专论与综述

　　分枝杆菌研究具有重大基础科学意义和实践价值。十余年来，全球以结核分枝杆菌为代表的分枝杆菌多组学研究取得了巨大进展。公共数据库至少有 30 种分枝杆菌的全基因组序列，细菌及其感染的宿主细胞、个体的转录组、结构基因组、蛋白质组、代谢组、翻译后修饰等研究不断深入。组学研究从描述性逐步深入到机理性认识，不断揭示结核菌重要生理和病理特征如持留和休眠的分子机理。这为认识分枝杆菌系统进化、发现更好的诊断标记、药物靶标和候选疫苗组分提供了基础。

<div style="text-align:right">谢建平</div>

全球分枝杆菌组学研究十年纵览：
以结核分枝杆菌为例

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摘　要：全球分枝杆菌组学研究取得了巨大进展，为进化、诊断、药靶等研究提供了基础。本文结合我们实验室工作从基因组、结构基因组、转录组、蛋白质组、代谢组、激酶组、免疫组、翻译后修饰等方面总结十余年来全球分枝杆菌多组学研究进展，对分枝杆菌生物学认识的深化以及未来研究重点进行展望。

关键词：分枝杆菌，组学，基因组，结构基因组，转录组，蛋白质组，代谢组，激酶组，免疫组

A survey of *Mycobacterium* omics studies over a decade: *M. tuberculosis* as a case study

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Abstract: Great strides of *Mycobacterium* multi-omics have been achieved by the global *Mycobacterium* consortium, which deepened the biology of mycobacterium and provided novel insights into the evolution, better diagnostic markers and drug targets. We extracted relevant data from publically available papers and database to define the progress of *Mycobacterium* omics over the last decade and envision further crucial directions.

Keywords: *Mycobacterium*, Omics, Genome, Structural genome, Transcriptome, Proteome, Metabolome, Kinome, Immunome

分 枝 杆 菌 (*Mycobacterium*) 属 于 放 线 菌 (Actinobacteria)。分枝杆菌研究具有重要的基础科学和应用价值, 对公共卫生、农业、工业、食品和环境等都具有极其重要的意义。结核分枝杆菌(*M. tuberculosis*, 以下简称结核菌)和麻风分枝杆菌(*M. leprae*, 以下简称麻风菌)分别是历史上和至今仍然困扰全球人类的两大公共健康威胁——结核病(Tuberculosis)和麻风病(Leprosy)的致病菌。一些原来不太引人注目的分枝杆菌如鸟分枝杆菌(*M. avium*)也日益威胁免疫功能减退的老龄化人群和 HIV 患者。牛分枝杆菌(*M. bovis*)、副结核分枝杆菌(*M. paratuberculosis*)和海分枝杆菌(*M. marinum*)是畜牧养殖业和水产业的威胁。*M. avium*、*M. kansasii* 和 *M. xenopi* 等耐去垢剂和氯的环境分枝杆菌是院内和自来水管网系统的感染源[1]。分枝杆菌也是甾醇类药物生物转化[2], 植物次生代谢产物前胡素(Decursin)转化为具有抗癌活性的前胡素醇(Decursinol)所需酯酶的来源[3]。分枝杆菌

也是降解环境污染物多环芳香烃的生力军[4]。食源性 *M. paratuberculosis* 感染日益引起关注[5]。研究分枝杆菌及其导致的疾病曾经并正在改变人类医/药学基础科研和公共卫生模式。药物研发临床实验中广泛采用的随机、双盲、对照试验模式[6], 抗生素研发理念的转变[7]都与结核菌感染带来的艰巨挑战密不可分。能够在人骨髓来源(Human bone marrow, BM) CD271(+)/CD45(−)间充质干细胞(Mesenchymal stem cell, MSC)内长期存活并耐药的结核菌[8], 劫持宿主 ERK1/2、MEK 和依赖 p56Lck 激酶调控人外周神经 Schwann 细胞增殖的麻风分枝杆菌[9]等启迪了干细胞、神经细胞发育、分化及其调控研究。免疫学发展与结核菌/结核病紧密相关[10]。从人类和哺乳动物遗传学的角度, 结核/麻风病易感基因的鉴定既是挑战[11−16], 也是学科发展的机遇[17−21]。许多研究工具也在探索解决结核病控制难题中不断更新。例如在组学基础上深化生物学认识不可或缺的结核菌基因突变、缺

失等遗传工程技术[22]。以构建糖肽脂生物合成途径中 *mmpL4b* 基因等位突变效率为指标, 发现 ts-*sac*B、噬菌体、重组工程(Recombineering) 3 个系统中, 重组工程效果最佳(效率为 7%)[23]。

作为非常成功的典型胞内致病菌, 结核菌感染的特征之一是持留(Persistence), 有时甚至持留达几十年。其间涉及致病菌-宿主间不同时空、多尺度相互作用网络。从几分钟到几十年, 涉及分子、细胞、组织、个体和群体。仅细胞就涉及不同类型如巨噬细胞、树突状细胞、不同 T 细胞亚型等。从认识结核菌致病机理和研发新抗生素的角度, 综合抗生素分子作用机理、在宿主体内的药代动力学、分布、以及抗生素对致病菌个体和群体的进化的影响[24], 应用整合系统生物学(Integrative systems biology)的理念非常关键。这在广泛应用的各种组学技术[25]不断产出大数据(Big data), 诸多学科都在发生革命性变革[26-30]的时代, 尤其迫切。本文以结核菌为主, 从微生物学角度综述分枝杆菌组学研究进展, 偶尔涉及宿主相关的研究, 并探索将来研究的重点。

1 分枝杆菌基因组、比较基因组和转录组

1.1 基因组和比较基因组

最早采用基于质粒 pBeloBAC11 的细菌人工染色体(Bacterial artificial chromosome, BAC)对结核菌 H37Rv 进行基因组作图、测序和比较基因组学研究[31]。1998 年实验室强毒株结核菌 H37Rv 的基因组序列公布[32]。截至 2013 年 2 月 11 日, 全球公共数据库至少有 30 种分枝杆菌的全基因组序列, 从基因组缩小、假基因大约一半的专性寄生的麻风菌到基因组较大的腐生、快生型耻垢分枝杆菌两个极端之间的多种分枝杆菌。如 *M. tuberculosis* Erdman (TMC 107; ATCC 35801)[33]、*M. tuberculosis* strain NCGM2209[34]、

M. bovis[35], 非结核分枝杆菌基因组[36]如草分枝杆菌(*M. phlei*)[37]、*M. xenopi*[38]、*M. avium* subspecies paratuberculosis (MAP)[39]、*M. abscessus* strain 47J26[40]、*M. abscessus* subsp. bolletii BD(T)[41]、*M. abscessus* strain M94[42]、*M. massiliense*[43]、*M. abscessus* strain M93[44]和 *M. massiliense* M172[45]。结核菌基因组演化呈现双向性, 一方面增加(基因获得或者增殖), 另一方面减少(缺失)。与非结核分枝杆菌基因组序列比较, 可以发现结核菌特异性的基因插入, 认识结核菌致病机理, 评估新诊断试剂和疫苗。

