The Role of SH2 Domain of Grb2 Protein in the Apoptosis Process in Cancer Cells

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SciTech 2017

SH3 | SH2 | SH3

Introduction

The leading cause of cancer deaths worldwide is lung cancer (1). It is caused by abnormal cell growth in the lung tissue. Melanoma skin Cancer is also responsible for numerous cancer-related deaths, in fact, its incidence is increasing. Melanoma is usually caused by exposure to ultra-violet radiation (2).

Growth Factor receptor bound protein 2 (Grb2) is an adapter protein that is instrumental in the ability of all cells to live and proliferate. It contains a single SH2 domain, flanked by two SH3 domains, thus having the capacity to couple tyrosine phosphorylated receptors and proteins to downstream effectors containing proline and arginine-rich motifs (3,4). This promotes association with a variety of signaling proteins which control cell survival and differentiation (5). Thus, we hypothesize that this protein may have an important role in cancer cells.

In this study, we aim to examine the effect of a mutated SH2 domain in the Grb2 protein on inducing apoptosis in Lewis Lung Carcinoma (LLC) cancer cells and B16 melanoma cells.

Methods

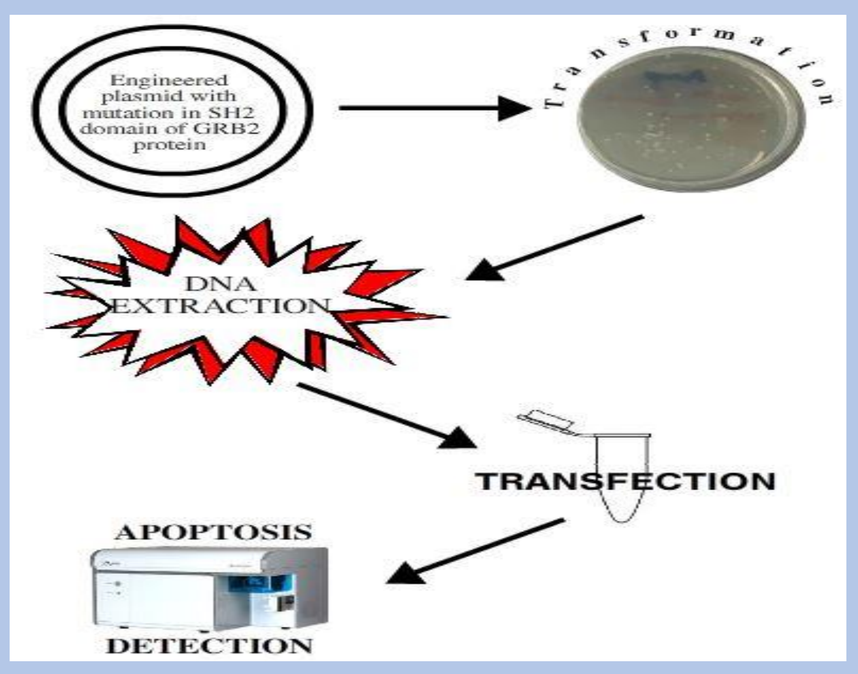

Figure 1: Process of Experiment
Engineered plasmid with mutation in SH2 domain of Grb2 protein was inserted into bacteria in order to replicate it. Plasmid was then extracted from bacteria using heat shock. Via a chemical reagent, plasmid was transfected into LLC and B16 cells. Flow cytometry was then employed in order to determine the levels of apoptosis in the cells.

Results

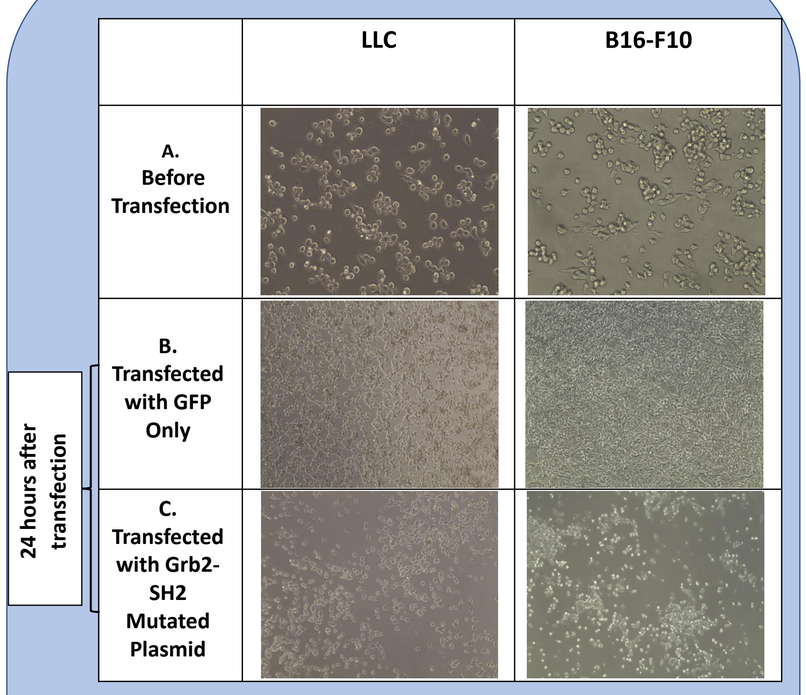

Figure 2: B16-F10 and LLC cells:
A) Before transfection **B)** 24 hours after transfection with GFP plasmid **C)** 24 hours after transfection with Grb2-SH2 mutated plasmid.

