

UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

ROLE OF TRANSFORMING GROWTH FACTOR-BETA1 (TGFB-1)
AND THE CELL SIGNALLING PATHWAY IN THE
DIFFERENTIATION OF SHED INTO EPITHELIAL-LIKE CELLS

PENYELIDIK

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PENYELIDIK BERSAMA

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ASSOC. PROF. DR. KHAIRANI IDAH MOKHTAR @ MAKHTAR
DR. ZURAIRAH BERAHIM
NUR IZYAN AZMI

2017



KEMENTERIAN
PENDIDIKAN
MALAYSIA

**FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)**

*Laporan Akhir Skim Geran Penyelidikan Fundamental (FRGS)
Pindaan 1/2015*

RESEARCH TITLE: Role of Transforming Growth Factor Beta1 (TGF β -1) and the cell signaling pathway in the differentiation of SHED into epithelial-like cells.

PHASE & YEAR: 3 & 2015

START DATE: 1 June 2012

END DATE: 31 Mei 2014

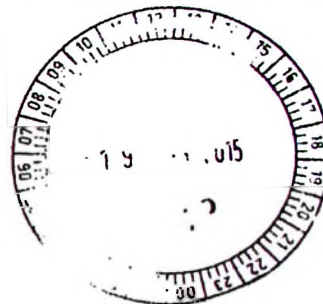
EXTENSION PERIOD (DATE): RMC LEVEL: 30 November 2014

KPM LEVEL: 31 May 2015

PROJECT LEADER: Dr Azlina Ahmad

C / PASSPORT NUMBER:

PROJECT MEMBERS: 1. AP Dr TP Kannan
(including GRA) 2. AP Dr Khairani Idah Mokhtar @ Makhtar
3. Dr Zurairah Berahim
4. Nur Izyan Azmi



PROJECT ACHIEVEMENT (*Prestasi Projek*)

ACHIEVEMENT PERCENTAGE

Project progress according to milestones achieved up to this period	0 - 50%	51 - 75%	76 - 100%
Percentage (please state #%)			90%

RESEARCH OUTPUT

Number of articles/ manuscripts/ books (Please attach the First Page of Publication)	Indexed Journal	Non-Indexed Journal
		1
Conference Proceeding (Please attach the First Page of Publication)	International	National
	1	2
Intellectual Property (Please specify)		

HUMAN CAPITAL DEVELOPMENT

Human Capital	Number				Others (please specify)
	On-going		Graduated		
Citizen	Malaysian	Non Malaysi an	Malaysian	Non Malaysian	
No. PHD STUDENT					
Student Fullname: IC / Passport No: Student ID:					
No. MASTER STUDENT	1				
Student Fullname: IC / Passport No: Student ID:	Nur Izyan binti Azmi 890221-11- 5432 P-SGM0005/13 (R)				
No. UNDERGRADUATE STUDENT					
Student Fullname: IC / Passport No: Student ID:					
Total	1				

EXPENDITURE (Perbelanjaan) as Borang K1(RMC)

C	Budget Approved (Peruntukan diluluskan)	: RM 153,100.00
	Amount Spent (Jumlah Perbelanjaan)	: <u>RM 153,001.10</u>
	Balance (Baki)	: <u>RM 98.90</u>
	Percentage of Amount Spent	: 99.9 %
	(Peratusan Belanja)	

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS (Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

D	International		
	Activity	Date (Month, Year)	Organizer
	(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1. International Conference On Medical & Health Sciences (ICMHS) 22-24 May 2013	1. College of Pathologists, Health Campus, USM.
	National		
	Activity	Date (Month, Year)	Organizer
	(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1. Health and Life Sciences Postgraduate Conference 2014 10-11 June 2014 2. 10 th Malaysia Genetics Congress 2013 (MGC10) 3-5 December 2013	1. Institute for Research in Molecular Medicine (INFORMM) 2. Persatuan Genetik Malaysia

E PROBLEMS / CONSTRAINTS IF ANY (Masalah/ Kekangan sekiranya ada)

1. Protein expression analysis takes longer times than expected.

RECOMMENDATION (Cadangan Penambahbaikan)

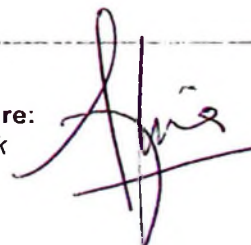
Future direction: Since this study showed that KGM media was not successfully to completely differentiated SHED into epithelial-like cells, we suggest that maybe addition of keratinocyte growth factor protein in the KGM media will help in the differentiation. However, we could not do this experiment because the growth factor is expensive.

