UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *CXCR4* SILENCING BY siRNA ENGRAFTED CATIONIZED DEXTRAN IN MOUSE MODELS OF COLORECTAL CANCER AND LIVER METASTASIS

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By

FATEMEH ABEDINI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2011

DEDICATION

This thesis is dedicated to the memory of my dear parents (Mohammad Abedini and Khadijeh Poorhady), my dear husband (Dr. Mohammad Ebrahimi) and my darling daughters (Niousha and Nadiya) for their endless support and continuous encouragement throughout the thesis work.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

EFFECTS OF *CXCR4* SILENCING BY siRNA ENGRAFTED CATIONIZED DEXTRAN IN MOUSE MODELS OF COLORECTAL CANCER AND LIVER METASTASIS

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Chairman: Professor Maznah Ismail, PhD

Faculty: Institute of Bioscience

Liver metastasis is the main cause of colorectal cancer related mortality. *CXCR4* is necessary for the outgrowth of colon cancer micrometastasis. *CXCR4* gene expression and serum total lactate dehydrogenase, creatine kinase and alkaline phosphatase levels are often increased in patients with colorectal cancer. RNA interference is a well recognized pathway involved in cellular defense against viral invasion and post transcriptional regulation. This technology has emerged as a promising new strategy for the study of functional genomics and drug target validation. It is currently being evaluated in clinical trials as a potential therapy for cancers. This study aims to evaluate the transfection efficiency of three biodegradable polymers as carriers for *CXCR4* siRNAI, II to treat liver metastasis from colorectal cancer *in vitro* on mouse colon cancer cells (CT26.WT) and human colon cancer cells (HT29) and *in vivo* balb/c mice. In this study, dextran spermine, pullulan spermine and dextran hexamine were used as non-viral vectors for *in vitro*

and *in vivo CXCR4* siRNAs. Characterization of the morphology, size and stability of *CXCR4* siRNAs cationized dextran were performed using transmission electron microscopy, particle sizer and zeta potential. *CXCR4* expression in human colorectal cell line HT29 was measured by real-time reverse transcription PCR and immunocytochemistry. Cell proliferation assay, cell cycle analysis, acridine orange/propidium iodide staining and ultrastructural changes of cells using transmission electron microscopy were also studied as biological evaluations. Among three carriers studied, dextran spermine showed smallest size 99.25±4.3 nm with suitable zeta potential 34.15±1.55 mV. These findings were further supported by RT PCR that showed more silencing has been achieved by *CXCR4* pool siRNA/dextran spermine in comparison to pullulan spermine and dextran hexamine. The percentage of viability for dextran spermine was higher than pullulan spermine and dextran-hexamine. Animal study demonstrated that inhibition of *CXCR4* gene with *CXCR4* siRNAI, II/dextran spermine was more efficient than naked *CXCR4* siRNAI, II and also post treatment follow transfection of tumor cells more efficient than just transfection cells treatment with naked *CXCR4* siRNAI, II or *CXCR4* siRNAI, II/dextran spermine.*CXCR4* expression was correlated with serum total lactate dehydrogenase, alkaline phosphatase and creatine kinase. These data show that *CXCR4* expression lactate dehydrogenase, alkaline phosphatase and creatine kinase may be useful markers to predict liver metastasis in colorectal cancer. Dextran spermine demonstrated improved transfection efficiency in siRNA therapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsfah

