Funded by the European Union
NextGenerationEU

SUMMIT
SOFIA UNIVERSITY
MARKING MOMENTUM
FOR INNOVATION AND
TECHNOLOGICAL TRANSFER

SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Nikolet Pavlova, Martina Traykovska and Robert Penchovsky
Laboratory of Synthetic Biology and Bioinformatics

Activity 3.4. Scientific research with potential for innovation and knowledge/intellectual property transfer

Design of Antisense Oligonucleotides Linked to Cell Penetrating Oligopeptides as New Antibacterial Agents Against Resistant Human Pathogenic Bacteria

INTRODUCTION

Antibiotic resistance (AR) is a huge problem affecting people all over the world, leading to their persistent and prolonged illness. According to an assessment by the World Health Organization covering the European Union and the European Economic Area, presented in 2022, it is clear that every year more than 670,000 cases of infection are due to bacteria that have already developed AR, of which 4.92% die (33,000 people). The annual mortality caused by the insensitivity to antibiotics of the various bacteria that infect humans is increasing exponentially and causes an urgent need to investigate new mechanisms of action, the selection of new targets from the bacterial genome and the application of molecules directed at them.

Among the successful strategies for discovering new antibiotics is the possibility of using new molecular targets that have not been used before, but are widely distributed in many pathogenic bacteria. When these targets control vital biochemical pathways for bacteria, without their presence in the human genome, they could be applied to regulate key metabolites and achieve a bactericidal or bacteriostatic effect. RNA is a preferred antibacterial drug target. It involves the creation of engineered molecular sensors such as RNA aptamers and allosteric ribozymes, which can be used for synthetic control of gene expression, discovery of specific molecules, drug design, etc. In the laboratory of Prof. Penchovsky, we are working on the application of RNA in the process of designing new antisense oligonucleotides (ASO) and creating new candidates for therapeutic agents against resistant human pathogenic bacteria and applying for an international patent.

The main goal of this project is to continue a very successful research in RNA synthetic and computational biology, medicine, and pharmacy started by the project leader Prof. Robert Penchovsky, Ph.D., to develop and patent new antibacterial agents based on ASOs. The project budget is 102,250 EUR.

PROJECT GUIDELINES

The design of ASOs allows them to be specifically targeted to various RNA targets of human resistant and multi-resistant bacterial pathogens. By regulating their critical metabolic pathways for synthesis or transport, the primary metabolites of the bacteria will not be synthesized and/or imported into the cell from the extracellular space. In this way, the bacteria will die and ASOs can be used as new antibacterial agents.

After identifying which bacterial RNA targets are suitable for drug purposes, we design ASOs against them. The antibacterial effect of ASOs is being tested in the laboratory by monitoring the cell growth of the bacteria. The bacteria that will be used during the study will be human clinical isolates from hospitals under the umbrella of the Medical University of Sofia. The project goals, hypotheses, and approaches are very relevant nowadays, due to the increasing number of resistant and multi-resistant pathogens. All the main goals of the project have already been experimentally proven. This is a massive advantage of the proposed project, which reduces the risk to a minimum. The main problem of the project is to apply the already established technology for ASO computer modeling to the maximum. This makes the current project very effective in terms of the planned results and their achievement and in terms of saving time and money.

METHODOLOGY

The methodology for achieving the above-described goals includes:

- Bioinformatic and genomic analysis of sequences of various RNAs that are present in human pathogenic bacteria such as Enterococcus faecalis, etc.
- Selection of suitable targets based on predefined criteria by Prof. Penchovsky and our team.
- Design of ASOs.
- Microbiological methods for growing pathogenic bacteria
- Antisense technology (Figure 1)