1.1.1 基因组揭示的分枝杆菌特征: 丰富的脂肪酸代谢基因、特有的 PE/PPE-PGRS 基因家族, 较多的重复序列(*M. leprae* 基因组的 2%为重复 DNA 序列)[46]等。麻风分枝杆菌基因组中编码蛋白质的基因少, 一半为假基因和非编码区。但 43%假基因有转录物[47]。这些转录的假基因在染色体上随机分布, 表达水平随感染阶段而异。用麻风分枝杆菌覆瓦芯片(Tiling microarray, 重叠的 60 聚体探针, 覆盖整个 3.3 Mb 基因组), 也证实了假基因转录物 RNA。虽然这些表达 RNA 的功能未知, 但提出了一个问题: 耗费大量能量产生不活跃 mRNAs 的价值何在? 也提示有多种翻译沉默机制。这为了解假基因形成的分子机理以及来自非编码区的 microRNAs 的功能提供了线索[48]。

1.1.2 比较基因组揭示进化、致病机理和流行病学特征: SNP 等比较基因组标记(Comparative-genome markers, CGMs)可以用来分析系统进化的阶段。CGM 提示结核菌的成簇性(Clonal)强, 检测不到基因水平转移。种群(Population)证据显示枝菌酸合成的关键酶 KasA 在选择压力下形成了 G312S 多态性[49]。先前认为主要分布于结核菌复合群的插入序列 IS6110 也在耻垢分枝杆菌中发现, 提示分枝杆菌基因库(Gene pool)

比预期大[50]。对 55 个公共数据库中的全基因组序列进行包括全局性地理系统进化(Phylogeography)、局部传播链和流行菌株多样性(Transmission chains and circulating strain diversity)、结核病患者成簇异质性(Clonal heterogeneity)、细菌自身进化(Evolutionary)等分析,也发现结核菌具有较大的遗传多样性[51]。寻找临床菌株特有序列是全球比较基因组研究的热点之一[52]。用 230 个同义/沉默单核苷酸多态性 [Synonymous (silent) single nucleotide polymorphisms, sSNPs]分析来自全球的 *M. tuberculosis* 复合群的 432 株菌全基因组序列,发现其毒力、传播性和宿主范围都存在差异[53]。在实验性研究基础上,构建数据库,收集整理基因组差异的复制错误、重复序列增减、重组和转位等基因组差异也很重要[54]。分枝杆菌最近共同祖先(The last common ancestor)和进化路径也是比较基因组研究的兴奋点之一[55]。比较结核菌和麻风分枝杆菌的基因组发现: 基因大规模缺失与假基因化同步进行,导致高度特化的致病菌[55]。比较古基因组学(Paleogenomics)可以追踪传染源[56]。比较 17 000 年前的欧洲野牛样品和 9 000 年前的人类遗骸中的分枝杆菌基因组发现: 结核菌先于牛分枝杆菌和其他相关物种出现,结核菌和麻风菌的最近共同祖先在 3 600 万年分化,结核菌复合群在 4 万年前分化。突破进化瓶颈后的结核菌在突变、重组和自然选择的作用下,快速分化为成功的致病菌[57]。许多强毒性致病菌都缺乏遗传多样性并有性生殖隔离。一般认为种群较纯的结核菌来自遗传漂移。比较 24 个基因组发现包括 VII 型分泌系统等适应所需关键功能区存在多态性,这可能和存在重组区域有关。部分重组区域也存在于 *M. canettii*。总体上,重组区域引入了大量非同义多样性,正选择或受到多样化选择的区域比

如细胞壁组分,基因多态性更明显。导致非同义突变的 SNPs 一般被有效清除,说明净化选择(Purifying selection)的力量非常强大。选择对于同义改变也非常有效,因为 MTBC 偏向 AT 核苷的突变并未被偏好的基因转换代偿。重组和选择对结核菌同义和非同义位置的影响都比较大。但也有观点认为结核菌基因进化研究中要谨慎使用[d(N)/d(S)]数据[58],因为结核菌选择偏爱同义取代。

1.1.3　基于基因组序列的全/部分基因组芯片也是寻找多态性的重要工具,这个方法发现了不少多态性: 俄罗斯临床菌株和实验室参考菌株之间具有插入缺失(Indel)多态性的两个预测膜蛋白可能参与宿主相互作用[60]。系统进化上较古老,与其亲代疫苗菌株最接近的 *M. bovis* BCG Russia 天然丢失 *recA*[61]。西非结核病的主要致病菌 *M. africanum* West African 2 是 *M. tuberculosis* 复合群中古老的株系,毒性较结核菌弱。相对于结核菌和牛分枝杆菌,这个菌株丢失 RD900[62]。海分枝杆菌进化而来的溃疡分枝杆菌(*M. ulcerans*)[59]具有多态性,其基因组还在变小[59]以适应环境[63]。海分枝杆菌和结核菌基因组比较也为洞悉结核菌进化提供了线索[64]。用来自不同实验室的 6 个 H37Rv 菌株比较发现结核菌实验室菌株也在演进(*In vitro evolution*)[65],其中 73 个位点具有多态性,包括插入/缺失、核苷酸替换、多个 IS6110 转座。有 2 株还丧失了合成分枝杆菌表面糖脂——二枝菌酸分枝杆菌蜡醇(Phthiocerol dimycocerosate, PDIM)的能力。它们基因组中的结核菌蜡酸合成酶(Mycocerosic acid synthase)基因发生移码突变。来自拉美的临床分离结核菌菌株中,不计 PPE 和 PG-PGRS 基因,430 个蛋白质至少具有 1 个氨基酸改变,新的 IS6110 位点,基因组缺失 3.6 kb,导致 *dosR* 调控单元(Regulon)基因

Rv1996 和 *Rv1997* 丢失和修饰[66]。临床菌株基因组比较有助于认识结核菌微进化[67], 提供社区结核菌暴发的流行病学信息。牛分枝杆菌 SNP 分析也为结核菌群体遗传学提供了借鉴, 有助于鉴定宿主范围和疾病表型[68]。

1.1.4　基因组研究耐药新机理: 代偿性突变(Compensatory mutation)可以降低耐药菌株的适应成本(Fitness cost)。携带 RNA 聚合酶基因代偿性突变的耐利福平结核菌临床分离株超过 30%, 说明这些代偿性突变确实提高了适应能力, 并有助于耐多药结核菌的全球传播[69]。比较基因组可以揭示结核菌突变率[70]。比较来自猴子感染模型中分别代表活动性、潜伏或者再激活结核病的 22 株结核菌基因组发现: 在相同时间内, 活动性结核病、潜伏和对数期生长的结核菌的突变率相同; 其多态性类型提示体内突变的诱因主要是氧化性 DNA 损伤; 潜伏期的结核菌也在突变。这也提示异烟肼单药治疗潜伏性结核病可能导致异烟肼耐药。

