CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Leukaemia is a cancer of the blood-forming tissues, including the bone marrow and lymphatic system causing an overproduction of abnormal white cells (WBC) that disseminate throughout the bloodstream (Tang et al., 2022). These abnormal cells overcrowd healthy blood cells, disrupting the body's ability to carry out vital tasks such as fighting infections, delivering oxygen and controlling bleeding (Haier & Nicolson, 2005). Leukaemia is categorised into different classifications based on the rate of advancement and the specific white blood cells implicated. For example, acute forms of leukaemia progress rapidly, but chronic ones develop more slowly. Cancer may also originate in different cell lines, such as myeloid or lymphoid cells (acute myelocytic leukaemia AML or chronic lymphoid leukaemia CLL respectively). These categories determine the disease's diagnosis, prognosis, and treatment plan (Tang et al., 2023). There are several factors contributing to the development of this disease. A combination of environmental and genetic variables might promote the ability to change from normal healthy cells to cancerous leukaemia cells. Moreover, the availability of genetic mutations, chromosomal translocations or epigenetic modifications can affect the normal cell function and biological pathways. Environmental factors. such as radiation, toxins, and viral infections, have been linked to causing these genetic changes, which can be acquired or inherited (Bavaro et al., 2019; Leak et al., 2023).

The signs and symptoms of leukaemia depend upon the type and phase of the illness. Common clinical symptoms include anaemia, tiredness, frequent infections, easy bruising or bleeding, bones or joints discomfort. The manifestation of these symptoms is a consequence of leukemic cells dominating the bone marrow, resulting in a reduction in the production of healthy blood cells (Kuan & Melaine Michael, 2018). Leukaemia has a substantial impact on the quality of life of patients and requires rigorous treatment approaches, thereby affecting various factors beyond only physical symptoms. The purpose of these treatments, such as chemotherapy, radiation, targeted drugs, and bone marrow transplantation, is to eliminate leukemic cells and restore normal blood cell production. Nevertheless, the highly combative characteristics of leukaemia and its propensity for relapse render it a formidable ailment to treat (Tang et al., 2023). This emphasises the significance of continuous investigation into the underlying causes and innovative treatment approaches.

Among the various types of leukaemia, Chronic Myeloid Leukaemia (CML) presents a unique molecular hallmark, the BCR-ABL1 fusion gene which provides a specific and traceable target for both diagnostic and therapeutic interventions. The existence of this fusion gene, resulting from a reciprocal translocation between chromosomes 9 and 22, allows researchers to explore targeted molecular mechanisms underlying leukemogenesis with greater clarity. Furthermore, while CML is well-managed by tyrosine kinase inhibitors (TKIs) such as imatinib, resistance and relapse remain clinically significant issues, highlighting the need for alternative or complementary therapeutic strategies. For these reasons, CML serves as a model disease to investigate gene regulation, resistance mechanisms, and potential microRNA-based therapeutic interventions.

1.1.1 Chronic Myeloid Leukaemia (CML)

In CML, a translocation between chromosome 9 and chromosome 22 leads to the formation of the Philadelphia chromosome, a distinctive abnormal chromosome with the *BCR-ABL1* fusion gene (Cilloni & Saglio, 2012; Navabi et al., 2022). In most cases, the p210 isoform is formed when the breakpoints in the BCR gene occur within the major breakpoint cluster region (M-bcr, exons b2 or b3) and join to the ABL1 gene at exon a2, resulting in e13a2 or e14a2 transcripts. The downstream ABL1 exons and its native 3′ untranslated region (3′UTR) are preserved in the fusion transcript. As a result, the ABL1 3′UTR is shared between both native ABL1 and the BCR-ABL1 fusion transcript, making it a regulatory site common to both molecules (Tamai et al., 2018).

