TARGETED GENE SILENCING OF THE MAPK PATHWAY IN ACUTE MYELOID LEUKEMIA CELLS USING RNAi

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Introduction

Acute myeloid leukemia (AML) is a type of blood disease resulting from an abnormal proliferation of myeloid cells in the bone marrow. Proliferation of the AML cells is frequently linked to gene mutations which lead to the activation of signal transduction pathways such as mitogen-activated protein kinase (MAPK) pathway [1]. Protein inhibitors have been designed to target the MAPK pathway, attempting to block it and stop proliferation [2].These synthetic drugs have demonstrated anti-tumor properties and increased survival of the patients [3]. However, as with most synthetic drugs, they exhibit undesirable side-effects [4]. RNA interference (RNAi) is a naturally occuring post-transcriptional gene silencing mechanism which has the ability to transiently inhibit the translation of specific mRNA in cells [5]. However, RNAi-based therapy is at an early stage and has many critical issues, mainly in delivery to target cells [6]. RNAi-based therapy is still considered an attractive prospect because it is induced by oligonucleotides such as short-interfering RNA (siRNA) which are subsequently degraded by the cells [7]. Hence, target-based therapies using RNAi shows promise for the development of novel treatment modalities for AML.

Methods

U937 leukemic cell line culture

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siRNA transfections (single & combinatorial target genes)

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Total RNA extraction & 1st strand cDNA synthesis

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qPCR was used to measure genes expression levels & validate knockdown of target genes

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Affymetrix® GeneChip ® Human Gene 1.0 ST Array was used to produce gene expression profiles for each sample

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Data mining & pathway analysis using Agilent ® Genespring 11.5 GX software

Results

We selected *raf1, mekk1* and *mlk3* as potential genes to be knocked down in the MAPK pathway using RNAi. The genes selected have been previously implicated in cancers [8,9,10] and due to the transient nature of siRNA, we carried out an experimental combinatorial RNAi knockdown of all three genes as well as single target knockdowns over a 24 hour period to observe the effects generated by the different approaches. Optimal knockdown levels, measured using reverse-transcription quantitative PCR (Figure 2) of *raf1, mekk1* and *mlk3* in single knockdown experiments were at 59.82%, 58.78% and 56.89% respectively. The knockdown levels for the combinatorial treatment were 53.16% for *raf1*, 58.32% for *mekk1* and 56.81% for *mlk3*.The treated cells were put through a microarray analysis to measure the gene expression profiles of each treatment.

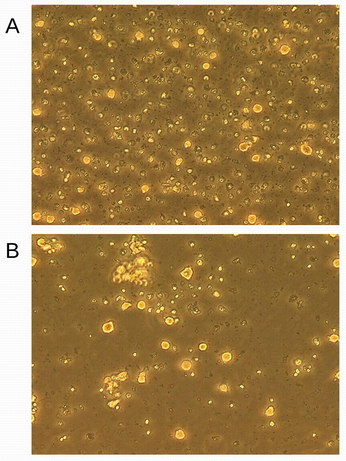
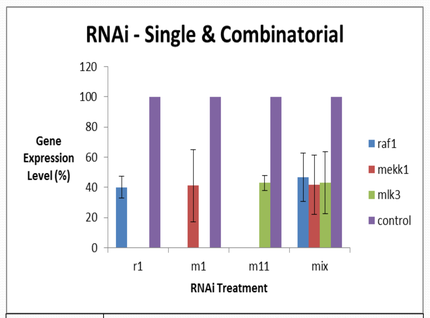


Figure 1. **A**. U937 cells at 0 hours mix RNAi treatment ; **B.** U937 cells at 24 hours mix RNAi treatment ; Magnification : 10 x 20.



TARGET GENE	RNAi TREATMENT (Gene expression level % ± standard deviation)			
	r1	m1	m11	mix
Raf1	40.18% ± 7.07	-	-	46.84% ± 15.89
Mekk1	-	41.22% ± 24.01	-	41.68% ± 19.70
Mlk3	-	-	43.11 ± 4.98	43.19% ± 20.46

Figure 2. Gene expression levels measured using RT-PCR ; r1 - raf1 RNAi ; m1 – mekk1 RNAi ; m11 – mlk3 RNAi; mix – raf1, mekk1 & mlk3 RNAi.

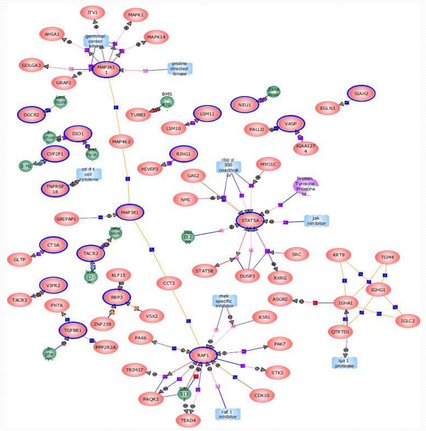


Figure 3. Expanded interaction pathway analysis from the mix treatment against negative control siRNA (lo & hi GC) gene list.

Gene Symbol	Regulation	Fold Change	p-value
TAOK2	UP	1.1	0.04524
RAF1	DOWN	1.7	0.00002
MAP3K1	DOWN	2.0	0.00155
MAP3K11	DOWN	1.5	0.00001
TGFBR1	DOWN	1.2	0.02256
STAT5A	UP	1.1	0.01016

Table 1. Gene list of significant (p-value < 0.05) up/down regulation and fold change in mix RNAi treatment against the negative control siRNA (lo & hi GC) gene expression profile averaged values.

Discussion

The combinatorial experiment displayed slightly similar levels of gene expression in comparison to the single target knockdowns. This indicates that the combinatorial knockdown potency is as effective as the single target gene RNAi treatment. Preliminary conclusions derived from the morphological observation of the mix treatment indicate that the cells might be undergoing apoptosis with the reduction of live cells (Figure 1). To further investigate the validity of this hypothesis, we carried out DNA microarrays to observe the gene expression profiles of the samples. Each different RNAi treatment was compared against its suitable negative control siRNA. Significant and differentially expressed genes were filtered from the raw data to form gene lists which have relevant ties to either MAPK signaling pathway, the apoptosis pathway or any pathway which may be of interest in cancer studies. By using the Agilent ® Genespring 11.5 GX software, we were able to obtain pathways (Figure 3) which contain genes from each gene list and sort it out according to pathway and regulation (Table 1). The mix treatment showed that when the 3 genes were simultaneously knocked down, *stat5a* was up regulated in response. The gene expression profile also suggests that the cells went into pro-survival mode with down regulation of *faslg* and *traf4*. However, *taok2,* which is involved in formation of apoptotic bodies, was up regulated, leading us to believe that the cells had initially started to undergo apoptosis but managed to bypass the MAPK pathway RNAi treatment and restart proliferation. This suggests that either the leukemic cells could not be induced into apoptosis completely using the combinatorial approach or that the gene set combination was not potent enough or sustained long enough within the cells to completely block cell survival.

Conclusion

A combinatorial approach to RNAi-based knockdown of the MAPK pathway revealed that even though more than one pivotal gene in the signaling transduction pathway was knocked down, the leukemic cells still had alternative pathways or mechanisms to restart proliferation.

Acknowledgements

We would like to thank the Ministry of Science and Technology (MOSTI) of Malaysia for the research grant, UiTM for the facilities and support, Mazatulikhma Mat Zain , Institute of Science, for permission to use the Tissue Culture lab, Dr. Faiz Foong Abdullah, Faculty of Applied Sciences, for permission to use the Molecular Biology (106) lab and instruments.

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