1.1.5　比较基因组研究寻找包括结核菌在内的其他分枝杆菌的分类鉴定和诊断标记、候选疫苗的组分或者药物靶标: 比较牛分枝杆菌(*M. bovis*) 和鸟分枝杆菌 (*M. avium*) 对结核菌素(Tuberculin)迟发型超敏反应的差异, 可以发现特异性和组分清楚的牛结核病诊断抗原[71]。比较 *M. avium* subsp. *avium* 和 *M. avium* subsp. *paratuberculosis* 基因组可寻找后者的诊断标记[72]。预测基因组序列中受到进化压力选择、变化较大的高变(热点)和低变(冷点)单核苷酸变异区(Single-nucleotide variations, SNVs), 并在临床菌株中得到了验证[73]。根据全基因组序列可以分析致病菌传播动力学[74]。生物信息学预测结核菌有 628 个必需基因, 其中 324 个在人体中缺乏相似性。手工去除假定蛋白后, 135 个必需蛋白是人体所缺乏的[75]。比较 27 个棒杆菌科

Corynebacterineae 菌株(18 *Mycobacterium*, 7 *Corynebacterium*, 1 *Nocardia* 和 *Rhodococcus*) 的基因组, 筛选候选诊断标记分子发现: *dna*K 位点的区分效果较现在广泛使用的 *hsp*65 更好, 长度变异较大的 *rpo*BC 基因间区域可以鉴定分枝杆菌种。用 *rpo*BC、*dna*K 和 *hsp*65 构成的多位点可以对分枝杆菌进行种的精确鉴定[76]。

1.1.6　基因组揭示结核菌的其他重要分子: 比如在细菌中分布广泛, 在进化、致病和胁迫应答中作用巨大, 可能作为广谱抗生素的毒素-抗毒素系统(Toxin-antitoxin, TA)[77–78], 特定转录因子在全基因组水平的调控特征[79]。结核菌全基因组中有 67 个 SigF 结合位点和 16 个表达被 SigF 启动子控制的基因。这些位点包括 sigF, 脂类、中间代谢和毒力基因, 一个转录调控因子 Rv2884、小 RNA F6。SigF 可能参与反义转录, Rv1358 和 Rv1870c 中, 依赖 SigF 的启动子位于预测的可读框内部。11 个基因的上游具有保守序列 GGTTT-N[(15-17)]-GGGTA。毒力因子 HbhA、非编码 RNAs 和反义转录物都受其调控。

1.1.7　基因组中调控小 RNA 日益受到关注[80]: RNA-Seq 发现结核菌基因组也存在大量非编码 RNA, 包括较长的 5′和 3′不译区、反义转录物、基因间小 RNA。部分 RNA 的丰度在稳定期增加, 在慢性感染小鼠的肺部积累量特别大, 说明可能与毒力相关。其转录后调控可能与结核菌极强的适应能力有关[81]。另一项 RNA-Seq 和生物信息学分析发现结核菌有 1 948 个候选 sRNAs[80]。指数期 *M. tuberculosis* H37Rv 发现 1 373 个 sRNA[82], 其中 258 个(19%) (22 个基因间 sRNAs, 84 个 sRNAs 位于 5′/3′ UTRs, 152 个反义 sRNAs)与芯片检测结果吻合。分析启动子和终止子序列发现 121 个 sRNAs (47%)具有 sigA 启动子保守序列, 22 个 sRNAs (8.5%)具有终止子保守序列。35 个 sRNAs (14%)兼具启动

子和终止子保守序列。反义 sRNAs 优先调控膜结合蛋白的转录。顺式编码的 sRNAs (*cis*-encoded sRNAs)多调控膜上氢运输和双组分系统编码基因[82]。

1.1.8 基因组定期再注释是确保信息准确的关键[83]: 有研究发现结核菌 5%−10%可读框的翻译起始位点预测和实验不符,269 需要重新注释[84]。蛋白质组或者转录组也是基因组再注释的重要手段(将在后面举例说明)。

1.2 转录组

芯片和 RT-qPCR 比较牛分枝杆菌减毒和强毒株的转录组,鉴定与宿主相互作用的分子发现: mce4D、Mb2607/Mb2608 和 Mb3706c 在强毒株中上调,而 alkB、Mb3277c 和 Mb1077c 在弱毒株中上调,在巨噬细胞内繁殖的结核菌也是如此[85]。分析耐多药结核病患者的肺切除样品中结核菌基因表达,并利用多种方法(Rosetta stone、系统进化谱、保守基因邻接法、操纵子计算方法)建立蛋白质连锁图,发现在肺部活跃转录的结核菌基因主要是加固自身防御,积极逃避宿主免疫应答[86]。

药物诱导转录组和结核菌特殊生理状体的转录组: 药物或者化合物诱导的转录组是研究药物作用机理,寻找新药物靶标的基础。利福平改变耐药结核菌编码外排泵、运输蛋白、毒力分子等基因的转录,明显上调 Rv0559c- Rv0560c[87]。卷曲霉素(Capreomycin)既诱导其特异性的靶标 16S rRNA,还上调新候选靶标,如作用在 DNA 水平的 Rv0054 (ssb)和 Rv3715c (recR)、细胞分裂相关的 Rv3260c (whiB2),并阻遏 *nuo* 基因簇和 ATP 合成酶基因簇的转 录[88]。第一个 Oxazolidinones 类药物 Linezolid 调控 729 个基因表达,其中上调 318 个,下调 411 基因[89]。Chelerythrine (天然的四 Benzophenanthridine 生物碱)调控 759 个基因表达,上调 372 个,下调 387 个[90]。抗生素如乙胺丁醇(Ethambutol)、利福平(Rifampin)、氯霉素(Streptomycin)和环丝氨酸(Cycloserine)都以剂量依赖方式特异性诱导 SigF 表达[91]。SigF 在稳定期的表达也比指数期高 100 倍,厌氧代谢诱导 SigF 约 150 倍,甲硝唑(Metronidazole)存在时,诱导倍数更高。冷休克也诱导 SigF, 热休克则无此功能。这些研究提示 SigF 可能与持留相关。结核菌持留被认为是结核菌耐药和疗程长的重要原因。D-环丝氨酸处理对数期生长的结核菌,离心收集裂解后存活的细胞可以视为持留子(Persister)。分析这些持留子与体外培养结核菌的转录组差异发现: 许多代谢和生物合成途径下调,明确了持留相关的核心基因[92]。利用时钟质粒(Clock plasmid)测定结核菌在胞内复制和死亡率,同时电镜观察细菌细胞的完整性,确定胞内结核菌在相对长期(14 d)培养的转录组特征与生理代谢之间的联系[93]。链特异性 RNA-seq 全局性转录组可以了解复制和转录复合体之间的配合[94]。ChIP-seq 监控 RNA 聚合酶(RNA polymerase, RNAP)和抗终止子(Anti-terminator) NusA 的全基因组动态。NusA 并不直接结合 DNA,而是结合 RNAP 和/或新转录产物(Transcript)。NusA 在整个染色体上都与 RNAP 相互作用。RNAP 在指数期或者稳定期分布特征是一致的。

