

**MOLECULAR MECHANISM OF SYNTHETIC MICRORNA
UNDERLYING CHRONIC MYELOID LEUKAEMIA CELLS
PROLIFERATION BY TARGETING THE 3'UTR OF *ABL1*
COMPONENT OF THE *BCR-ABL1* FUSION GENE**

Syarifah Faezah Syed Mohamad

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ABSTRAK

Leukemia Myeloid Kronik (CML) adalah malignan hematologi yang didorong oleh gen gabungan BCR-ABL1, yang menyebabkan proliferasi sel myeloid yang tidak terkawal. Walaupun terdapat kemajuan dalam rawatan, rintangan terhadap perencat tirosin kinase (TKI) masih merupakan cabaran kritikal. Kajian ini menyiasat mekanisme molekul mikroRNA (miRNA) sintetik dalam mengawal proliferasi sel dengan menasaskan kawasan tidak diterjemah 3' (UTR) gen *ABL1* sebagai komponen dalam gen gabungan *BCR-ABL1*. Melalui analisis in-silico menggunakan DIANATOOLS, psRNATarget, RNA22, dan TargetRank, tiga calon miRNA telah dikenal pasti, iaitu hsa-miR-3131, hsa-miR-891a-3p, dan osa-miR1858a/b. Ketiga-tiga miRNA ini telah dianalisis lanjut melalui analisis rangkaian dan laluan menggunakan STRING dan Cytoscape untuk meramalkan kesan selular yang lebih luas. Melalui in-vitro, kecekapan transfeksi dan interaksi miRNA-mRNA telah dikaji dalam garis sel CML K562-s (sensitif) dan K562-r (resisten) menggunakan ujian TaqMan dan teknik penarikan turun biotin miRNA. Ekspresi gen ABL1 dan BCR-ABL1 selepas transfeksi dinilai melalui qPCR, manakala viabiliti sel dan kesan kitaran sel diukur melalui ujian MTS dan sitometri aliran. Ekspresi protein ABL1 pula dinilai melalui ujian kolorimetri (ELISA), dan analisis mikroarray dilakukan untuk mengenal pasti gen yang diekspresikan secara berbeza (DEG) akibat transfeksi miRNA. Keputusan menunjukkan transfeksi yang berkesan, dengan osa-miR1858a/b menunjukkan ekspresi tinggi dalam sel k562-r dan hsa-miR-3131 dalam sel k562-s, mencadangkan kecekapan transfeksi spesifik kepada jenis sel. Ujian penarikan turun biotin miRNA mengesahkan interaksi pengikatan antara hsa-miR-891a-3p dan hsa-miR-3131 dengan mRNA sasaran dalam k562-r, tetapi tidak dalam sel k562-s. Ujian Mann-Whitney menunjukkan bahawa hsa-miR-3131 menurunkan ekspresi *BCR-ABL1* secara signifikan dalam sel k562-s ($p < 0.05$), namun tiada penurunan ketara berlaku dalam k562-r. Walau begitu, tiada perubahan ketara dalam tahap protein ABL1 selepas transfeksi. Secara fungsinya, daya hidup sel K562-r menurun kepada $50.5\% \pm 1.53$, $63.4\% \pm 0.25$ dan $61.3\% \pm 0.23$ apabila ditransfeksi dengan osa-miR1858a/b, hsa-miR-3131 dan hsa-miR-891a-3p masing-masing berbanding kumpulan kawalan ($p < 0.05$). Daya hidup sel k562-s turut menurun kepada $84.45\% \pm 0.673$ (hsa-miR-3131), $81.45\% \pm 0.816$ (miR-891a-3p), dan $83.86\% \pm 0.733$ (osa-miR-1858a) dengan $p < 0.05$ berbanding kumpulan kawalan. Selain itu, osa-miR1858a/b dan hsa-miR-891a-3p menyebabkan perencatan kitaran sel pada fasa G2/M dalam sel k562-r, dengan peratusan masing-masing $6.5\% \pm 0.9$ dan $8.6\% \pm 1.1$, berbanding dengan kumpulan kawalan ($p < 0.05$). Tambahan pula, osa-miR1858a/b menyebabkan perencatan kitaran sel pada fasa S dan G2/M dalam sel k562-s, dengan $59.74\% \pm 1.34$ dan 7.80 ± 0.58 masing-masing, berbanding dengan kawalan ($p < 0.05$). Analisis mikroarray tidak menunjukkan (DEGs) yang signifikan antara kumpulan yang dirawat dan kawalan namun, gen terpilih yang berkaitan dengan CML telah disahkan semula menggunakan qPCR. Osa-miR1858a/b secara signifikan menurunkan IL6 dan MAP4K1 dalam sel K562-s dan K562-r. Kedua-dua gen ini terlibat dalam laluan berkaitan proliferasi seperti MAPK dan JAK/STAT. Penemuan ini menonjolkan keupayaan luar biasa miRNA berasaskan tumbuhan osa-miR1858a/b dalam menurunkan kebolehan hidup sel, merencatkan kitaran sel, serta menurunkan pengawalan gen utama seperti IL6 dan MAP4K1, sekali gus membuktikan potensi pengawalseliaan merentas kerajaan secara in-vitro. Penyelidikan ini memberikan pandangan baru tentang potensi terapeutik miRNA sintetik dalam rawatan CML,

