•综述•

结核分枝杆菌生物素合成途径是抗结核菌药物开发的新靶点

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摘要: 结核分枝杆菌 (*Mycobacterium tuberculosis*) 感染导致的结核病仍然是全球主要的公共卫生难题。生物素即维生素H是脂肪酸生物合成、糖异生及氨基酸代谢等途径所需的辅因子。结核分枝杆菌无法从外界吸收生物素, 生物素的合成是分枝杆菌生物素的唯一来源。不同于经典的BioC-BioH、BioI-BioW途径及非经典的BioZ途径, 结核分枝杆菌早期阶段通过“BioC-BioH(2)”途径合成生物素。本综述重点总结结核分枝杆菌独特的生物素合成途径及其关键基因, 尤其是该途径及生物素依赖性羧化酶对结核病一二线药物的响应, 以及靶向生物素合成的抑制剂和天然产物。

关键词: 结核分枝杆菌; 生物素; 生物素依赖性羧化酶; 生物素合成; 抗结核一二线药物

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The biotin synthesis pathway in *Mycobacteria tuberculosis* is a new target for the development of anti-tuberculosis drugs

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Abstract: *Mycobacterium tuberculosis*, responsible for tuberculosis (TB), remains a major health problem worldwide and is one of the infectious diseases causing increased morbidity and mortality worldwide. Biotin, namely vitamin H, is an important cofactor necessary for fatty acid biosynthesis, gluconeogenesis and amino acid metabolism in organisms including *Mycobacterium tuberculosis*. Due to its inability to ingestion biotin from outside, *Mycobacterium tuberculosis* can only obtain biotin through biotin biosynthesis. Different from the classical BioC-BioH, BioI-BioW and non-classical BioZ pathways, *Mycobacterium tuberculosis* synthesized biotin by "BioC-BioH(2)" pathway in the early stage. This review focuses on the unique biotin synthesis pathway of *Mycobacterium tuberculosis* and its key genes, especially the response of this pathway and biotin-dependent carboxylase to tuberculosis first-and second-line drugs, as well as inhibitors and natural products targeting biotin synthesis.

Key words: *Mycobacterium tuberculosis*; biotin; biotin-dependent carboxylase; biosynthesis; first-line and second-line anti-tuberculosis drug

结核分枝杆菌感染引起的结核病是第二大传染病杀手, 也是单一感染源导致死亡的主要原因, 其死亡率甚至高于艾滋病。世界卫生组织发布的《2022年全球结核病报告》中指出, 耐药结核仍然是全球公共卫生危机, 在发现和治疗方面存在着一定的差距。迄今为止, 中国仍是30个结核病高负担国家之一, 且仅次于印度和印度尼西亚, 因此结核病的研究刻不容缓[1]。

生物素, 即维生素H或维生素B7, 又称为辅酶R, 化学名称是 (3a*S*,4*S*,6a*R*)-六氢-2-氧代-1-噻吩并[3,4-*d*]咪唑-4-戊酸, 是结核分枝杆菌生长和致病性所必需的。1901年生物素发现时被称为“Bios”, 人们认为其

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是一种在酵母的基本培养基中的未知微生物生长因子[2]。其分子式 ($C_{10}H_{16}N_2O_3S$) 和结构式分别于1941年和1942年被 du Vignaued 等[3-5]所确定。生物素由一个咪唑环、一个四氢噻吩环和一个正戊酸侧链构成, 分子量为244.31 g·mol^{-1}, 通常生物素十分稳定, 不易被酸碱所破坏。且生物素含有3个手性碳原子, 能产生8种可能的立体异构体。然而, 只有天然存在的顺式*d*-生物素具有维生素活性[6]。生物素是羧化酶的辅酶, 参与脂肪酸生物合成、糖异生及氨基酸代谢等过程中的羧化、脱羧和反羧化作用[7]。生物素缺乏可能会导致细胞增殖速度降低, 使生物免疫功能受损, 甚至能直接影响基因的表达[8]。所有细胞都需要生物素, 但哺乳动物自身并不能合成, 只能通过饮食和肠道微生物的作用获得生物素[9]。人体内如果缺乏生物素, 可能会引发癫痫、皮炎、脱发、智力低下、代谢性酸中毒、有机尿酸症及胎儿畸形等[10]。

