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Can Genetically Engineered Tuberculosis Cell of Kill its Own Kind?

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Background

Tuberculosis (TB), a contagious disease, is the tenth major cause of death worldwide according to world health organization and over 10 million people had been reported with TB, and 1.6 million people had died from TB in 2017 [1]. TB is caused by gram positive bacteria, *Mycobacterium tuberculosis* that primarily affects the lungs. Healthy individuals get infected with TB if they inhale aerosols sneezed, spit or coughed by an infected TB patient. On invasion, this bacterium is phagocytosed by alveolar macrophages, where they reside without being affected by host immune responses and cause pathogenesis [2]. *M. tuberculosis* is able to establish an asymptomatic latent infection that can later reactivate to cause active diseases [3-5]. Despite, many worldwide uses of vaccine, antibiotics and ongoing immense researches, still it is a major enigma for clinicians and researchers to combat this disease. Additionally, foremost concern is with poor management, illiteracy, incorrect diagnosis and poor treatment leading to development of multidrug resistant TB (MDR-TB), extensively drug resistant TB (XDR-TB) and totally drug resistant TB (TDR-TB).

With the advancement in medical sciences and therapeutics, different modes of drug delivery has been discovered, still, to defeat the challenges posed by antituberculosis drugs, there is a need to envision new ways to treat this disease. Genetic engineering in phages has successfully treated patients with multi antibiotic resistant pathogen [6]. Another excellent ongoing example of cancer cells where engineered cancer cells made through CRISPR technique can fight their own tumor cells [7]. In this paper we have discussed ways of cell-cell communication in mycobacteria and put forth an idea of how this information can be applied to kill cells of own kind using genetic engineering.

Cell-Cell interactions in Mycobacteria

Mostly mycobacteria multiply through binary fission, although some may show sporulation too when exposed to stresses such as starvation and oxygen deficit [8]. Like in case of *M. Marinum* and *M. bovis* BCG, sporulation occurs in late stationary phase [9]. Sporulation capability in *M. tuberculosis* is still not known. Asexual reproduction in bacteria will not give any survival advantage in the course of evolution as it delivers no variations. Horizontal gene transfer and cell to cell fusion limits the state of inbreeding by exchanging the genetic material between the bacterial cells.

a) Conjugation Based Interaction

Horizontal gene transfer (HGT) is the process through which more extensive segments of DNA are transferred between species and even kingdom [10-13]. HGT is mediated through three fundamental processes: conjugation, transformation and transduction [14]. Conjugation is considered as the major contributor of HGT to create variation and drives evolution. It transfers DNA unidirectionally from donor cells to recipient cells via direct cell to cell contact or through bridge like connection called pilus. Pilus is particularly associated with the donor cell which carries plasmid. Traditionally conjugal processes are plasmid encoded or encoded by discrete genetic elements integrated into the chromosome. Plasmid carries a unique origin of transfer (oriT) which guides the DNA into the recipient cell [15]. When oriT recombined into the chromosome (Hfr strain), it can mediate transfer of chromosomal DNA. Mycobacteria shows distributive conjugal transfer (DCT) which is chromosomal and not plasmid based and genetic elements responsible for transfer have yet to be identified [16-18]. Like conjugation in *Escherichia coli,* DNA transfer in *Mycobacterium smegmatis* displays all of the criteria of conjugation as it requires stable and extended contact between a donor and a recipient strain, is DNA resistant and transferred DNA into the recipient incorporate into the chromosome through homologous recombination [19]. Mycobacteria displays no initiation site like OriT in *E. coli*, all regions of the chromosome are transferred with equivalent efficiencies [20,21]. Equivalent transfer of kanamycin- resistant markers regardless of its chromosomal location depicts multiple initiation sites within the chromosome [22]. This distributive mechanism of conjugation in mycobacteria creates genome wide mosaicism in a single event where donor DNA segments range from 0.05 kb to ~250 kb. From the genome sequence analysis, it was postulated that randomly donor chromosomal DNA are generated,

