IDENTIFICATION OF MICRORNA-21 AS A PRO-INVASIVE TARGET IN UROTHELIAL CELL CARCINOMA

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IDENTIFICATION OF MICRORNA-21 AS A PRO-INVASIVE TARGET IN UROTHELIAL CELL CARCINOMA

By

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

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IDENTIFICATION OF MICRORNA-21 AS A PRO-INVASIVE TARGET IN UROTHELIAL CELL CARCINOMA

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Chair: Abhimanyu Veerakumarasivam, PhD
Faculty: Medicine and Health Sciences

MiRNAs are short non-coding endogenous RNA molecules that play substantial roles in human development and cell lineage decisions. It has been shown to regulate gene expression by controlling messenger RNA (mRNA) translation efficiency. There are emerging evidences suggesting that miRNA plays a critical role in cancer initiation and progression, acting either as tumor suppressors or oncogenes. This study is aimed at identifying mRNA expression patterns associated with the invasiveness of Urothelial Cell Carcinoma (UCC) cell lines and functional targeting of specific miRNAs that potentially regulate these target genes. Microarray-based global gene expression profiling of EJ28 (invasive) and RT112 (non-invasive) cells was performed to identify differentially regulated genes (P<0.01). Loess normalization using non-differentially expressed genes was performed by a rank invariant selection method to normalize the logarithmic expression ratios. Non-parametric Wilcoxon rank-sum test was used to identify top differentially expressed genes. Gene ontology was assigned to the top dysregulated genes using GeneDecks V3 online software (p<0.01) and the comprehensive set of functional annotation
tools of DAVID v6.7. Genes linked to metastasis were identified as amongst the top
dysregulated genes, and they were correlated to miR-21 and other miRNAs based on
*in silico* prediction. Several genes such as *SERPINB5, TIMP3* and *TPM1* were
predicted to be potentially regulated by miR-21. Several phenotype assays (matrigel
invasion, migration and cell proliferation) were conducted to characterize the
phenotypic effects of miR-21 expression modulation. The relative proliferation rate
at 144 hours of RT112 cells transfected with miR-21 inhibitor decreased dramatically,
at 33.23% and 36.96% as compared to untransfected sample control and mock
transfection control, respectively. Consistently, the relative proliferation rate at 96
hours of EJ28 cells transfected with miR-21 inhibitor decreased by 10.20% and
12.13% as compared to untransfected and mock transfected controls, respectively. In
the cell migration assay, knockdown of miR-21 in RT112 cells showed a 30.44%
decrease in cell migration rate at 27 hours. The migration rate was reduced more
significantly in knockdown EJ28 cells (47.38% at 27 hours). RT112 miR-21
knockdown cells demonstrated an invasion potential decrease of 3.41 and 3.29 fold
as compared to untransfected and mock transfection controls, respectively. As for
EJ28 cells, the invasion potential decreased by 2.53 and 2.33 fold as compared to
untransfected and mock transfection controls, respectively. Silencing of miR-21 in
both non-invasive and invasive bladder cancer cell lines was then demonstrated to
have an effect on cell proliferation, migration and invasion. In conclusion, miR-21 is
a potential key regulator in UCC progression and invasion, making it a likely
biomarker in the future.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENAL PASTIAN MIKRORNA-21 SEBAGAI SASARAN PRO-INVASIF DALAM KARCINOMA SEL UROTELIIUM

Oleh

TAN KEAI SINN

Disember 2011

Pengerusi: Abhimanyu Veerakum arasivam, PhD

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MiRNA merupakan molekul RNA endogenus tidak mengekod yang pendek dan memainkan peranan penting dalam perkembangan manusia dan ketentuan susur galur sel. Ia mampu mengawal ekspresi gen dengan mengawal kecekapan translasi RNA pengutus (mRNA). Pelbagai bukti baru telah ditemui membayangkan bahawa miRNA memainkan peranan penting dalam permulaan dan perkembangan kanser, iaitu bertindak sebagai penahan tumor atau onkogen. Kajian ini bertujuan untuk mengenal pasti kaitan di antara corak ekspresi mRNA dengan sifat invasif titisan karsinoma sel urotelium (UCC) dan sasaran berfungsi bagi miRNA khusus yang berpotensi mengawal atur gen sasaran. Pemprofilan ekspresi gen berasaskan mikroatur bagi sel-sel EJ28 (invasif) dan RT112 (tidak invasif) telah dijalankan untuk mengenal pasti gen-gen yang menunjukkan perbezaan dari segi pengawalaturannya (P<0.01). Normalisasi Loess menggunakan gen-gen, yang dikenal pasti melalui kaedah pemilihan pangkat tidak berubah serta tidak mengalami perubahan dari segi ekspresinya, telah dilakukan untuk menormalkan nisbah ekspresi.
pengembangbiakan sel, migrasi dan pencerobohan sel. Kesimpulannya, miR-21 berpotensi sebagai pengawal atur utama dalam perkembangan dan pencerobohan UCC, menjadikannya sebagai bio-penanda yang berpotensi pada masa yang akan datang.
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I certify that a Thesis Examination Committee has met on 14 December 2011 to conduct the final examination of Tan Keai Sinn on her thesis entitled “Identification of Microrna-21 as a Pro-Invasive Target in Urothelial Cell Carcinoma” 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 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.

___________________
TAN KEAI SINN

Date: 14 December 2012
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