分枝杆菌细胞包被是一种具有独特脂质的选择通透性屏障, 赋予结核分枝杆菌耐药性, 并保护其免受宿主免疫系统的影响[11]。结核分枝杆菌具有250多个脂质代谢相关基因, 而在大肠埃希菌中只有50个基因, 这表明了分枝杆菌脂质代谢的重要性。潜伏期的结核分枝杆菌依赖于脂肪酸生物合成途径, 通过对抗潜伏性结核分枝杆菌的药物筛选, 部分脂质生物合成酶(如三酰甘油合酶1) 可以作为治疗潜伏性结核分枝杆菌感染药物的靶点[12]。分枝杆菌脂质都是以丙二酰辅酶A为构建模块, 这些构建模块又由酰基辅酶A羧化酶 (acetyl-CoA carboxylase, ACC) 组成。每个ACC都依赖于辅因子生物素进行翻译后修饰, 才能变得有活性[13]。因此, 阻断从头生物素的生物合成或生物素化有可能抑制分枝杆菌中所有的脂质生物合成[14-16]。生物素合成缺陷会严重阻碍结核分枝杆菌的生长, 降低毒力。结核分枝杆菌缺乏转运外源生物素的高亲和力蛋白, 只能通过自身合成生物素。宿主体内的生物素能以非生理浓度扩散到分枝杆菌中, 但不能通过高亲和力的转运蛋白输入[14,17]。综上, 生物素的生物合成对结核分枝杆菌来说是必不可少的, 将是新药物研发的重点之一。

1 分枝杆菌生物素合成途径

生物素合成主要分为两大步, 形成前体庚二酸单酰CoA, 和通过庚二酸单酰CoA形成生物素 (图1A)[14]。大肠埃希菌、枯草芽孢杆菌和球状芽孢杆菌等细菌中生物素的合成途径研究较多, 许多微生物的生物素合成基因形成操纵子。生物素的合成途径大致分为经典的BioC-BioH途径、BioI-BioW途径及非经典的BioZ途径。大肠埃希菌生物素的合成途径为BioC-BiH途径, 参与生物素合成的基因包括*bio*B、*bio*F、*bio*C、*bio*D、*bio*A和*bio*H。枯草芽孢杆菌生物素合成途径为BioI-BioW途径, 主要参与生物素合成的基因包括*bio*I、*bio*B、*bio*D、*bio*F、*bio*A和*bio*W[18]。*α*-变形细菌有独特的受到“BirA-BioR”二元系统负调控的*bio*BFDA/Z基因簇, 即BioZ途径[19]。

分枝杆菌生物素的合成完全依赖于从头合成, 结核分枝杆菌存在BioC和BioH同系物, 推测结核分枝杆菌采用与大肠埃希菌相同的途径合成生物素[14,20,21]。具体过程如下, 首先丙二酸单酰CoA被BioC (*O*-甲基转移酶) 甲基化, 产生甲酯[22,23], 其作用是延长脂肪酸合成途径的烷基链, 产生庚烯酰CoA甲基酯; 庚烯酰CoA甲基酯随后被BioH (羧酸酯酶) 水解, 生成庚烯酰单酰CoA, 用于装配生物素[24]。分枝杆菌生物素合成的第一阶段BioH有3种同工酶, 为分枝杆菌生物素合成的早期阶段建立了“BioC-BioH(n)”途径 (n, 结核分枝杆菌为2, 非结核分枝杆菌为3)[25]。装配生物素可分为4个反应, 且这4个反应在微生物和植物中高度保守, 首先庚二酸单酰CoA和*L*-丙氨酸在5-磷酸吡哆醛依赖的酶BioF (7-酮-8-氨基壬酸合酶, KAPAS) 作用下生成7-酮-8-氨基戊酸 (KAPA)[26]; 然后BioA催化KAPA与*S*-腺苷甲硫氨酸 (7,8-二氨基壬酸合酶, DAPAS) 生成7,8-二氨基戊酸 (DAPA)[9]; 随后DAPA在BioD (脱硫生物素合成酶, DTBS) 的催化下消耗ATP生成脱硫生物素 (DTB)[9]; 最后BioB (生物素合成酶) 将DTB转化为生物素[14,27]。

