3. Menguji Doktor

KODE K06

DESKRIPSI	:	Penguji Tertutup	Halaman
		a.n Andrianto, dr., SpJP(K)	
BUKTI	:	SK Dekan No 238/UN3.1.1/HK.04/2020, tanggal 15 Juli 2020 ……...	02
		Bukti kinerja yaitu hal sampul, hal pengesahan dll …...	05

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SALINAN

KEPUTUSAN
DEKAN FAKULTAS KEDOKTERAN
NOMOR 238/UN3.1.1/HK.04/2020

TENTANG

PANITIA UJIAN TAHAP PERTAMA (TERTUTUP)
PROGRAM DOKTOR PROGRAM STUDI ILMU KEDOKTERAN
FAKULTAS KEDOKTERAN ATAS NAMA ANDRIANTO, dr., Sp.JP(K).

DEKAN FAKULTAS KEDOKTERAN,

Menimbang :

a. bahwa sehubungan dengan telah siap dilakukan ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran, maka perlu dibentuk panitia ujian tahap pertama (tertutup) tersebut;

b. bahwa nama-nama yang tersebut di bawah ini telah memenuhi syarat dan bersedia untuk diangkat sebagai panitia ujian dimaksud;

c. bahwa berdasarkan pertimbangan sebagaimana dimaksud pada huruf a dan huruf b, perlu menetapkan Keputusan Dekan Fakultas Kedokteran tentang panitia ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran.

Mengingat :

1. Undang-Undang Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional (Lembaran Negara Republik Indonesia Tahun 2003 Nomor 78, Tambahan Lembaran Negara Nomor 4301);
2. Undang-Undang Republik Indonesia Nomor 14 Tahun 2005 tentang Guru dan Dosen (Lembaran Negara Republik Indonesia Nomor 157, Tambahan Lembaran Negara Nomor 4586;
3. Undang-Undang Nomor 12 Tahun 2012 tentang Pendidikan Tinggi (Lembaran Negara Republik Indonesia Tahun 2012 Nomor 158, Tambahan Lembaran Negara Nomor 5336);
4. Undang-Undang Nomor 5 Tahun 2014 tentang Aparatur Sipil Negara (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 06, Tambahan Lembaran Negara Nomor 5494);
5. Peraturan Pemerintah Republik Indonesia Nomor 57 Tahun 1954 tentang Pendirian Universitas Airlangga Di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah Nomor 3 Tahun 1955 tentang Pengubahan Peraturan Pemerintah Nomor 57 Tahun 1954. (Lembaran Negara Republik Indonesia Tahun 1954 Nomor 99 Tambahan Lembaran Negara Nomor 695 juncto Lembaran Negara Republik Indonesia Tahun 1955 Nomor 4 Tambahan Lembaran Negara Nomor 748);

6. ...

6. Peraturan Pemerintah Nomor 4 Tahun 2014 tentang Penyelenggaraan Pendidikan Tinggi dan Pengelolaan Perguruan Tinggi. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 16, Tambahan L embaran Negara Nomor 5500);
7. Peraturan Pemerintah Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 100, Tambahan Lembaran Negara Nomor 5535);
8. Peraturan Rektor Universitas Airlangga Nomor 27 Tahun 2018 tentang Peraturan Pendidikan Universitas Airlangga;
9. Peraturan Rektor Universitas Airlangga Nomor 21 Tahun 2014 tentang Pedoman Pendidikan Program Doktor (S3) Universitas Airlangga;
10. Keputusan Rektor Universitas Airlangga Nomor 1947/H3/KR/2011 tentang Penetapan Ruang Lingkup Program Studi dalam Kategori Monodisiplin, Interdisiplin dan Multidisiplin untuk Pengelolaan Program Magister dan Program Doktor;
11. Keputusan Rektor Universitas Airlangga Nomor 1732/UN3/2015 tentang Pengangkatan Dekan Fakultas dan Direktur Sekolah Pascasarjana Periode 2015-2020.

