EVALUATION OF HYBRID PROTEINS WITH APOPTOSIS ACTIVITY (A1-GM CSF) RECEPTORS ON VARIOUS CELL LINES IN IN VITRO GMCSF

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ABSTRACT
One of the new solutions targeted to treat diseases such as cancer, the use of substances is known as immunotoxins. Immunotoxins consist of two parts tracking and exterminators. In recent years a new mechanism has been established for this purpose and the use of a cytotoxic molecule that is carried out by the fusion technology. In this study, a hybrid protein that is composed of two catalytic Shiga toxin A1 and GMCSF is human, and cytotoxic activity induced by arabinose GMCSF receptor on cells was tested. The data indicate that the hybrid proteins were cytotoxic to GMCSF receptor cell lines and different concentrations of the proteins induces apoptosis.

KEYWORDS: apoptosis, protein GM CSF, immunotoxins, cancer.

INTRODUCTION
Directions for cancer treatment, typically include surgery, radiotherapy, chemotherapy and gene therapy, and despite the efforts of oncologists and geneticists in the fight against cancer, the disease is still one of the major causes of death in humans.[1] Even with the development of anti-cancer treatment efficacy of new drugs, is not satisfied, so a different approach is proposed for the treatment of cancer.[2] Certain drugs with unique mechanisms of action that can prevent multi-drug resistance to cancer, is required.[3]
This fusion toxins is a group of health factors that consist of two parts tracking and Slayers is on the cell. The first part of the inhibition of protein synthesis, a ligand for binding to the cancer cells. At first, the ligand directs the toxin to the target cells and bind to their corresponding receptors, endocytosis to stream throws. The toxin into the cytosol and then transferred to disable the catalytic protein synthesis. This leads to cell death or apoptosis is physiological. Plant and bacterial toxins, especially Racine, diphtheria toxin and exotoxin A of Pseudomonas toxin have been used as factors.

**MATERIALS AND METHODS**

Protein expression: plasmids Pbad / giii in the host cell E. coli strain Top10 GMCSF-A1 was used to express recombinant protein. Periplasmic protein purification GM CSF-A1: This
plasmid has the primary signal in order to direct the protein to the periplasmic is activated. Protein purification was performed by polymyxin B so that sediment bacteria in PBS 100μL, polymyxin B solution and then by 1% was added and incubated at 37 °C for 1 hour were used. Then, at 10000rpm for 12 minutes, was centrifuged and the supernatant was collected. Recombinant protein identification GCSF-A1: activated protein with sample buffer at 90 °C for 5 minutes was heated and was placed on SDS-PAGE gels and by water Kumasi, were stained. Assess the cytotoxic effect of cytotoxic proteins GM CSF-A1 receptors on the cell lines GMCSF-A1 was evaluated by measures of neutral red and blue Trypan.

![Figure 1](image1.png)

**Figure 1**: Purification of GM CSF-A1 protein in E. coli bacteria on human cell receptors.

Investigate the mechanism of cell death: To investigate the mechanism of cell death, Flow cytometry was used to measure the ELISA specific kit. In order to evaluate Flowcytometric, 5 × 105 cells K562 cells after treatment with 100μg concentration of the protein sample, washed in PBS and 100μl colored solution was kept for 15 minutes at room temperature. The cells were analyzed by flow cytometry.

![Figure 2](image2.png)

**Figure 2**: Formation of GM CSF-A1 protein in response PCR.
DISCUSSION AND CONCLUSION

Today, fusion toxins of many different plant and bacterial toxins is synthesized as part of the patient's cells are cytotoxic ligand. For example, fusion toxin GMCSF-A1 in which A1, the cytotoxic and GMCSF, the ligand is attached to it.

Figure 3: GM CSF protein bond formation in terms of time in the presence of caspase 3.

The results of PCR

Figure 4: Schematic view of the transmission frequency GM CSF protein in different time periods.
Figure 5: Schematic view of the impact of GM CSF protein Neutrophil blood cells.
REFERENCES