dengan menekankan peranan unik hsa-miR-3131, hsa-miR-891a-3p dan terutamanya osa-miR1858a/b dalam mengganggu isyarat proliferaatif penting CML.



ABSTRACT

Chronic myeloid leukaemia (CML) is a hematologic malignancy driven by the BCR-ABL1 fusion gene, leading to uncontrolled proliferation of myeloid cells. Despite advancement in treatment, resistance to tyrosine kinase inhibitors (TKIs) remains a critical challenge. This study investigates the molecular mechanisms of synthetic microRNAs (miRNAs) in regulating cell proliferation by targeting the 3' untranslated region (UTR) of the *ABL1* as the components of the *BCR-ABL1* fusion gene. In-silico analyses using DIANATOOLS, psRNATarget, RNA22, and TargetRank, identified three candidate miRNAs namely, hsa-miR-3131, hsa-miR-891a-3p, and osa-miR1858a/b. These miRNAs were further explored via network and pathway analyses using STRING and Cytoscape to predict their broader cellular impact. Through in-vitro, miRNA transfection efficiency and miRNA-mRNA interactions were determined in K562-s (sensitive) and K562-r (resistant) CML cell lines using TaqMan assays and biotin miRNA pull-downs. Gene expression of *ABL1* and *BCR-ABL1* post-transfection was assessed by qPCR, while cell viability and cell cycle were measured via MTS assays and flow cytometry, respectively. Additionally, ABL1 protein expression was evaluated through a colorimetric enzyme-linked immunosorbent assay (ELISA), and microarray analysis was performed to identify differentially expressed genes (DEGs) affected by miRNA transfection. Results indicated effective transfection, with osa-miR1858a/b exhibiting high expression in k562-r cells and hsa-miR-3131 in k562-s cells, suggesting cell-type-specific transfection efficiency. The miRNA pull-down assay confirmed binding interactions of hsa-miR-891a-3p and hsa-miR-3131 with target mRNA in k562-r but not in k562-s cells. Notably, Mann-Whitney test shows that hsa-miR-3131 significantly downregulated target *BCR-ABL1* in k562-s cells ($p < 0.05$), while no substantial downregulation occurred in k562-r cells. Despite this, no significant changes in ABL1 protein levels were observed post-transfection. Functionally, cell viability of k562-r cells was lowered by over $50.5\% \pm 1.53$, $63.4\% \pm 0.25$ and $61.3\% \pm 0.23$ when transfected with osa-miR1858a/b, hsa-miR-3131 and hsa-miR-891a-3p respectively compared to the control group ($p < 0.05$). Nevertheless, the viability of k562-s cells was decreased to $84.45\% \pm 0.673$ (hsa-miR-3131), $81.45\% \pm 0.816$ (hsa-miR-891a-3p), and $83.86\% \pm 0.733$ (osa-miR-1858a) with $p < 0.05$ compared to the control groups. Additionally, osa-miR1858a/b and hsa-miR-891a-3p induced a cell cycle arrest at the G2/M phase in k562-r cells, with $6.5\% \pm 0.9$ and $8.6\% \pm 1.1$ respectively, compared to the control group ($p < 0.05$). Furthermore, osa-miR1858a/b induced a cell cycle arrest at the S and G2/M phases in k562-s cells, with $59.74\% \pm 1.34$ and 7.80 ± 0.58 , respectively, compared to controls ($p < 0.05$). Microarray analysis revealed no (DEGs) between miRNA-treated and control groups; however, selected genes relevant to CML were further validated by qPCR. Notably, osa-miR1858a/b significantly downregulated IL6 and MAP4K1 in K562-s and K562-r cells, respectively. Both genes are involved in proliferation-related pathways such as MAPK and JAK/STAT. These findings highlight the exceptional ability of the plant-based miRNA osa-miR1858a/b in decreasing cell viability and inducing cell cycle arrest, as well as downregulating key genes such as IL6 and MAP4K1, demonstrating inter-kingdom regulatory potential in-vitro. This research provides novel insights into the therapeutic potential of synthetic miRNAs in CML treatment, emphasizing the unique role of hsa-miR-3131, hsa-miR-891a-3p and especially osa-miR1858a/b in disrupting essential proliferative signalling cascades of CML.