2 生物素合成基因对抗结核药物的响应

生物素对结核分枝杆菌生长和致病性十分重要, 在结核分枝杆菌各个感染阶段起关键作用[13,28-30]。生物素合成途径是新抗生素研发的潜在靶标。结核分枝杆菌参与生物素合成的基因在染色体上的排列如图1B。其中*bio*A、*bio*F1、*bio*D以及功能未知的*rv1571*组成保守的操纵子[31], *bio*C基因位于该操纵子的上游约1.7 Mbp处; 该操纵子下游约2 kbp处是*bio*B基因, 这2 kbp被认为是噬菌体phiRv1的插入序列[32]; *bio*H1基因和*bio*H2基因分别位于*bio*B基因下游1.7 Mbp处以及*bio*C基因0.7 Mbp处, *bio*H1基因和*bio*H2基因与*bio*C有相同的作用, 主要负责庚二酸的合成[33]。

异烟肼 (isoniazid) 是结核病治疗的一线药物, 抑制结核分枝杆菌细胞壁枝菌酸的合成[34-36]。异烟肼处理下*bio*A基因 (*Rv1568*) 表达上调2.5倍 (处理浓度见图1C)[37]。乙硫异烟胺 (ethionamide) 为异烟酸的衍生物, 是结核病治疗中的二线药物, 和异烟肼一样都抑制细胞壁枝菌酸的合成[38-40]。*bio*A基因在其处理下上调4.1倍。PA824 (pretomanid) 于2019年被美国FAD批