1.3 基因组规模突变库的建立和功能基因筛选

构建结核菌全基因组水平突变菌株库是研究特定功能基因的重要手段。用该库寻找在巨噬细胞内存活所需基因发现: 磷酸运输相关基因是其关键,而且很多基因组成性表达,而非调控表达。这提示通过进化选择的结核菌已经适应了宿主体内生活[95]。荧光显微镜技术结合 10 100 个功能缺失的结核菌转座子突变菌株库,

在小鼠巨噬细胞株中检测 12 个宿主免疫应答基因的启动子构建的启动子-报告基因的表达变化, 获得 364 个候选免疫调控基因。其中 35 个在原代巨噬细胞中进行验证。其中功能未知的 Rv0431 突变后, 诱导巨噬细胞产生更多 TNF-alpha、IL-12p40 和 IL-6, 在小鼠中的毒力降低[96]。分泌蛋白是诊断标记分子和候选疫苗分子研究的重点。体外 Tn552'phoA 转座系统可以鉴定结核菌分泌蛋白[97]。寻找疫苗候选抗原, 用 PPD 阳性、ESAT6/CFP10 应答的患者的免疫细胞筛选 2 780 个体内表达的结核菌抗原分子[98]。Beta-内酰胺酶报告基因转座子(Beta-lactamase reporter transposon)技术也被用来在基因组水平寻找结核菌在胞内生长所需的分泌蛋白[99]。从 177 个抗 Beta-内酰胺的转座子突变菌株中, 筛选获得了 111 个分泌蛋白。在巨噬细胞中逐个测定突变菌株存活力发现: mce1A、mce1B、mce2F、rv0199、ctaC 和 lppX 突变影响在巨噬细胞内的存活力。这个方法还可以研究功能未知的可读框。

　　除了以上研究, 生物信息学和计算生物学在基因组、转录组研究中的应用也值得关注。用随机偏最小二乘回归技术(Random partial least squares regression, r-PLS), 从芯片同时获得的数千个基因的表达水平改变中筛选共表达基因和同一代谢途径中相互作用的蛋白质, 可以发现功能联系, 也可以研究功能未知的蛋白质, 在系统水平分析生物的共表达网络[100]。收集现有文献中的结核菌转录组数据, 构建转录调控网络(Transcriptional regulatory network), 则可以弥补蛋白质组实验数据的缺乏[101]。

2　蛋白质组

　　蛋白质一直被认为是生命功能的主要执行者。结核菌和其他分枝杆菌的蛋白质组研究迅速发展。通过分离亚细胞组分、富集细胞膜

组分, 利用液相色谱-质谱鉴定了结核菌细胞壁、细胞膜、细胞浆、裂解物和分泌产物, 得到 1 051 个蛋白质[102]。质谱数据和基因组数据参照鉴定了 3 176 个蛋白质[103]。代谢标记鉴定了结核菌低氧应答蛋白质组(包括细胞组分和分泌蛋白质)[104]。双向电泳发现 *M. tuberculosis* (H37Rv 和 CDC 1551)蛋白质组约有 1 750 个点的差异[105]。异烟肼耐药和敏感临床菌株之间差异蛋白多为膜蛋白, 耐药菌株上调 5 个蛋白质: Rv1446c、Rv3028c、Rv0491、Rv2971 和 Rv2145[106]。比较卡介苗和结核菌的蛋白质组差异[107], 强毒株和卡介苗分泌蛋白的蛋白质组差异[108]。结核菌在好氧和厌氧条件下的蛋白质组差异点 50 个(某一条件特有或者高丰度), 质谱鉴定了其中的 16 个[109]: 结核菌休眠诱导约 1% 基因表达。使用了 60 多年的诊断潜伏结核病和体外免疫检测感染结核菌与否的纯化蛋白质衍生物(Purified protein derivative, PPD)的蛋白质组组成最近也用美国食品药品局标准品 PPD-S2 得以阐明[110]: 含有许多已知的结核菌 T 细胞抗原, 热休克蛋白(Heat shock proteins, HSPs) GroES、GroEL2、HspX 和 DnaK 的比例较大。推测原因可能是其分子伴侣特性以及影响免疫应答的特征导致 PPD 的高溶解性。PPD 中是否存在早期分泌系统(Early secretory system, Esx)蛋白及其数量差异是导致迟发型超敏反应(Delayed type of hypersensitivity, DTH)差异的关键。蛋白质组方法学(双向电泳和同位素亲和标签 Isotope-coded affinity tag)之间具有互补性[111]。

2.1　蛋白质组学技术也用来鉴定结核病药物靶标和降解特定污染物的突变

　　异烟肼(Isoniazid, INH)的蛋白质组水平的药物靶标。异烟肼是结核病治疗的核心药物之一, 其杀菌成分是嘧啶核苷辅酶(Pyridine nu-

cleotide coenzyme)的异烟肼加合物(INH adducts [INH-NAD(P)])。这是体内 INH 激活、结合、抑制必需酶形成的分子。纳摩尔水平的 INH-NAD(P)加合物也抑制依赖 NADH 的烯酰基-ACP 还原酶(NADH-dependent enoyl-ACP reductase, InhA)和依赖 NADPH 的二氢叶酸还原酶(NADPH-dependent dihydrofolate reductase, DfrA)。将 INH-NAD 和 INH-NADP 加合物偶联到固体基质，发现了另外 16 个与其高亲和力结合的蛋白质。这些蛋白质多预测为依赖嘧啶核苷的脱氢酶/还原酶(Pyridine nucleotide-dependent dehydrogenases/reductases)，参与多种细胞活动如依赖 S-腺苷甲硫氨酸的甲基转移反应，嘧啶和缬氨酸代谢，精氨酸降解途径、质子和钾离子运输、胁迫应答、脂类代谢、核黄素生物合成等。异烟肼良好的杀菌效力可能与其多靶标有关[112]。

用杀菌浓度的 RNIs (Reactive nitrogen intermediates, RNIs)处理结核菌，可以检测亚硝基化(S-nitrosylation)蛋白质组变化[113]。从摩尔效力而言，宿主对结核菌免疫应答的关键酶——一氧化氮合成酶[Nitric oxide (NO) synthase, NOS] 2 合成的 NO 体外杀菌效力优于许多常规抗生素。29 个被 RNI S-亚硝基化的蛋白质都是酶，分别参与中间代谢、脂类代谢和抗氧化防御。S-亚硝基化抑制硫辛酰胺脱氢酶(Lipoamide dehydrogenase)和分枝杆菌蛋白酶体 ATP 酶活性。

分离自 PAH 污染河口的 *M. gilvum* PYR-GCK 可以降解烃类污染物。鸟枪法比较蛋白质组学分析发现了该菌与降解烃类相关的途径。乙醛酸、莽草酸途径的蛋白质在降解时表达增加。Pyrene 降解中间产物经部分糖异生途径进入戊糖磷酸途径，生成核苷酸和氨基酸合成所需的前体[114]。