ملخص

سرطان الدم النخاعي المزمن (CML) هو ورم خبيث دموي مدفوع بجين الاندماج BCR-ABL1، الذي يسبب تكاثرًا غير منضبط لخلايا نخاع. بينما تم إحراز تقدم كبير في علاج CML، لا يزال مقاومة مثبطات كيناز التيروسين (TKIs) تمثل تحديًا كبيرًا، مما يبرز الحاجة إلى أساليب علاجية جديدة. تبحث هذه الدراسة في الآليات الجزيئية للميكروRNAs الاصطناعية (miRNAs) في تنظيم التكاثر من خلال استهداف المنطقة غير المترجمة 3' UTR (UTR) لجين BCR-ABL1. من خلال التحليلات الحاسوبية باستخدام DIANA TOOLS و psRNA Target و RNA22 و TargetRank، تم تحديد miRNAs البشرية والنباتية المحتملة التي تستهدف 3' UTR لجين ABL1، مع وجود مرشحين بارزين مثل hsa-miR-3131 و hsa-miR-891a-3p و osa-miR1858a/b. تمت دراسة هذه الجزيئات الصغيرة من خلال تحليلات الشبكات والمسارات باستخدام STRING و Cytoscape و للتنبؤ بتأثيرها الخلوي الأوسع. من خلال التجارب في المختبر، تم تحديد كفاءة نقل miRNA وتفاعلات miRNA-mRNA في كل من خطوط خلايا K562-s (الحساسة) و K562-r (المقاومة) باستخدام اختبارات TaqMan وسحب البيوتين miRNA. تم تقييم التعبير الجيني لـ ABL1 و BCR-ABL1 بعد النقل الجيني باستخدام qPCR، بينما تم قياس حيوية الخلايا وتأثيرات دورة الخلية عبر اختبارات MTS والتدفق الخلوي على التوالي. بالإضافة إلى ذلك، تم تقييم تعبير بروتين ABL1 من خلال اختبار الامتصاص المناعي المرتبط بالإنزيم الملون (ELISA)، وتم إجراء تحليل المصفوفة الدقيقة لتحديد الجينات المعبر عنها بشكل مختلف (DEGs) المتأثرة بنقل miRNA. أشارت النتائج إلى نقل فعال، حيث أظهر osa-miR1858a/b تعبيرًا عاليًا في خلايا k562-r و hsa-miR-3131 و hsa-miR-891a-3p في خلايا k562-s، مما يشير إلى كفاءة نقل نوعية للخلايا. أكد اختبار سحب miRNA تفاعلات الربط بين hsa-miR-891a-3p و miR-891a-3p مع mRNA المستهدف في خلايا k562-r ولكن ليس في خلايا k562-s. من الجدير بالذكر أن hsa-miR-miR-3131 خفض بشكل كبير الهدف BCR-ABL1 في خلايا k562-s ($p < 0.05$)، بينما لم يحدث أي انخفاض كبير في خلايا k562-r. على الرغم من ذلك، لم تُلاحظ أي تغييرات كبيرة في مستويات بروتين ABL1 بعد النقل الجيني. وظيفيًا، قللت osa-miR1858a/b من حيوية خلايا k562-r بأكثر من 50.5% ($1.53 \pm 63.4\%$) و hsa-miR-3131 ($0.25 \pm 61.3\%$) و hsa-miR-891a-3p ($0.673 \pm 84.45\%$) مقارنة بمجموعة التحكم ($p < 0.05$). ومع ذلك، انخفضت حيوية خلايا k562-s إلى $0.733 \pm 83.86\%$ و hsa-miR-3131 ($0.816 \pm 81.45\%$) و osa-miR-1858a ($0.733 \pm 83.86\%$) مع $p < 0.05$ مقارنة بالمجموعات الضابطة. بالإضافة إلى ذلك، فإن hsa-miR-891a-3p و osa-miR1858a/b قد تسببا في توقف دورة الخلية عند مرحلة G2/M في خلايا k562-r، بنسب $0.9 \pm 6.5\%$ و $1.1 \pm 8.6\%$ على التوالي، مقارنة بمجموعة التحكم ($p < 0.05$). علاوة على ذلك، أدى osa-miR1858a/b إلى توقف دورة الخلية في مراحل S و G2/M في خلايا k562-s، بنسبة $1.34 \pm 59.74\%$ و $0.58 \pm 7.80\%$ على التوالي، مقارنة بالمجموعة الضابطة ($p < 0.05$). أظهر تحليل المصفوفة الدقيقة عدم وجود جينات معبرة بشكل مختلف (DEGs) بين المجموعات المعالجة بـ miRNA والمجموعات الضابطة؛ ومع ذلك، تم التحقق من صحة الجينات المختارة ذات الصلة بـ CML باستخدام qPCR. من الجدير بالذكر أن osa-miR1858a/b خفض بشكل كبير من مستوى IL6 و MAP4K1 في خلايا K562-s و K562-r على التوالي. كلا الجينين متورطان في مسارات متعلقة بالتكاثر مثل JAK/STAT و MAPK. تسلط هذه النتائج الضوء على القدرة الاستثنائية للـ miRNA النباتي osa-miR1858a/b في تقليل حيوية الخلايا وإحداث توقف في دورة الخلية، بالإضافة إلى تقليل التعبير عن الجينات الرئيسية مثل IL6 و MAP4K1، مما يظهر الإمكانات التنظيمية بين الممالك في المختبر. توفر هذه الدراسة رؤى جديدة حول الإمكانات العلاجية للـ miRNAs الاصطناعية في علاج CML، مع التأكيد على الدور الفريد لـ hsa-miR-3131 و hsa-miR-891a-3p و osa-miR1858a/b وخاصة في تعطيل مسارات الإشارات التكاثرية الأساسية لـ CML.

