SciTech summer science camp

Technion Israel Institute of Technology

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Mutated Protein SHC1 and it's Influence on the Apoptosis Process in Cancer cells

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Introduction

Acute Myeloid Leukemia (AML) is a cancer of the myeloid line of blood cells from the bone marrow. Characterized by rapid proliferation of abnormal white blood cells. This cancer is often fatal and difficult to cure [1]. SHC1 is a protein expressed ubiquitously in mammalian cells which has been found to be important in the regulation of apoptosis. This protein plays an essential role in cell signaling such as; growth factor receptors, antigen receptors, hormone receptors, etc. [2,3]. It is also involved in growth factor signaling in major networks such as mitogen-activated protein kinase (MAPK) [4]. SHC1 has been found to be associated with the progress of many cancer types and within acute myeloid leukemia blasts [5].

Our project aim is to examine the influence of mutant SHC1 protein in inducing apoptosis in AML cell line.

Method

1. Transformation and Plating

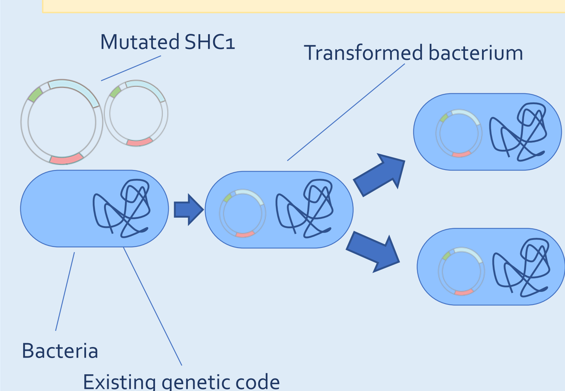
In order to produce the amount of the plasmid that is necessary for our experiment, we inserted the mutant plasmid into competent bacteria via heat shock technique. The bacteria plated into agar dishes with antibiotics, where they proliferated in ideal condition (Figure 1) . With the bacteria, the volume of plasmid increased as well.

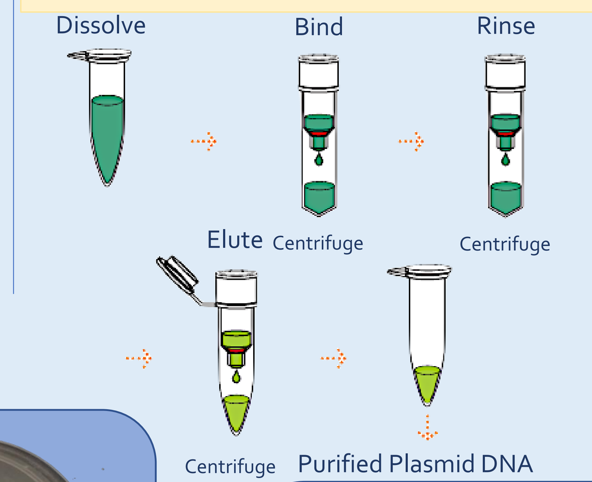
2. Plasmid Extraction

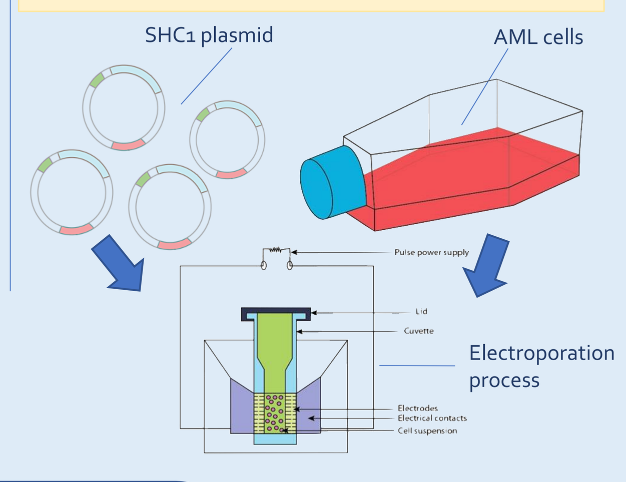
After we had cultured our bacteria, we centrifuged the bacteria and drew out excess medium. Then, we exploded the membrane of the bacteria. We then centrifuged the bacteria in order to eradicate the unnecessary components and purified the DNA thus receiving only the final plasmid.

3. Culture and Transfection

In parallel to producing our plasmid, we matured and cultured OCI-AML3 cancer cells. In order for the cells to thrive, they were given nutrients and warm living conditions (Figure 2). Once the cancer cells were mature and we had produced enough of our Shc1 plasmid, we transfected the plasmid into the cells via electroporation and detected the apoptosis process through Flow-cytometry (FACS).







