Aberrant expression of TNRC6a and miR-21 during myocardial infarction

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Abstract

In the present study, aberrant expression of trinucleotide repeat-containing gene 6a (TNRC6a) and miR-21 was noted and documented in rat myocardial infarction. Briefly, Sprague–Dawley rat model was used for the development of myocardial infarction. Experiments such as histological analysis were carried out to confirm the histopathology of the myocardial infarction. The expression profile of TNRC6a and miR-21 was identified by using quantitative real-time PCR. In addition, immunoblotting was performed to validate the expression profile of TNRC6a and phosphatase and tensin homolog (PTEN). The histological analysis confirmed the progress of myocardial infarction in rat model. As the disease progresses, the protein TNRC6a expresses abnormally which in turn up-regulates the miR-21 after 3rd and 5th week of infarction. Interestingly, miR-21 binds with its specific target genes PTEN and thereby degrades the target mRNA; as a result, its expression was down-regulated progressively and paved the development of myocardial infarction. The present study concludes that the aberrant expression of TNRC6a and miR-21 was documented during myocardial infarction. These findings play an important role in the diagnosis as well as pave a way for the development of drug targets for treating myocardial infarction.

Keywords Myocardial infarction · TNRC6a · miR-21 · PTEN · qRT-PCR · Immunoblotting

Introduction

Cardiovascular disease is a most commonly occurring myocardial infarction, considered as a major health problem globally due to its high morbidity and mortality rate (White and Chew 2008). Myocardial infarction is a myocardial necrosis entrenched by rigorous hypoxia–ischemia of coronary artery which finally leads to heart failure (Lisowska et al. 2016; Davies 2016). A survey report indicates that around 15 and 20 million people will be affected by myocardial infarction during the year 2020 and 2030, respectively (Moran et al. 2010). Therefore, early and accurate diagnosis as well as identifying the molecular mechanisms of myocardial infarction is important to reduce the progression of myocardial infarction and thereby increase the endurance of the patients.

MicroRNAs (miRNAs) are small non-coding RNA that regulates the expression of the target genes (Yu and Li 2015). These miRNA binds with the analogous sequence in the target mRNA and thereby suppresses or modifies the expression of the protein which in turn involved in the progression of diseases (Bartel 2009). Abnormal expressions of these miRNAs were observed in the patients suffering from cancer (Li et al. 2015a, b; Yang et al. 2014; Niu et al. 2015), acute myocardial infarction (Wang et al. 2010), coronary artery disease (Fichtlscherer et al. 2010), acute coronary syndrome (Widera et al. 2011), etc. Many studies implicated the association of miRNA in various cardiovascular diseases.

Aberrant elevation of miR-208 (Ji et al. 2009), miR-499 (Olivieri et al. 2013) and miR-1 (Cheng et al. 2010) was found to be associated with acute myocardial infarction. It has been illustrated that miR-29 inhibits the synthesis of collagen and is involved in the progression of myocardial infarction (Van Rooij et al. 2008). Reports confirm the up-regulation of miR-21 in the blood of atherosclerosis patients (Li et al. 2015a, b). But the actual mechanism relying on the up-regulation of miRNA was still unclear. A recent study identified that a protein trinucleotide repeat-containing gene

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6a (TNRC6a) which is a module of posttranscriptional gene silencing miR machinery is responsible for the up-regulation of miRNAs (Jessica et al. 2018). Hence, the current study specifically aimed to examine the abnormal expression of TNRC6a and miR-21 in myocardial infarction.

Materials and methods

Animal model

The experiments were carried out in SPF grade Sprague–Dawley (SD) adult rat model with prior approval from the institutional animal and ethical committee board. For this experiment, 40 healthy SD rats (20 males and 20 females) with age group 8–10 weeks and body weight ranging between 200 and 250 g were used. The rats were fed freely and maintained at normal room temperature for 1 week prior to testing.

Establishment of myocardial infarction model

Animals were anesthetized intraperitoneally using 10% chloral hydrate. Tracheal intubation was carried out before exposing the heart. Myocardial infarction was established by ligating the descending branch of the left anterior coronary artery, whereas in sham group, the rats were subjected to surgery, but ligation was not carried out. The rats were randomly allotted into four groups ($N=10$). The myocardial infarction-established rats were killed at 1st, 3rd and 5th week, and the tissue samples obtained were subjected for histology, RNA extraction, qPCR and immunoblot analysis.

Histological analysis

Heart tissue samples were dissected and washed with normal saline followed by formaldehyde fixation and paraffin embedded as per standard protocols. Thin sections of 4 μm each were deparaffinized using xylene and hydrated. The sections were then stained using hematoxylin followed by rehydration and stained using cytoplasmic stain eosin. The sections were then mounted and observed under light microscope and documented.