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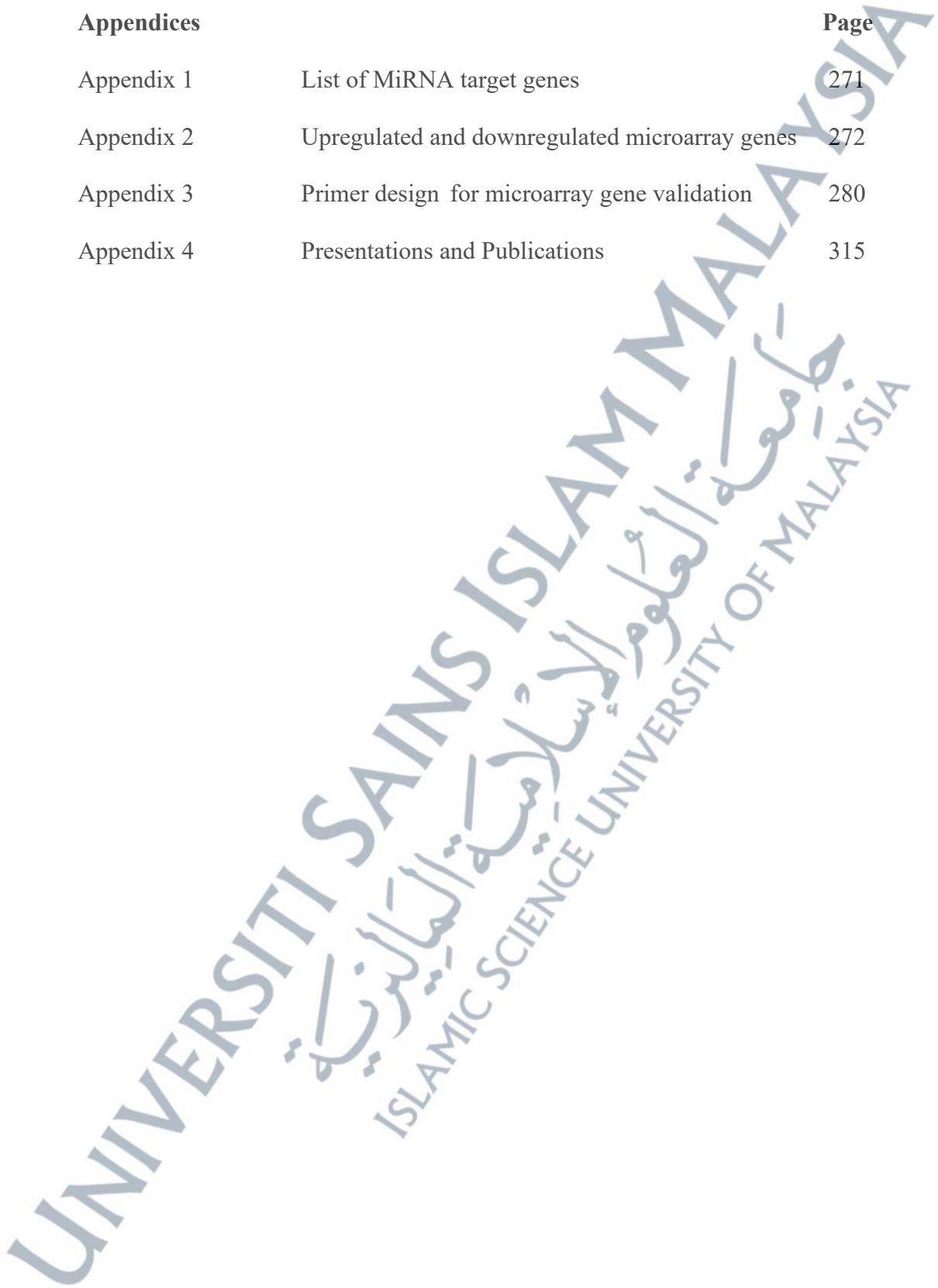
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LIST OF ABBREVIATIONS