2.1.1 在宿主如豚鼠体内的结核菌蛋白质组[115]： 这个工作鉴定了 500 种独特蛋白质。细胞壁、细胞壁过程、中间代谢、呼吸是肺部主要表达的蛋白质。适应所需的蛋白质如硝酸/亚硝酸还原存在于任何时间。研究颇少的 PE-PPE 是体内第三高丰度的蛋白质。

2.1.2 蛋白质组补充和完善基因组注释： 全基因组翻译对麻风分枝杆菌全细胞提取物凝胶-质谱分析结果进行比对，鉴定了 1 046 个蛋白质，包括 5 个先前注释的假基因[116]。质谱进行结核菌蛋白质组水平的翻译起始位点的鉴定[117]。13 个已知蛋白质中，准确鉴定了 11 个，2 个重新确定，发现 3 个蛋白质被乙酰化。表位标记结合移码突变法也用来确定翻译起始位点并用 3 个结核菌分子(LexA、SigC 和 Rv1955)进行了验证[118]。

2.1.3 结构基因组学研究： 2003 年左右，来自全球 13 个国家的 31 所大学和科研机构成立了结核病结构基因组学研究团队(TB Structural Genomics Consortium)，目标是解析 400 个结核菌药物靶标的结构[119]。通过生物信息学、物理和物种特异性、蛋白质系统进化谱、Rosetta Stone 方法、生化途径相关性、蛋白质溶解性、蛋白质或者功能域大小、缺乏真核同源性等标准，确定研究对象，分析药物靶标、药物-蛋白质相互作用。到 2011 年，解析了结核菌基因组中大约 8.5%可读框的分子结构[120]。结核菌蛋白质组比较数据库[121] (*Mycobacterium tuberculosis* Proteome Comparison Database, MTB-PCDB) http://www.bicjbtdrc-mgims.in/MTB-PCDB/。高阶聚类方法建立全基因组水平连锁图，建立结核菌蛋白质网络图谱并可视化[122]。同步众包(Crowd sourcing)和社交网络法(Social networking methods)建立从"联系到解码" (Connect to Decode, C2D)的方法，获得第一个，也是最大的手工的结核菌相互作用组-相互作用组途径(Interactome pathway, IPW)，包括通过 2 575 个

相互作用功能联系起来的 1 434 个蛋白质。分为基因调控、信号传递、代谢、结构复合体形成等类别。功能注释量为基因组的 87%。IPW 与 STRING 为基础的网络寻找核心蛋白, 得到副作用可能最小的候选药物靶标 17 个。其中 5 个是已经生化或者遗传实验证实的[123]。结核菌 4 000 多个可读框中, 有结构的 312 个。蛋白质结构有助于高分辨率理解生物学。计算方法可以进行结构注释。大约 2 877 个 ORFs 用基于 Fold 的功能和新结合位点配体偶联方法和新算法进行了注释。219 Fold 即可完成细胞代谢(http://proline. physics.iisc.ernet.in/Tbstructuralannotation)。在基因组水平基于结构进行功能注释。

3 代谢组

结核菌代谢生理, 尤其是感染过程中的代谢是深入认识结核菌生物学, 研发控制结核菌新手段的基石之一。GSMN-TB (Genome-scale metabolic model of *M. tuberculosis*)涉及 849 个独特反应, 739 个代谢物和 726 个基因。用连续培养的卡介苗和稳定期生长, 代谢流平衡分析计算底物消耗率等验证了该模型。模拟代谢流、结核菌体外生长的全局性代谢数据可以分析结核菌基因的必需性。该模型预测发现: 已知的结核病药物靶标都是必需基因, 异柠檬酸裂合酶是分枝杆菌慢生长所需。该模型可以预测细菌代谢可塑性和结核菌代谢新特征[124]。计算系统生物学(Computational systems biology)寻找结核菌代谢和致病之间的联系可拓宽研究对象, 不在局限于已知的几个酶[125]。卡介苗可以作为模型研究结核菌在碳源受限制(甘油作为碳源)的恒化器(Chemostat)中的生长。代时 23 h [D=0.03 h (−1)]模拟急性感染期, 代时 69 h [D=0.01 h (−1)]模拟持续感染期。测定各时期菌体中的大分子(RNA、DNA、碳水化合物和脂类)、元素

(C、H 和 N)的含量。脂类含量受生长阶段影响较大。恒化器适合研究结核菌基因组学、生理和系统生物学[126]。代谢组学揭示了结核菌中间代谢(Intermediary metabolism)特殊的空间分布(Topologic organization)特征[127]。结核菌致病离不开代谢适应。详细研究其代谢网络的生化特征有助于揭示其致病机理。很多细菌具有典型的二次生长特征(Diauxic kinetics), 优先利用不同碳源。结核菌则可以同时利用多种碳源, 获得增强的单相生长。糖酵解、戊糖磷酸途径、三羧酸循环等与不同碳源的命运有关。这些研究也提示中枢代谢是结核菌生理和致病机理研究的新重点之一[128]。基于 ^{13}C-的代谢组研究发现: 负责磷酸化葡萄糖的两个激酶(ppgK、glkA)导致的葡萄糖磷酸化可能与结核菌在小鼠体内持留相关[129]。基于 ^{1}H 核磁共振的代谢组学(NMR-based metabolomics)发现了被实验室强毒株感染的小鼠组织和血清中代谢物变化特征: 膜磷脂前体、磷脂酰胆碱、磷脂酰乙醇胺、糖酵解、氨基酸代谢、核酸代谢、抗氧化胁迫应答[130]。这个方法也用来考察临床流行毒株对小鼠代谢组的改变[131]。气相色谱-质谱联用可以通过代谢物鉴定分枝杆菌属[132]。代谢流分析异烟肼处理后, 结核菌代谢适应[133]。测定 3-硝基丙酸和 5′-O-(N-salicylsulfamoyl)腺苷对结核菌生长的影响, 建立系统生物学框架[134]。基于代谢的系统生物学框架, 模拟药物诱导的结核菌在小鼠巨噬细胞中的生长抑制, 发现了异柠檬酸裂合酶是 3-硝基丙酸(3-Nitropropionate, 3-NP)靶标[135]。用接近度指数(Nearness index)分析蛋白质之间、途径之间、某一蛋白质对代谢网络的影响, 寻找有效中断代谢的策略[136]。代谢模型与体内数据的兼容问题已经提上日程[137]。在分枝杆菌降解污染物的途径和酶的鉴定方面, 包括代谢组、基因组和蛋白质组在内

的系统生物学方法确定 *M. vanbaalenii* PYR-1 降解高分子多环芳烃(Polycyclic aromatic hydrocarbon, PAH)芘(Pyrene)涉及 27 个酶[138]。芘经邻苯二甲酸(O-phthalate)和 Beta 酮己二酸(Beta-ketoadipate)途径进入中枢代谢。18 个酶的表达升高两倍。三拷贝的羟基化环的加氧酶(Ring-hydroxylating oxygenase, NidAB2, MvanDraft_0817/0818 和 PhtAaAb)、二氢二醇脱氢酶(Dihydrodiol dehydrogenase, MvanDraft_0815)、开环双加氧酶(Ring cleavage dioxygenase, MvanDraft_3242)只在生长于芘中的细胞中发现。