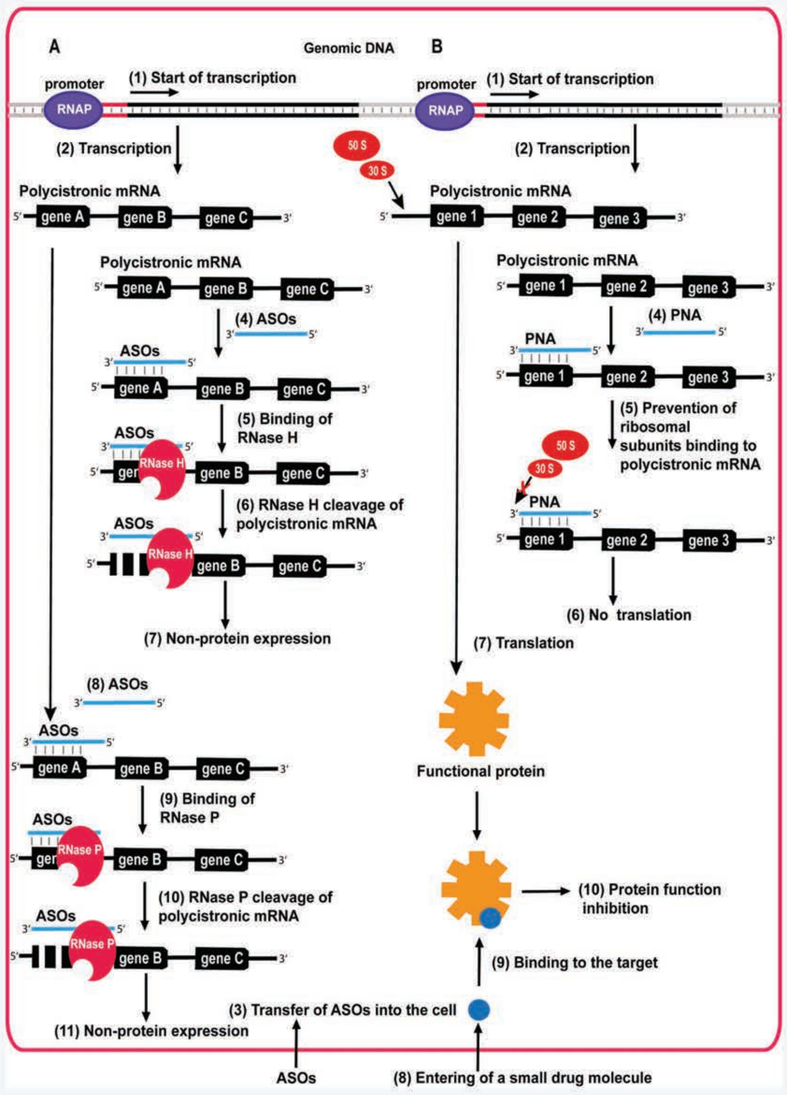

Figure 1. Antisense technology.

A. (1) Binding of RNAP. (2) Transcription of genes A, B, C. (3) Penetration of ASO into the cell. (4) Complementary binding of ASO to mRNA and formation of a double-stranded molecule. (5) Binding of RNaseH. (6) Cleavage of the double-stranded molecule - lack of synthesis of a functional protein. (7) Recognition of the double-stranded molecule (8) by RnaseP. (9), which leads to cleavage of mRNA (10).
B. (B). (1) Binding of RNAP. (2) Transcription of genes 1,2,3. (3) Entry of ASO and binding to mRNA (4). PNA blocks the binding site of ribozymes (5) leading to prevention of translation (6). Without ASO - translation of a functional protein (7). Introduction of a small molecule drug (8) whose target is a functional protein (9) leads to inhibition of its function.

RESULTS

To date, extensive bioinformatic and genomic analysis have been performed for rational target selection based on pre-selected and validated criteria, with 100% efficiency and accuracy in selecting appropriate targets among human pathogenic bacteria. Bacterial targets have been chosen as suitable ones for ASO design and targeting. As a result, novel ASOs have been designed, targeting the selected parts in the genome of antibiotic-resistant human pathogenic bacteria. The designed ASOs have been synthesized, and laboratory tests have been conducted to confirm their effectiveness as bacteriostatic and/or bactericidal agents. A patent examination request has been submitted to verify the feasibility of using the targets we have selected as targeting elements for the newly synthesized ASOs.

Due to the nature of the work and the subsequent patent application, in this poster we will present exemplary results for the design of ASO that do not violate the principles of confidential, innovative and unique development and drug design.

Riboswitches are functional domains found in the untranslated regions (5′-NTP) of many bacterial mRNA, responsible for controlling gene expression by binding the metabolite. Most of them control gene expression through transcription termination and translation prevention. Only the glmS riboswitch controls gene expression by destabilizing mRNA. The glmS riboswitch controls gene expression of an essential metabolite for the cell, namely GlcN6P.

It is one of the suitable targets for drug design and ASO technology. It is found in various bacteria like S. aureus, *E. faecalis, B. subtilis and E. coli.* We have designed different ASOs, which have been tested on other bacteria. The binding of pVEC_glmS_ASO_1 with the complementary sequence of the glmS riboswitch of *S. aureus* leads to enzymatic degradation of the glmS mRNA by the endonuclease RNase H (Figure 2). Bioinformatics research has found two biochemical pathways for synthesizing the essential metabolite glmS. When glucose enters the cell, it is converted into glucose-6-phosphate (Glc-6-P), which is converted into fructose-6-phosphate (Fru-6-P). This process is carried out with fructose-6-phosphate aminotransferase, regulated by the glmS riboswitch. Fru-6-P is part of glycolysis. The enzyme glucosamine-6-phosphate (GlcN6P) deaminase, encoded by the gene nagB, catalyzes the reverse reaction of GlcN6P to Fru-6-P. When the deamination reaction takes place, ammonia is released. The imported glucosamine taken up inside the cell with ATP consumption is converted into GlcN6P. nagA regulates the deacetylation of N-acetylglucosamine-6-phosphate by N-acetylglucosamine-6-phosphate deacetylase. It is an alternative pathway for the synthesis of glmS.

