UNIVERSITI TEKNOLOGI MARA

LSD1 REGULATES ALDOSTERONE: MINERALOCORTICOID RECEPTOR SIGNALING IN ENDOTHELIAL CELLS.

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AUGUST 2012

ACKNOWLEDGEMENTS

After a year of training, it is time to finish my thesis and have a chance to acknowledge and thank the people who have me in all kinds of ways.

First and foremost, I would like to thank my beloved parents and family members, who always give me the greatest support and care all the way.

Next, bunch of thanks to my supervisor and mentor, Dr. Amanda E. Garza, who supported, guided and inspired me. I will never forget every single moment of being her mentee as it benefited me greatly in the process of doing research. Without her guidance and generosity to share the idea, my thesis could never be done. Every discussion we had inspired me to go further and see more clearly.

I would also like to thank the lab members, Dr. Luminita Pojoga, Paul, Tham, Dr. Rene, Dr. Alex, Mina, Elijah and Siti for every precious moment that we spend together like a family. I really appreciated it. Besides, many thank to Dr. Gail Adler for her inspiring idea and concern.

Then, I feel immense gratitude to my advisor, Professor Dr. Gordon H Williams. The experiences that we shared during the lab meeting and personal meeting, were really valuable and insightful, and helped me a lot refine my thesis.

Also many thanks to my scholarship body, MARA, which sponsored me for the entire year in Boston; UiTM that gave me a chance in a life time to further my study in Advanced Medical Science; Dean of Medical Faculty of UiTM, Professor Dato' Dr. Khalid, who has inspired me all the time; Dr. Norashikin, who helped me a lot when I'm in USA; and anyone that helped me all the way direct and indirectly.

Finally, a thousand thanks again to all people those who helped me all the time. With all the supports given, I have the strength and courage to turn a new leaf and stride forward.

ABSTRACT

The mineralocorticoid receptor (MR) is a well studied steroid hormone receptor known to be a key modulator of cardiovascular function and disease. Recent studies demonstrate that lysine specific demethylase 1 (LSD1) interacts with another steroid receptor, the androgen receptor, to modulate its transcription function. In our laboratory we have demonstrated MR co-immunoprecipitates with LSD1, and potentially modulates MR's transcriptional activity. Furthermore, our lab has identified polymorphic variants in the LSD1 gene in a subset of humans with salt sensitive hypertension, who have on a high salt (HS) diet decreased aldosterone responsiveness to infused angiotensin II, decreased aldosterone excretion on, and suppressed plasma renin activity (PRA) and on a low salt (LS) diet normal renin status. In addition heterozygous LSD1 knockout ($LSD1^{+/-}$) mice on a high-salt diet have salt sensitive hypertension associated with alterations in vascular contraction and the NO-cGMP pathway.

Therefore, I tested the hypothesis that LSD1 expression is modulated by aldosterone (ALDO) and that LSD1 modulates ALDO:MR genomic signaling in an endothelial cell hybrid line, EA.hy926 cells. Initially, cell culture environment parameters were optimized to obtain the appropriate conditions by which EA.hy926 cells would respond to ALDO. The levels of LSD1 in response to ALDO were assessed at multiple time points and at 10 nM and 100 nM ALDO concentrations. Next, endogenous LSD1 protein levels in EA.hy926 cells were suppressed by siRNA and ALDO:MR genomic and non-genomic signaling was evaluated. My conclusions are: 1) ALDO appears to induce significant changes in LSD1 protein expression at 24 hours *in vitro* as tested in EA.hy926 cells; 2) LSD1 appears to be involved in the ALDO: MR genomic signaling as it increases MR activation, as measured by ENaC, SGK1 and Wnk gene expression, when endogenous LSD1 is suppressed. Together these data support a novel mechanism where LSD1 regulates ALDO:MR signaling and ALDO modulates LSD1 levels.

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CHAPTER I

INTRODUCTION

Cardiovascular (CV) disease accounts for more deaths in the developed world than any other major disease (WHO 2009). Pathogenesis of cardiovascular disease is complex and attributed to numerous risk factors such as hypertension (Kannel 2000), hyperlipidemia (Bolton, Sweetnam et al. 1988), smoking (Holbrook, Grundy et al. 1984) and epigenetic modifications (Baccarelli, Rienstra et al. 2010). A major problem in effectively treating CV disease is marked by the complexity of multiple interacting systems and many signalling pathways.

Mineralocorticoid receptor (MR) is a well studied steroid hormone receptor known to be a key modulator of CV function and disease. Clinical and experimental studies have provided direct evidence for the involvement of aldosterone (ALDO), signalling through MR, in the development of CV disease induced by endothelial vascular injury. Large-scale studies have demonstrated that the addition of MR blockade to standard therapy 1) reduces CV morbidity and mortality in patients with heart failure, either chronic or after acute myocardial infarction(Zannad, McMurray et al. 2010); 2) reduces left ventricular hypertrophy and proteinuria in individuals with hypertension(Tirosh, Garg et al. 2010) and 3) reduces proteinuria in patients with diabetes(Nishiyama, Kobori et al. 2010). Identifying the mechanism(s) by which MR mediates vascular dysfunction is essential to prevent and treat CV disease.

Robin Holliday defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms"(Holliday 1990). Epigenetic modifications are induced by multi-factorial mechanisms and mediated by several factors and processes including, development *in utero*, environmental chemicals, pharmaceuticals, and diet (Perera and Herbstman 2011). Some epigenetic modifications can increase the exposure of the molecular substrates to the harmful exogenous stimuli that initiate various diseases such as cancer (Baylin and Jones 2011), cardiovascular disease (Lorenzen, Martino et al. 2012), diabetes mellitus (Ng, Lin et al. 2010) and obesity (Yazbek, Spiezio et al. 2010). Identifying the causes and consequences of epigenetic changes is essential to understanding disease development and more importantly, how these changes can be targeted either by the pharmacological or genetic intervention to activate or suppress gene transcription related to disease development (Cai, Kohler et al. 2011; Radpour, Barekati et al. 2011).