zhang et al., 2015a). Our previous study showed that endosulfan could induce endothelial dysfunction by inhibition of cell growth and induction of inflammation in human umbilical vein endothelial cells (HUVECs) (Li et al., 2016). We also utilized gene expression profile analysis to reveal the genetic mechanism in endothelial toxicity of endosulfan and potential relevant disease outcomes (Xu et al., 2016). However, it is still unknown what is the epigenetic control of gene expression when exposed to endosulfan.

microRNAs (miRNAs) are an abundant class of small noncoding RNAs and function as post-transcriptional regulators by base-pairing with the complementary sites in the 3′-untranslated region (3′-UTR) of the mRNA. miR-22 was originally identified from HeLa cells as a 22-nucleotide miRNA, and was subsequently shown to be ubiquitously expressed in various tissues (Lagos-Quintana et al., 2001; Neely et al., 2006). miR-22 has been connected to a great number of activities that encompass tumorigenesis, epigenetic modification, skeletal metabolism, senescence and so on (Iliopoulos et al., 2008; Jazbutyte et al., 2013; Liu et al., 2010; Xu et al., 2011). Recently, miR-22 has been implicated in cardiac pathology and vascular diseases (Dong and Yang, 2011; Huang and Wang, 2014; Qin and Zhang, 2011; Urbich et al., 2008). It is reported that miR-22 was prominently upregulated during cellular senescence and aging, involved in age-associated cardiac changes, such as cardiac fibrosis (Jazbutyte et al., 2013; Xu et al., 2011). miR-22 functions as an integrator of Ca^{2+} homeostasis and myofibrillar protein content during stress in the heart (Gurha et al., 2012). Microarray analysis of miRNA expression profiles showed that miR-22 was highly expressed in the peripheral blood of coronary heart disease patients and high-risk patients (Chen et al., 2015), implying the possible role of miR-22 in cardiovascular diseases. Endothelial dysfunction is associated with a variety of cardiovascular diseases, such as atherosclerosis and hypertension. However, the underlying mechanism of miR-22 in endothelial function was still obscure.

Serum response factor (SRF) is a transcription factor that regulates the activity of many genes, and thereby participates in cell proliferation, apoptosis and cytoskeleton integrity in different cell types (Schratt et al., 2002, 2004). SRF is a key endothelial cell regulator and contributes to endothelial dysfunction (Chen et al., 2015). HUVECs have considerably been used for the investigation of endothelial dysfunction (Chen et al., 2015; Zhang et al., 2015b). In the present study, we provide evidence that miR-22 acts as a key regulator in endosulfan-induced apoptosis and inflammation in HUVECs. Furthermore, we identified that SRF was a novel target gene for miR-22 by luciferase reporter assay and knockdown of SRF could cause endothelial dysfunction. This study provides new insights into the miR-22-mediated mechanism in endosulfan-induced endothelial dysfunction.

2. Materials and methods

2.1. Cell culture and endosulfan exposure

HUVECs (ATCC, Manassas, VA, USA) were cultured in RPMI-1640 medium (KeyGen, Nanjing, China) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in culture incubator with 5% CO_2. Endosulfan exposure experiments were performed as previously described (Li et al., 2016). Endosulfan (Jiangsu Anpon Electrochemical Co., Huaian, China) caused cytotoxicity in HUVECs at the concentrations of 20, 40 and 60 μM within the detected concentration range of endosulfan (0.69–176.2 μg/ml) in human blood of people exposed to endosulfan (Singh et al., 2007).

2.2. Transient miRNA/siRNA transfection

Negative control siRNA (NC) were purchased from Invitrogen (Carlsbad, CA, USA). siRNAs targeting SRF and STAG2, pre-miR-22 and anti-miR-22 were obtained from Biomics (Nantong, China). Cells were transfected with 10 nM siRNA or 40 nM miRNA using LipofectamineRNAiMax (Invitrogen) according to the manufacturer's protocol.

2.3. Apoptosis analysis

Cells were stained with Annexin-V-FITC and PI according to the manufacturer's protocol of the Annexin V-FITC Apoptosis Detection Kit (KeyGen). The fluorescence intensity of cells was evaluated by flow cytometry (BD Biosciences, San Jose, CA, USA) using quadrant statistics for necrotic and apoptotic cell populations. PI was used for the detection of late apoptosis and necrosis, and Annexin-V was consumed for the detection of early and late apoptosis.

Caspase-3 activity was measured using the Caspase-3 Colorimetric Assay Kit (KeyGen) according to the manufacturer's protocol. Briefly, cell lysates were prepared in cell lysis buffer. Total protein was quantified by the Bradford method (Sangon, Shanghai, China). The protein lysate was mixed with the reaction buffer and incubated at 37 °C for 4 h in the dark. The colour developed was measured at 405 nm using a microplate reader (SpectraMax M5, Molecular Devices, CA, USA).

2.4. Real-time qPCR

Total RNA was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The expressions of miRNAs and mRNAs were quantified by TaqMan miRNA assays (Applied Biosystems, Foster City, CA, USA) and SYBR green (Invitrogen), respectively. Real-time qRT-PCR was performed using an ABI PRISM 7300 system (Applied Biosystems). qRT-PCR reactions were performed in triplicate from all the samples in different groups. Primers used were listed in Table S1. The relative