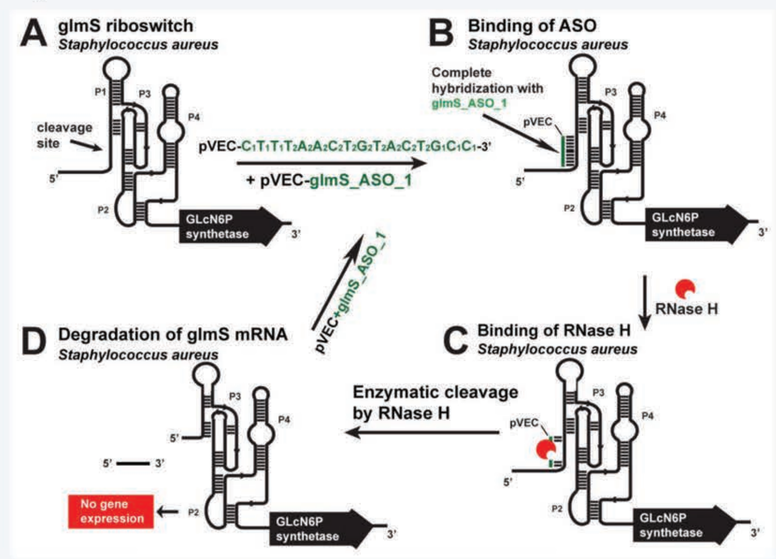

Figure 2. Targeting the *S. aureus* glmS riboswitch with a specific chimeric antisense oligonucleotide. (**A**) The chimeric pVEC_glmS_ASO_1 complex with the cell-penetrating oligopeptide pVEC binds to the complementary sequence of the glmS aptamer domain. (**B**) After their binding, a double-stranded molecule is formed. (**C**) The double-stranded molecule is recognized by RNase H, which binds it and triggers the enzymatic cleavage of the glmS mRNA. (**D**) The enzymatic cleavage causes a degradation of the glmS mRNA part and, as a result, prevents gene expression and glmS synthesis. The results demonstrate that glmS riboswitch is a suitable target, and pVEC_glmS_ASO_1 has a bacteriostatic effect because it stops the main pathway for the synthesis of glmS in *S. aureus*. These ASO works as narrow-spectrum antibiotics. This figure is part of our collective's article: Targeting FMN, TPP, SAM-I, and glmS Riboswitches with Chimeric Antisense Oligonucleotides for Completely Rational Antibacterial Drug Development, https://www.mdpi.com/2079-6382/12/11/1607.

CONCLUSION

Based on postulated and carefully selected criteria, different mRNAs have been classified as suitable targets for drug discovery. The ASOs with modifications from the first and second generations were specifically designed to target different targets (riboswitches and etc) to avoid hybridization with human RNAs. All designed ASOs have proved the high fidelity of our rational approach to drug design, including the estimation of drug targets. We can conclude that our bioinformatics methodology for selecting suitable targets works precisely. It allows us to select suitable targets that can then be used in laboratory assays. It also allows us to easily create a specific design of ASO, which will be aimed at a specific target. The approach demonstrated 100% accuracy in four different procedures until now in both parts, RNA target selection and ASO design. It saves a lot of time. The approach is universal, applicable to any RNA target for antibacterial drug development, and easily adaptable to AR. Due to bioinformatics and genomic analyses applied, we can develop ASOs to target one or more bacteria. In this way, we could create either specific narrow-spectrum candidate antibacterial agents or broad-spectrum therapeutics.

Contacts:

Laboratory of Synthetic Biology and Bioinformatics,
Biology Faculty, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria
room 363
Phone:+3592 816 73 40
E-mail: robert.penchovsky@biofac.uni-sofia.bg
https://penchovsky.atwebpages.com/research.php?page=19

Contract BG-RRP-2.004-0008 for the financing of project "Sofia University - Marking Momentum for Innovation and Technological Transfer" under pillar 2 "Establishing a network of research higher education institutions in Bulgaria", component "Innovative Bulgaria" from National Recovery and Resilience Plan part of the program to accelerate economic recovery and transformation through science and innovation