Up-regulation of hsa-miR-608, in response to *Bcl-xL* silencing, increases cell death in A549 and SK-LU1 human lung adenocarcinoma cells.

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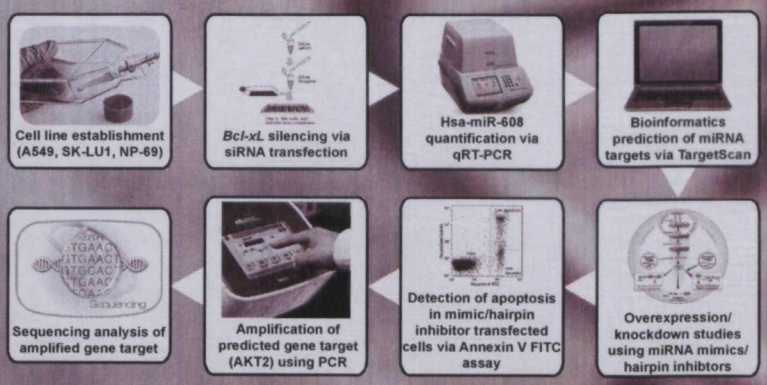
Introduction

Evasion of apoptosis is an important hallmark of tumor proression. *Bcl-xL*, an anti-apoptotic *bcl-2* gene, has been shown to be over-expressed in NSCLCs [1]. Over-expression of Bcl-xL counteracts the pro-apoptotic functions of Bax and Bad by preventing their translocation from the cytosol to the mitochondria, inhibiting apoptosis by maintaining the permeability status and stabilization of the outer mitochondrial membrane, which subsequently prevents cytochrome c release and pro-caspase-9 activation [2].

MicroRNAs (miRNAs) are small non-coding RNAs of about 19 to 23 nucleotides that regulate gene expression post-transcriptionally, by either inhibting mRNA translation or by inducing mRNA degradation [3]. These regulatory elements play a role in a wide range of biological processes including cell proliferation, differentiation and apoptosis [4-6]. Alterations in miRNA function and expression may disorganize cellular processes and eventually contribute to disease progression, including cancer [7]. This study describes the succesful determination of hsa-miR-608 dysregulation in response to *bcl-xL* silencing in lung adenocarcinoma NSCLC cells, with the aim to elucidate the role this miRNA plays in the induction of cell death.

Methodology



Results & Discussion

Upregulation of hsa-miR-608 increases cell death in A549 and SK-LU1 cells

Figure 1A

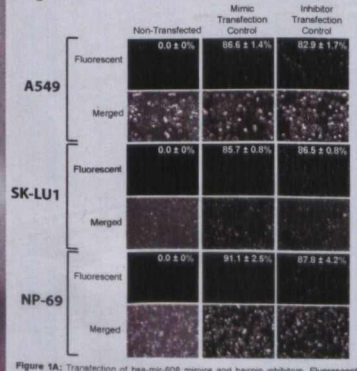


Figure 1A: Transfection of hsa-mir-608 mimics and hairpin inhibitors. Fluorescent and merged images of A549, SK-LU1 and NP-69 cells transfected with miRIDIAN microRNA Mimic/Hairpin Inhibitor Transfection Control with Dy547. Percentage of mean transfection efficiency is indicated, and all images shown are a representative of triplicates independent experiments.

Figure 1B

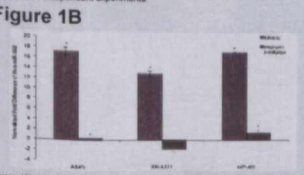


Figure 1B: Quantification of hsa-miR-608 mimics/ hairpin inhibitors via RT-qPCR RT-qPCR of hsa-miR-608 presented as normalized fold difference in mimic/ hairpin inhibitor-transfected A549, SK-LU1 and NP-69 cells, in comparison to cells transfected with scrambled negative controls. All experiments were carried out as triplicates and presented as mean ± S.D. Statistically significant differences in fold difference between scrambled negative controls and transfected samples were indicated by (*) where *p*-value < 0.05. NTC denotes non-transfected cells. MTC denotes cells transfected with transfection reagent only.

Figure 1C

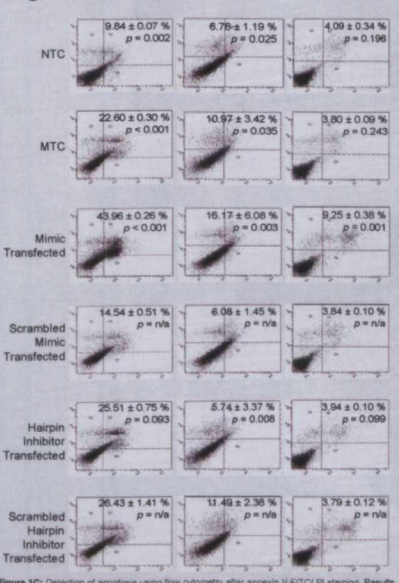


Figure 1C: Detection of apoptosis using flow cytometry after annexin V-FITC/ PI staining. Results indicated that up-regulation of hsa-miR-608 expression potentiates apoptosis-mediated cell death after 72 h in A549 and SK-LU1 cells, while NP-69 cells were minimally affected by hsa-miR-608 over-expression. Viable cells are in the lower left quadrant, early apoptotic cells are in the lower right quadrant, late apoptotic cells are in the upper right quadrant and non-viable necrotic cells are in the upper left quadrant. Dot plots are representative of 1.0 × 10 cells from triplicates with percentage of apoptosis indicated. NTC denotes non-transfected cells, while MTC denotes cells transfected with transfection reagent only. Scrambled miRNA mimics/ anti-sense hairpin inhibitor sequences were used as negative controls.