不同建模算法的评价和优化。同一生物的代谢网络可以用不同算法建模。究竟哪种算法的结果更好？如何优化这些算法？MetaMerge 是一种半自动协调不同代谢网络重建的算法，可以合并不同模型，得到单一模型无法预测的反应，提高必需基因预测的准确率[139]。

4　激酶组

激酶组研究旨在发现结核菌感染和持留关键的宿主特异性激酶，为研发激酶调节剂提供基础。哺乳动物磷特异性抗体筛选结核菌感染应答发现了 31 个被磷酸化的分子[140]。RNA 干扰筛选发现抑制胞内致病菌的激酶集中在围绕 AKT1 (也称 PKB)的网络。致病菌可以劫持这些宿主激酶。靶向这些激酶的抑制剂可以控制胞内致病菌，包括耐多药结核菌[141]。用牛特异性肽段构成的芯片分析激酶组(Kinome)发现，牛结核病的致病菌 *M. avium* subsp. *paratuberculosis* 不影响 JAK-STAT 途径的激活，增加负调控因子 SOCS1 (Stimulator of cytokine signal)和 SOCS3 表达，降低 Gamma 干扰素受体链(IFN-gamma receptor chains) 1 和 2 表达[142]。*M. avium* subsp. *paratuberculosis* 感染改变牛单核细胞对 TLR9 刺激的应答。尽管摄取 CpG ODNs，增加 TLR9 表达 10 倍，*M. avium* subsp. *paratu-*

berculosis 仍然抑制经典的 TLR9 介导的应答。TLR9 介导的其他应答，如经过非经典途径的氧化暴发，仍然具有功能。激酶组分析发现 TLR9 信号传递被抑制，信号由 Pyk2 介导。这可能反映了该菌特殊的主动改造宿主环境，适合细菌生长的特征[143]。

5　免疫组及其计算机建模

免疫组和激酶组有一定重叠。但免疫组研究范围更大，集中在致病菌感染后，在组学水平研究宿主免疫应答反应，包括从转录组、蛋白质组和代谢组等内容。全基因组水平 siRNA 筛选调控人巨噬细胞内致病菌数量的宿主因子，获得了 275 个分子，其中多数为自噬(Autophagy)相关[21]。抗性和敏感小鼠对结核菌 H37Rv 感染的应答不同[144]。分析第 14 天小鼠肺部表达谱变化显示两种小鼠在抗原递呈，NK、T 和 B 细胞激活途径方面不同。抗性小鼠较敏感小鼠显示更复杂、更强的宿主防御途径。敏感小鼠的嗜中性粒细胞应答(Neutrophil response)上升，也包括特异性增加半胱氨酸蛋白酶(Cysteine protease)抑制剂。在蛋白质组水平分析与结核菌感染后果相关的抗体应答，确定结核菌免疫蛋白质组(Immunoproteome)[145]，发现 10 个结核菌抗原引起的抗体应答影响猴子和人体中结核菌感染的结果[146]。系统生物学分析结核菌 T 细胞表位识别的免疫特征[147]。芯片技术获得卡介苗免疫和牛分枝杆菌感染的 BALB/c 小鼠肺部、脾脏转录组特征对候选疫苗进行评价。Th17 相关的细胞因子谱与疫苗诱导的保护性免疫相关，嗜中性粒细胞生物学和炎症相关的分子也被上调[148]。另外的方法也用来在全基因组水平筛选 *M. tuberculosis* 的 HLA-B*3501 T 细胞表位。预测的 479 个表位中，合成了得分最高的 13 个，刺激来自自然接触的 HLA-B*3501 个体的淋巴细胞，酶联

免疫斑点(Enzyme-linked immunospot, ELISPOT)测定 Interferon (IFN)-gamma 水平[149]。卡介苗吞噬体蛋白质组的研究有助于全面揭示分枝杆菌影响的宿主应答[150]。在深度测序测定感染分枝杆菌的斑马鱼的转录组变化[151]的基础上,用数学、计算模型在斑马鱼中模拟分枝杆菌感染早期过程[152]。代谢的可能性调控(Probabilistic regulation of metabolism, PROM) 构建基因组水平的调控-代谢网络。该模型整合的数据包括 1 300 芯片结果, 2 000 转录因子-靶标相互作用, 3 300 代谢反应, *E. coli* 和 *M. tuberculosis* 1 905 个 KO 表型[153]。相互作用组(Interactome)、反应组(Reactome)和基因组水平的结构分析用来寻找结核菌药物靶标[154]。

6 国内研究概述

国内最近十年在结核菌研究领域进展颇大。比较了结核菌实验室强毒株和弱毒株基因组差异[155], 测定了耐药临床分离株中小 RNA (与毕利军研究员会议交流), 调查了全国耐药结核菌概况[156], 耐药结核菌如何在结核病患者群体中定殖[157], 建立了结核菌体外蛋白质-蛋白质相互作用网络[158], 理清了结核菌吞噬体蛋白质组涉及的信号传递网络[159], 分析卡介苗的蛋白质组[160], 建立了结核菌持留相关分子网络[161]。在具体基因研究方面, 对氟喹诺酮类药物相关的研究较多[162-165]。

数据整合与界定病原生物学关键科学问题在未来需要特别关注。深入认识结核菌基础生物学需要综合来自不同模型的知识[166]。*M. bovis* BCG、*M. marinum*、*M. smegmatis* 等非常重要; 遗传操作所需诱导型启动子、重组工程, 分子水平和毒力发挥过程中的分泌系统, 潜伏感染和宿主免疫, 不同模型的相互参照也不可或缺。结核菌蛋白的翻译后修饰是值得加强的一个方面: 甲基化的结核菌结合肝素的凝集素(Heparin-binding hemagglutinin, HBHA)诱发小鼠更强的 T 细胞免疫[167], 包括 PknB 等几个结核菌丝/苏氨酸蛋白质激酶负责磷酸化结核菌 MabA(Beta-ketoacyl-acyl carrier protein reductase), 负责合成枝菌酸(Mycolic acid)的直接前体(Meromycolic acid)[168], 调控枝菌酸合成。酰基化(Acylation)和 O-糖基化(O-glycosylation)影响结核菌 19 kD 脂蛋白的免疫功能[169]。结核菌乙酰辅酶 A 合成酶的可逆乙酰化和失活需要 cAMP[170]。紧密围绕分枝杆菌, 尤其是结核菌重要基础科学问题和临床实践难题, 进行整合生物学研究非常迫切。

参 考 文 献

[1] Vaerewijck MJ, Huys G, Palomino JC, et al. Mycobacteria in drinking water distribution systems: ecology and significance for human health[J]. FEMS Microbiology Reviews, 2005, 29(5): 911−934.

[2] Wei W, Wang FQ, Fan SY, et al. Inactivation and augmentation of the primary 3-ketosteroid-{delta}1-dehydrogenase in *Mycobacterium neoaurum* NwIB-01: biotransformation of soybean phytosterols to 4-androstene-3,17-dione or 1,4-androstadiene-3,17-dione[J]. Applied and Environmental Microbiology, 2010, 76(13): 4578−4582.