out of which some are co-transferred into the recipient strain and replace recipient chromosomes through homologous recombinations that generates large scale transconjugants diversity as seen in meiotic products of sexual reproduction [16]. Based on sequence comparisons, *M. canettii* and smooth-colony *Mycobacterium tuberculosis* complex (MTBC) strains showed HGT and genome wide mosaicism and it was proposed that MTBC progenitor species, *M. protuberculosis* underwent HGT and give rise to *M. canettii* and rough colonies of *M. tuberculosis* [23-26]. Through genome sequencing comparison it has been known that some form of genetic exchange has occurred between *M. tuberculosis* and *M. canettii* [27]. Transposon mutagenesis has shown that ESX-1 is the key component for the conjugation in mycobacteria. During DCT kanamycin resistance gene and esx1 locus segregates [17].

b) Plasmid Encoded ESX System Based Interaction

There is dichotomy regarding the presence of plasmid in *M. tuberculosis.* Some reports have cited presence of plasmid in association to antibiotic resistance phenomenon but there are no clear evidences for the same and assumed that only mutations will be the cause of variations in clonally expanded TB cells [28, 29]. Recently, existence of plasmid encoded ESX system has been reported in addition to chromosomal based ESX loci in many mycobacterium species namely, *M. kansasii, M. abscessus, M. chubuense, M. gilvum, M. marinum, M. smegmatis, M. yongonense [27].* For *M. marinum* it is reported that plasmid encoding elements (type VII and IV) and relaxase are responsible for conjugation event [30]. From the phylogeny and synteny data evidences it is envisioned that plasmid-encoded ESX system is substantially contributing to ESX diversification and is the driving force to mycobacterial pathogenesis evolution leading to adaption in host environment.

c) Cell fusion-based Interaction

Cell to cell fusion is another way through which variations occur in bacterial genome. As in case of *E. coli* not only classical conjugation occurs for the exchange of material but there are evidences of spontaneous zygogenesis leading to diploidy in *E. coli* cells resulting through a mechanism of cell fusion, or, atleast, close contact between parental cells at the cytoplasmic level [31]. Z-mating appears to be a form of true sexuality in prokaryotes. Bacterial dynamin like proteins disclose mechanism of membrane fusion [32]. Mitochondria which are the descendants of bacteria acquired by eukaryotic cells through symbiotic relationship also show fusion mechanism. In response to metabolic or environmental stresses, fusion and fission occur to maintain functional mitochondria [33]. Key proteins requisite for mitochondrial fusion are mitofusins (Mfn1, Mfn2), OPA1 (optic Atrophy protein1) and large GTPases belonging to dynamin family of proteins. Within the eukaryotic cell one more example is of *Chlamydia trachomatis* bacterium which can propagate only in human cells and causes fusion of its inclusion bodies to increase its pathogenecity. Homotypic fusion of the inclusions is mediated by IncA proteins and it is consistent with the fact that dissimilar IncA proteins do not fuse [34]. Similar research is also going on mycobacteria, where membrane fusion is key to tuberculosis.

SIGNIFICANCE

From the gathered information it is manifested that some mycobacterial species do have plasmids and have an evidence of a unique distributive conjugal transfer mechanism for the exchange of material. Also, some bacterial species can show cell fusion mechanism but particularly to *M. tuberculosis* there are no clear evidences.

If we come to know about any mechanism by which *M. tuberculosis* can exchange their genetic material (say by fusion or by conjugation) then we can exploit this property to deliver a drug, a protein or any killing switch in form of plasmid in a tuberculosis cell making it an engineered TB cell. Promoter of which will activate only after entering into macrophage and the killing genes attached downstream to promoter will transcribe in engineered TB cell and enter into other persisting TB cells, causing death of TB cells without harming normal cells. Also, if TB cells show any re-homing property, characteristic feature of cancer cells [35, 36], then this engineered TB cell can be used to cure pulmonary as well as extra-pulmonary tuberculosis.

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Conflict of Interest

There is no conflict of interest.

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