DprE1 and MmpL3

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DprE1 is an enzyme that works in concert with DprE2 to synthesize the unique arabinose precursor for lipoarabinomannan and arabinogalactan, essential building blocks of the mycobacterial cell-wall [1]. To date, more than 15 pharmacophores were found to inhibit DprE1 activity. MmpL3 is the only Mtb transporter of trehalose monomycolate, required for the formation of the mycolic acid layer of the cell wall [2], and has been found to be affected by several molecules. Recently, direct inhibition of MmpL3 by BM212, the first compound found to hit MmpL3, was shown using spheroplasts [2], while the dissipation of proton motive force is the proposed mechanism for the other molecules [3].

Keywords: mycobacteria ; tuberculosis ; multi-drug resistance ; drug discovery ; promiscuous targets

1. Introduction

One of the biggest problems in fighting *Mycobacterium tuberculosis* (Mtb), the etiologic agent of tuberculosis (TB), is the development and spread of multi-drug resistant strains [4]. Therefore, there is an urgent need to identify novel druggable targets for the development of more efficient anti-TB agents [5][6]. In this context, the medicinal chemistry efforts made in the last years led to the discovery of new antimycobacterial compounds, and the identification of novel targets [6][7][8].

High-throughput screenings (HTS), based on Mtb whole cells, were developed to identify hit compounds with potent inhibitory activity, and consequently, new targets emerged. The HTS had the merit of fueling the scarce TB drug development pipeline, since many molecules under preclinical and clinical development came out from this approach. Indeed, the first new TB drug, Bedaquiline, approved by the Food and Drug Administration (FDA) since 1971, was discovered in a whole-cell HTS campaign [9], and the same origin had both Delamanid [10] and Pretomanid [11], other two drugs recently approved for multidrug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) treatment [12][13].

Curiously, an unforeseen outcome of many phenotypic screens against Mtb is the finding that the same targets have been frequently found in many different screening assays, despite the use of different compound libraries [14]. These targets were named “promiscuous” targets, for their nonspecific susceptibility to being inhibited by different scaffolds [5][15]. Although this term was initially given with a negative connotation, the value of these potential pharmacological targets has been reevaluated. Indeed, the high vulnerability of these essential targets, reflecting the biggest vulnerabilities of Mtb, can provide new opportunities to be explored for the development of TB drugs [15]. Among them, there are DprE1, MmpL3, QcrB and Psk13 [6][16]. It is noteworthy that several research groups independently identified these promiscuous targets. These proteins are embedded in pathways that have key roles for Mtb growth and survival under the screening conditions, but even more important during infection. Their essentiality represents an Mtb Achilles' heel that is important to exploit for the development of new effective drug regimens able to shorten the TB treatment. Interestingly, all these promiscuous targets are localized within the cell envelope, emphasizing that the cell wall still represents a fruitful source of drug targets.

2. The First Discovery of DprE1 and MmpL3 as Drug Target

Dr. Vadim Makarov and collaborators, including our Laboratory, published in 2009 the first report in which DprE1 was described as the target of 1,3-benzothiazin-4-ones (BTZ), a new class of agents endowed with antimycobacterial activity [17]. To find the BTZ target, two genetic approaches were performed in parallel using both a *Mycobacterium smegmatis* cosmid library for resistance to BTZ, and the selection and characterization of *M. smegmatis*, *Mycobacterium bovis* BCG and *M. tuberculosis* spontaneous resistant mutants. Both methods revealed that dprE1 gene was responsible for BTZ resistance. It is noteworthy that all the mutants carried mutations in the same codon of dprE1, leading to the replacement of Cys387 with a Ser or Gly residue [17]. DprE1 was known to be involved in the arabinogalactan biosynthesis, a key precursor required for the synthesis of the cell-wall [1][18], and it was demonstrated that its inhibition abolishes the formation of decaprenylphosphoryl arabinose (DPA), thus provoking cell lysis and bacterial death [17]. Later, new

antimycobacterial compounds targeting DprE1 were identified by independent whole cell-based screens, like dinitrobenzamides (DNB) and bromoquinoxaline (VI-9376) [19][20], and afterward, numerous DprE1 inhibitors have come out since that groundbreaking report in 2009.

MmpL3 was firstly identified in our laboratory as the cellular target of BM212, the hit compound of pyrrole-derivative class of antimycobacterial agents by screening the *M. smegmatis* cosmid library for BM212 resistance, as well as the selection and characterization of spontaneous resistant mutants [21]. Immediately after this first publication, MmpL3 was demonstrated to be the target of SQ109, an antitubercular in clinical trials, for which the mechanism of action was still unclear, and adamantyl urea AU1235, a compound displaying potent bactericidal activity against Mtb [22][23]. Genetic and biochemical studies indicated a clear effect of inhibiting MmpL3 on the translocation of trehalose monomycolate (TMM) in Mtb, thus abolishing the trehalose dimycolate (TDM) formation and mycolic acid transfer onto arabinogalactan [22][23]. Subsequently, several papers have reported the discovery of new MmpL3 inhibitors.