RESEARCH ABSTRACT – Not More Than 200 Words (Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan)

Epithelial cells play a crucial role in the human body and in the oral cavity. Epithelial formation is important during the healing process after injuries. Previous study had shown that primary cells from human exfoliated deciduous teeth (SHED) can be differentiated into epithelial-like cells when cultured in Keratinocyte Growth Medium (KGM). This study aims to determine the effects of TGF- β 1 and ALK-5 inhibitor in the differentiation of SHED into epithelial-like cells cultured in specific differentiation medium (KGM). MTT and alamarBlue assay was done to investigate and determine the best concentration to be used in the study. Protein expression using flow cytometer was conducted to identify the presence of stem cell markers, CD105, and epithelial marker, E-Cadherin. The cell was positive for stem cell protein marker, but we still need to optimize the protein expression level of E-Cadherin. Then, gene expression of mesenchymal, epithelial, and TGF- β signaling marker was carried out on SHED cultured with KGM treated with 1.25 ng/ml of TGF- β 1 or 0.625 μ M of ALK-5 inhibitor. The treated SHED was harvested at day 1, 3, 7, 14, and 21 and subjected to RNA extraction. Then, the study was proceeded with two-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The presence of mesenchymal marker, *Nanog* on all samples except on SHED cultured in KGM + 0.625 μ M on day 21. Based on epithelial marker, *Keratin5* showed there is no expression on all treated samples. For TGF- β signaling marker which were *ALK-5* and *Smad4*, *Smad4* showed expression on day 1 until 7 for all treatments and only SHED in KGM on day 14 showed a mild expression. *ALK5* expressed in most of samples analysed but only SHED in KGM with TGF- β 1 showed no expression. In conclusion, this study showed that TGF- β 1 and ALK-5 inhibitor might give a little effect to the differentiation of SHED into epithelial-like cells but not enough to induce the differentiation process.

Date :
Tarikh

Project Leader's Signature:
Tandatangan Ketua Projek



H COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

Pembentangan,

Name:

Nama:


PROF. DR LEE KEAT TEONG

Pencarah

Pejabat Pengurusan & Kreativiti Penyelidikan
Universiti Sains Malaysia

Signature:

Tandatangan:


18/8/14

Date:

Tarikh:

Title: Role of Transforming Growth Factor Beta1 (TGFβ-1) and the cell signaling pathway in the differentiation of SHED into epithelial-like cells.

ABSTRACT

Epithelial cells plays crucial role in human body and in oral cavity, epithelial formation is important during the healing process after injuries. Previous study had shown that primary cells from human exfoliated deciduous teeth (SHED) can be differentiated into epithelial-like cells when cultured in Keratinocyte Growth Medium (KGM). This study aim to determine the effects of TGF-β1 and ALK-5 inhibitor in the differentiation of SHED into epithelial-like cells cultured in specific differentiation medium (KGM). MTT and alamarBlue assay was done to investigate and determine the best concentration to be used in the study. Then, gene expression of mesenchymal, epithelial, and TGF-β signaling marker was carried out on SHED cultured with KGM treated with 1.25 ng/ml of TGF-β1 or 0.625 μM of ALK-5 inhibitor. The treated SHED was harvested at day 1,3,7,14, and 21 and subjected to RNA extraction. Then, study was proceeded with two-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The presence of mesenchymal marker, *Nanog* on all samples except on SHED cultured in KGM + 0.625 μM on day 21. Based on epithelial marker, *Keratin5* showed there is no expression on all samples treatment except control which is keratinocyte cells. For TGF-β signaling marker which were *ALK-5* and *Smad4*, *Smad4* showed expression on day 1 until 7 for all treatments and only SHED in KGM on day 14 showed a mild expression. *ALK5* expressed in most of samples analysed but only SHED in KGM with TGF-β1 showed no expression. As conclusion, this study showed that TGF-β1 and ALK-5 inhibitor might give a little effects to the differentiation of SHED into epithelial-like cells but not enough to induce the differentiation process.