This translocation event causes a constitutively active tyrosine kinase activity, which later activates signalling pathways and drives abnormal cell proliferation and survival (Goldman & Melo, 2003). Moreover, the BCR-ABL1 protein abnormally stimulates several important signalling pathways such as RAS, PI3K/AKT and JAK/STAT, which are responsible for regulating cell growth, division and differentiation and leads to uncontrolled myeloid cell proliferation (Al-Rawashde et al., 2022). The uncontrolled cell proliferation eventually leads to the accumulation of immature WBC in the bone marrow and blood, impairing haematopoiesis.

Current treatments for CML include the tyrosine kinase inhibitor (TKI) with Imatinib (IM) as the front-line treatment. TKI functions by targeting the BCR-ABL1 kinase, thus reducing CML cell proliferation. If the patients are not responding well to IM, second-generation TKIs such as Dasatinib and Nilotinib will be administered. These second-generation TKIs are more potent for patients who experience IM resistance.

CML usually progress through three phases: the chronic phase, the accelerated phase, and the blast phase. Each phase is characterised by the accumulation of leukemic cells and the increasing severity and aggressiveness of the disease. Most patients (approximately 85-90%) are diagnosed during the chronic phase of the disease; however, without treatment, the disease progresses to the accelerated phase and eventually to a blast crisis within 3-5 years (Bee et al., 2012; Lim et al., 2017). Furthermore, the accelerated phase can rapidly transform into the blast phase, and prognosis is usually poor in this phase. Hence, a more aggressive treatment, including a higher dosage of TKIs, chemotherapy, and haematopoietic stem cell transplantation (HSCT), might be needed to manage the disease.

CML accounts for about 15% of adult leukaemia cases and is characterised by its slow progression, affecting both blood and bone marrow (Deininger et al., 2000). Although CML can occur at any age, it is predominantly diagnosed in older adults, typically between the ages of 60 and 65. Despite the development of tyrosine kinase inhibitors (TKIs) to target the BCR-ABL1 pathway, challenges such as drug resistance and the persistence of leukemic cells remain, leading to ongoing disease and potential relapse. Although TKIs like imatinib initially show effectiveness, resistance can develop due to mutations in the BCR-ABL1 kinase domain or other compensatory mechanisms, highlighting the need for alternative therapies that can more effectively target CML (Deininger et al., 2000; Walz & Sattler, 2006; Yap et al., 2017).

1.1.2 MicroRNA

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression by binding to complementary sequences in the 3' untranslated regions (3'UTRs) of target mRNAs, leading to either mRNA degradation or translational

repression (Rath et al., 2016; Saiyed et al., 2022). In cancer, including CML, miRNAs can act as oncogenes or tumour suppressors by modulating the expression of genes involved in cell proliferation, differentiation, and apoptosis (Zhu et al., 2022). Dysregulation of miRNA expression can contribute to the CML pathogenesis, making them potential therapeutic targets (Radhi et al., 2022).

The therapeutic strategies involving synthetic or mimic miRNAs offers a promising approach to modulating oncogenic pathways in CML. In the case of p210 *BCR-ABL1*, the *ABL1* 3′UTR sequence is preserved in the fusion transcript, providing a shared regulatory region for both native *ABL1* and oncogenic *BCR-ABL1*. This shared 3′UTR underpins the rationale for identifying miRNAs that bind to this region, as they could simultaneously regulate both transcripts. Consequently, exploring human and plant-derived miRNA mimics that target the *ABL1/BCR-ABL1* 3′UTR presents a novel therapeutic approach, although their molecular mechanisms require further elucidation.

1.2 Problem Statement

Chronic Myeloid Leukaemia (CML) is a hematologic malignancy characterised by the uncontrolled cell proliferation of myeloid cells, driven by the BCR-ABL1 fusion protein. Introducing of tyrosine kinase inhibitors (TKIs) such as Imatinib revolutionised CML treatment, offering patients a targeted approach that dramatically improved survival rates. Nonetheless, despite the effectiveness of TKIs in attaining remission, resistance to this treatment is an ongoing challenge for patients, primarily due to modifications in the *BCR-ABL1* gene or the persistence of leukemic stem cells. Although second and third-generation TKIs have been developed to address resistance, they are associated with specific side effects, and their inability to eliminate leukemic

cells highlights the necessity for alternative therapeutic approaches that target the fundamental molecular mechanisms of CML.

