Title

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Abstract

Due to antimicrobial resistance, current treatments for tuberculosis (TB) are very limited and have very low efficacy. Existing therapeutics are inadequate for the ongoing epidemic of drug resistance TB. The evolutionary push of mutations created is destabilizing global TB control, thereby needing new novel therapies for treatment and screening purposes. In this paper, we propose two potential pathways that target TB through IFN-I signaling and the AhR pathway which allows for more accurate and efficient early screening of TB. Targeting these pathways impacts TB outcome by increasing treatment efficacy and strengthening host defense. IFN-I signaling and the AHR pathway can be seen as potential targets for host directed therapies.

Introduction

Tuberculosis

Tuberculosis (**TB**) is a serious, contagious infectious disease caused by the bacteria Mycobacterium tuberculosis (**Mtb**). The bacteria predominantly attacks the lungs and can spread to other parts of the body such as the kidneys, brain and spine. M. tuberculosis is transmitted through particles called droplet nuclei in the air, making it an airborne disease. Prolonged exposure to someone with the illness will lead to inhalation of droplet nuclei which can traverse through the respiratory tract, ultimately finding its way to the alveoli of the lungs.

TB is typically divided into two types of infections: latent TB infection and active TB infection. Most individuals infected with M. tuberculosis have an immune system with the capabilities of keeping the bacteria in check. Their infection remains dormant and the typical symptoms encountered in active tuberculosis patients fail to manifest. However, once tuberculosis becomes active, a patient's situation can escalate rapidly. Without discriminating

between age groups and gender, the clinical characteristics of a person with active TB will show symptoms of persistent cough for 3 weeks or longer (with blood or mucus), chest pain when breathing or coughing, unintentional weight loss, and drenching night sweats (Center for Disease and Control). Failure to treat active tuberculosis can be fatal, and a combination of different antibiotics for a period ranging between 6-12 months (American Lung Association, 2020). This itself presents a major drawback as patients often quit therapy prematurely, ultimately heightening the risk of drug-resistant tuberculosis strains emerging. A pragmatic, cluster-randomized trial study across 780 patients in China observed that 5.1% of participants discontinued treatment after 60 days and 14.4% discontinued by 120 days. (Stagg et. al, 2019)

Due to these characterizations, TB remains the second-most common infectious disease (after COVID-19), accounting for 1.5 million lives in 2020 and increasing the number of claimed lives entering 2021 and 2022 (World Health Organization, 2021). The COVID-19 reversed and disrupted the global process of addressing TB and presented itself with an increased number of cases in over 30 countries.

Drug Discovery for Tuberculosis

Increased public recognition on the repercussions and impacts of tuberculosis have driven biomedical research and development on the disease. Tools conducting such research only existed for the past few decades, yet several breakthroughs have been established on the diagnosis and immunization of the disease. Despite these efforts, several challenges still remain in tuberculosis drug discovery. There are two main complementary approaches in TB drug discovery (Lage, 2018). The first of which is a drug-based approach using a drugs-to-genes model. The second of two approaches is classical pharmacology - also known as phenotypic drug

screening - which utilizes a drug-based (drug-to-genes) model. Meanwhile, the second approach is reverse pharmacology which utilizes a target-based (genes-to-drug model). In target-based drug discovery, a specific drug target is purified and utilized to recognize molecules that regulate in-vitro function. These in-vitro projects typically involve assaying - determining the amount of a particular constitution of the pharmacological potency of a drug - for inhibition (Rock, 2019).

Both models have yielded underwhelming advances in the treatment of antibacterials. This is due to limited chemical diversity in screening libraries. Antibiotics like tuberculosis often do not act in accordance with Lipinski's rule of five. Lipinski's rule of five defines a molecule's "druggability" and analyzes integral molecular properties for a drug's pharmacokinetics, observing how a drug is absorbed, distributed, metabolized, and excreted in the human body. A drug hence cannot be made since the target molecule does not have the physical and chemical properties to be biologically available (Drugbank, 2022). Furthemore, Mtb has a distinct outer membrane mainly composed of mycolic acids, lipids with long-chain and branched fatty acids. These lipids create a permeability barrier that makes small drug-like molecules unable to penetrate bacterial cell walls and makes Mtb able to survive extremely hostile surroundings. They are able to evade efflux and avoid xenobiotic metabolism. Xenobiotics, defined as chemical substances that are foreign like drugs, are unable to work on Mtb (Favrot, 2013).