Isolation of RNA from cardiac tissues

Total RNA was isolated from the myocardial tissues using TRIzol reagent as per standard protocol. The integrity as well as the purity of the RNA was analyzed by NanoDrop and agarose gel electrophoresis. The RNA samples were stored at $-80\text{ }^{\circ}\text{C}$ for further experimental analysis.

Analyzing the target gene for miR-21

The target of miR-21 was predicted using Bioinformatics approaches. The prediction of target gene was carried out using three databases such as TargetScan (www.targetscan.org), miRTarBase (www.mirtarbase.mbc.nctu.edu.tw/php) and miRDB (www.mirdb.org).

Quantitative RT-PCR for TNRC6a and miR-21

Quantitative RT-PCR for TNRC6a and miR-21 was performed using mirVana qRT-PCR miRNA Detection Kit (P/N: 1556, Ambion). RT-PCR for TNRC6a and miR-21 was carried out using standard procedure (Chen et al. 2005). From the RNA samples, cDNA was transcribed at $37\text{ }^{\circ}\text{C}$ for 30 min followed by inactivating the reverse transcriptase at $95\text{ }^{\circ}\text{C}$ for 10 min. Quantitative RT-PCR was executed with threshold cycle (CT), and the number of cycles exceeds with fluorescence with a fixed threshold of 0.2. Quantitative TNRC6a and miR-21 expression was analyzed using Ct methods ($2^{-\Delta\Delta\text{CT}}$), where $\Delta\Delta\text{CT} = [\text{CT}(\text{miR-24/miR-34/TGF-}\beta 1/\text{E2F3}) - \text{CT}(5\text{S rRNA})]$ (Schmittgen et al. 2004). The experiments were repeated thrice to obtain accurate data. Primers used for qRT-PCR analysis are shown in Table 1.

Immunoblot analysis of TNRC6a and PTEN

Protein samples were prepared using mirVana PariS Kit. Proteins samples were estimated using Lowry's method of protein estimation. Protein lysate was resolved in 12% SDS-PAGE and transferred to PVDF membrane followed by blocking using 5% BSA (Cat. No. 05470, Sigma-Aldrich). The PVDF membrane was incubated with anti-TNRC6a (Cat. No. HPA017869) and anti-PTEN (Cat. No. P3487) primary antibodies. Non-specific binding was washed using

Table 1 Primer sequences

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
TNRC6a	TCACATCATATCACATTGCCAGG	TATGGTTGTCTTGCTCTCTGTCTC
miR-21	TGTAACAGACACTCCATGTGG	GCTGTCAACAGTACGCTACG
U6	ATTGGAACGATACAGAGAAGAT	GGAACGCTTCACGAATTTG
β -Actin	AACAGTCCGCCTAGAAGCAC	CGTTGACATCCGTAAAGA

PBST followed by incubated using secondary antibodies. The membranes were once again washed in PBST. The signals obtained were detected using chemiluminescent detection system. β -Actin was used as a loading control.

Statistical analysis

Statistical analyses were performed using SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL, USA). Measurement data were expressed as mean $\pm$ standard deviation. The intensity of bands obtained via immunoblot was analyzed by scanning in gel documentation system (Bio-Rad, USA). Statistical significance was evaluated using *t* test, which was performed by measuring the mean intensity of the immunoblot using Bio-Rad imaging software. All the experiments were carried out in triplicates, and the level of significance was set at $P < 0.05$.

Results

Development of myocardial infarction in rat model

Abnormal variations were observed in rat myocardial tissue samples within 5th week of cardiac infarction (Fig. 1).

Cellular arrangement with clear and pointed muscle stripes was noticed in the control tissue samples (Fig. 1a). Disappearance and uneven arrangement of cells in the boundary of myocardial tissues were noticed at 1st and 3rd week of myocardial infarction (Fig. 1b, c). After 5th week, patchy and clumsy arrangement of tissues along with widening of intra-myocardial cleft was observed and is shown in Fig. 1d.

Up-regulation of TNRC6a and miR-21 in myocardial infarction

Quantitative RT-PCR analysis showed an elevated expression of TNRC6a and moderate increase in miR-21 at 1st and 3rd week of myocardial infarction. Interestingly, during 5th week of myocardial infarction both TNRC6a and miR-21 showed an abnormal expression. From the reports, it has been noted that the expression of the protein TNRC6a was found to be abnormal even at the 1st week of myocardial infarction, whereas the expression of miR-21 was found to be high at 5th week. It confirms that the protein TNRC6a plays a significant role in augmenting the regulation of miR-21 in myocardial infarction (Fig. 2).

