CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Research Findings

Chronic Myeloid Leukaemia (CML) remains a significant challenge in the field of haematological malignancies, primarily driven by the *BCR-ABL1* fusion gene. This study aimed to elucidate the molecular mechanism by which synthetic microRNAs influence CML cell growth by targeting the 3'UTR of the *ABL1* gene.

Through *in-silico* analysis, molecular biology approaches, and functional assays, we successfully identified and experimentally validated miRNAs with confirmed or predicted targeting of *ABL1/BCR-ABL1* genes, assessing their influence on gene expression, cell proliferation and cycle progression. In addition, transcriptome analysis enabled us to understand better the global gene expression changes triggered by miRNA transfection in CML cells. Key findings of this study are:

1) In-silico analysis predicted five human and five plant-based miRNAs with potential binding to the 3'UTR of the ABL1 gene. From these, three miRNAs (hsa-miR-3131, hsa-miR-891a-3p, and osa-miR1858a/b) were selected for further experimental validation based on optimal miTG scores, folding energy, and p-values.
2) The TaqMan assay demonstrated efficient miRNA transfection in both k562-s and k562-r cells, with k562-r exhibiting a higher miRNA transfection efficiency.
3) Biotin pull-down assay successfully validated the interaction of hsa-miR-3131 and hsa-miR-891a-3p with *ABL1* in k562-r cells, confirming their

direct targeting. However, due to limitations in the biotin-based method for plant miRNAs, osa-miR1858a/b target interaction could not be verified in this system.

4) Transfection of the miR-3131 induced significant downregulation of *ABL1* and *BCR-ABL1* genes in k562-s cells, whereas in k562-r cells, only ABL1 expression was reduced when transfected with all miRNAs. Interestingly, no significant changes were observed in ABL1 protein expression.
5) Functional assays revealed a substantial reduction in cell viability, with osa-miR1858a/b reducing viability by ~50% in k562-r cells, alongside significant cell cycle arrest at the G2/M phase. Meanwhile, in k562-s, all miRNAs reduced cell viability by ~80% with osa-miR1858a/b inducing a cell cycle arrest at the S and G2/M phases.

Additionally, while microarray analysis did not identify statistically significant differentially expressed genes (DEGs) in the miRNA-transfected groups compared to controls, qPCR validation demonstrated the downregulation of IL6 in k562-s cells and MAP4K1 in k562-r cells post-transfection with osa-miR1858a/b.

However, as direct binding between osa-miR1858a/b and *ABL1*/*BCR-ABL1* could not be confirmed in this study, further target validation is necessary to elucidate their precise molecular mechanisms and potential indirect effects within the signalling network. While the direct binding of osa-miR1858a/b to the 3' UTR of IL6 remains unverified, the observed IL6 downregulation in K562-s cells suggest possible modulation of the IL6/JAK/STAT pathway. Based on previous reports, such modulation could reduce JAK/STAT activation, thereby lowering STAT3 phosphorylation and nuclear translocation, which in turn may diminish the expression of STAT3-regulated genes associated with cell survival, proliferation, and

inflammatory response. This mechanism if validated, could contribute to reduced inflammatory signalling and cell viability in k562-s cells.

In k562-r cells, osa-miR1858a/b downregulates MAP4K1, an upstream regulator in the MAPK pathway, specifically the JNK signalling cascade. Literature evidence indicates that reduced MAP4K1 expression can decrease MAPK pathway activation, which may impair proliferation and promote cell cycle arrest, particularly in resistant CML cells.

Despite the lack of interaction with the *ABL1/BCR-ABL1*, these findings indicate that osa-miR1858a/b warrants further investigation as a potential candidate for miRNA-based strategies targeting alternative pathways in CML. Figure 6.1 summarises important findings in this study and the suggested molecular mechanism of osa-miR1858a/b in k562-s and k562-r are shown in Figure 6.2 and Figure 6.3, respectively.

