SYNERGISTIC EFFECTS OF CISPLATIN AND APIGENIN ON TELOMERASE EXPRESSION AND TELOMERASE ACTIVITY IN TNBC CELLS

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Letter of Report Submission

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Dear Professor Dr. Hadariah Bahron,

Submission of Final Report for MAKNA Grant

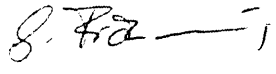
Herewith we are submitting the final report for the MAKNA Grant entitled **"Synergistic Effects of Cisplatin and Apigenin on Telomerase Expression and Telomerase Activity in TNBC cells "** with the project code number 100-RMI/PRI 16/6/2 (6/2013) under the basic medical sciences category. The grant was awarded to Noorfaiza binti A. Aziz with a total amount of RM30,000.00.

Here we are reporting the synergistic effect of apigenin and cisplatin on the expression and activity of the reverse transcriptase telomerase and its consequence for the viability of two triple negative breast cancer and one non-tumorigenic epithelial cell line.

All researchers have seen and agreed with the content of this report. Lastly, we thank the Research Management Institute (RMI) of UiTM for supporting our research in the field of breast cancer and natural products.

Thank you.

Best regards,



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3.2 Enhanced Executive Summary

Almost 80-90% of cancer cells show a highly active telomerase while normal, differentiated cells have no or very little activity. Telomerase activity in cancer cells depends on several factors like increased hTERT expression, modification of telomerase structural subunits and telomere-associated proteins. Treatment options for triple negative breast cancer (TNBC) are limited due to a lack of possible targets. Therefore, in this work, the effect of apigenin with and without cisplatin on telomerase activity and structure was investigated. Two different types of TNBC (MDA-MB-231 and HCC1806) and one control cell line (MCF-10A) were evaluated with regards to the cytotoxicity of the compounds mentioned above. The transcriptional and translational level of active telomerase including hTERT, Hsp90, p23 and p53 were measured using RT-PCR and ELISA. The gene and protein expression of telomerase and its activity highly depended on the cell type investigated. The reduction of telomerase activity in TNBC cells by cisplatin and apigenin was associated with a reduction of Hsp90 and p23 levels. The combined treatment of cisplatin and apigenin inhibited Hsp90 expression in MCF-10A. However, no significant change was detected in p23 expression as compared to control cells. The level of p53 protein expression in TNBC was inhibited after the combined treatment of cisplatin and apigenin. No significant changes were observed after cisplatin and apigenin single treatment in HCC1806. In MDA-MB-231 cisplatin-treated cells showed a significant increase in p53 protein expression as compared to control. The expression of p53 was up-regulated at the higher concentration of cisplatin as well as the combined treatment in MCF-10A cells. In conclusion, the inhibitory effect of cisplatin and apigenin telomerase activity in TNBC involves complex regulatory mechanisms. Nevertheless, telomerase activity remains a potential target for the treatment of TNBC, and the combination of cisplatin and apigenin offers increased efficacy in cancer cells but unfortunately less protective in normal cells.

3.3 Introduction

Triple Negative Breast Cancer (TNBC) is a highly heterogeneous and metastatic breast cancer form with a low five-year survival rate of diagnosed patients. As TNBC is negative for the expression of estrogen (ER), progesterone (PR) and Her2neu receptors it has limited treatment options due to a lack of molecular targets and the heterogeneity of deregulated pathways. Various therapeutic treatments for breast cancers have been developed including surgery, adjuvant chemotherapy, radiotherapy, hormonal therapy or targeted therapy. Combination therapy is a new approach whereby two or more pathways are targeted at the same time.

Cisplatin belongs to the class of platinum-containing anticancer drugs which is used to treat various types of cancer including lung cancer, ovarian cancer, germ cell tumor and lymphoma. However, the usage of this drug is reduced due to its high toxicity and many side effects to normal cells. Cisplatin induces cytotoxicity in tumour cells by binding to genomic and non-DNA targets and therefore inhibiting transcription and DNA replication that leads to the activation of either necrosis or apoptosis (Cepeda *et* al., 2007). Another limiting factor is the development of cisplatin-resistant cancer cells after prolonged treatment, or in re-emerging cancer. Any compound that can decrease cisplatin toxicity in normal cells while increasing its toxicity in cancer cells without triggering the development of cisplatin resistance would be of immense value for the treatment of TNBC.

Various natural products or bioactive compounds isolated from various plant parts have shown potential anticarcinogenic abilities in inhibiting the progression of cancer cells. Apigenin (4', 5, 7-trihydroxyflavone) is a flavone and belongs to the group of flavonoids that are commonly found in fruits and vegetables (Miean et al., 2001). Previous investigations have reported that apigenin has anti-inflammatory, anticancer and free-radical scavenging properties (Soares et al., 2006, Singh et al., 2004, Romanova et al., 2001).

One of the characteristics of malignant cells is the reactivation of the reverse-transcriptase telomerase. Shay et al. (1997) have reported that 85 - 90 percent of all cancer cells have telomerase activity while the expression and, therefore, the activity is very low or undetectable in normal cells. Lu et al. (2011) observed that the expression of telomerase appeared to be slightly higher in tumours with longer telomeres as well as in larger tumours or aggressive diseases. However, a few