MEMUTUSKAN:

Menetapkan : KEPUTUSAN DEKAN FAKULTAS KEDOKTERAN TENTANG PANITIA UJIAN TAHAP PERTAMA (TERTUTUP) PROGRAM DOKTOR PROGRAM STUDI ILMU KEDOKTERAN FAKULTAS KEDOKTERAN ATAS NAMA ANDRIANTO, dr., Sp.JP(K).

PERTAMA : Membentuk panitia ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran atas nama Andrianto, dr., Sp.JP(K) yang dilaksanakan pada tanggal, 15 Juli 2020 dengan susunan nama-nama sebagai berikut:

Ketua : Prof. Dr. Fedik Abdul Rantam, drh
Anggota :
1. Prof. Dr. Budi Susetyo Pikir, dr., Sp.PD., Sp.JP(K), FIHA
2. Dr. Ferdiansyah, dr., Sp.OT(K)
3. Prof. Mohammad Yogiarto Rohman, Sp.JP(K), Ph.D
4. Mohammad Saifur Rohman, dr., Sp.JP(K)., Ph.D
5. Dr. H. Budi Utomo, dr., M.Kes
6. Dr. Gondo Mastutik, drh., M.Kes

KEDUA : Dalam menjalankan tugasnya sebagaimana dimaksud dalam diktum PERTAMA, berpedoman pada peraturan dan ketentuan yang berlaku serta mempertanggungjawabkan tugasnya kepada Dekan Fakultas Kedokteran.

KETIGA : Biaya untuk keperluan tersebut dibebankan pada dana Rencana Kegiatan dan Anggaran Tahunan (RKAT) Fakultas Kedokteran.

KEEMPAT: ...

KEEMPAT : Keputusan ini mulai berlaku pada tanggal ditetapkan.

Ditetapkan di Surabaya
pada tanggal 15 Juli 2020

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2. KPS S3 Ilmu Kedokteran
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DISERTASI

PENGARUH MIKRO RNA *miR-1* DAN *miR-133a* TERHADAP EKSPRESI HDAC4 DAN SRFBP1 DALAM PROSES INDUKSI TRANSDIFERENSIASI SEL CD34+ DARAH PERIFER MENJADI KARDIOMIOSIT MATUR

ANDRIANTO

PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
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DISERTASI

Untuk memperoleh Gelar Doktor
dalam Program Studi Ilmu Kedokteran Jenjang Doktor
pada Fakultas Kedokteran Universitas Airlangga
dan dipertahankan di hadapan Panitia Ujian Akhir Tahap 1 (Tertutup)

Oleh:

ANDRIANTO
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PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2020

LEMBAR PENGESAHAN

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TELAH DISETUJUI

PADA TANGGAL 6 JULI 2020

Oleh
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Disertasi ini telah disetujui untuk diuji dan dinilai oleh panitia penguji Ujian Tahap 1 (Tertutup) pada Tanggal 15 Juli 2020

Panitia penguji:

Ketua : 1. Prof. Dr. Fedik A. Rantam, drh.

Anggota : 2. Prof. Dr. Budi S. Pikir dr., SpPD., SpJP (K)

3. Dr. Ferdiansyah, dr., Sp.OT (K)
4. Prof. Mohammad Yogiarto, dr., SpJP (K)
5. M. Saifur Rohman, dr., PhD., SpJP (K)
6. Dr. Budi Utomo, dr., M.kes
7. Dr. Gondo Mastutik, drh., M.Kes

SUMMARY

The Effect of miR-1 and miR-133a on HDAC4 and SRFBP1 Expression in the Processes of Inducing Transdifferentiation of CD34+ Peripheral Blood Cells into Mature Cardiomyocytes

Coronary heart disease can cause myocardial infarction characterized by cardiomyocyte death. Until now, the aim of managing myocardial infarction is limited to restoring coronary blood flow and reducing the burden on the heart muscle. However, these treatments could not replace dead cardiomyocytes. Myocardial infarction is also a leading cause of heart failure. To date, there is no cure for advanced heart failure except heart transplantation, which still has many limitations, such as the small number of donors, and the possibility of immune rejection (Burchfield & Dimmeler, 2008; Dimmeler et al., 2005). Innovative therapy is needed to overcome these problems, one of which is through regenerative treatment. Regeneration of dead myocardium can be done by replacing dead cardiomyocytes with new cells originating from stem cells using a technique called cellular cardiomyoplasty (Hanson et al., 2012; Srivastava, 2017). Cellular cardiomyoplasty aims to replace damaged myocardial tissue and restore myocardial function (Chachques et al., 2005; Hamano et al., 2001).