6.2 Recommendations for Future Research

The primary limitation of this study was the inability to examine the effects of miRNA transfection on Primary Peripheral Blood Mononuclear Cells (PBMCs) owing to technical challenges in cell culture. Future studies should concentrate on evaluating the potential of these miRNAs in primary CML patient samples to confirm their therapeutic efficacy in a clinical setting. Although osa-miR1858a/b did not demonstrate direct binding to the BCR-ABL1 3′UTR in the pull-down assay, functional assays indicated notable effects on CML cell proliferation and cell cycle regulation. These findings suggest that osa-miR1858a/b may act through alternative or indirect molecular mechanisms, potentially involving off-target binding or regulation of genes upstream or downstream of BCR-ABL1 signalling. To clarify these pathways, further studies should include comprehensive target validation approaches, such as transcriptome-wide

binding analysis, luciferase reporter assays, and protein phosphorylation profiling, to identify the precise molecular targets and signalling networks modulated by osa-miR1858a/b. Understanding these alternative mechanisms could provide new insights into the broader regulatory roles of plant-derived miRNAs in CML biology.

This study provides new insights of miRNA-mediated regulation in chronic myeloid leukaemia (CML) and highlights osa-miR1858a/b as a promising candidate for therapeutic development, particularly against drug-resistant CML cells.

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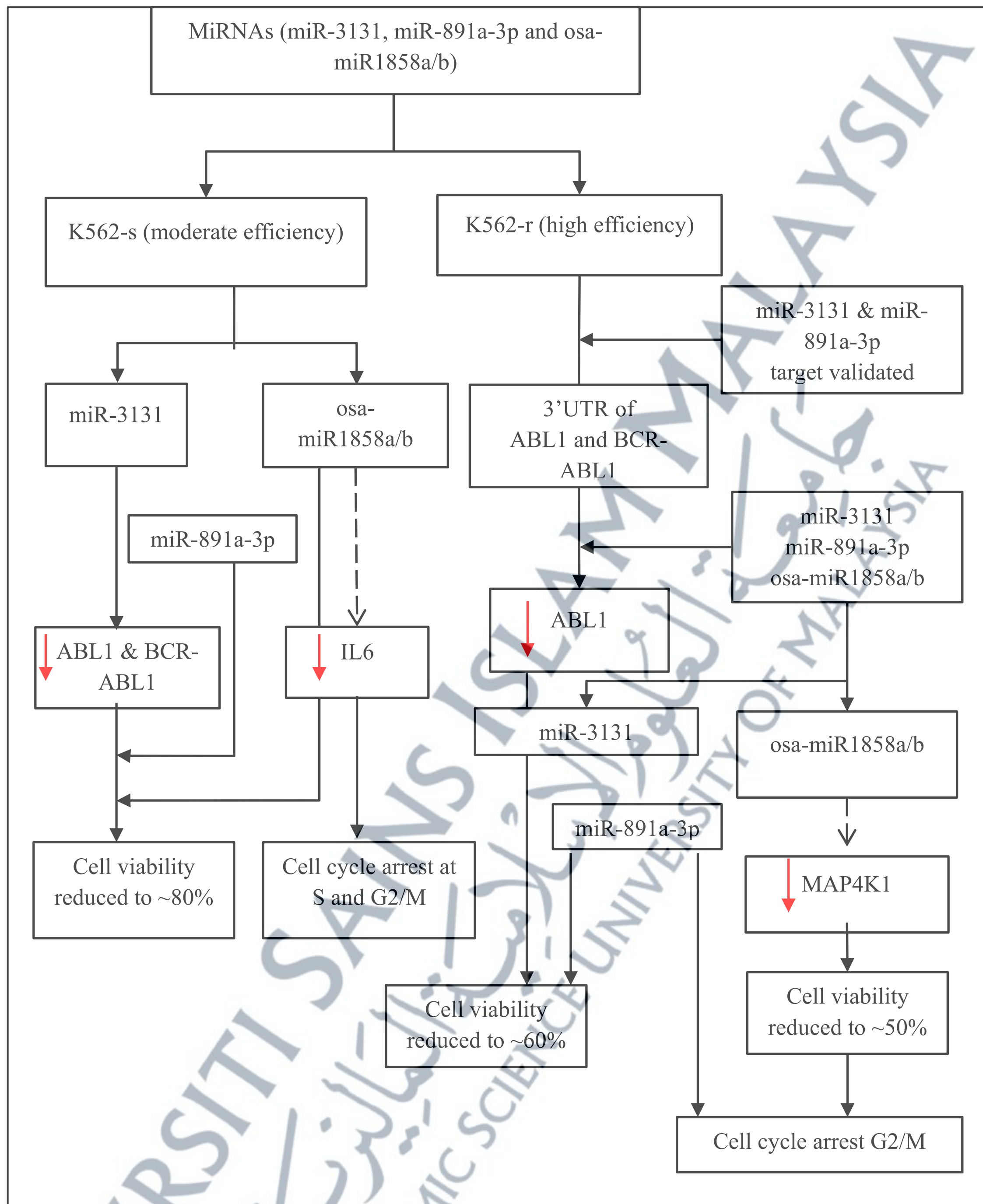

Figure 6.1: Key findings from miRNA transfection in CML cells; k562-s and k562-r.

