Knock-Down Punch to Tuberculosis

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Story of development of a "marvel" tool for TB research.

We, as humans, have come a long way from being cavemen to a species capable of space travel to a point where we can incorporate intelligence into things. But would you believe me if I will tell you that there is a force which kills two people every three minutes and that we are unable to stop it? Well, that force is known as Tuberculosis (TB). TB is not merely an infectious disease but a social phenomenon. Putting up numbers won't make a significant difference here as the impact of TB on our society is very much palpable. But, in spite of all the biomedical advancements, why do we still fail? Well, the causes are numerous; first being the character of the bug. *Mycobacterium tuberculosis* (causative organism for TB, which is usually referred as Mtb) is tremendously resilient and intelligent at the same time. Humans and TB bacterium have a historic relationship and this co-evolution has given TB an upper hand. Secondly, our weaponry of vaccines and drugs against it is too scarce to be called as sufficient. The main problem is that we don't understand the enemy we are fighting and this makes us incapable of developing any effective countermeasures.

Physiological wisdom about any given organism is usually derived from a number of well-performed genetic studies. This makes genetic manipulation as the single most important tool for a successful biologist. Mtb is resilient not only to the therapeutic agents but also to the genetic manipulations. Conventional "Gold Standard" for genetic studies is usually based on swapping the desired gene out by technique called as recombination (results into "Knock-out" of the desired

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gene). But this tool is particularly cumbersome in terms of designing and application. Although most effective, the technique suffers a blow as we cannot examine genes which are quintessential for the survival of bacteria (known as essential genes). Likewise, other popular techniques for genetic engineering also fall short from being ideal. Fresh attempts were made by our lab at Translational Health Science and Technology Institute (THSTI) under the supervision of Dr NisheethAgarwal (Associate Professor, THSTI) to address this problem and create a near-ideal platform for genetic manipulation in Mtb. Our efforts substantiated into successful implementation of "most ideal tool" CRISPRi in *Mycobacterium tuberculosis* and were published in the journal, *Nature Communications.*

What is CRISPR?

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and is responsible for adaptive bacterial immunity against invading viruses. Let's understand the concept of CRSIPR with the help of a simple example. Have you ever visited a police station? Even if you have not, you must be aware that every police station has a notice board with pictures of the "MOST WANTED" star criminals of the locality (courtesy: Bollywood movies). The purpose of this board is to make all police officers and anyone who visits police station aware of the wanted criminals and catch them anyhow. So recognition, entrapment and execution are the steps to catch and prosecute a thief, right? CRISPR also works the same way, whenever any virus attacks bacteria (yes, they do!), bacteria cleave attackers genetic material into small pieces and incorporates it into its own genetic material (pictures of criminals) as a memory for subsequent attacks. Police here is a protein known as Cas9, which with the help of these pictures (known as crRNA and trRNA) recognises the invading organism, catch him (Cas9-guideRNA complex goes and binds with the foreign genetic material) and execute (Cas9 has an ability to cleave DNA). sThe whole system very well resonates with

"Keep your friends close, but your enemies closer".

Although existence of CRISPR was long known but "marvel" happened when scientists were able to repurpose it as an effective genetic engineering tool. The trick was to redesign crRNA and trRNA into a new sgRNA (photo of the criminal) so that Cas9 (the police) recognises its "own" (designated) gene instead of "foreign" gene. Another trick was played by taking away the ability of Cas9 (the police) to cleave the DNA to create new Cas9 called as deadCas9 or dCas9. Hence, dCas9-sgRNA complex now can only recognise and bind (catch) the desired gene in turn "knocking down" its expression inside the cell (even if you don't execute the thieves, putting them in jail would also curb the mayhem, right!). This adaptation of using CRISPR to suppress the expression of target gene is known as CRISPRi (interference).

Our job was to break the resilience of *Mycobacterium* and successfully adapt CRISPRi as the new "Gold Standard". We chose deadCas9 (dCas9) from *streptococcus pyogenes*as our police. Use of dCas9 required codon optimisation for about 191 amino acids from the primary sequence (which simply means that we made it "suitable" to work in Mtb). Next, we constructed a plasmid (independent replicative DNA) as our delivery vehicle for the target sgRNA ("wanted" pictures).

Once the system was ready, we tested its effect on expression of variety of genes to observe the resultant "Knock-down" of target genes. We were also able to determine and optimise the critical factors responsible for maximal suppression. The fundamental advantage that our system creates is the reversibility as expression of dCas9 and sgRNA are under the control of an inducible promoter (which actually means that we can decide when and where to introduce both the police and the picture of the criminal). This function endows us with a remarkable ability to study and understand the workings of essential genes which most other tools lack. Not only this, CRISPRi was found to be effective in suppression of an operon (which means genes which are expressed together) i.e., if it's a gang of thieves and you catch one, you eventually get all of them. CRISPRi also allows seamless multiplexing (suppression of multiple genes at the same time) which means you can really deliver multiple photographs at once.

Our study has established CRISPRi as "most effective tool" in all aspects for genetic manipulation in Mtb. CRISPRi provides us incomparable ease for designing, using and maintaining this system at the lowest possible cost. Concrete tool like CRISPRi usually lays the solid foundation and paves the way for assimilating necessary and comprehensive insight into the workings of an organism. Our aspiration would always be to turn these insights into wisdom which will expedite our dream of better therapeutics against TB and possibly deliver that "knock-down" punch.