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Mini Review

Why Some Riboswitches are Suitable Targets for Antibacterial Drug Discovery?

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Abstract

The global spread of a growing number of antibiotic-resistant strains of human pathogenic bacteria imposes the pressing need for the development of novel antibiotics using novel antibacterial drugs. One promising strategy is to use novel targets for antibacterial drug discovery such as bacterial riboswitches. They have conservative and well-known RNA sequences and structures and regulate the synthesis of essential metabolites in different pathogenic bacteria by sensing their presence in the cell. In this review, we summarized three different bioinformatic-based criteria for the assessment of the suitability of riboswitches as suitable drug targets. They enable us to focus our research on the most suitable and promising riboswitches as antibacterial drug targets.

Keywords: *Antibiotics; Antibacterial Drug Discovery; Bioinformatics Analyses; Drug Targets; Riboswitches*

Introduction

According to the report by the American Centers for Disease Control and Prevention (CDC) entitled Antibiotic Resistance Threats for 2019 more than 2.8 million antibiotic-resistant infections occur in the United States each year and as a result, more than 35,000 people die annually. To mitigate this problem, different strategies are being proposed and new substances with antibacterial properties are being sought to be developed targeting novel antibacterial drug targets such as riboswitches [1,2]. The riboswitches are highly conservative gene control elements, mostly found in the 5'-untranslated region of messenger RNA in many bacteria, and archaea and some fungi and plants [3-5]. The structural organization of riboswitches is well known and usually consists of a specific metabolite-binding aptamer and a regulatory expression platform [6-8]. The riboswitches regulate the biosynthesis of some vitamin's precursors, such as riboflavin, thiamin, and cobalamin; the metabolism such amino acids such as methionine, lysine, the synthesis of some nucleotides such as adenine, and guanine, and other essential metabolites by two main regulatory mechanisms such as termination of transcription, and prevention of translation. In addition, bacterial riboswitches are found to regulate gene expression by destabilization of mRNA, and by trans-acting mechanisms. Regardless of gene control mechanisms, the riboswitches regulate the expression of genes responsible for the biosynthesis of many different metabolites in bacteria. Most of them are essential for the bacterium. However, not every bacterial riboswitch is a suitable target for antibacterial drug discovery because of several different reasons explained below.

To classify the suitability of each of the different riboswitch classes for targeting for antibacterial drug development, we introduced three main criteria. The most promising classes of riboswitches as antibacterial targets must fulfill all three criteria for a particular bacterium using pure bioinformatics methods [9].

Criterion: Riboswitches to be found in human pathogenic bacteria

The first criterion for the suitability of a particular class of riboswitches as an antibacterial drug target is whether it is found in human pathogenic bacteria. For example, the Flavin mononucleotide (FMN) riboswitch is one of the most widespread riboswitches in human pathogenic bacteria. According to information published in the Rfam database, it is found in 3281 different types of species, including in 41 human bacterial pathogens. Eight of these 41 human bacterial pathogens are on the World Health Organization's (WHO) list of priorities for developing and researching novel antibacterial drugs (https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed). In fact, riboswitches of any class are found in various numbers of human pathogenic bacteria. Therefore, different riboswitches may be used for the development of broad-spectrum or narrow-spectrum antibiotics.

Criterion: Riboswitches to control the biochemical pathway(s) for the synthesis of essential/key metabolites in human pathogenic bacteria, which do not have an alternative biosynthetic pathway, not under riboswitch control

If the riboswitch regulates the synthesis of a key metabolite, its inhibition can lead to critical and deathly deficiencies in the bacterium [10,11]. For instance, the synthesis of FMN in the bacteria is a five-step biochemical process carried out by five different enzymes produced by a single polycistronic mRNA transcribed from the RibD operon, which is under a riboswitch control [12].

In addition, there are not alternative pathways for the biosynthesis of FMN that are not under the control of the FMN riboswitch. This is a great advantage, as antibacterial drugs targeting the FMN riboswitch can completely block the biosynthesis FMN, which is also an essential metabolite.

If the bacterium uses an alternative biosynthetic route, it should be checked if it's under the control of a riboswitch or not. If it is not under the control of a riboswitch, it should be investigated whether the amount of the alternatively synthesized metabolite is sufficient to compensate for the inhibition of the pathway controlled by the riboswitch.

For instance, the adenine-sensitive riboswitches and the guanine-sensing riboswitch regulate the biosynthesis of adenine and guanine. However, there are alternative biosynthetic pathways, which are not under their control, therefore, they may very suitable targets for antibacterial drug development.

Criterion: The synthesis of transporter protein for the essential metabolite should also be under riboswitch control

The last criterion for the suitability of the riboswitches as antibacterial drug targets is whether the bacteria has an active transporter protein importing the key metabolite from outside into the cell. If there is such a transporter protein, it should be checked by bioinformatic analyses whether the synthesis of the protein is controlled by the riboswitch or not. This criterion is also related to the first criterion because if the synthesis is blocked the bacteria will be in a critical deficiency of the key metabolite and it will try to compensate the amounts of it by active transport if possible. In the presence of an active extracellular metabolite transport, it is necessary to verify that the amount of the imported metabolite is not sufficient to compensate for the inhibition of the biosynthetic pathway of metabolite, which is controlled by the riboswitch. Some classes of riboswitches are found to regulate the synthesis of transporter protein as well.

Conclusion

The application of bioinformatics and biochemical approaches led to the identification may different riboswitch classes, which number is still increasing [13-15]. After performing analyzes based on the three criteria, we have classified the riboswitches into four different categories based on their suitability to serve as targets for antibacterial drug development. The most suitable classes of riboswitches are found in many species of human pathogenic bacteria to control unique, essential, and vital biosynthetic pathways, including the FMN, and

the S-adenosyl methionine (SAM) riboswitches. They also control the active transport of the metabolite into the cell, if one is found. In these cases, it is necessary to inhibit only the riboswitch and this will lead to the inhibition of the cell growth.

Other classes of riboswitches control the synthesis of metabolites important for the bacterium but do not control the active transport of the same, including the glmS, TPP, and Lysine riboswitches. In some of these cases, in the presence of active transport of the metabolite in the bacterium, it may be necessary to inhibit the expression of the transporter protein. In the third category are riboswitches that have an alternative biosynthetic pathway(s) of the key metabolite that are not under the control of the riboswitch. In these cases, it may be necessary to inhibit not only the function of the riboswitch but also the alternative biosynthetic pathways in the bacterium. In the last category are the unsuitable targets are those riboswitches that control metabolite degradation pathways.

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