MicroRNAs (miRNAs) are a critical regulator of various cellular processes, including cell proliferation, apoptosis, and differentiation through their post-transcriptional control of gene expression. In CML, miRNAs constitute a promising treatment strategy, especially owing to their capacity to regulate oncogenes such as *BCR-ABL1* post-transcriptionally. Targeting the 3′UTR of oncogenic transcripts like *ABL1*/*BCR-ABL1* is a particularly attractive strategy because it allows miRNAs to suppress gene expression in a highly specific and regulated manner. The 3′UTR contain miRNA binding site, and successful targeting at this region can lead to mRNA destabilization or translational inhibition without affecting the upstream coding sequences. This approach is especially relevant in CML, where abnormal kinase activity is driven by the fusion transcript that retains the *ABL1* 3′UTR, providing a potential regulatory entry point for miRNA-based intervention.

Nevertheless, the precise role of miRNAs in targeting the 3' untranslated region (UTR) of *BCR-ABL1* and *ABL1* is inadequately investigated. The efficiency of miRNA transfection into CML cells and the molecular details of miRNA-mRNA interaction still need to be fully understood, further complicating efforts to harness miRNA-based therapies. Moreover, the precise molecular mechanism by which synthetic miRNAs may influence the expression of BCR-ABL1 and ABL1, alter CML cell proliferation and survival, and impact cell cycle progression requires deeper investigation.

Addressing such gaps is essential for advancing of miRNA-based therapeutics that complement or provide an alternative to TKIs, particularly for CML patients exhibiting TKI resistance. The capacity of synthetic miRNAs to reduce BCR-ABL1 expression and influence CML development at the molecular level is a promising but

underexplored research domain. Comprehending the exact molecular processes via which miRNAs affect CML cells may result in innovative, focused therapy approaches that surmount resistance, reduce side effects, and improve patient outcomes.

1.3 Research Questions

1. Which miRNAs are predicted to target the 3' UTR of the *ABL1* gene using in-silico analysis?
2. How efficient is the transfection of the selected miRNA mimics in CML cells?
3. Do the selected miRNAs directly bind to the 3'UTR of the *ABL1* gene in CML cells?
4. How does the selected miRNA transfection affect the expression levels of *BCR-ABL1* and *ABL1* genes in CML cells?
5. How does miRNA transfection impact ABL1 protein expression in CML cells?
6. How does miRNA transfection influence cell viability and cell cycle progression in CML cells?
7. What are the differently expressed genes (DEGs) in CML cells following miRNA transfection, and how do they contribute to the molecular mechanism of CML?

1.4 Objective of Study

This study aims to elucidate the molecular mechanism of miRNA transfection underlying CML cell proliferation by targeting the 3'UTR of the ABL1 gene. Specifically, the objectives of this study are:

1. To identify miRNAs that target the 3'UTR of the *ABL1* gene using *in-silico* analysis.
2. To determine the transfection efficiency of miRNA mimics in CML cells.
3. To validate miRNA-mRNA binding on the 3'UTR of *ABL1* in CML cells.
4. To assess the *BCR-ABL1* and *ABL1* gene expression in CML cells transfected with miRNA mimics.
5. To determine the effects on ABL1 protein expression following miRNAs transfection in CML cells.
6. To evaluate cell proliferation and cell cycle following miRNA transfection in CML cells.
7. To determine differentially expressed genes (DEGs) and the pathways regulated in CML cells transfected with miRNA.