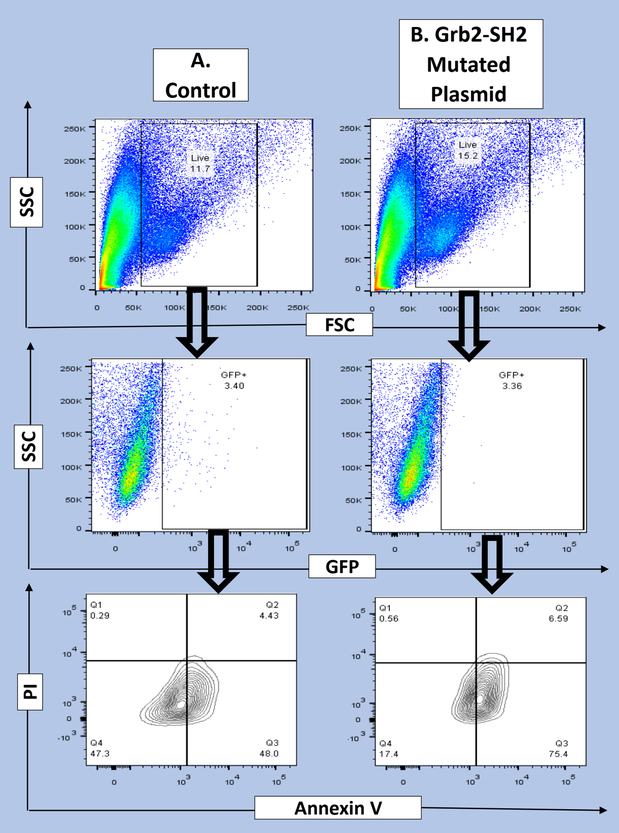

Figure 3: FACS–Flow Cytometry analysis of LLC Cell Line 24 hours after transfection with:
A) GFP-control plasmid. **B)** Grb2-SH2 mutated Plasmid. Examining apoptosis using Annexin-V and Propidium Iodide (PI) Biomarker Staining for live GFP+ successfully transfected cells.

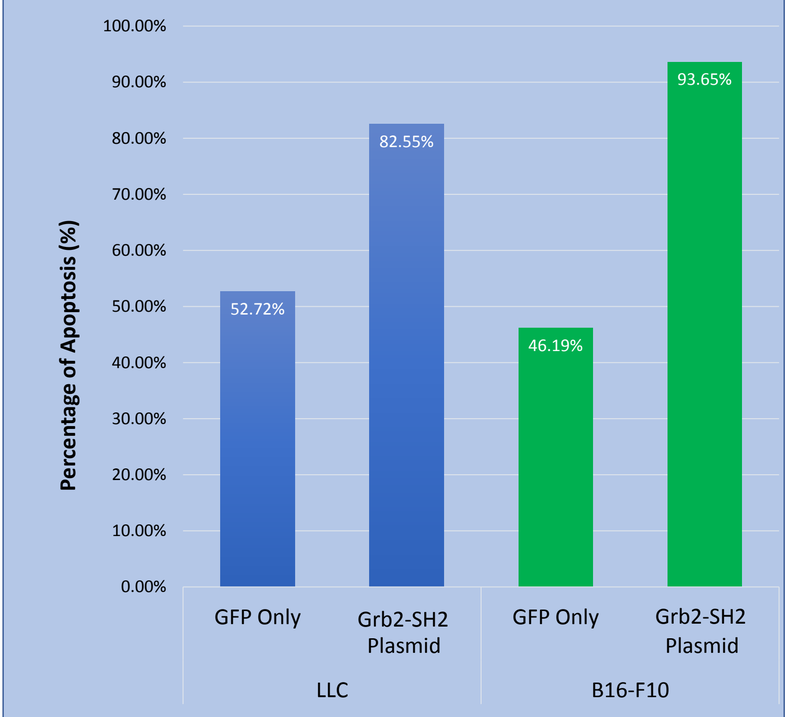

Figure 4: The sum of cells in apoptosis process in LLC and B16 cell lines
Apoptosis incidence is greater in both cell lines with Grb2-SH2 plasmid compared to the control of GFP only. In both cell lines the apoptosis in the GFP only was about 50%, whereas, for the plasmid group there was a significant increase.

Conclusions

- The Grb2-SH2 mutated plasmid increased the total apoptosis in both cell lines comparing with the control, as shown in the results section.
- According to the results, we can conclude that the SH2 domain in the Grb2 protein is essential to the downstream signaling pathways that are involved in the survival of the cells.
- In order for the results to be statistically significant, we recommend further investigations.
- The SH2 domain in Grb2 protein can be a future target for gene therapy in the treatment of cancer.

References

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Acknowledgements

We would like to thank Yasmine Khier, Msc student and Dr. Igal Louira-Hoian, Ph.D. and Dr. Yishai Ofran, Ph.D. M.D. for hosting and guiding us through our research in the Clinical Research Institute at Rambam Hospital.

We would also like to thank the foundations and donors for their generous support of the SciTech Program.