3. DprE1: The Hot TB Target of the Moment

DprE1 (Decaprenylphosphoryl-β-d-ribofuranose 2-oxidoreductase, EC 1.1.98.3), is involved along with DprE2 (Decaprenylphosphoryl-2-keto-β-d-erythro-pentose reductase, EC 1.1.1.333), in the two-step epimerization of decaprenylphosphoribose (DPR) to decaprenylphosphoarabinose (DPA), a key arabinosyl donor essential for the biosynthesis of cell-wall arabinan polymers [24]. The conversion of DPR to DPA is a two steps reaction, in which the product of DprE1 decaprenyl-phospho-2′-keto-d-arabinose must be reduced to finalize the epimerization of the substrate. This second step is catalyzed by the enzyme DprE2 (decaprenylphosphoryl-2-keto-β-d-erythro-pentose reductase) [1]. As DprE1, also DprE2 is essential for mycobacterial growth [25]. Moreover, the two enzymes have been supposed to strongly interact, and sometimes they were considered two subunits of a single enzyme named decaprenylphosphoryl-β-d-ribofuranose 2-epimerase.

Currently, at least 11 different scaffolds have been reported as effective DprE1 inhibitors, either covalent and noncovalent, showing different efficacy in vitro, ex vivo and in vivo [26]. It is noteworthy that the majority of these compounds have been identified through phenotypic screening, then subjected to optimization processes.

The first DprE1 inhibitors identified is the BTZ043, belonging to the class of the benzothiazinones [17], and extremely potent against *M. tuberculosis* with an MIC of 1 ng/ml (2.3 nM) [17]. SAR study of piperazine benzothiazinone derivatives afforded the PBTZ-169 [27], with improved *in -vitro* and *in-vivo* potency, which is currently in clinical trial under the name of macozinone [28].

The high vulnerability of DprE1 as an antitubercular drug target was confirmed by the number of screening campaigns that identified effective non-covalent inhibitors, including thiadiazoles [29], carboxy-quinoxalines [30], aminoquinolones [31], pyrazolopyridones [32], and azaindoles [33][34]. The latter are the most promising drug candidates among the non-covalent DprE1 inhibitors. Currently, the azaindole TBA-7371 has started phase 1 clinical trial [35], further confirming the great potential of DprE1 inhibitors in anti-tubercular drug discovery.

4. Mmpl3 Transporter: The Other Hot TB Target of the Moment

MmpL3 is a membrane protein, member of the resistance-nodulation-cell division (RND) superfamily of transporters. In mycobacteria, MmpL transporters have specialized in the export of several lipids and glycolipids across the plasma membrane to the cell surface, namely, trehalose monomycolates (TMM), di- and poly-acyltrehaloses, sulfolipids, phthiocerol dimycocerosates, monomycolyldiacylglycerol, glycopeptidolipids and mycobactins [23][36][37][38][39][40]. Genetic studies with transposon mutant libraries and the inability to knock-out the gene using different strategies suggested that MmpL3 was essential for the survival of M. tuberculosis [23][41]. An alternative strategy, utilizing knock-down strains both in vitro and in vivo had confirmed that MmpL3 is indeed essential for survival. Silencing of MmpL3 in mice, both during the acute or persistence phase of infection, led to a complete clearance of bacteria from lungs and spleens. These studies not only reinforce the idea of MmpL3 as an attractive drug target but also the potential of MmpL3 inhibitors to shorten TB treatment [42].

In recent years, a diversity of scaffolds have been reported to inhibit MmpL3: SQ109 (diamine), DA5 (SQ109 related compound), BM212 (diarylpyrrole), Au1235 (adamantyl urea), C215 (benzimidazole), NITD-349 (indolecarboxamides), THP P (tetrathydropyrazolo pyrimidine), Spiro (N-benzyl-6′,7′-dihydrospiro[piperidine-4,4′-thieno[3,2-c]pyrans]), PIPD1 (piperidinol), E11 (acetamide) and HC2091 (carboxamide) [21][22][23][43][44][45][46][47][48].

These findings will lead to a new era in MmpL3 inhibitor drug discovery since structure-guided molecules can now be designed with better anti-tubercular efficacy and pharmacokinetic/pharmacodynamic (PK/PD) properties.

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