Fig. 1 Histological analysis of rat tissue samples after myocardial infarction. **a** Histology of control rat (sham group) tissue sections. **b** Histology of rat tissue sections after 1st week of myocardial infarction. **c** Histology of rat tissue sections after 3rd week of myocardial infarction. **d** Histology of rat tissue sections after 5th week of myocardial infarction

Fig. 2 Quantitative RT-PCR analysis of TNRC6a and miR-21. Expression analysis of TNRC6a and miR-21 after 1st, 3rd and 5th week of rat myocardial infarction, respectively

Protein profile of TNRC6a and PTEN in myocardial tissue samples

In order to confirm the regulation of target genes, immunoblotting experiment was carried out. Expression of the target gene PTEN was reduced significantly; moreover, the protein profile of TNRC6a was increased abnormally (Fig. 3). From this study, we hypothesized that the accomplishment of miR-21 relies on the expression of TNRC6a. As the disease progress, the protein TNRC6a expresses abnormally which in turn up-regulates the miR-21. Interestingly, miR-21 binds with its specific target genes PTEN and thereby degrades its mRNA; as a result, its expression was down-regulated progressively and paved the development of myocardial infarction.

Fig. 3 Immunoblot analysis of TNRC6a and PTEN. Gene expression profile of TNRC6a and PTEN after 1st, 3rd and 5th week of myocardial infarction

Discussion

As a budding regulator, miRNAs are associated with cardiovascular diseases and myocardial infarction is a type of cardiovascular disease which critically affects the health of human and is considered as one of the most common causes of heart failure (Lim et al. 2016). Numerous studies indicate a strong connection between miRNAs and injury in endothelium because activation of endothelium is the first step in the development of myocardial infarction (Tedgui and Mallat 2006).

Recent studies reported that a protein TNRC6a is considered as a key regulator for miRNAs (Jessica et al. 2018). This finding was correlated with our study which indicates that aberrant expression of TNRC6a induces the up-regulation of miR-21 in myocardial infarcted rat.

In this study, rats exposed to myocardial infarction were successfully identified by histological analysis (Fig. 1) and from those further experiments were carried out. The expression pattern of TNRC6a and miR-21 was analyzed in the myocardial infarcted rats, and it has been found that the expression was elevated significantly when compared with sham group.

In order to confirm the expression pattern, the target for miR-21 was identified by means of bioinformatics approaches. The target gene of miR-21 was found to be PTEN. Our results illustrated the abnormal expression of TNRC6a and miR-21 in myocardial infarcted rats (Fig. 2). The immunoblot analysis proved that the expression of the protein TNRC6a was up-regulated abnormally, whereas the target gene of miR-21, PTEN, was down-regulated even after prolonged time of infarction (Fig. 3).

As the disease progresses, the protein TNRC6a expresses abnormally which in turn up-regulates the expression of miR-21. As a result, miR-21 binds with PTEN mRNA and degrades the target and hence the expression of PTEN mRNA was down-regulated progressively and paved the way for the development of myocardial infarction.

In vitro analysis in murine hearts confirmed the up-regulation of TNRC6a which in turn augmented the expression of various miRNAs such as miR-15b, miR-26, miR-34a and miR-140, but interestingly their particular target genes SIRT1, SMAD1, ALDH2 and MFN1 were down-regulated (Jessica et al. 2018). PTEN was considered as an important signaling pathway for myocardial infarction. Previous studies validate that the elevated level of miR-21 during myocardial infarction influences the production of collagen, by interfering PTEN signal pathway mediated through vascular endothelial growth factor (VEGF) (Feng et al. 2016). Activation of PTEN by VEGF improves the expression of MMP-2 and promotes the invasion of tumor cell (Boosani 2013). In addition, miR-21 regulates the PTEN signaling pathway in human liver cancer and skeletal muscle cells (Meng et al. 2007; Ye et al. 2015). A previous study states that activation of PTEN in myocardial tissues showed a significant improvement in cardiac fibrosis (Konopka et al. 2013).

It has been well documented that TNRC6a is one of the major regulators for miRNAs to partake in myocardial infarction. The current study confirms that TNRC6a augmented the regulation of miR-21 which down-regulated the target protein PTEN. However, further studies are necessary to understand the detailed molecular mechanism of the process. On the other hand, this study affords a new approach by exemplifying the role of TNRC6a and miR-21 as novel therapeutic biomarkers in identifying the prognosis of myocardial infarction.

Conclusion

The present study demonstrated the aberrant expression of TNRC6a and miR-21 during myocardial infarction. The findings play a significant role in the diagnosis as well as pave a way for the development of drug targets for treating myocardial infarction.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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