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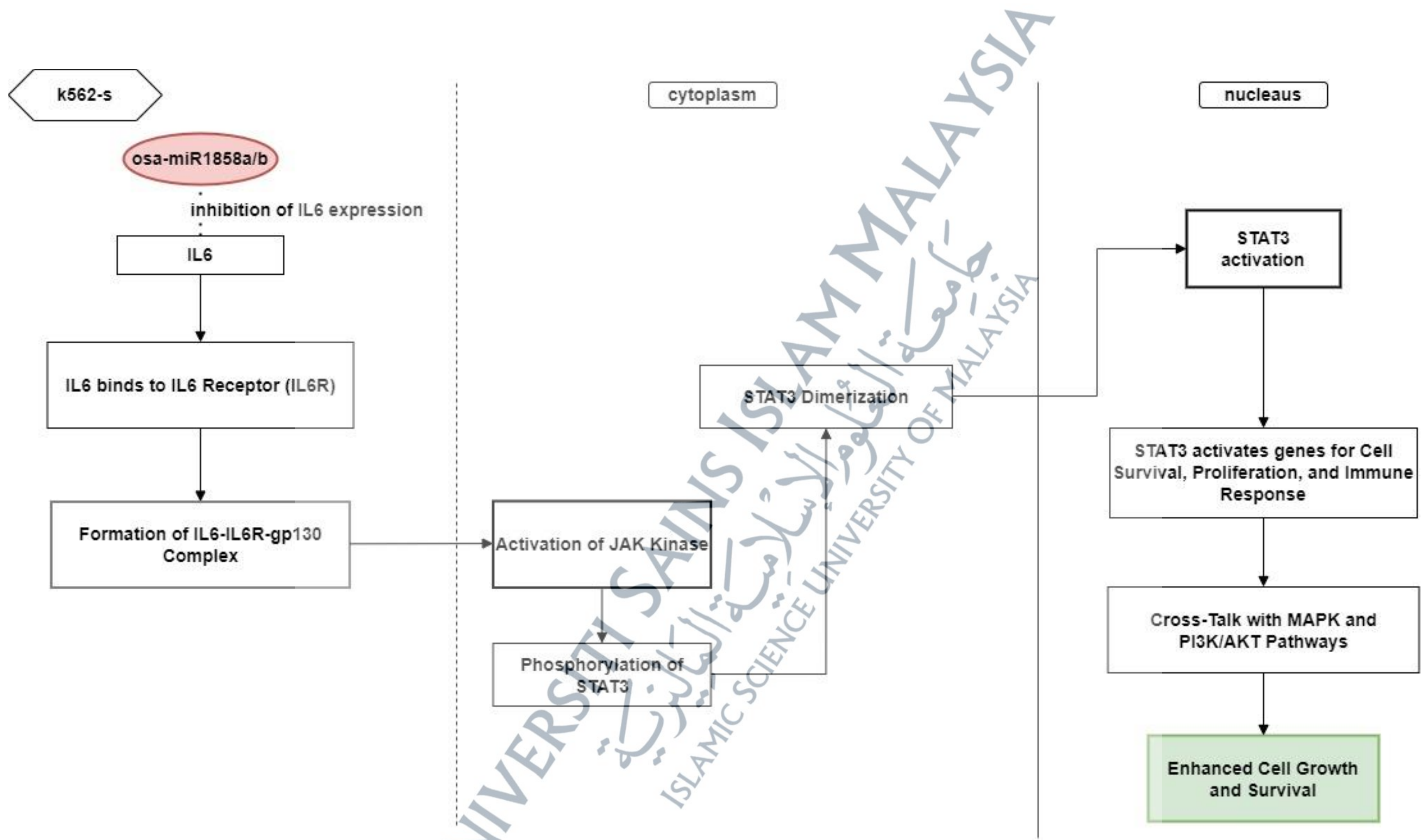

Figure 6.2: Proposed molecular mechanism of osa-miR1858a/b in regulating the IL6/JAK/STAT Pathway in K562-S Cells.

