UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF B-LYMPHOBLASTOID CELL LINE FROM PERIPHERAL BLOOD MONONUCLEAR CELL AND THE CHARACTERIZATION OF *FCGR3B* COPY NUMBER VARIATION IN NEGRITOS AND MALAYS

MOHD HELMY BIN YUSOF

Thesis submitted in fulfilment
of the requirements for the degree of
Master of Science

Faculty of Medicine

May 2016

CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 20th November 2015 to conduct the final examination of Mohd Helmy Bin Yusof on his Master of Medical Science thesis entitled 'Development of B-Lymphoblastoid Cell Line from Peripheral Blood Mononuclear Cell and the Characterization of *FCGR3B* Copy Number Variation in Negritos and Malays' in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

Azian Abdul Latiff, PhD
Professor
Faculty of Medicine
Universiti Teknologi MARA
(Chairman)

Vijay Kumar, PhD
Associate Professor
Biotechnology Research Institute
Universiti Malaysia Sabah
(External Examiner)

Farida Zuraina Mohd Yusof, Phd
Associate Professor
Faculty of Applied Science
Universiti Teknologi MARA
(Internal Examiner)

SITI HALIJJAH SHARIFF, PhD
Associate Professor
Dean
Institute of Graduates Studies
Universiti Teknologi MARA
Date: 29th April, 2016

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	:	Mohd Helmy Bin Yusof
Student I.D. No.	:	2012650436
Programme	:	Master of Science (MD 780)
Faculty	:	Medicine
Thesis Title	:	Development of B-Lymphoblastoid Cell Line from Peripheral Blood Mononuclear Cell and the Characterization of *FCGR3B* Copy Number Variation in Negritos and Malays
Signature of Student	:	
Date	:	May 2016

ABSTRACT

Obtaining a continuous source of normal cells or DNA from a single individual has always been a rate limiting step in biomedical research. The availability of Lymphoblastoid cell lines (LCLs) as a surrogate for replacement of isolated peripheral blood mononuclear cell (PBMC) has substantially accelerated the process of biological investigations. LCLs can be established by *in vitro* infection of B lymphocytes from peripheral blood with Epstein Barr Virus (EBV) resulting in continuous biomaterials, bearing minor genetic and phenotypic alterations. Fc Gamma Receptor 3B (FcγRIIIB, encoded by the gene *FCGR3B*) plays a crucial role in immunity triggered by cellular effectors and regulatory functions. Copy number variation (CNV) of this gene has been previously reported to affect susceptibility to several diseases such as autoimmune diseases and chronic inflammatory conditions. Here the first generation of LCL in indigenous populations from Peninsular Malaysia were reported and the method for immortalization B-lymphocytes cell line was establish. First, the most appropriate blood collection tube to collect the peripheral blood was selected. ACD tube was found to be most appropriate to collect *Orang Asli* blood sample in field trip while CPT tube was used to collect Malay (control) samples. PBMC from Malay sample and *Orang Asli* samples were isolated by using centrifugation and modified method of CPT (MMC) respectively. The best EBV supernatant to transfect PBMC was produced from B95-8 marmoset cell line treated by 40ng/ml of tetradecanoyl phorbol acetate (TPA). From 89 PBMC sample isolated including Malay (n=25) and *Orang Asli* (n=64), a total of 84 samples was successfully transformed into LCL by transfect with EBV supernatant in a single attempt with a success rate 100% (n= 25) for Malay and 92.2% (n=59) for *Orang Asli*. Copy number variation (CNV) was characterized by using the Paralogue Ratio Test-Restriction Enzyme Digest Variant Ratio (PRT-REDVR) for *FCGR3B*. Reliability of using LCLs for Copy number variation (CNV) genotyping were tested by comparing CNV call of primary PBMC with the same sample from LCL where CNV calling was 100%, identical in both PBMC and LCL. A total of 84 of DNA extracted from LCL samples were performed on PRT-REDVR assay. Eighty-two samples (25 Malays, 50 *Orang Asli* Negrito, and 7 *Orang Asli* Senoi) were successfully genotyped for *FCGR3B* CNV. From 50 *Orang Asli* Negrito samples, 88% of them had two copy numbers (2) of *FCGR3B*. Meanwhile, there was no low copy number (<2) for *Orang Asli* Negrito. Then again, 12% of *Orang Asli* Negrito had high copy number (>2) of *FCGR3B*. Analysis revealed that no significance difference (p=0.558) for CNV of *FCGR3B* between Malay and *Orang Asli* Negrito.

ACKNOWLEDGEMENT

Firstly, praise is to Allah S.W.T for giving me grace, strength, good health, motivation, and patience in completing my master's research. I would like to express my sincere appreciation and the deepest gratitude to my supportive supervisor, Associate Professor Dr. Hoh Boon Peng for his patience, guidance, and support along the journey to complete my master research. I would like to express my sincere gratitude to my co-supervisor Dr. Sharaniza Binti Ab Rahim and Dr. Siti Hamimah Binti Sheikh Abdul Kadir for their guidance and advice.

Special thanks to MOSTI GRANT [100 - RMI/ BIOTEK 16/6/2 B (1/2011)] for funding this project. Thanks to Tenaga Pengajar Muda (TPM) scholarship for supporting me throughout my study.

I would like to acknowledge the fellow staffs from Institute Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA Sungai Buloh campus, for all their technical support. I also would like to thank all my colleagues, especially those under Dr. Hoh's research team for their support and cooperation throughout my study. Their support, guidance and wonderful friendship have made my research life interesting.

A very deep gratitude to my beloved wife, Salwa Binti Shawan and both my family and family in law for their understanding, cooperation, prayers and for allowing me to further my study makes this whole adventure possible. Lastly, thanks to all who have directly or indirectly contributed in this study.