[3] Kim KY, Lee S, Cha CJ. Biotransformation of plant secondary metabolite decursin by *Mycobacterium* sp. PYR1001[J]. Journal of Agricultural and Food Chemistry, 2010, 58(5): 2931−2934.

[4] Kim SJ, Song J, Kweon O, et al. Functional

robustness of a polycyclic aromatic hydrocarbon metabolic network examined in a *nidA* aromatic ring-hydroxylating oxygenase mutant of *Mycobacterium vanbaalenii* PYR-1[J]. Applied Environmental Microbiology, 2012, 78(10): 3715−3723.

[5] Bannantine JP, Barletta RG, Stabel JR, et al. Application of the genome sequence to address concerns that *Mycobacterium avium* subspecies *paratuberculosis* might be a foodborne pathogen[J]. Foodborne Pathogens and Disease, 2004, 1(1): 3−15.

[6] Nathan C, Gold B, Lin G, et al. A philosophy of anti-infectives as a guide in the search for new drugs for tuberculosis[J]. Tuberculosis (Edinb), 2008, 88(Sup. 1): S25−33.

[7] Nathan C. Fresh approaches to anti-infective therapies[J]. Science Translational Medicine, 2012, 4(140): 140sr2.

[8] Das B, Kashino SS, Pulu I, et al. CD271+ bone marrow mesenchymal stem cells may provide a niche for dormant *Mycobacterium tuberculosis*[J]. Science Translational Medicine, 2013, 5(170): 170ra13.

[9] Tapinos N, Rambukkana A. Insights into regulation of human Schwann cell proliferation by Erk1/2 via a MEK-independent and p56Lck-dependent pathway from leprosy bacilli[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(26): 9188−9193.

[10] Kaufmann SH. How can immunology contribute to the control of tuberculosis?[J]. Nature Reviews Immunology, 2001, 1(1): 20−30.

[11] Kondratieva E, Logunova N, Majorov K, et al. Host genetics in granuloma formation: human-like lung pathology in mice with reciprocal genetic susceptibility to *M. tuberculosis* and *M. avium*[J]. PLoS One, 2010, 5(5): e10515.

[12] Tabarsi P, Marjani M, Mansouri N, et al. Lethal tuberculosis in a previously healthy adult with IL-12 receptor deficiency[J]. Journal of Clinical Immunology, 2011, 31(4): 537−539.

[13] Rezaei N, Aghamohammadi A, Mansouri D, et al. Tuberculosis: a new look at an old disease[J]. Expert Review of Clinical Immunology, 2011, 7(2): 129−131.

[14] Fortin A, Abel L, Casanova JL, et al. Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis[J]. Annual Review of Genomics and Human Genetics, 2007, 8: 163−192.

[15] Abel L, Casanova JL. Genetic predisposition to clinical tuberculosis: bridging the gap between simple and complex inheritance[J]. The American Journal of Human Genetics, 2000, 67(2): 274−277.

[16] Misch EA, Berrington WR, Vary JC, et al. Leprosy and the human genome[J]. Microbiology and Molecular Biology Reviews, 2010, 74(4): 589−620.

[17] Thye T, Owusu-Dabo E, Vannberg FO, et al. Common variants at 11p13 are associated with susceptibility to tuberculosis[J]. Nature Genetics, 2012, 44(3): 257−259.

[18] Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease[J]. Nature, 2012, 491(7422): 119−124.

[19] Barreiro LB, Tailleux L, Pai AA, et al. Deciphering the genetic architecture of variation in the immune response to *Mycobacterium tuberculosis* infection[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109(4): 1204−1209.

[20] Baker AR, Zalwango S, Malone LL, et al. Genetic susceptibility to tuberculosis associated with cathepsin Z haplotype in a Ugandan household contact study[J]. Human Immunology, 2011, 72(5): 426−430.

[21] Kumar D, Nath L, Kamal MA, et al. Genome-wide analysis of the host intracellular network that regulates survival of *Mycobacterium tuberculosis*[J]. Cell, 2010, 140(5): 731−743.

[22] Lamrabet O, Drancourt M. Genetic engineering of *Mycobacterium tuberculosis*: a review[J]. Tuberculosis (Edinb), 2012, 92(5): 365−376.

[23] Medjahed H, Reyrat JM. Construction of *Mycobacterium abscessus* defined glycopeptidolipid mutants: comparison of genetic tools[J]. Applied Environmental Microbiology, 2009, 75(5): 1331−1338.

[24] Young D, Stark J, Kirschner D. Systems biology of persistent infection: tuberculosis as a case study[J]. Nature Reviews Microbiology, 2008, 6(7): 520−528.

[25] Boshoff HI, Lun DS. Systems biology approaches to understanding mycobacterial survival mechanisms[J]. Drug Discovery Today: Disease Mechanisms, 2010, 7(1): e75−e82.

[26] Garrison LP Jr. Universal health coverage-big thinking versus big data[J]. Value in Health, 2013, 16(1 Suppl): S1−3.

[27] Schatz MC. Computational thinking in the era of big data biology[J]. Genome Biology, 2012, 13(11): 177.

[28] Schadt EE. The changing privacy landscape in the era of big data[J]. Molecular Systems Biology, 2012, 8: 612.

[29] Praneenararat T, Takagi T, Iwasaki W. Integration of interactive, multi-scale network navigation approach with Cytoscape for functional genomics in the big data era[J]. BMC Genomics, 2012, 13(Suppl 7): S24.

[30] Fox B. Using big data for big impact. How predictive modeling can affect patient outcomes[J]. Health Management Technology, 2012, 33(1): 32.

[31] Brosch R, Gordon SV, Billault A, et al. Use of a *Mycobacterium tuberculosis* H37Rv bacterial artificial chromosome library for genome mapping, sequencing, and comparative genomics[J]. Infection and Immunity, 1998, 66(5): 2221−2229.

[32] Cole ST, Brosch R, Parkhill J, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence[J]. Nature, 1998, 393(6685): 537−544.

[33] Miyoshi-Akiyama T, Matsumura K, Iwai H, et al. Complete annotated genome sequence of *Mycobacterium tuberculosis* Erdman[J]. Journal of Bacteriology, 2012, 194(10): 2770.

[34] Miyoshi-Akiyama T, Matsumura K, Kobayashi N, et al. Genome sequence of clinical isolate *Mycobacterium tuberculosis* NCGM2209[J]. Journal of Bacteriology, 2011, 193(23): 6792.

[35] Garnier T, Eiglmeier K, Camus JC, et al. The complete genome sequence of *Mycobacterium bovis*[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100(13): 7877−7882.

[36] Veyrier FJ, Dufort A, Behr MA. The rise and fall of the *Mycobacterium tuberculosis* genome[J]. Trends in Microbiology, 2011, 19(4): 156−161.

[37] Abdallah AM, Rashid M, Adroub SA, et al. Complete genome sequence of *Mycobacterium phlei* type strain RIVM601174[J]. Journal of Bacteriology, 2012, 194(12): 3284−3285.