1.5 Significance of Study

Findings from this study will elucidate the molecular pathways involved in the transfection of selected miRNAs in CML cells, offering important insight into the function of miRNAs in cancer progression and treatment. Understanding the molecular mechanism of gene expression in CML is crucial for improving therapy strategies. CML is induced by the BCR-ABL1 fusion gene, resulting in the unregulated proliferation of cells. Despite the efficacy of tyrosine kinase inhibitors (TKI) in managing CML,

specific individuals may develop resistance over time, resulting in disease persistence and relapse. In CML, the molecular pathways driving cell proliferation, survival, and resistance to therapies are poorly understood, especially in resistant strains. By examining these pathways at a molecular level, researchers can pinpoint specific intervention points where they can implement novel therapies.

Synthetic miRNAs have emerged as a promising tool in gene regulation due to their ability to target mRNA for gene expression suppression selectively. The present study uses miRNA mimics to target the 3'UTR of *BCR-ABL1*, the cause of CML pathogenesis. MiRNA mimics are engineered for stability and specificity, facilitating precise gene expression and making them suitable for therapeutic applications. This study uses miRNA mimics to investigate a new way to lower *BCR-ABL1*, which could be an alternative to traditional treatments for people who aren't responding to TKIs.

The main point of this study is the investigation of CML cell proliferation, which is the hallmark of cancer progression. By examining these miRNA effects towards on cell proliferation, the current study hopes to determine whether miRNA-based treatments can help in inhibit or decrease the growth of CML cells. It is very important to test cell proliferation because inhibiting uncontrolled cell division may show how well miRNA therapy works at slowing down the progression of CML.

Targeting the 3'UTR of the *BCR-ABL1* gene is crucial as it significantly regulates post-transcriptional gene expression. Moreover, targeting this region allows for precise regulation of BCR-ABL1 protein production, potentially reducing the oncogenic signalling that causes the disease. This will also enhance our understanding of how miRNAs can impact gene expression, creating of customised and effective treatments for CML, especially for patients with Imatinib resistance.

This research, which explores both human and plant-based miRNAs, uncovered novel therapeutic strategies for CML, potentially leading to the development of alternative targeted therapies. These therapies could be cost-effective options or additions to existing treatments, markedly enhancing patient outcomes. Furthermore, the study could open new research opportunities, enhancing the understanding of miRNA applications in oncology and other fields.

1.6 Scope of Study

This study aims to elucidate the molecular mechanisms by which miRNA mimics influence CML cell proliferation and viability, with a particular focus on the challenge of Imatinib resistance. This research utilises two well-characterised CML cell lines: K562-s (ATCC® CRL-3343), representing Imatinib-sensitive cells, and K562-r (ATCC® CRL-3344), representing Imatinib-resistant cells. Specifically, by comparing the effects of miRNA transfection between these two cell types, this study seeks to uncover the potential of miRNAs to modulate cell viability and overcome drug resistance in CML. The findings from this research could provide insights into new therapeutic strategies for CML, particularly in patients who have developed resistance to Imatinib.

1.7 Conceptual Framework

The conceptual framework provides a clear explanation of the essential components and their interactions that guide the research, therefore acting as the basic framework for this work. This design links the fundamental theories and current literature with research objectives. This guarantees a logical and focused strategy to

investigate the molecular mechanisms of microRNA (miRNA) in the proliferation of CML cells. Figure 1.1 illustrates the conceptual framework of the study.

MiRNA mimics derived from both humans and plants that target the 3' UTR of the *ABL1* gene constitute the most critical compound of this framework. The framework integrates biological processes such as *ABL1* and *BCR-ABL1* gene expression, protein activity, and their impacts on cell viability and the cell cycle. The introduction of synthetic miRNA mimics of human and plant targeting the 3'UTR of *ABL1* was evaluated by analysing its impact on gene and protein expression. The downstream effects of miRNA binding were assessed by measuring cell viability and cell cycle progression. We hypothesise that targeting the *ABL1* gene will decrease cellular viability and proliferation. Furthermore, the efficacy of miRNA transfection was assessed by quantifying the miRNA level post-transfection.