According to the World Health Organization’s 2019 Global TB report, the current treatments are inefficacious due to drug resistance. This repeated course of antibiotic use has very limited benefits and is a good biological marker for adverse outcomes which helps continue their reign as the number one public health threat. Reduced antibiotic efficacy developed from frequent usage makes it very crucial to understand the host immune response and Mtb. Mtb is an

intracellular parasite that invades macrophages and inhibits the host cells' apoptosis. Besides their innate ability to withstand bacterial host defenses and to resist the attacks of the body's immune system, Mtb can survive through most antimicrobial agents currently available. Due to these selective pressures in pushing Mtb evolution, there are outcomes of strains of TB that are now classified as extensively drug-resistant (XDR-TB) and totally drug resistant (TDR-TB).

Methods

Gene Editing

In the field of genome-editing technology, there has been rapid innovation over the past two decades. The gene modification first emerged primarily using homologous recombination in the 1970s. To perform homologous recombination in the laboratory, DNA fragments that are similar to the target sequence need to be delivered into the cell (Cupta, 2014). Once inside the cell, these fragments can recombine and replace the targeted portion of the genome. This approach has been widely used in mouse embryonic stem cells, but it remains inefficient in its application in many cell types, with a one-in-a-million success rate. It typically takes more than a year to generate a genetically modified mouse. These shortcomes have accelerated the development of more effective methods of gene modification such as zinc finger nucleases (ZNFs) and transcription activator-like effector nucleases (TALENs).

ZFNs are engineered by fusing proteins containing site-specific DNA binding domains to the endonuclease domain of the bacterial FokI restriction enzyme. The ZFNs cut the genome at the targeted location and induce cellular repair systems, including nonhomologous end joining (NHEJ) and homology-directed repair (HDR). The error-prone nature of NHEJ can introduce knock-in or knock-out in the genome. HDR can repair the break using the traditional

homologous recombination or by using an exogenous single-stranded DNA oligonucleotide as a repair template. Nonetheless, both cases show high efficiency in gene modification. But it is not straightforward to construct zinc finger domains, and it can take non-specialists months to obtain optimized ZFNs. Other disadvantages include limited target site selection and potential off-target events.

TALENs are also engineered from naturally occurring proteins that bind and recognize specific DNA sequences. TALENs generate double stranded breaks (DSBs) at target sites and introduce knock in and knock out mutations in the same ways as ZFNs. The repeat-variable di-residue (RVD) in TALENs confers higher specificity and affinity to the desired genomic sequences. In addition, it takes a shorter time to design and construct TALENs in comparison to ZFNs. Some disadvantages of TALENs include the off-target effects and the larger size of TALENs, making it difficult to deliver TALENs in cells and for therapeutic purposes.

Starting in the 2000s, a cheaper and more effective genome editing method, clustered regularly interspaced short palindromic repeats (CRISPR), has gained increasing popularity and is now utilized in a wide range of applications. CRIPSR refers to a specialized group of DNA sequences adapted from the genomes of prokaryotic organisms including bacteria and archaea. These microorganisms utilize CRISPR-derived RNA (crRNAs) and numerous CRISPR-associated enzymes (Cas9 proteins) establish the basis of the CRISPR-Cas9 system. This system has successfully allowed for efficient, site-specific genome editing and has led to a diverse array of applications: curing genetic diseases including sickle cell anemia, developing biotechnological products, creating drought-resistant crops, etc. The Cas9 proteins act as "molecular scissors". They are specifically responsible for locating and cleaving target DNA in CRISPR systems.

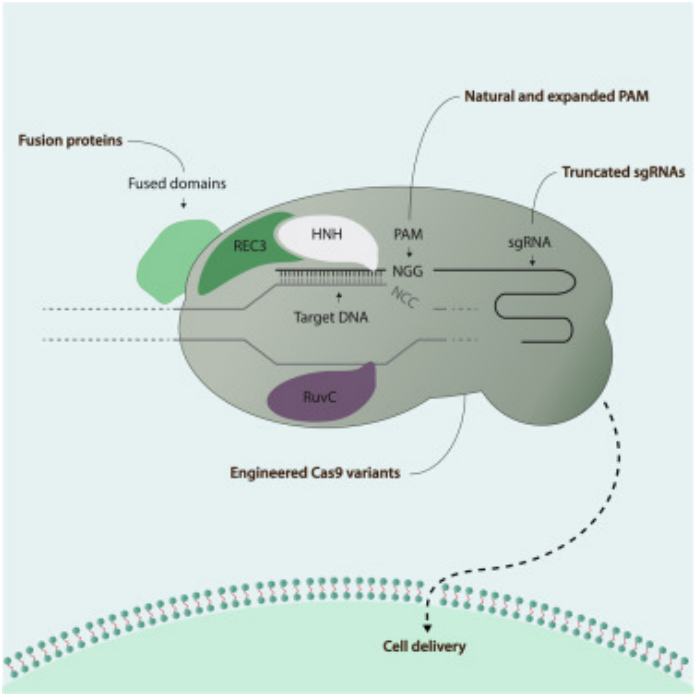

Figure 1: delivery of CRISPR into target cell and the general components of CRISPR protein (Broeders, 2020).

