UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ZINC SUPPLEMENT ON TUMORIGENIC (MCF-7) AND NON-TUMORIGENIC (MCF-10A) MAMMARY EPITHELIAL CELLS UNDER DIFFERENT OXYGEN CONDITIONS

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Faculty : Biotechnology and Biomolecular Sciences

Zinc is an important trace element which is obtained through our daily food intake. Its imbalance causes a range of human diseases from simple headaches to cancer. Insufficient zinc is addressed with the use of zinc supplements mostly in the form of tablets. The importance of zinc level in the normal homeostasis of the human body highlights the need for further understanding of zinc involvement in cellular regulation. Changes in human physiology also affect the outcome of zinc functions. These changes in women are more common due to conditions such as pregnancy,

lactation and menopausal changes. Earlier studies reported that malignant breast tissues were commonly found to correlate with high cellular zinc concentration. Further studies on the outcome of high cellular zinc concentration on the cellular functionhowever were never reported until now. To address this issue, this study was conducted to investigate the effects of zinc supplement on tumorigenic MCF-7 and non-tumorigenic MCF-10A mammary epithelial cells. It was hypothesized that zinc supplement will cause the cell cycle control mechanisms of breast epithelia to be deregulated. Results obtained showed that the cells responded differently to zinc treatment beginning at 100 µM zinc, but not at lower concentrations. MCF10-A was found to be arrested at the G_2/M cell phase at higher level compared to the MCF-7 cells. Conditions of normoxia and hypoxia did not drastically affect the way that the MCF-7 and MCF-10A responded to zinc treatment. The G_2/M arrest was found to be associated with the increase in Cyclin A in both cell lines. In MCF-10A, $p21^{CIP1/WAF1}$ protein was increased but not in MCF-7. This perhaps contributed to the higher G_2/M population in zinc-treated MCF-10A. Interestingly, a hypoxia-inducible factor alpha (HIF-1α), a monomer of the HIF-1 transcription factor also became accumulated in the presence of zinc, in both MCF-7 and MCF-10A cells. The HIF-1 complex was found to be active via detection of the CAIX expression, its specific transcriptional target. Overall, data obtained from this study contribute to further understanding of zinc in the regulation of breast epithelial cells. The information can be used to assist future research in the correlation of zinc and the development of breast cancer as well as other types of cancers in human.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ZINK TAMBAHAN TERHADAP SEL EPITELIUM MAMARI YANG BERBARAH (MCF-7) DAN TIDAK BERBARAH (MCF-10A) BAWAH KANDUNGAN OKSIGEN YANG BERBEZA

Oleh

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Zink merupakan unsur surih penting yang dapat diperolehi melalui pemakanan harian. Ketidakseimbangan zink mengakibatkan pelbagai penyakit dari simptom ringan seperti sakit kepala ke penyakit barah yang kronik. Kekurangan zat zink diatasi dengan pengambilan zink tambahan selalunya dalam bentuk pil. Kepentingan zink dalam pengawalaturan homeostasis tubuh manusia menekankan keperluan pemahaman lanjut terhadap penglibatan zink dalam pengawalaturan sel. Perubahan fisiologi manusia juga menjejaskan fungsi zink. Perubahan ini adalah lebih ketara di

kalangan wanita disebabkan oleh faktor-faktor seperti kehamilan, laktasi dan perubahan putus haid. Kajian sebelum ini melaporkan bahawa tisu payu dara malignan biasanya dihubungkaitkan dengan kepekatan selular zink yang tinggi. Walaubagaimanapun, tiada kajian lanjut mengenai kesan kepekatan selular zink yang tinggi terhadap fungsi selular pernah diterbitkan. Oleh itu, kajian ini dijalankan untuk mengkaji kesan zink tambahan pada sel mamari epitelium yang berbarah (MCF-7) dan tidak berbarah (MCF-10A). Zink tambahan dihipotesiskan akan menyebabkan penyahkawalan dalam mekanisme kitaran sel. Keputusan yang diperolehi menunjukkan bahawa sel-sel bertindakbalas secara berbeza terhadap rawatan zink dengan kepekatan zink pada 100 μM dan ke atas, tetapi tidak pada kepekatan yang lebih rendah. Kitaran sel MCF-10A didapati ditahankan pada fasa G_2/M dengan magnitud yang lebih tinggi berbanding MCF-7. Keadaan normosia dan hipoksia tidak menjejaskan tindakbalas MCF-7 dan MCF-10A terhadap rawatan zink secara drastik. Penahanan kitaran sel pada fasa G_2/M didapati berhubungkait dengan peningkatan dalam Cyclin A dalam kedua-dua sel yang digunakan dalam kajian ini. Peningkatan protein $p21^{CIP1/WAF1}$ dalam MCF-10A tidak diperhatikan dalam MCF-7. Peningkatan ini mungkin menyumbang kepada populasi G_2/M yang lebih tinggi dalam MCF-10A yang menerima rawatan zink. Menariknya, peningkatan subunit factor induksi hipoksia 1α (HIF-1α) juga diperhatikan dalam MCF-7 dan MCF-10A dengan kehadiran zink. Kompleks HIF-1 tersebut dibuktikan aktif dari segimen jalankan fungsi transkripsi melalui ekspresi CAIX yang merupakan sasaran khusus transkripsi HIF-1α. Secara keseluruhan, data kajian ini menyumbang kepada pemahaman lanjut tentang peranan zink dalam pengawalaturan sel epithelium mamari. Maklumat kajian ini juga dapat membantu penyelidikan masa depan yang berkaitan hubungan zink

tambahan dengan pembentukkan kanser manusia secara am dan kanser payu dara secara khusus.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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LEE SZE YEN

Date: 29 November 2013

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