Since, miRNA can simultaneously target multiple gene through sequence complementarity, it is possible that, in addition to ABL1, other genes involved in cell proliferation, viability, or cell cycle control may also be affected. These potential interconnections were investigated through a series of experiments aimed at elucidating the role of miRNAs in regulating key molecular pathways, particularly in the context overcoming imatinib resistance in CML

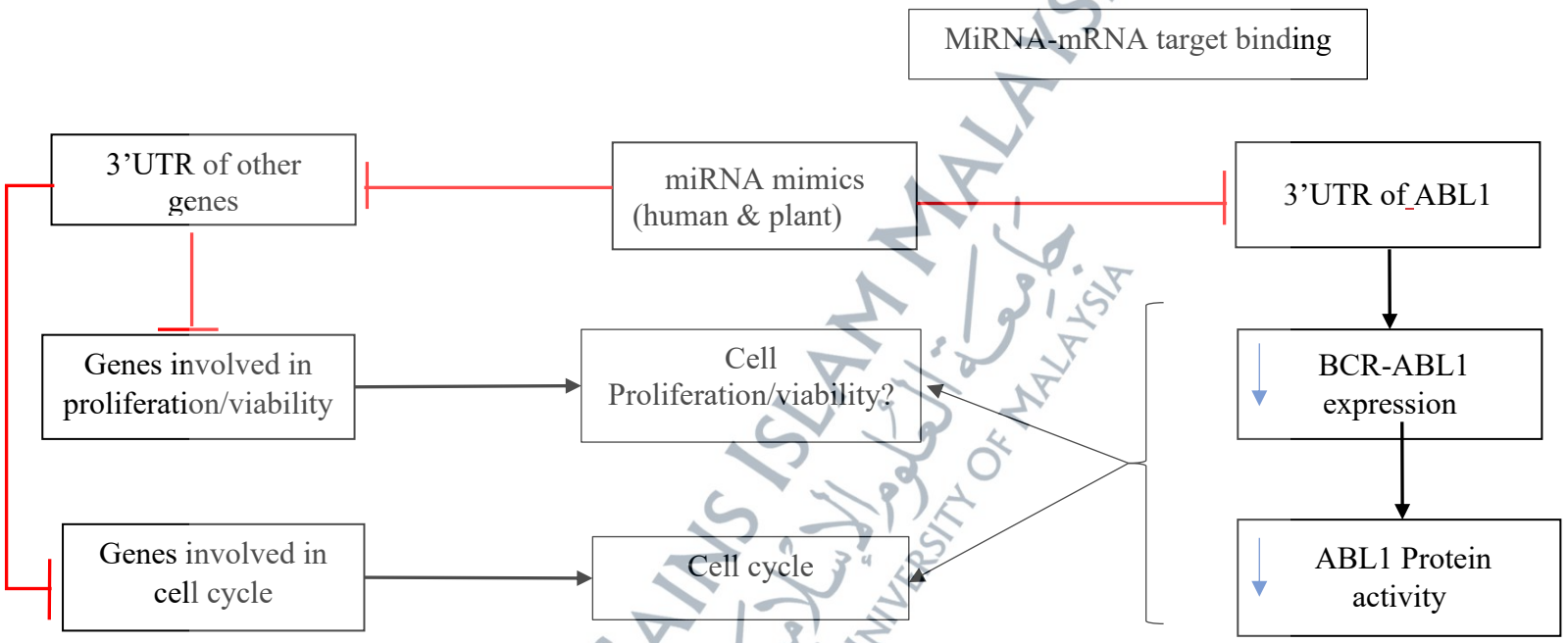

Figure 1.1: Study Conceptual Framework. The diagram uses two types of lines to represent the nature of interactions: black arrows illustrate a causal or promoting effect between variables, while red lines without arrowheads represent inhibition or repression, particularly in the context of miRNA targeting the 3′UTR of mRNA to suppress gene expression.