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Review Article

Mechanism of Drug Resistance in Mycobacterium Tuberculosis

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Summary

Mycobacterium tuberculosis the causative agent of tuberculosis has many intrinsic features which enable it to evade the activity of antibiotics. Many studies have been carried out to understand the mechanisms of drug resistance by this organism. An attempt was made in this write up to elucidate the various mechanism of drug resistance in M. tuberculosis, including its innate impermeable cell wall and mutation of specific genes. Drug resistance in Mycobacterium tuberculosis is not a product of a single homogeneous genetic unit. Rather it is as a result of frequent mutation in various genes which encode for resistance to antibiotics. Also, the slow metabolism during a prolonged dormant stage greatly enhances it resistance to drug, the waxy impermeable cell wall with the presence of numerous efflux pump are essential for withstanding the potency of antibiotics. Having an adequate knowledge on the molecular mechanisms of drug resistance in M. tuberculosis may be helpful in exploring new targets for drug development.

Keywords: Tuberculosis, Drug resistance, Antibiotic, Mycobacterium, Mechanism

Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. Streptomycin, with high bactericidal activity was the earliest curative agent used in its treatment. However, drug-resistant strains began to emerge few years later. Initially, this was thought to result mainly from using only single drug, streptomycin, to treat the infection, prompting the use of multi-drug therapy for controlling the infection, but in recent decades multi-drug resistant (MDR) has emerged [1]. Furthermore, the reports gave by World Health Organization in 2010 reveal two other more classes which are, extensively- drug resistant (XDR), which is resistant to at least 4 of the core anti-TB drugs and totally-drug resistant (TDR) strains of TB. A type of TB resistance in people who are originally infected by the antibiotics resistant strain but have not used any anti-TB chemotherapy is regarded as the primary resistance, while a situation in which resistance developed due to inadequacy of treatment is referred to as acquired resistance [2,3].

The complexity of the mechanisms used by M. tuberculosis in drug resistance has led scientists to studying M. tuberculosis at the molecular level. Various researchers have been able to identify some molecular features which have been attributed to drug resistance in the organism. When considering the mechanisms of drug resistance in M. tuberculosis, it is imperative to understand the interplay between the molecular mechanisms, adaptive features and the innate attribute which play crucial role in resistant-MTB strain. Both [4] and Blair (2015), accentuated that drug resistance in Mycobacterium tuberculosis is not a single homogeneous biological unit, thus suggesting that the complexity and severity of resistance in the pathogen is due to some other intrinsic factors.

This study aims to present findings on the mechanism of drug resistance in M. tuberculosis, presenting a comparative review of the molecular mechanism, the adaptive features and the innate mechanisms through which the organism develop resistance to antitubacular drugs.

Mechanistic Innate Features of Drug Resistance

Impermeable Cell Wall

The fundamental characteristics of passive resistance to antibiotics in M. tuberculosis are due to its impermeable cell wall [5]. M. tuberculosis has a cell wall that is composed of three main components, these are: mycolic acids, Wax-D and the cord factors [6]. The hydrophilic arabinogalactan layer ensures the impermeability of the cell wall to hydrophobic chemicals. This layer is further contained in hydrophobic mycolic acids which extremely impede the entrance of hydrophilic molecules [7]. This impermeability result in accumulation of antibiotics slowly around the cell, the accumulated drugs around the cell wall are slowly detoxify by different cellular component or by release of enzyme. [8], showed that β-lactams, which are inhibitive to the incorporation of peptidoglycan (rigidity structure) into the cell wall, are degraded by M. tuberculosis because it possesses β-lactamases, an enzyme that effectively degrade β-lactam antibiotics.

Danilchanka O et al. [7], reported the presence of an outer membrane channel protein CpnT in both M. tuberculosis and M. bovis, which performs a dual role of nutrient uptake and selective susceptibility to antibacterial agents. The report showed that the CpnT mutant of M. bovis is more resistant to most antitubercular drugs, including the bactericidal nitric oxide, which is utilized in controlling M. tuberculosis infection in mice.

Slow Metabolism Mechanism

Bacteria with slow metabolic processes and long generation time are difficult target for most antibiotics i.e., bacteria that are metabolically active and replicate quickly are good target for antibiotics [9]. However, in M. tuberculosis, it is not still clear if the long generation time corroborate its drug resistance. According to [10], the long generation time in M. tuberculosis does not impact drug resistance ability on the organism. [11]. Also reported a negative association between drug resistance and generation time in the organism. However, the slow growth rate of M. tuberculosis has been reported to play a key role in its drug resistance, for example, unstable antibiotics such as carbapenems loses their activity at a faster rate than the mycobacterial growth rate [12], identified some specific genes which allow M. tuberculosis to grow in oxygen-deprived (stress) conditions, most of which are directly involved in triacylglycerol production. Triacylglycerol causes slowdown in the metabolic processes of Mycobacterium tuberculosis. This is mainly because triacylglycerol synthesis uses up acetyl CoA, which is an essential component in tricarboxylic acid cycle, a fundamental metabolic pathway [12]. This triacylglycerol is extensively produced by Mycobacterium tuberculosis when responding to diverse stress conditions such as oxygen deprivation, acidic pH, and iron deficiency [13,14], observed that the ability of the organism to grow under acidic condition is aided genetically and not only limited to physiological adaptations.