Cellular reprogramming has the potential to obtain stem cell availability through pluripotent induction or transdifferentiation techniques (Takahashi & Yamanaka, 2006). Transdifferentiation is the technique of converting certain differentiated cell types into other cells. Transdifferentiation has been proven safer and has a more straightforward process than pluripotent induction (Wang et al., 2015). The success of the transdifferentiation process is determined by the source cell type. Hematopoietic stem cell marked CD34+ has the potential to become a source cell because these cells can differentiate into cardiomyocytes. This differentiation process can be done because CD34+ cells originate from the same embryonic layer as the heart called the mesoderm layer (Lim et al., 2013). Other than that, CD34 + cells can be extracted from large numbers of peripheral blood through minimally invasive procedures (Romagnani et al., 2006).

Cell transdifferentiation can be induced by microRNA (miRNA). MicroRNA is a non-coding RNA that works in the post-transcription stage by degrading or inhibiting the translation of messenger RNA (mRNA) that results in the modification of gene expression (Serradifalco et al., 2013). Transdifferentiation using miRNA is easier to do in mature cells and has no risk of permanent genome change. MicroRNA has a crucial role in the stage of differentiation of cardiomyocytes, especially microRNA-1 (miR-1) and microRNA-133a (miR-133a). miR-1 is known to have the most dominant amount in heart tissue up to 45%, while miR-133a is a miRNA derived from the same cluster as miR-1 whose functions and actions are interconnected (Malizia & Wang, 2011; Wang et al., 2015).

miR-1 is known to have the ability to inhibit the expression of the histone deacetylase 4 (HDAC4) gene, which then increases the expression of the myocyte enhancer factor 2 (MEF2) gene. Increasing MEF2 can trigger cell differentiation into mature cardiomyocytes characterized by the expression of cardiac troponin (Al-Maqtari et al., 2017; Chen et al., 2015). miR-133a can inhibit the Serum

Response Factor Binding Protein 1 (SRFBP1) gene, which is a cofactor of the serum response factor (SRF) gene (Sepulveda et al., 2002). Inhibition of SRFBP1 and SRF in cells will trigger proliferation and inhibit cell differentiation into mature cardiomyocytes (Al-Maqtari et al., 2017). This study aims to prove the effect of miR-1 and miR-133a on HDAC4 and SRFBP1 expression in the process of inducing transdifferentiation of CD34+ peripheral blood cells into mature cardiomyocytes, which is indicated by the expression of cardiac troponin.

CD34+ cells isolated from the peripheral blood was successfully done by magnetic bead isolation method. CD34+ cell culture was successfully expanded and was adherent and also confluent by 75% on the 7th day of culture. After the expansion, the CD34+ cell was transfected with miR-1 (P1) and miR-133a (P2). CD34+ cells also treated using cardiomyocyte differentiation medium (P3). Cells were harvested on the 2nd-day post-transfection to measure HDAC4 and SRFBP1 gene expression using RT-qPCR. The remaining cells are collected on the 5th-day post-transfection to measure the expression of cardiac troponin using immunocytochemistry.