Utilizing CRISPR Cas9 Systems for Mtb Screening

Since MtB is considered one of the most successful pathogens, ongoing treatments include using CRISPR-Cas9-mediated genome screens and CRISPR interference (CRISPRi) screens to study host-pathogen interactions. The CRISPRi screens that were used require host cell survival. This is crucial for understanding which processes are targeted by Mtb in live host cells. By analyzing these CRISPR screens, it was found that Type I interferons (IFN-I) signaling was the main contributor to cell death in response to Mtb. IFN-I plays an essential role in triggering the antiviral immune responses and allowing for non-immune cells to go into an antiviral state. However, IFN-I signaling is associated with the susceptibility of Mtb because there are high transcription levels of IFN inducible genes in the blood of active TB patients.

Therefore, disturbances made to the IFN-I pathway helps eliminate more Mtb and enhances host cell survival. Such phenomena can be reversed by repressing the Interleukin-1 receptor antagonist (IL-1Ra) and having the TYK2 inhibitor, Cerdulatinib, to protect the host cells from Mtb (Lai, 2020).

Another pathway that was observed in a study was the Aryl hydrocarbon receptor (AhR) pathway. AhR is a ligand-activated transcription factor that serves an essential role during immune responses and mediating pathological development. The aryl hydrocarbon receptor nuclear translocator protein (ARNT) that is encoded by the ARNT gene binds to AhR, creating a complex. This complex is required for the receptor to function. By utilizing CRISPR-Cas9 knockout and CRISPRi screens, it is observed that AhR and ARNT enhances host cell death during Mtb infection. It is observed in AhR-deficient mice that there is a decreased resistance to Mtb infection (Lai , 2020). However due to the complexity of Mtb, the defense against Mtb through AhR knockout is not distinguished enough yet in humans. Nevertheless, this is not discouraging news as the IFN-I and AhR, the top positive genetic hits, are marked as prospective targets for advancing host directed therapies (HDTs) to treat TB.

Discussion

While there have been significant advances in streamlining the tuberculosis screening process, the controlling and management of tuberculosis has been an issue in various parts of the world. The gaps in the accessibility of screening and treating tuberculosis results in a public health crisis and a health security threat. Current tuberculosis screening methods are costly and ineffective, presenting a significant barrier to reducing tuberculosis in a region. Rapid and inexpensive diagnostic methods are urgently needed for screening tuberculosis, especially in

high-risk populations. Although screening for tuberculosis is essential to elimination of tuberculosis, the accessibility and affordability of receiving tuberculosis treatment has been prioritized to control the current infected population. Most populations with high tuberculosis morbidity have reported inadequate resources to screen entire populations as well as prioritizing their quality of service. In order to execute population wide screening, efforts to collaborate with health-care providers and agencies must be made to ensure proper treatment for identified tuberculosis individuals (Centers for Disease Control and Prevention, 1995). However, with the application of CRISPR-based methods, tuberculosis screening could be streamlined to transform population health. With a more efficient approach to tuberculosis screening, both the government and various health institutions could allocate more resources to those who are infected with tuberculosis and in need of treatment and therapy.

Conclusion

TB, along with Malaria and HIV/AIDS, has been considered as the "Big Three" killer diseases due to their deadly infections. Millions of people all over the world suffer from these infections every year. The addition of COVID-19 has expanded the category into "Big Four" as well as increased the number of infection cases by disrupting the global process of addressing TB. (Makam, 2021) Current treatment of TB relies largely on drug-based therapies and drug screening. However, the misuse and overuse of antibiotics have led to increasing antibiotic resistance in the pathogenic bacteria. (Christaki, 2020) The rising public health issues are urging for more effective and efficient treatment of the disease that can overcome or avoid drug resistance, thus providing more enduring control and clearance of the disease. The breakthrough in the field of gene editing by CRISPR has opened a variety of possibilities in infectious disease

treatment. Latent TB can be controlled by individuals' immune system, but once it becomes active, the infection escalates rapidly and can be fatal without prompt treatment. Given these characteristics of TB, early Mtb screening may be a more promising way of TB intervention. CRISPRi screens allow for high accuracy and efficiency that simplifies and promotes early diagnosis of TB and thus preventing TB from activation or further development of infections. Two potential pathways target IFN-I signaling and the AhR pathway, respectively. With the assistance of CRISPR knock out and CRISPRi, the production and application of Mtb screens could be made a lot faster and cheaper. Early diagnosis leads to early intervention of the disease, which would be easier to treat and confer less drug reliance. Yet the drug discoveries of TB are relatively recent. More studies need to be done on TB as well as the proposed two pathways.

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