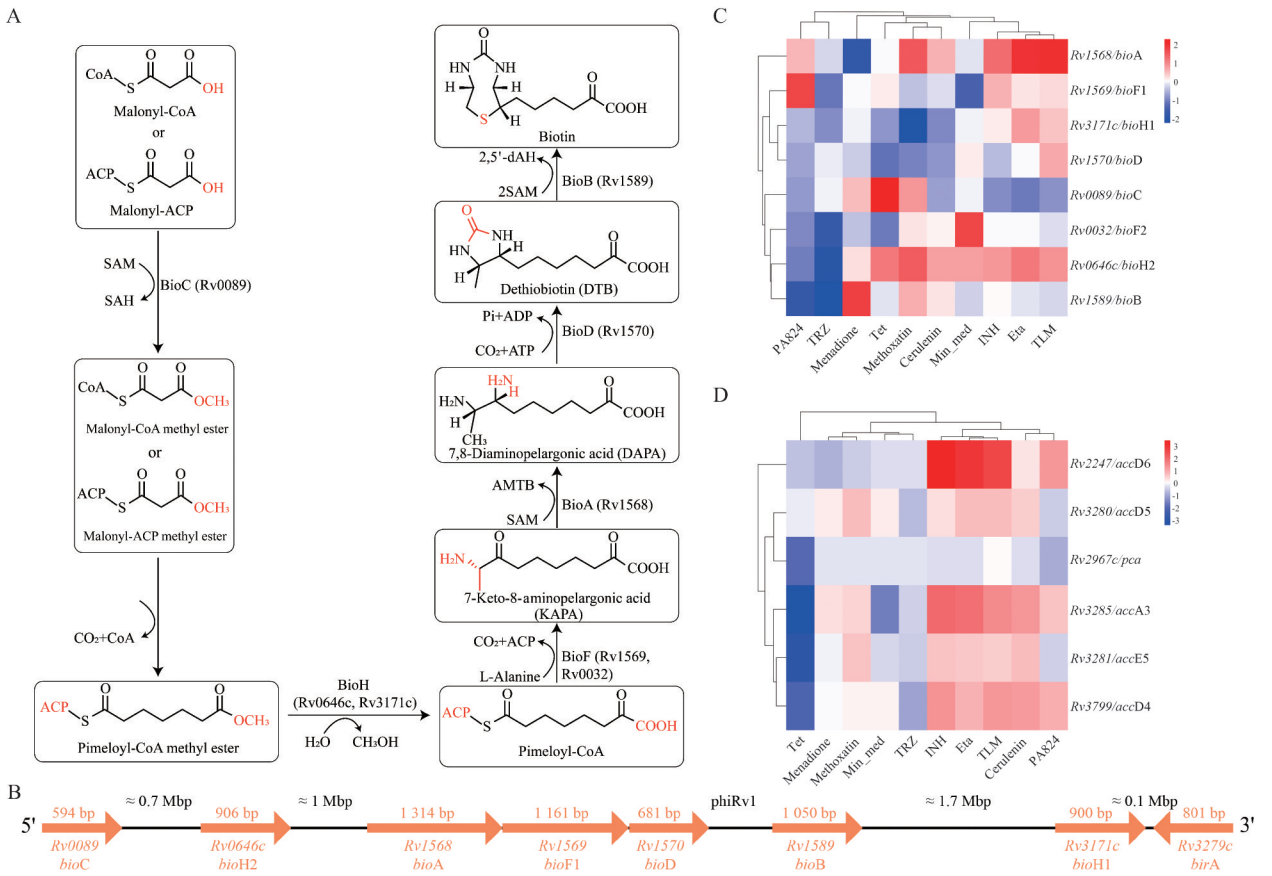

Figure 1 Biotin metabolism pathway of *Mycobacterium tuberculosis* and the response of its coding gene to drug therapy. A: Synthetic pathway of biotin in *Mycobacterium tuberculosis*; B: Genetic map of biotin biosynthetic pathway genes in the genome of *Mycobacterium tuberculosis* (not to scale); C: Response of gene expression of biotin synthetic pathway to first- and second-line drugs treatment of tuberculosis. Tet: 5–10 μg·mL^{-1} tetracycline; Eta: 12–40 μg·mL^{-1} ethiopiamine; TLM: 0.1–0.2 mg·mL^{-1} thiolactomycin; Methoxatin: 10–20 μg·mL^{-1} pyrroloquinoline quinone; Cerulenin: 0.32–5 μg·mL^{-1} pale blue colistin; INH: 0.2–0.4 μg·mL^{-1} isoniazid; Min_med: Minimal medium with succinate as sole carbon source as compared to growth in 7H9/ADC/Tween/glycerol; PA824: 0.2–2 μg·mL^{-1} PA824; TRZ: 10–25 μg·mL^{-1} thioridazine; Menadione: 6–10 μg·mL^{-1} naphthoquinone, or vitamin K3; D: Expression of gene encoding biotin-dependent carboxylase in first- and second-line drugs treatment of tuberculosis

准用于治疗广泛耐药结核病, 具有双重作用机制, 能够抑制生长状态和持留状态的结核分枝杆菌枝菌酸和蛋白的合成[41-45]。PA824 上调 *bio*F1 基因 (*Rv1569*) 3.5 倍。硫乳霉素 (thiolactomycin)[46]和浅蓝菌素 (cerulenin) 是通过对脂肪酸和枝菌酸合成的抑制来达到抗菌活性的天然物质[47-50]。在硫乳霉素的处理下, *bio*A 基因上调 4.3 倍, 在浅蓝菌素的处理下该基因表达差异不明显。*bio*C 基因 (*Rv0089*) 在四环素 (tetracycline) 的处理下表达上调 5 倍。综上, 靶向脂肪酸或枝菌酸合成的药物影响 *bio*A 基因表达, 而靶向细菌核糖体 30S 亚基的药物 (如四环素) 影响 *bio*C 基因表达[51,52]。靶向细胞壁的药物抑制细胞壁的合成导致细胞壁受损, 推测细菌感应到该信号后, 促进合成细胞壁相关基因的表达去修复细胞壁, 而生物素作为细胞壁合成酶的辅因子, 也将大量增加[14]。*bio*A 基因是生物素合成途径的关键基因 (结核分枝杆菌缺失 *bio*A 导致细菌死亡), 其表达变化最为明显[31]。

