UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF A CELL-BASED REPORTER ASSAY FOR SCREENING OF HYPOXIA-INDUCIBLE FACTOR ACTIVITIES

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ESTABLISHMENT OF A CELL-BASED REPORTER ASSAY FOR SCREENING OF HYPOXIA-INDUCIBLE FACTOR ACTIVITIES

By

LIEW SIEN YEI

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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

ESTABLISHMENT OF A CELL-BASED REPORTER ASSAY FOR SCREENING OF HYPOXIA-INDUCIBLE FACTOR ACTIVITIES

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November 2013

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Hypoxia-inducible factor (HIF) is one of the most important transcription factors involves in cells survival under low oxygen concentrations, termed hypoxia. Hypoxia is associated with various human diseases from ischemic injuries to cancer. The central role of HIF in these conditions has made it as one of the main target molecules for drug discovery. Demand for HIF assay system is high. Cell-based HIF assay systems are preferred since they can provide direct data for intracellular biochemical changes. HIF assay systems which are currently available in the market offer limited sensitivity with restricted reproducibility at a high cost. Therefore, in the present study, a HIF assay was succesfully developed. The system used a Saos-2 human osteosarcoma cell line which is one of the most studied cell lines to express a luciferase protein in response to HIF activation. The luciferase gene construct which was previously cloned downstream of four copies of a hypoxia response element (HRE) of the e*rythropoietin* (*EPO*) gene. *EPO* is one of the specific target genes of HIF. Therefore this assay can be used to

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measure HIF activation upon induction by candidate drugs of interest, by measuring the luciferase expression. This highly sensitive assay system offers a 40-times increased sensitivity compared to the currently available systems. This aspect would be highly beneficial to researchers since the increase in sensitivity can contribute towards result consistency and reproducibility leading to reduced wastage of precious drug candidates.

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

PEMBINAAN ASAI BERASAS SEL UNTUK PEMERIKSAAN AKTIVITI FAKTOR INDUKSI HIPOKSIA

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Faktor induksi hipoksia (HIF) merupakan faktor transkripsi yang amat penting dengan penglibatannya dalam kemandirian sel pada kepekatan oksigen yang rendah, juga dikenali hipoksia. Hipoksia dikaitkan dengan pelbagai jenis penyakit manusia dari kecederaan iskemia kepada kanser. HIF yang memainkan peranan penting dalam keadaan ini telah menjadikannya sebagai salah satu sasaran molekul utama dalam penemuan dadah. Permintaan sistem pengasaian HIF adalah tinggi. Sistem pengasaian HIF berasas sel adalah diutamakan kerana sistem ini dapat membekalkan data langsung kepada perubahan biokimia intrasel. Sistem pengasaian HIF yang kini didapati di pasaran menawarkan sensitiviti terhad dan kebolehasilan yang tersekat pada kos yang tinggi. Maka, satu asai HIF telah berjaya dibina dalam kajian ini. Sistem ini menggunakan titisan sel osteosarkoma manusia, Saos-2 yang merupakan salah satu jenis titisan sel yang meluas dikaji selidik untuk mengekspreskan protein lusiferase yang

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bergerak balas kepada pengaktifan HIF. Konstruk gen lusiferase ini adalah diklon di bawah empat salinan elemen respon hipoksia dalam gen *eritropoietin* (*EPO*). *EPO* adalah salah satu sasaran khusus HIF. Oleh itu, asai ini dapat digunakan untuk mengukur pengaktifan HIF semasa induksi oleh calon dadah yang dipentingkan dengan mengukur ekspresi lusiferase. Sistem pengasaian yang sangat peka ini menawarkan 40-kali kenaikan dalam sensitivitinya jika dibandingkan dengan sistem-sistem yang sedia ada. Ini akan banyak mendatangkan manfaat kepada penyelidik-penyelidik kerana kenaikkan sensitiviti ini dapat menyumbang kepada keputusan yang lebih konsisten dan kebolehasilan tinggi, dan juga mengurangkan pembaziran calon dadah yang berharga.

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I certify that a Thesis Examination Committee has met on 11 November 2013 to conduct the final examination of Liew Sien Yei on her thesis entitled "Establishment of a Cell-based Reporter Assay for Screening of Hypoxia-inducible Factor (HIF) activities" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends the student be awarded the Master of Science.

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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 institution.

_____

LIEW SIEN YEI

Date: 11th November 2013

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