KESAN PERENCATAN CXCR4 siRNA PELEKAP DEXTRAN TERKATION PADA KANSER KOLORAKTEL DAN METASTASIS HATI DI DALAM MODEL TIKUS

Oleh

FATEMEH ABEDINI

July, 2011

Pengerusi: Maznah Ismail, PhD

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Metastasis hati adalah penyebab utama yang menyumbang kepada kanser koloraktel (CRC) yang boleh menyebabkan kematian. *CXCR4* diperlukan untuk perkembangan kanser kolon metastasis mikro. Expresi gen *CXCR4* dan serum total laktat dehidrogenase, kreatin kinase dan tahap alkalin fosfatase selalunya meningkat pada pesakit yang menghidapi kanser koloraktel. RNA interference (RNAi) adalah rantaian tindakbalas biokimia yang diperakui terlibat di dalam tindakbalas pertahanan selular terhadap serangan virus, pengembangan transposon dan regulasi pasca-transkripsi. Teknologi ini telah berkembang sebagai teknologi baru yang berpotensi sebagai strategi untuk sasaran validasi ubat dan kajian terhadap fungsi genomik, dan sedang dinilai di dalam percubaan klinikal sebagai terapi yang berpotensi untuk penyakit etiologi genetik. Kajian ini bertujuan untuk menilai keberkesanan transfeksi tiga polimer yang boleh diuraikan secara biologi sebagai pembawa *CXCR4* siRNA ke atas sel kanser koloraktel secara *in vitro* manakala

metastasi hepatik telah dilancarkan dengan sel karsinoma kolon tikus CT26.WT pada tikus balb/c secara in vivo.

Dalam kajian ini, polisakarida terkation seperti dekstranspermin, pullulan-spermin, dan dekstran-hexamin, telah digunakan sebagai vector bukan virus untuk *CXCR4* siRNA. Pencirian morfologi, saiz dan kestabilan dextran terkation-*CXCR4* siRNA (dekstran-spermin) telah dilakukan dengan menggunakan mikroskopi electron transmisi, portikel sizer dan potensi zeta. Ekspresi *CXCR4* di dalam garisan sel kolorektal manusia HT29 telah diukur dengan menggunakan real time reverse transcription PCR dan immunocytochemistry. Asai proliferasi sel, analisis kitaran sel, pewarnaan acridine orange and propidium iodide dan perubahan saiz nanopartikel untuk kegunaan ke atas binatang dan manusia haruslah dalam skala 10-100nm dengan cas permukaan yang minimum. Dextran spermin menunjukkan saiz paling kecil (99.25±4.3 nm) di antara ketiga-tiga pembawa yang digunakan dalam kajian ini yang mana sesuai dengan keupayaan zeta 34.15±1.55 mV. Ini dibuktikan lagi melalui RT-PCR yang menunjukkan keputusan knock down yang lebih tinggi oleh *CXCR4* siRNAI, II/ dextran-spermine berbanding pullulan spermine dan dextran hexamine. Dextran spermin tidak mempengaruhi viabiliti sel pada 7.5 g/ml melalui asai viabiliti MTS.

Kajian *in vivo* menunjukkan "knock down" *CXCR4* siRNAI, II/ dextran spermine lebih efisyen berbanding naked *CXCR4* siRNA dan rawatan susulan selepas pemindahan sel tumor adalah lebih efisyen berbanding hanya melalui rawatan pemindahan sel dengan naked *CXCR4* siRNA atau *CXCR4*. Kajian ini menunjukkan dekstran terkation telah meningkatkan tahap ekspresi relative *CXCR4* siRNA dalam sel HT29 secara signifikan berbanding *CXCR4* siRNA tanpa dekstran terkation, pullulan-spermin dan dekstran-

hexamine. Keberkesanan perencatan yang tinggi dengan kadar toksik yang rendah telah dicapai dengan menggunakan *CXCR4* siRNAs/dekstran-spermin, dan telah dibuktikan dengan ekspresi gen *CXCR4* hati. Ekspresi *CXCR4* adalah berkait-rapat dengan serum total LDH, CK, dan ALP enzym. Data ini menunjukkan *CXCR4,* LDH, CK dan ALP boleh dijodikan penunjuk untuk menentukan matastasis hati dalam kanser kolorektal.

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I certify that a Thesis Examination Committee has met July 2011 to conduct the final examination of Fatemeh Abedini on her thesis entitled "Effects of Silencing CXCR4 siRNA Engrafted Cationized Dextran in Mouse Models of Colorectal Cancer and Liver Metastasis" 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 that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. 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 institution.

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FATEMHE ABEDENI

Date:

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