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DEVELOPMENT OF A GENETICALLY ENGINEERED SYSTEM FOR BACTOFECTION BASED ON PROBIOTIC STRIAN ESCHERICHIA COLI NISSLE 1917

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Abstract: Conventional anti-tumor therapy approaches face numerous difficulties, including the difficulty to penetrate deep tumor tissue, toxicity to normal cells (lack of targeting), and cells eventually developing drug resistance. However, there is growing interest in using living, genetic modified bacteria as carriers for genetic material and therapeutic drugs. Probiotic bacteria can be manipulated using genetic engineering methods to have the ability to penetrate eukaryotic cells and deliver genetic material. Thus, the probiotic chosen for this study is *E. coli* Nissle 1917 (EcN).

Introduction: "The process of active transfer of genetic material into mammalian cells via bacteria" is known as Bactofection, and throughout the previous years, *Salmonella* and *Listeria* were used in most studies for their ability to invade eukaryotic cells. Pathogenic bacteria must be modified and attenuated to become a human-applicable delivery system. A safer and more straightforward approach would be to use probiotic bacteria that can be modified using genetic engineering tools. the probiotic chosen for this study to change is *E. coli* Nissle 1917 (EcN). With the addition of two genes from two pathogenic. *Inv* from *Yersinia enterocolitica* which will give EcN the ability to invade eukaryotic cells, and *hly* from *Listeria monocytogene*s, which will ensure the bacteria escape from the phagosome and reach the cytosol.

Main part: Delivery levels of EcN carrying pAL:Ptrp-*inv*, obtained previously were checked. The system showed the ability of the modified EcN with pAL:Ptrp-*inv* to enter the cells after gentamicin test as well as fluorescence and confocal microscopy with using fluorescent dyes DAPI (Thermo Fisher Scientific, USA) and PI (Thermo Fisher Scientific, USA). Moreover, modified ECN was transformed with pTurboGFP-C (evrogen, Russia). The ability of the modified strain to deliver the reporter gene to HCT-116 cell line was quantified using CytoFlex cytometer (Beckman Coulter, USA), and then the data obtained was compared to level of transfection using Lipofictamine 2000 (ThermoFisher Scientific, USA) and pTurboGFP-C on the same cell line, where the florescence levels in the FITC-A channel passed 10^6. This transfection was done to serve as a positive control. While the results of flowcytometry of HCT-116 cell line infected with modified EcN with different multiply of infection (MOI) (100, 500, 2000) showed that the population of eukaryotic cells with fluorescence in the FITC channel increased with increasing MOI. The maximum value was achieved at MOI equal to 2000 with approximately fluorescence levels 10^4.

Building on the previous experiments, we designed a new system that relies on adding *hly* gene to the previous system to increase the efficiency of the delivery.

A Ribosomal binding site has been added to *inv* gene, the construct was then cloned into pAl plasmid and followed by cloning of *hly* using Xba I and Aat II restriction enzymes.

Conclusion: The results of flow cytometry showed that the population of eukaryotic cells with fluorescence in the FITC channel increased with increasing MOI. The maximum value was achieved at MOI (multiply of infection) equal to 2000. However, when compared to lipofectamine transfection results, the histogram clearly shows populations with greater fluorescence, indicating a low efficiency of delivery. Thus, the addition of hly gene is necessary to increase the efficiency of the Bactofection process. The new system has not been tested yet, but based on literature we expect an increase in the efficiency of the delivery.