Measurement results of gene expression using RT-qPCR showed that the transfection of miR-1 decreased HDAC4 gene expression by -0.54 fold while transfection of miR-133a decreased SRFBP1 gene expression by -0.55 fold at 2nd-day post-transfection. Treatment using cardiomyocyte differentiation medium decreased HDAC4 gene expression by 0.60 fold and increased SRFBP1 gene expression by 5.17 fold at 2nd-day post-transfection. Transfection of miR-1 (P1) (median 31.34) and treatment using cardiomyocyte differentiation medium (P3) (median 21.06) caused a significant increase in the percentage of cardiac troponin when compared to P0 (median 12.13) ($p < 0.05$) at 5th-day post-transfection. Transfection of miR-133a (P2) (median 7.15) did not change the percentage of cardiac troponin when compared to P0 (median 12.13) ($p > 0.05$). Transdifferentiation efficiency of CD34+ peripheral blood cells into mature cardiomyocytes through miR-1 transfection was 32%, while the treatment using the cardiomyocyte differentiation medium was 21.4%.

The regression analysis showed that miR-1 transfection had a significant positive relationship with the percentage of cardiac troponin (B= 0.701, p=0.001). The analysis also showed that miR-1 transfection had a significant negative relationship with HDAC4 gene expression (B= -1.000; p=0.001). The transfection of miR-133a showed no significant relationship to the percentage of cardiac troponin (B= 0.181; p=0.265) but had a significant negative relationship with SRFBP1 gene expression (B= -1.000; p=0.001). The addition of cardiomyocyte differentiation medium had a significant positive relationship with percentage cardiac troponin (B= 0.505; p=0.001), a significant negative relationship with HDAC4 gene expression (B= -1.000, p=0.001) and also a significant positive relationship with SRFBP1 gene expression (B= 1.000, p=0.001). HDAC4 gene expression has been shown to had a negative and significant relationship with percentage of cardiac troponin (B= -0.701; p=0.001) whereas SRFBP1 gene expression did not correlate with percentage of cardiac troponin (B= -0.181; p=0.265).

This study concludes that miR-1 transfection reduced HDAC4 gene expression, and miR-133a transfection reduced SRFBP1 gene expression. Transfection of miR-1 more effective than the administration of cardiomyocyte

differentiation medium in the process of inducing transdifferentiation of peripheral blood CD34+ cells into mature cardiomyocytes. While miR-133a transfection did not influence the process of inducing transdifferentiation of peripheral blood CD34+ cells into mature cardiomyocytes. HDAC4 gene expression has been shown to had a negative and significant relationship with cardiac troponin expression whereas SRFBP1 gene expression had no relationship percentage of cardiac troponin. Transdifferentiation efficiency of CD34+ peripheral blood cells into mature cardiomyocytes through miR-1 transfection was 32% and through the addition of cardiomyocyte differentiation medium was 21.4% This study also suggests a new mechanism of miR-1 and cardiomyocyte differentiation medium in the process of inducing transdifferentiation of peripheral blood CD34+ cells into mature cardiomyocytes by decreasing HDAC4 gene expression.

ABSTRAK

Pengaruh Mikro RNA *miR-1* dan *miR-133a* terhadap Ekspresi HDAC4 dan SRFBP1 dalam Proses Induksi Transdifrensiasi Sel CD34+ Darah Perifer menjadi Kardiomiosit Matur

Andrianto

Latar belakang: Regenerasi miokardium yang telah mati dapat dilakukan dengan teknik kardiomioplasti seluler. Salah satu cara untuk menyediakan sumber sel baru untuk kardiomioplasti seluler adalah transdiferensiasi. Sel CD34+ darah perifer berpotensi berdiferensiasi menjadi kardiomiosit matur dengan induksi *miRNA* spesifik jantung yaitu *miR-1* dan *miR-133a*. Penelitian ini bertujuan untuk menganalisis peran *miR-1* dan *miR-133a* dalam menginduksi transdiferensiasi sel CD34+ darah perifer menjadi kardiomiosit matur.

Metode: Penelitian ini merupakan penelitian eksperimental *in vitro*. Sel CD34+ diisolasi dari darah perifer menggunakan metode *magnetic beads*. Kultur sel dibagi menjadi empat kelompok perlakuan yaitu : kontrol negatif (P0), perlakuan transfeksi *miR-1* (P1), perlakuan transfeksi *miR-133a* (P2); dan perlakuan dengan pemberian medium diferensiasi kardiomiosit (P3). Kultur sel dipanen pada hari ke-2 pasca perlakuan untuk mengukur ekspresi gen HDAC4 dan SRFBP1 menggunakan RT-qPCR dan pada hari ke-5 pasca perlakuan untuk mengukur prosentase *cardiac troponin* menggunakan metode imunositokimia.