3 生物素依赖性羧化酶

生物素依赖性羧化酶广泛存在于生命领域 (古菌、细菌和真菌) 中, 十分古老且多样。该家族由一组利用共价结合的辅基生物素作为辅因子的酶组成, 包括酰基辅酶 A 羧化酶、丙酮酸羧化酶 (pyruvate carboxylase, PC)、丙酰辅酶 A 羧化酶 (propionyl-CoA carboxylase, PCC) 和尿素羧化酶 (urea carboxylase, UC) 等成员[53]。它们含有生物素羧化酶 (biotin carboxylase, BC)、羧基转移酶 (carboxyltransferase, CT) 和生物素–羧基载体蛋白 (biotin carboxyl carrier protein, BCCP) 3 种成分[54], 该家族具有类似的催化机制, 即将 CO_2 分子固定在生物素羧基载体肽的生物素羧化酶结构域上, 以及将 CO_2 部分转移到每种酶的特

定底物上的羧基转移酶结构域[55]。

生物素依赖性羧化酶在结核分枝杆菌的致病机制和耐药性方面也十分重要。结核分枝杆菌中有两类生物素依赖性羧化酶, 分别是ACCs和PC。ACCs中的乙酰辅酶A羧化酶是脂肪酸合成第一步所需要的酶, 为分枝杆菌中的脂肪酸、枝菌酸和复合脂质生物合成提供构建模块, 也被视为开发新抗结核药物的潜在靶点[56]。结核分枝杆菌ACC具有BC/BCCP-α亚基及CT-β亚基, 分别由*acc*A1～*acc*A3和*acc*D1～*acc*D6编码 (表1), 其他细菌通常只有1或2个基因编码ACC[57]。编码结核分枝杆菌的6种CT基因中, 只有*acc*D4、*acc*D5和*acc*D6对分枝杆菌的生存必不可少, 非必需的*acc*D1/2/3在整个结核分枝杆菌的生长阶段的表达水平没有显著变化, 而*acc*D4/5/6在指数生长阶段的表达水平较高[58,59]。*acc*D6和*acc*A3将乙酰辅酶A作为底物形成一种具有活性的ACC全酶, 在乙硫异烟胺、异烟肼和硫乳霉素的处理下上调, 且*acc*D6的上调倍数高于*acc*A3 (图1D)[37]。ACCβ6亚基能够向FAS II复合物提供丙二酰辅酶A影响枝菌酸的合成, 推测在枝菌酸合成受阻的情况下, 结核分枝杆菌通过某种代偿的机制上调*acc*D6和*acc*A3的表达, 增强FAS II复合物的活性, 促进枝菌酸的合成[59]。

4 调控生物素合成的转录因子

调控生物素合成的转录因子在不同菌中存在多样性, 有3种主要的转录因子BirA[60]、BioR[61]和BioQ[62]。其中BirA的研究最多, 它由生物素蛋白连接酶 (biotin protein ligase, BPL) 和DNA结合结构域 (DNA binding domain, DBD) 两部分组成, 因此它既可作为转录因子用于调控生物素合成, 又可以作为连接酶催化生物素依赖性酶的生物素化[63,64]。根据BirA是否具有DBD结构, 可分为N-末端缺失DBD的I型BirA以及具有DBD结构的II型BirA[63]。I型BirA只有BPL, 无法调控生物素合成相关基因。α-变形菌 (*Alphaproteobacteria*) 和放线菌 (*Actinobacteria*) 都为I型BirA, 但分别由GntR家族的BioR以及TetR家族的BioQ接替BirA的DBD功能, 根据细胞生物素水平降低生物素响应基因的转录[61,65-67]。