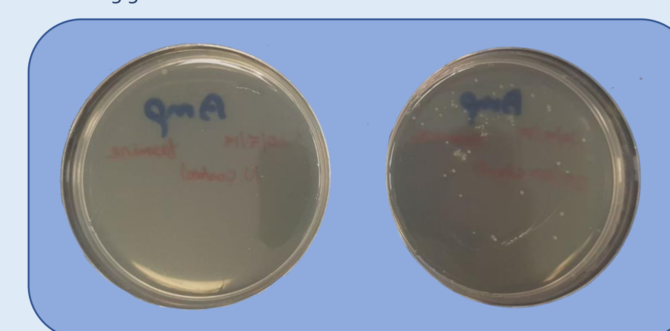


Figure 1: Bacteria plated into agar dishes with antibiotics; left plate – negative control, right plate – with plasmid.

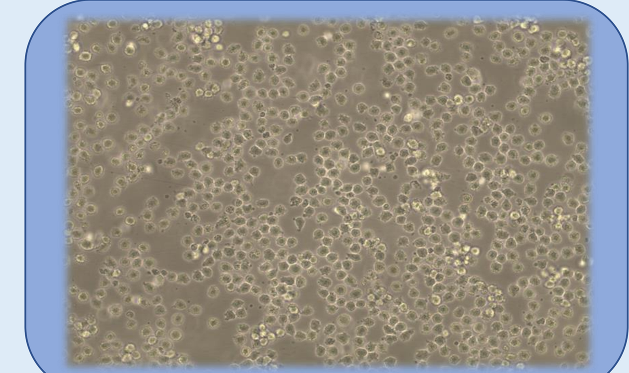


Figure 2: Cultured OCI-AML3 cancer cells before transfection - under microscope.

Results

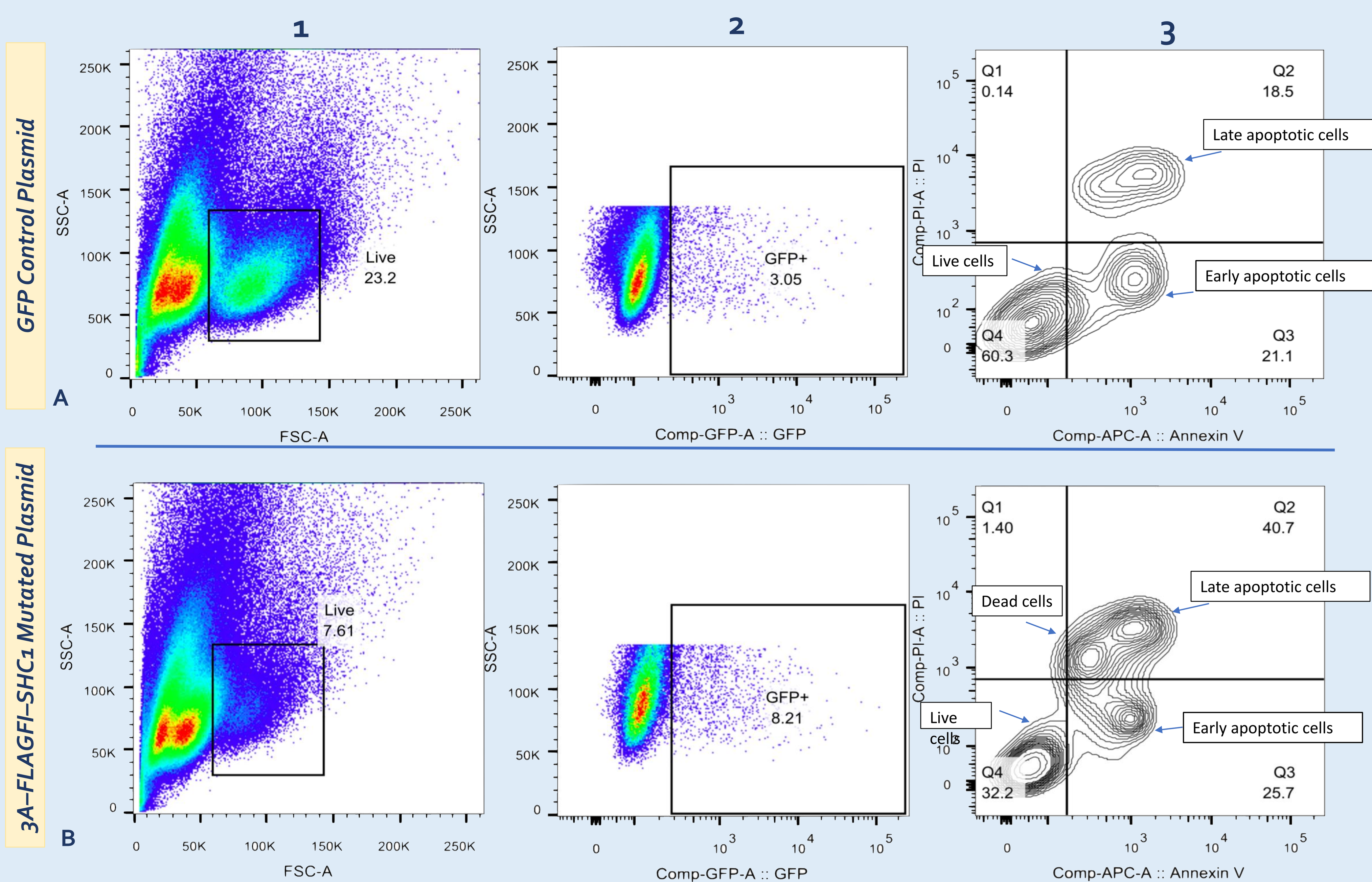


Figure 3: Flow cytometry analysis of apoptotic OCI-AML3 cell line 24 hours after transfection with 3A-FLAG-SHC1 plasmid
A. GFP control plasmid B. 3A-FLAG-SHC1 mutated plasmid: 1. Percentage of live cells. 2. Percentage of GFP+ from live cells. 3. Analysis of annexin-V and propidium iodide (PI) staining of apoptotic cells from GFP+ cells.