Hasil: Ekspresi gen HDAC4 menurun -0,54 kali pada P1. Ekspresi gen SRFBP1 menurun -0,55 kali pada P2. Prosentase *cardiactroponin* meningkat signifikan ($p<0,05$) pada P1 dan P3, namun tidak pada P2. Efisiensi transdiferensiasi P1 sebesar 32%, sedangkan P3 sebesar 21,4%. Ekspresi gen HDAC4 memiliki hubungan negatif dan signifikan dengan prosentase *cardiac troponin* (B= -0,701; p=0,001), sedangkan ekspresi gen SRFBP1 tidak berhubungan dengan prosentase *cardiac troponin* (B= -0,181; p=0,265).

Kesimpulan: Transfeksi *miR-1* menurunkan ekspresi gen HDAC4 dan transfeksi *miR-133a* menurunkan ekspresi gen SRFBP1. Transfeksi *miR-1* lebih efisien dibandingkan dengan pemberian medium diferensiasi kardiomiosit dalam proses transdiferensiasi. Penelitian ini menghasilkan temuan baru bahwa *miR-1* dapat menginduksi transdiferensiasi sel CD34+ darah perifer menjadi kardiomiosit matur melalui penurunan ekspresi gen HDAC4.

Kata kunci: transdiferensiasi, sel CD34+, *microRNA-1*, *microRNA-133a*, kardiomiosit

ABSTRACT

The Effect of miR-1 and miR-133a on HDAC4 and SRFBP1 Expression in the Processes of Inducing Transdifferentiation of CD34+ Peripheral Blood Cells into Mature Cardiomyocytes

Andrianto

Background *Regeneration of the dead myocardium can be done with cellular cardiomyoplasty. Transdifferentiation is a technique for providing cell sources for cellular cardiomyoplasty. CD34+ cell has the potential to differentiate into cardiomyocytes by induction of cardiac-specific miRNA, namely miR-1 and miR-133a. This study aimed to analyze the role of miR-1 and miR-133a in inducing transdifferentiation of peripheral blood CD34+ cells into mature cardiomyocytes.*

Methods *This research was an in vitro study. CD34+ cells were isolated from peripheral blood using the magnetic beads method. Cell culture was divided into four treatment groups, negative control (P0), miR-1 transfection (P1), miR-133a transfection (P2), and treatment with cardiomyocyte differentiation medium (P3). Cell culture was harvested on the second-day post-treatment to measure HDAC4 and SRFBP1 gene expression using RT-qPCR and on the fifth-day post-treatment to measure the percentage of cardiac troponin using immunocytochemistry.*

Results *HDAC4 gene expression decreased by -0.54 fold in P1. SRFBP1 gene expression decreased by -0.55 fold in P2. The percentage of c-troponin increased significantly ($p < 0.05$) in P1 and P3, but not in P2. Transdifferentiation efficiency in P1 was 32%, while P3 was 21.4%. HDAC4 gene expression had a negative and significant relationship with cardiac troponin percentage ($B = -0.701$; $p=0.001$), whereas SRFBP1 gene expression was not related to cardiac troponin percentage ($B = -0.181$, $p = 0.265$).*

Conclusion *miR-1 transfection decreased HDAC4 gene expression and miR-133a transfection decreased SRFBP1 gene expression. miR-1 transfection is more efficient than the treatment of cardiomyocyte differentiation medium in the transdifferentiation process. This research also found a new finding that miR-1 can induce transdifferentiation of peripheral blood CD34+ cells into mature cardiomyocytes through a decrease of HDAC4 gene expression.*

Keywords: *transdifferentiation, CD34+ cell, microRNA-1, microRNA-133a, cardiomyocyte*