耻垢分枝杆菌 (*M. smegmatis*) BioQ结合*bio*FD、*bio*B的启动子区域以及其自身编码基因, 通过生物素配体非依赖性过程抑制生物素操纵子的转录[25,66,68]。BioQ需要47位的赖氨酸乙酰化来激活蛋白质与抑制生物素响应基因所需的DNA操纵基因序列的结合[67,68]。BioQ通过识别一个13 bp保守的回文反向重复序列 (5'-TGAACnnnGTTCA-3') 去抑制*bio*F、*bio*D和*bio*B基因的转录, 其中对*bio*B抑制最强, 对*bio*D和*bio*F的抑制弱[68]。尽管*bio*F、*bio*D和*bio*B都受到BioQ的阻遏调控, 但*bio*H同源异构体和*bio*C都没有典型的BioQ结合位点。在脓肿分枝杆菌 (*M. abcessus*) 等6种分枝杆菌中也存在*bio*Q, 但在结核分枝杆菌中并未发现该基因, 这一定程度上与结核分枝杆菌中缺少一种BioH同工酶的情况相符。这种不受BioQ调控的优势可能确保足够多的酶参与分枝杆菌生物素合成的第一阶段, 以及提供生物素代谢以外的活性。结核分枝杆菌在致病过程中需要更多的生物素, BioQ的缺乏可能有利于结核分枝杆菌中生物素的产生, 吞噬体逃逸, 以及在宿主体内存活[25]。

5 靶向生物素合成的化合物

哺乳动物自身不能合成生物素, 因此靶向生物素合成的化合物能在不改变宿主细胞机制的情况下提供选择性治疗[69]。在大多数研究中, 酶抑制剂都是根据已知底物、反应中间体或产物的化学结构来设计的。虽然大多数研究都将这些化合物的体外特性描述为酶抑制剂, 但只有少数的抗菌活性被报道。

BioA是依赖于5'-磷酸吡哆醛 (PLP) 的转氨酶, 不仅在生物素合成中发挥关键作用, 而且在动力学和结构表征方面对其机制有广泛的见解, 是抗结核药物开发的新靶标。氨基丁酸霉素 (amiclenomycin, ACM) 是从链霉菌中分离出来的抑制BioA破坏结核分枝杆

Table 1 Biotin-dependent carboxylase component

Name	Locus	Product
*acc*A1	*Rv2501c*	Acetyl-/propionyl-CoA carboxylase alpha chain (alpha subunit) AccA1
*acc*A2	*Rv0973c*	Acetyl-/propionyl-CoA carboxylase alpha chain (alpha subunit) AccA2
*acc*A3	*Rv3285*	Acetyl-/propionyl-CoA carboxylase alpha chain (alpha subunit) AccA3
*acc*D1	*Rv2502c*	Acetyl-/propionyl-CoA carboxylase (beta subunit) AccD1
*acc*D2	*Rv0974c*	Acetyl-/propionyl-CoA carboxylase (beta subunit) AccD2
*acc*D3	*Rv0904c*	Acetyl-CoA carboxylase carboxyl transferase (subunit beta) AccD3
*acc*D4	*Rv3799c*	Propionyl-CoA carboxylase beta chain 4 AccD4 (pccase)
*acc*D5	*Rv3280*	Propionyl-CoA carboxylase beta chain 5 AccD5 (pccase)
*acc*D6	*Rv2247*	Acetyl-/propionyl-CoA carboxylase (beta subunit) AccD6
*acc*E	*Rv3281*	Acetyl-/propionyl-CoA carboxylase (epsilon chain)
pca	*Rv2967c*	Pyruvate carboxylase