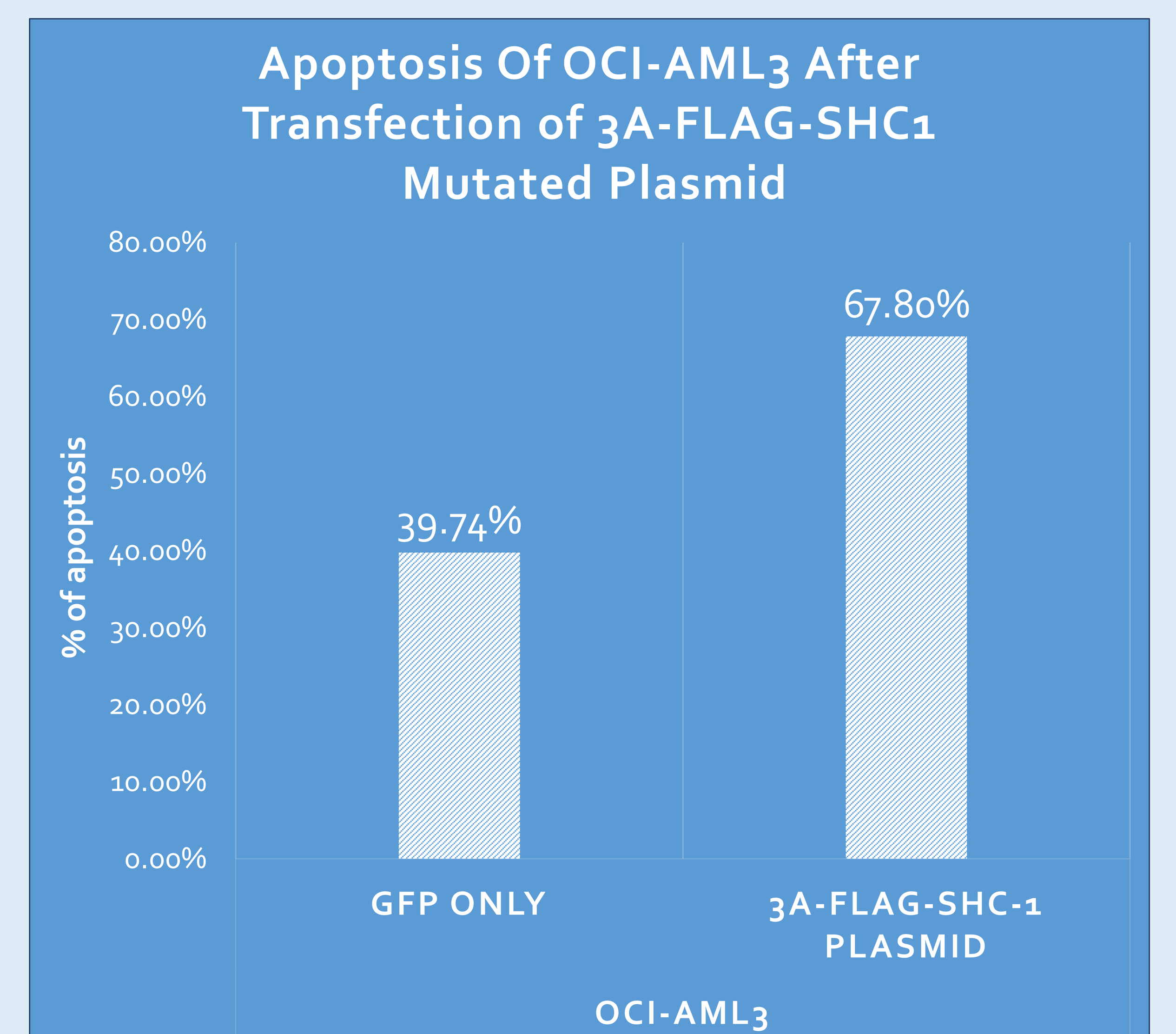


Figure 4: Present the percentage of apoptotic of OCI-AML3 cells after Transfection of 3A-FLAG SHC1 mutated plasmid vs. control cell with GFP plasmid only.

Conclusion: Mutated Protein Shc1 affected the apoptosis process in OCI-AML3 cell line. Our results present major differences between the control GFP AML cells and the AML cells treated with mutated SHC1 plasmid (Figure 3). The AML cells treated with the plasmid showed more apoptosis than the control (Figure 4). Further research is necessary in order to determine the statistical significance of our results, making this technique suitable for future cancer therapy.

References:

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