UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF EXTRACELLULAR MATRIX GLYCOSYLATION INHIBITION ON OSTEOSARCOMA INVASIVENESS

SARMILA HANIM BINTI MUSTAFA

MSc

September 2020

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 Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	:	Sarmila Hanim binti Mustafa
Student I.D. No.	:	2016280618
Programme	:	Master of Medicine (Biochemistry) – MD751
Faculty	:	Medicine
Thesis Title	:	The Effects of Extracellular Matrix Glycosylation Inhibition on Osteosarcoma Invasiveness
Signature of Student	:	
Date	:	August 2020

ABSTRACT

Osteosarcoma (OS) is a rare malignant bone cancer affecting children and young adults. To date, OS still has a poor prognosis despite the use of advanced multimodal therapy in its treatment. Recurrence cases has also shown to be increased in metastatic patients especially to the lung. A possible cause for this poor prognosis could be because of lack of understanding in the underlying mechanism of OS progression and metastasis. Previously, loss of Wnt/β-catenin pathway activity during osteoblast differentiation was suggested to contribute to the OS development. In addition, a metastatic condition of OS was shown to be established through the adhesion of OS cells to blood vessel mediated by $\alpha_4\beta_1$ integrin. It has been known that aberrant glycosylation is associated with metastasis in various cancer by modulating the cells invasiveness and their dissemination through the extracellular matrix (ECM) layer. However, the effect of aberrant glycosylation, mainly during *N*-glycan processing in OS has yet to be elucidated. Therefore, the objective of this study is to investigate the effects of aberrant glycosylation towards OS invasiveness via the cell-ECM interaction. In this study, the inhibition of glycosylation was carried out by the treatment of OS cell line (MG-63) with 1-deoxynojirimycin (1-DNJ); an α-glucosidase-I/II inhibitor, and 1-deoxymannojirimycin (1-DMJ); an α-mannosidase I inhibitor. Then, its effects on glycosylation pattern, invasion ability, protein expression of $\alpha_4\beta_1$ integrin and the expression level of ECM degradative enzymes genes; MMP-2, MMP-9, TIMP-1, TIMP-2 and β-catenin genes were determined. In this study, the normal osteoblast cell (hFOB1.19) was also treated with 1-DNJ and 1-DMJ and both samples were subjected to the same method as MG-63 cells except for cell invasion assay. The results showed that the inhibition of *N*-glycosylation by 1-DMJ enhanced the invasion of MG-63 cells through the ECM layer significantly by 3.87 fold ($p<0.05$). It was also found that 1-DMJ significantly ($p<0.05$) regulates the expression of $\alpha_4\beta_1$ integrin protein in hFOB1.19 cells. qRT-PCR analysis showed that 1-DMJ downregulate the expression MMP-9 gene (2.42 fold ± 0.284; $p<0.05$) in MG-63 cells. On the other hand, TIMP-1 (1.54 fold ± 0.035), TIMP-2 (1.22 fold ± 0.041) and β-catenin (1.24 fold ± 0.07) genes were significantly ($p<0.05$) upregulated in MG-63 cells when compared to control. However, in hFOB1.19 cells treated with 1-DMJ, MMP-2 (48.32 fold ± 0.16), MMP-9 (4.38 fold ± 0.389), and TIMP-1 (1.45 fold ± 0.064) genes were significantly ($p<0.05$) downregulated when compared to control. Whereas, the expression of TIMP-2 (1.2 fold ± 0.03), and β-catenin gene (2.22 fold ± 0.232) in hFOB1.19 cells were significantly ($p<0.05$) upregulated. 1-DNJ was shown to downregulate the expression of MMP-2 in both cells; MG-63 (1.29 fold ± 0.067) and hFOB1.19 (55.25 fold ± 0.097) significantly ($p<0.05$) when compared to control. However, the expression of β-catenin gene in 1-DNJ treated hFOB1.19 cells was significantly ($p<0.05$) increased by 2.4 fold ± 0.103 when compared to control. This study suggested that 1-DMJ may promote the invasion of MG-63 cells through the ECM layer via the regulation of MMP-9, TIMP-1, and TIMP-2 genes. Whereas the overexpression of β-catenin genes in 1-DMJ and 1-DNJ treated hFOB1.19 cells might give an insight on the epithelial-to-mesenchymal transition (EMT) of normal osteoblast cells. This study might provide more information on the underlying mechanism of OS therefore contributes to OS prognosis improvement.

ACKNOWLEDGEMENT

Alhamdulillah. Firstly, I wish to thank God for giving me the opportunity to embark on my MSc and for completing this long and challenging journey successfully.

My deepest and sincere gratitude go to my supervisor Assoc. Prof. Dr. Sharaniza Ab. Rahim and Assoc Prof. Dr. Mudiana Muhamad @ Mohd. Saufi. Thank you for accepting me as a part of SYBER group. I am grateful for having both of you as my supervisors. In this challenging journey, both of you have always been patients towards me and you have taught me on how to be a better student and a better person.Your guidance and endless support was the key to this project and I will never forget your kindness and sincere advices to me and to the rest of the students. Both of you are my role models and I will always pray for your success in this world and hereafter.

To my groupmates from SYBER, thank you so much for sharing your knowledge and skills with me. I have learnt a lot from all of you. Only God can repay your kindness. To my friends that is always there for me through thick and thin, thank you so much for your support. Thank you for helping me creates good memories while we are here and making this journey less stressful. All of you are the best and I hope nothing but the best for all of you.

I would also like to thank all the Institute of Medical Molecular Biotechnology (IMMB) staff which are very friendly and helpful from the first day I was here until today.

Finally, this thesis is dedicated to my loving parents; En. Mustafa bin Arof and Pn. Arbaiah bt Mohd Yusof. Thank you so much for having faith in me and be an understanding parent. You have always been my motivation to finish this study. Without you, I am nothing. To Mohd. Firdaus, you are my best friend and you are the best support system I have ever had during the completion of this study. Thank you so much.

This research was supported by Ministry of Education, Malaysia; 600-IRMI/MyRA 5/3/LESTARI (0029/2016) and 600-IRMI/MyRA 5/3/GIP (074/2017) grants.

TABLE OF CONTENTS