菌中生物素代谢的化合物, 具有显著的选择性抗分枝杆菌活性 (耻垢分枝杆菌 MIC=12.5 μg·mL^{-1}), 但也具有不稳定性和高极性, 无法在体内使用[70,71]。ACM 的氨基醇类似物 (ACM-OH) 也是 BioA 的抑制剂。在结构上, ACM 和 ACM-OH 都具有对称的顺式-1,4-环己二烯环, 能够形成外部醛胺中间体, 通过 α-脱质子化共价连接到辅因子上, 导致 BioA 的 PLP 辅因子不可逆失活, 该过程也称为芳构化。ACM 和 ACM-OH 固有的化学稳定性较差, 这导致快速芳构化为非活性苯胺衍生物。为了增加其稳定性, 在 ACM 的基础上, 利用迈克尔加成反应 (Michael addition reaction), 衍生出 4 个抑制剂 M-1/2/3/4[72]。利用一种全细胞高通量筛选方法, 筛选出 *N*-芳基、*N*'-苯甲酰哌嗪作为 BioA 抑制剂支架, 进一步优化结构, 得到化合物 **36**[69,73]。通过对 BioA 的活性位点进行虚拟筛选, 7 种化合物对 BioA 酶活起抑制作用。A65 是 7 种化合物中最有效的, 能够抑制病原体的生长与 BioA 活性, 并具有类似药物的特性[74]。然而, 这些化合物具有许多化学惰性 (如反应性官能团和泛分析干扰化合物) 的官能团, 因此不应该被认为是“类药物”[75]。

MtBPL (由 *bir*A 编码) 和 BioA 一样都是分枝杆菌生存的关键, 负责生物素翻译后附着到生物素依赖酶活性位点的特定赖氨酸残基上, 催化脂肪酸和丙酮酸辅酶 A 羧化酶合成的第一步[29]。设计 BPL 抑制剂的主要策略是通过模拟 Bio-AMP 中间体的分子来破坏蛋白质的生物素化。对 8 000 种海洋天然产物进行了包括基于结构的虚拟筛选、分子对接和分子动力学模拟分析在内的全面的计算机模拟, 找到了 CMNPD10112 和 CMNPD10113 两种能够以高亲和力持续结合 BioA 和 MtBPL, 从而抑制生物素合成的化合物[76]。Bio-AMS (5'-[*N*-(*d*-生物素酰) 氨磺酰氨基]-5'-脱氧腺苷) 化学抑制 MtBPL, 从而杀死结核分枝杆菌[13]。

BioF 也是一种 PLP 依赖型酶, 具有较高的底物立体特异性。利用 *D*-丙氨酸反应的产物 *D*-KAPA 同时抑制 BioF 和 BioA[77]。利迪链霉菌中分离的天然产物 α-甲基生物素和 α-甲基去硫生物素可抑制偶发分枝杆菌、耻垢分枝杆菌、鸟分枝杆菌以及草分枝杆菌的 BioB 活性[78]。酸霉素 (actithiazic acid) 通过竞争性抑制 BioB 从而抑制生物素合成, 对结核分枝杆菌菌株有活性 (MIC=0.096～6.2 μmol·L^{-1}), 但对非结核分枝杆菌、革兰阳性和革兰阴性病原体无活性 (MICs > 1 000 μmol·L^{-1})[79,80]。

6 总结与展望

生物素在脂质生物合成中起着至关重要的作用, 生物素及其底物对结核分枝杆菌来说至关重要。生物素合成途径以及生物素依赖性羧化酶的部分编码基因已经明朗, 这些基因对抗结核一二线药物的响应, 表明生物素在结核分枝杆菌耐药机制中发挥重要作用, 为开发靶向分枝杆菌生物素代谢的抗结核药物提供了基础。利用全细胞高通量筛选、表型筛选等手段, 找到了一系列对生物素生物合成和连接酶具有良好抑制活性的化合物, 但都尚未进行临床试验。鉴于细菌在人类巨噬细胞中缺氧和营养缺乏的内环境, 这些化合物的效果应在相似的生长环境中进行验证, 同时还需减轻药物不良反应、增强对结核分枝杆菌的选择性以及避免药物的相互作用等。尽管面临这些挑战, 但开发针对这一途径的抗结核药物仍具有较大的前景。

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