Transfection with hsa-miR-608 anti-sense inhibitors blocks si*Bcl-xL* induced cell death

Figure 2

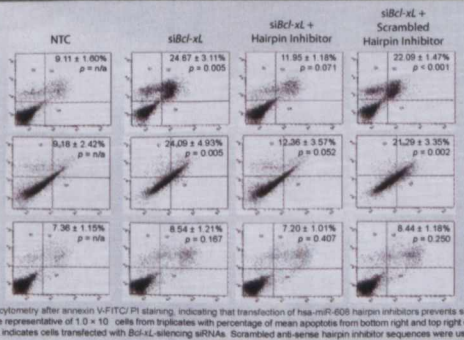


Figure 2: Detection of apoptosis using flow cytometry after annexin V-FITC/ PI staining, indicating that transfection of hsa-miR-608 hairpin inhibitors prevents si*Bcl-xL* induced cell death in A549 cells and SK-LU1 cells. Dot plots are representative of 1.0 × 10 cells from triplicates with percentage of mean apoptosis from bottom right and top right quadrants indicated. NTC denotes non-transfected cells while si*Bcl-xL* indicates cells transfected with *Bcl-xL*-silencing siRNAs. Scrambled anti-sense hairpin inhibitor sequences were used as negative controls.

Hsa-miR-608 is predicted to bind to *Gene 1* 3'UTR

Figure 3A

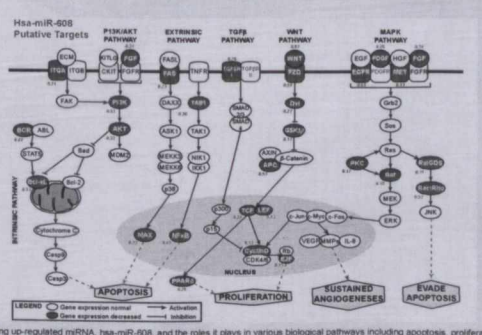


Figure 3A: Scheme depicting up-regulated miRNA, hsa-miR-608, and the roles it plays in various biological pathways including apoptosis, proliferation, and angiogenesis.

Figure 3B

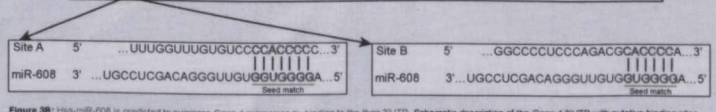


Figure 3B: Hsa-miR-608 is predicted to suppress *Gene 1* expression by binding to the their 3'UTR. Schematic description of the *Gene 1* 3'UTR with putative binding sites for hsa-miR-608

Figure 3C

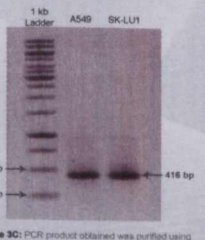


Figure 3C: PCR product obtained was purified using the QIAquick Gel Extraction Kit. Agarose gel electrophoresis image of *Gene 1* bands observed after DNA purification from the agarose gel.

Figure 3D

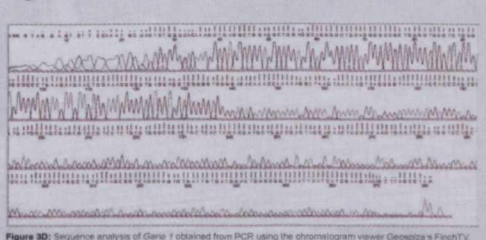


Figure 3D: Sequence analysis of *Gene 1* obtained from PCR using the chromatogram viewer Geospiza's FinchTV. The full sequence of *Gene 1* was analyzed and compared to sequences in the Human Standard Nucleotide BLAST, and the sequence was found to have a match (Identity: 99%) candidate gene.

Conclusion

This study describes the succesful determination of hsa-miR-608 dysregulation in response to bcl-xL silencing in lung adenocarcinoma NSCLC cells, with the aim to elucidate the role this miRNA plays in the induction of cell death. Hsa-miR-608 was found to be linked to several apoptotic signaling pathways including the PI3K/AKT, WNT, TGF-β, MAPK/ERK and the intrinsic pathway,and all were implicated as those directly affected by Bcl-xL levels. With further studies carried out to determine the specifc gene targets of hsa-miR-608, our study has provided a platform for anti-sense treatment whereby miRNA expression can be exploited to increase the apoptotic properties in lung adenocarcinoma cells as demonstrated by our over-expression and knockdown studies.

Acknowledgements

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