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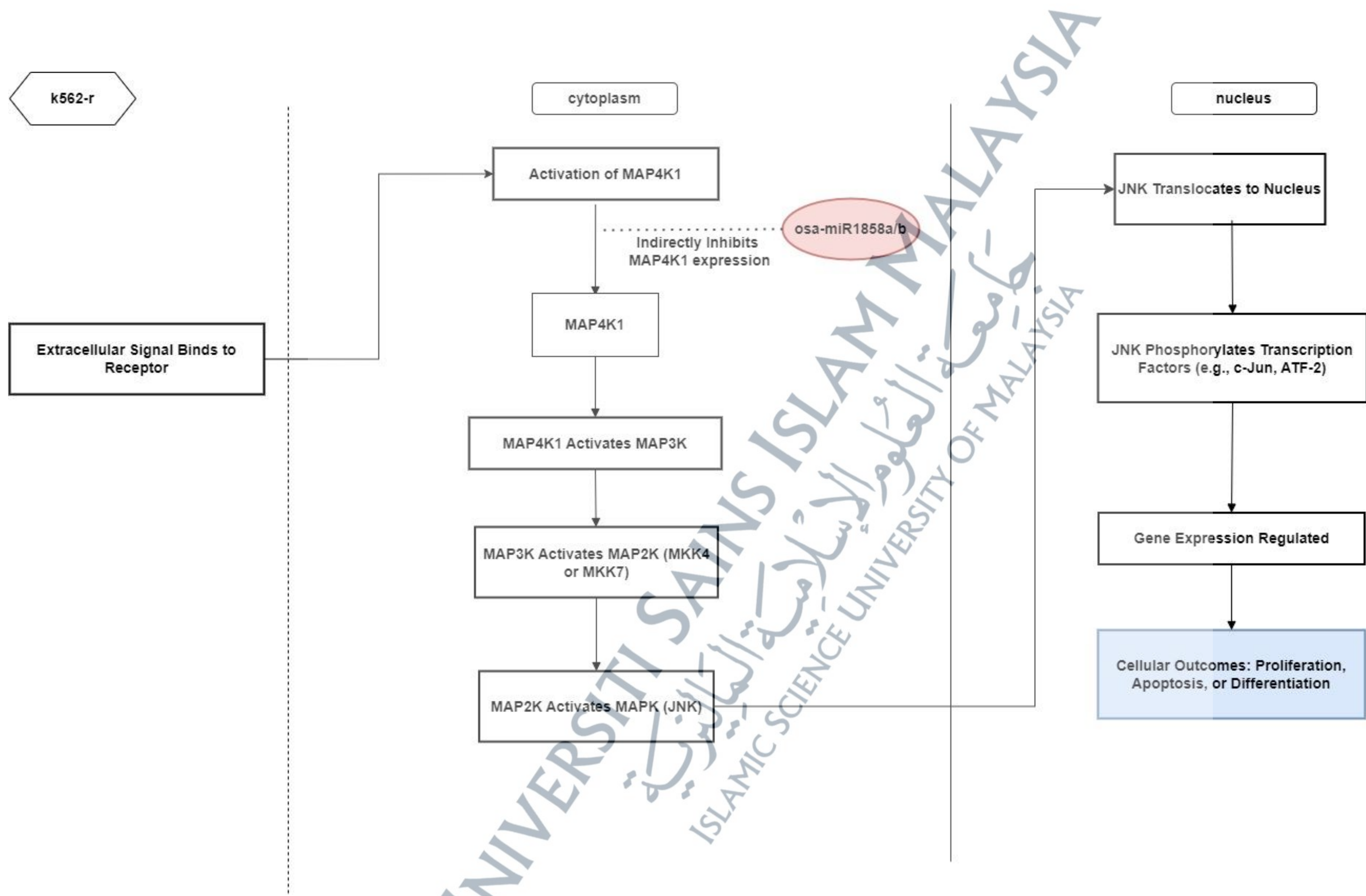

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Figure 6.3: Proposed molecular mechanism of osa-mir1858a/b in regulating MAPK pathway via MAP4K1 in k562-r cells.