Circulating MicroRNA

A Biomarker for Antibiotic Resistance

Abstract

Antimicrobial resistance (AMR) remains a threat to health care in developed and developing nations alike that continues to only develop in severity as time passes. With the rising difficulty in effective antibiotic conception and the escalating obsolescence of conventional drugs, preventative measures must be taken to halt AMR's progression. Serum miRNAs present a possible alternative to AMR identification, compared to slower, gold-standard culturing methods, with results generated from noninvasive blood samples. This proposal aims to experimentally investigate differences in murine serum miRNA composition following pan-sensitive *Mycobacterium tuberculosis* (Mtb) versus multidrug-resistant Mtb infection to identify putative biomarkers of AMR. The findings of this venture may prompt rectification of the deficit in literature studying the connection between miRNA and bacterial drug resistance.

Key Words

microRNA, *Mycobacterium tuberculosis*, biomarker, antibiotic resistance, diagnosis

Abbreviations:

AMR - antimicrobial resistance
CDC - Center for Disease Control and Prevention
MDR - multidrug-resistant
Mtb - *Mycobacterium tuberculosis*
TB - tuberculosis
WHO - World Health Organization

Background

Bacterial infections are afflictions that can occur anywhere in the body and are principally transmitted through droplets, contact, and airborne particles[1]. Given the increasingly prevalent use of antimicrobial products, the development of AMR has been identified as a growing global health crisis by the WHO[2]. The CDC[3] has outlined infection prevention and proper administration of antibiotics as potential methods of avoiding the approaching epidemic.

With regards to the proper administration of antibiotics, two major obstructions can be inferred that limit a physician's ability to administer the right antimicrobial with the correct dosage: uncertainty of the specific infection and uncertainty of the bacteria's level of AMR. The first ambiguity arises from the non-specific presentations of many bacterial infections, whose similar symptoms render making an accurate diagnosis difficult. Furthermore, the bacteria's susceptibility cannot be anticipated with an infection's clinical presentation[4]. This leads to cycling antimicrobials and substantial amounts of unnecessary antimicrobial exposure that could foster the development of AMR.

A solution to inaccurate diagnosis are biomarkers -- a broad sub-category of medical signs that exist of parameters that can be accurately measured and reproduced[5]. They can be any substance, structure or process that can be measured in the body or its products that indicate, influence or predict the incidence or outcome of a disease[6]. The accuracy and specificity of biomarkers may aid in the detection of bacterial infections diseases and their treatment.

Circulatory miRNAs have been hypothesized to be effective diagnostic and prognostic biomarkers in various diseases[7]. miRNAs are small, non-coding RNAs that mediate post-transcriptional gene regulation[8]. It was discovered that these miRNAs could exist in the extracellular space in microvesicles or RNA-protein complexes and could be produced to have intercellular functions, such as inflammation regulation, that could be regulated by the presence of a bacterial infections.[7] Although the precise mechanism of extracellular miRNA release is unknown, it is hypothesized that miRNA is released through exosomes or microvesicles to function as bodies of intercellular communication. It has also been hypothesized that the release of extracellular circulating miRNA could be in response to the organism's pathophysiological conditions[9].

Hypothesis & Rationale

We hypothesize the differential expression of miRNA profiles in mouse models infected with pan-sensitive Mtb and multidrug-resistant Mtb. The identification of a reliable biomarker of MDR TB would not only be of great benefit to the fight against the leading cause of death by an infection agent[11], but also serve as a compelling proof of concept for the application of miRNA detection in the diagnosis of drug resistance in other bacterial infections.

Although much work has been done investigating the potential of miRNA to act as a biomarker of bacterial infection[12,13], comparatively little has been explored in the use of miRNA profiles in assessing antimicrobial resistance. Cui et al.[10] and Wagh et al.[14] have reported associations between serum levels of specific miRNAs with patient infection of MDR TB or pan-sensitive TB. Validating these putative clinical findings in an experimental setting would act as a jumping-off point for future studies on the connection between miRNA and AMR.

Methodology

A mouse model will be selected based on susceptibility to mycobacterial infection and housed individually under the same conditions with controlled access to food and water[15]. Multiple strains of pan-sensitive and multidrug resistant Mtb will taken from clinical samples and stored at −80 °C until time of experiment, before being thawed and diluted in sterile saline. Infection of mice with Mtb will be performed by delivering bacteria into nasal cavities under anaesthesia. After 24 hours, infection will be confirmed via the bacterial load in lungs[16].

Blood samples will be taken from the control mice as well as animals infected with each strain of *M. tuberculosis* via cardiac puncture[16]. Serum will be extracted and processed for RNA isolation. Using size-exclusion chromatography as well as Qiagen's miRNeasy kits, small RNAs will be isolated from the serum samples; yield, purity, and quality of miRNA will be determined using Small RNA chips (Agilent Technologies)[13]. Following Illumina TruSeq™ Small RNA protocol, small RNA samples will be prepared using reverse transcription, PCR amplification, and gel purification to generate an RNA-seq library. These libraries will be subsequently sequenced and checked for quality by passing raw sequence data through miRDeep2, which quantifies reads matching known mature miRNAs. Additional filters will be used to eliminate results with low abundance hits that still remain from miRDeep2 analysis[12].

In order to compare the expression of miRNA between the pan-sensitive and MDR strains of Mtb, processed RNA-seq data will be statistically analyzed for differential expression. RNA-seq data generated from the control mice miRNA samples will also be compared to sequence data from

infected mice. The differential expression data will be used to form functional annotations of the RNA-seq library, so that specific miRNA molecules can be categorized as up-regulated or down-regulated for each Mtb strain based on statistical significance[12]. Overlapping miRNA profiles will be identified and serve to either validate or disprove the hypothesis.

Conclusion

The use of circulating miRNA in the diagnosis of antimicrobial resistance remains a largely unstudied field, and therefore represents a potential goldmine of overlooked data. In this paper, we propose an experiment to justify future research into this application of serum miRNA, using Mtb as a model bacterium given the previous identification of differentially expressed miRNAs. The findings that result from this proposal may serve as a stepping stone towards a possible future where patient-miRNA screening is extensively consulted as a means of guiding individualized treatment in a future of rising AMR.

Citations

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