Also, evaluating the metabolic status of the organism at each stage of its pathogenesis is challenging since the organism can adapt to various microenvironment within the host. Furthermore, the extent of heterogeneity of infecting population in various cellular compartment within the same host adds to the challenges of targeting the metabolism processes [15] for therapeutic purpose.

Possession of Numerous Efflux Pumps

This protein channels play crucial role in the normal metabolism and physiology of the organism such as signaling molecules across the cell wall, and toxins, waste, and nutrient transportation [16]. Efflux pumps have also been shown to be adapted to drug resistance in M. tuberculosis. Multidrug efflux pumps pass through the inner and outer membranes and serve as an outlet for antibiotics from the cell [17]. Drug efflux pumps in M. tuberculosis have been found to possess regulatory protein systems; which controls efflux pump expression and thus specializing them for drug resistance roles [16].

Molecular Mechanisms

Acquisition of antibiotics resistance in M. tuberculosis has been shown to result from spontaneous mutation in several chromosomal genes, this frequent mutation has been found to confer alteration to the required interaction between each anti-tuberculosis drug and their specified target.

Rifampicin

This is a fundamental lipophilic annamycin chemotherapeutic agent initiated into the multi-drug treatment scheme of tuberculosis in the 1970s. It is usually combined with isoniazid as the first line chemotherapy in the treatment of tuberculosis [18]. Rifampicin is known to have an inhibitory effect against slow and actively growing tuberculosis [19]. Rifampicin mode of action is by binding to the β-subunit of the RNA polymerase, inhibiting the elongation of messenger RNA [20]. Rifampicin resistant strain of Mtb, usually serve as an indicator for multi-drug resistant tuberculosis, because these strains are not sensitive to all other tubercular drugs Comas et al., (2011) [21].

Resistance to rifampicin in M. tuberculosis have been found to be due to mutation in rpoB of RNA polymerase retarding affinity for rifampicin [20]. Some studies have been able to identify specific codon which can causes rifampicin resistance when mutation occur in them [22,23]. Report by [24] observed that most of the rifampicin resistant isolates, have a missense mutation and substitution

of nucleotide at codon 526 and 531 of rpoB. [25], was also able to identify resistant strains with a form of mutation at 69-bp region and point mutation in rpoB alleles. [26] in his review work concluded that rifampicin resistance in tuberculosis is associated with nucleotide substitution at point 516, 526, or 531of rpoB locus. The potency for rifampicin cross-resistance with rifamycin has been reported by several researchers, this has been characterized with conformational changes in codons (518 or 529) [27-29].

Pyrazinamide

Pyrazinamide is a first-line drugs, potent against non-replicating persistent MTB [30]. PZA is also perform crucial function in reducing the relapse rate in tuberculosis; shortened the course of treatment from one year to six months [31] effective where there is resistance to rifampicin and isoniazid [32]. The proposed mechanism of action of pyrazinamide involves conversion of pyrazinamide to pyrazinoic acid, by the enzyme pyrazinamidase/nicotinamidase coded by the pncA gene. The pyrazinoic acid disrupts the bacterial membrane energetics; membrane transport; formation of CoA, and acidification of the cytoplasm [32]. Most resistance to pyrazinamide has been associated with mutation in the pncA gene [30,33]. The mutation in pncA accounts for most of the resistance cases reported in MTB. Other targets and mechanism include: efflux pump [34] ribosomal protein S1 (RpsA) involved in trans-translation [31], Yang et al. (2015), identified mutation at the C-terminus of RpsA which is responsible for retarding the binding of POA to RpsA, thus making PZA inactive.0

Isoniazid

Isoniazid and rifampicin form the core antibiotics in the treatment of tuberculosis. Isoniazid is usually present in the inactive form but their metabolism in the body convert them into a pharmacologically active form [35], Rouse et al. (2005). The metabolism of isoniazid to the active form occur via the activities of catalase/peroxidase enzyme KatG, coded by the KatG gene [36]. Once activated, it has been found to be a strong bacteristatic agent on metabolically and physiologically active M. tuberculosis, with a minimum inhibitory range of (0.02 µg/ml to 0.06 µg/ml) [37,38].