3'UTR	3' Untranslated Region
ABL	Abelson Murine Leukemia Viral Oncogene Homolog
AML	Acute Myeloid Leukemia
ARE	AU-Rich Element
ASO	Antisense Oligonucleotide
ATP	Adenosine Triphosphate
antimiRs	Antagonistic MicroRNAs
BCR	Breakpoint Cluster Region
CCyR	Complete Cytogenetic Response
CDK	Cyclin-Dependent Kinase
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
DNA	Deoxyribonucleic Acid
GTP	Guanosine Triphosphate
HSCT	Hematopoietic Stem Cell Transplantation
IM	Imatinib Mesylate
kD	Kilodalton
LNA	Locked Nucleic Acid
LSCS	Leukemic Stem Cells
m-bcr	Major Breakpoint Cluster Region
μ -bcr	Micro Breakpoint Cluster Region
miRNA	MicroRNA
miTG	miRNA-Target Gene Interaction
MMR	Major Molecular Response
MRE	MicroRNA Recognition Element
mRNA	Messenger RNA
ncRNA	Non-Coding RNA
oncomiRs	Oncogenic MicroRNAs
Ph	Philadelphia Chromosome
RiSC	RNA-Induced Silencing Complex

RNA	Ribonucleic Acid
SRC	Proto-Oncogene Tyrosine-Protein Kinase Src
TKI	Tyrosine Kinase Inhibitor
TRBP	Trans-Activation Response RNA-Binding Protein
WBC	White Blood cells

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