The mode of action of isoniazid resistance is complex and remains unclear, however, most isoniazid Mtb-resistant strains have been associated with mutation in KatG and inhA [39,40]. Mutations of the S315T of KatG is more common in isonazid resistant strains, mutation at this point causes the formation of isoniazid product with little affinity for isoniazid aduct [36]. Also, the conformational changes in inhA active site retard efficient binding to drug, drugs such as Ethionamide which have inhA as their target site are also affected (Ho et al., 2009). [40] who studied 11,411 Mtb isolates from 49 countries reported that 64% of all isoniazid resistant strain studied has mutation in katG315 while 19% having mutation in the inhA-15 region. [41] reported that 88% of Mtb strains from patients with multidrug-resistant (MDR), and extensive drug resistant (XTR) tuberculosis in the Republic of Moldova had a katG 315T mutation. However, [38] was able to identify isoniazid resistant strains which do not harbor conformational changes in the KatG nor inhA.

Ethambutol

Part of the four-drug regimen anti-tuberculosis drug, it is combined with PZA, rifampicin, and isoniazid to prevent rifampicin resistance especially in cases where their isoniazid resistance is not detectable [42]. The mode of action of ethambutol involved interfering with synthesis of cell wall in the Mycobacterium tuberculosis [43]. However, several other formulated hypotheses on the mechanism of action of ethambutol include inhibition of the synthesis of spermidine [44], blockage of mycolic acid transfer to the cell wall [45], interfering with RNA metabolism [46], inhibition of the synthesis of phospholipid [45]. There is no clarity on the mechanism to ethambutol resistance, with the resistance mechanism initially related to mutation of the codon 306 in embB, [47,48], however, certain studies have found M. tuberculosis with mutation at this same point to be susceptible to ethambutol [49,50]. Isoniazid resistant-MTB with mutation at the katG Ser315 were also found to concurrently exhibit resistance to ethambutol as well [48]. Polymorphism in embA, embC, and Mutations in embB497 and embB406, mutation in the codon 306 in embB have all been implicated in ethambutol resistance [48]. In 2013, Safi et al. proposed that mutation in ubiA (Rv3806c) resulted in high level of ethambutol resistance. [51], found mutations in ubiA in all the Ethambutol resistant Mtb he worked on, while [52], observed that ubiA mutations in Mycobacterium tuberculosis varies from one geographical location to another.

Fluoroquinolones

Fluoroquinone majorly the ciprofloxacins are chemotherapeutic agents used in the treatment of tuberculosis Rustomjee et al. (2008) [53], they have been found to demonstrate high antibacterial activity on Mycobacterium complexes and are majorly employed as combined therapy, it is inhibitory to DNA gyrase and topoisomerase IV [54]. Resistant to fluoroquinolone occur via mutation in the gyrA or gyrB [55]. Efflux pumps, pentapeptide proteins (MfpA) mediated regulation of gyrase, and the bacterial cell wall have been also implicated in fluoroquinone resistance in Mtb [34].

Ethionamide

These are antitubacular drugs that are structurally like isonaizid, in their normal state, they are inactive chemotherapeutic agents, and however, their metabolisms make them to be pharmacologically active, this activation requires a monooxygenase coded by ethA gene [56]. The mode of action of ethionamide by their inhibitory effect on the synthesis of mycolic acid in M. tuberculosis [57], the mechanism of resistance to ethionamide is largely unclear, however several report has associated the conformational changes in orf1 gene of inhA locus with ethionamide resistant strains [58],

although certain resistant strains have also been found to harbor mutation at etaA / ethA , ethR [59].

Kanamycin, Capreomycin, Amikacin, Viomycin

These are second line antitubacular drugs which inhibit protein synthesis. Viomycin and Capreomycin are cyclic polypeptides, while, amikacin and kanamycin are aminoglycoside antibiotics [60]. Capreomycins and Viomycin are bacteriostatic antibacterial agents with a known mechanism of action of inhibiting translation reactions through biding to the 50S ribosomal subunit [61,56]. The exact mode of acquisition of resistance to Capreomycin and Viomycin is not totally clear; however, cross-resistance between Capreomycin and Viomycin has been reported [59].

[60, 62], reported mutations in tlyA gene plays key role in Viomycin and Capreomycin resistance. Amikacin and Kanamycin alters the 16S rRNA configuration, thus inhibiting protein synthesis, amikacin and kanamycin resistance are associated with mutational alterations in the rrs gene [63-66] conformational changes in the aminoglycoside acetyltransferase gene (eis), also contribute to Kanamycin resistance [58].

Conclusion

Drug resistance in Mycobacterium tuberculosis is not a product of a single homogeneous genetic unit. Rather it is as a result of frequent mutation in various genes which encode for resistance to antibiotics. Also, the slow metabolism during a prolonged dormant stage greatly enhances it resistance to drug, the waxy impermeable cell wall with the presence of numerous efflux pump are essential for withstanding the potency of antibiotics. Having an adequate knowledge on the molecular mechanisms of drug resistance in M. tuberculosis may be helpful in exploring new targets for drug development.

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