[38] Abdallah AM, Rashid M, Adroub SA, et al. Complete genome sequence of *Mycobacterium xenopi* type strain RIVM700367[J]. Journal of Bacteriology, 2012, 194(12): 3282−3283.

[39] Bannantine JP, Wu CW, Hsu C, et al. Genome sequencing of ovine isolates of *Mycobacterium avium* subspecies paratuberculosis offers insights into host association[J]. BMC Genomics, 2012, 13: 89.

[40] Chan J, Halachev M, Yates E, et al. Whole-genome sequence of the emerging pathogen *Mycobacterium abscessus* strain 47J26[J]. Journal of Bacteriology, 2012, 194(2): 549.

[41] Choi GE, Cho YJ, Koh WJ, et al. Draft genome sequence of *Mycobacterium abscessus* subsp. *bolletii* BD(T)[J]. Journal of Bacteriology, 2012, 194(10): 2756−2757.

[42] Choo SW, Wong YL, Leong ML, et al. Analysis of the genome of *Mycobacterium abscessus* strain M94 reveals an uncommon cluster of tRNAs[J]. Journal of Bacteriology, 2012, 194(20): 5724.

[43] Choo SW, Wong YL, Tan JL, et al. Annotated genome sequence of *Mycobacterium massiliense* strain M154, belonging to the recently created taxon *Mycobacterium abscessus* subsp. *bolletii* comb. nov.[J]. Journal of Bacteriology, 2012, 194(17): 4778.

[44] Choo SW, Wong YL, Yusoff AM, et al. Genome sequence of the *Mycobacterium abscessus* strain M93[J]. Journal of Bacteriology, 2012, 194(12): 3278.

[45] Choo SW, Yusoff AM, Wong YL, et al. Genome analysis of *Mycobacterium massiliense* strain M172, which contains a putative mycobacteriophage[J]. Journal of Bacteriology, 2012, 194(18): 5128.

[46] Cole ST, Supply P, Honore N. Repetitive sequences in *Mycobacterium leprae* and their impact on

genome plasticity[J]. Leprosy Review, 2001, 72(4): 449−461.

[47] Williams DL, Slayden RA, Amin A, et al. Implications of high level pseudogene transcription in *Mycobacterium leprae*[J]. BMC Genomics, 2009, 10: 397.

[48] Akama T, Suzuki K, Tanigawa K, et al. Whole-genome tiling array analysis of *Mycobacterium leprae* RNA reveals high expression of pseudogenes and noncoding regions[J]. Journal of Bacteriology, 2009, 191(10): 3321−3327.

[49] Alland D, Whittam TS, Murray MB, et al. Modeling bacterial evolution with comparative-genome-based marker systems: application to *Mycobacterium tuberculosis* evolution and pathogenesis[J]. Journal of Bacteriology, 2003, 185(11): 3392−3399.

[50] Coros A, DeConno E, Derbyshire KM. IS6110, a *Mycobacterium tuberculosis* complex-specific insertion sequence, is also present in the genome of *Mycobacterium smegmatis*, suggestive of lateral gene transfer among mycobacterial species[J]. Journal of Bacteriology, 2008, 190(9): 3408−3410.

[51] Ford C, Yusim K, Ioerger T, et al. *Mycobacterium tuberculosis*—heterogeneity revealed through whole genome sequencing[J]. Tuberculosis (Edinb), 2012, 92(3): 194−201.

[52] Fleischmann RD, Alland D, Eisen JA, et al. Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains[J]. Journal of Bacteriology, 2002, 184(19): 5479−5490.

[53] Gutacker MM, Smoot JC, Migliaccio CA, et al. Genome-wide analysis of synonymous single nucleotide polymorphisms in *Mycobacterium tuberculosis* complex organisms: resolution of genetic relationships among closely related microbial strains[J]. Genetics, 2002, 162(4): 1533−1543.

[54] Vishnoi A, Srivastava A, Roy R, et al. MGDD: *Mycobacterium tuberculosis* genome divergence database[J]. BMC Genomics, 2008, 9: 373.

[55] Gomez-Valero L, Rocha EP, Latorre A, et al. Reconstructing the ancestor of *Mycobacterium leprae*: the dynamics of gene loss and genome reduction[J]. Genome Research, 2007, 17(8):

1178−1185.

[56] Djelouadji Z, Raoult D, Drancourt M. Palaeogenomics of *Mycobacterium tuberculosis*: epidemic bursts with a degrading genome[J]. The Lancet Infectious Diseases, 2011, 11(8): 641−650.

[57] Namouchi A, Didelot X, Schock U, et al. After the bottleneck: Genome-wide diversification of the *Mycobacterium tuberculosis* complex by mutation, recombination, and natural selection[J]. Genome Research, 2012, 22(4): 721−734.

[58] Wang TC, Chen FC. The evolutionary landscape of the *Mycobacterium tuberculosis* genome[J]. Gene, 2012, 518: 187−193.

[59] Rondini S, Kaser M, Stinear T, et al. Ongoing genome reduction in *Mycobacterium ulcerans*[J]. Emerging Infectious Diseases, 2007, 13(7): 1008−1015.

[60] Azhikina T, Gvozdevsky N, Botvinnik A, et al. A genome-wide sequence-independent comparative analysis of insertion-deletion polymorphisms in multiple *Mycobacterium tuberculosis* strains[J]. Research in Microbiology, 2006, 157(3): 282−290.

[61] Keller PM, Bottger EC, Sander P. Tuberculosis vaccine strain *Mycobacterium bovis* BCG Russia is a natural *recA* mutant[J]. BMC Microbiology, 2008, 8: 120.

[62] Bentley SD, Comas I, Bryant JM, et al. The genome of *Mycobacterium africanum* West African 2 reveals a lineage-specific locus and genome erosion common to the *M. tuberculosis* complex[J]. PLOS Neglected Tropical Diseases, 2012, 6(2): e1552.

[63] Stinear TP, Seemann T, Pidot S, et al. Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of *Buruli ulcer*[J]. Genome Research, 2007, 17(2): 192−200.

[64] Stinear TP, Seemann T, Harrison PF, et al. Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*[J]. Genome Research, 2008, 18(5): 729−741.

[65] Ioerger TR, Feng Y, Ganesula K, et al. Variation among genome sequences of H37Rv strains of *Mycobacterium tuberculosis* from multiple laboratories[J]. Journal of Bacteriology, 2010,

192(14): 3645−3653.

[66] Isaza JP, Duque C, Gomez V, et al. Whole genome shotgun sequencing of one Colombian clinical isolate of *Mycobacterium tuberculosis* reveals DosR regulon gene deletions[J]. FEMS Microbiology Letters, 2012, 330(2): 113−120.

[67] Walker TM, Ip CL, Harrell RH, et al. Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study[J]. The Lancet Infectious Diseases, 2013, 13(2): 137−146.

[68] Joshi D, Harris NB, Waters R, et al. Single nucleotide polymorphisms in the *Mycobacterium bovis* genome resolve phylogenetic relationships[J]. Journal of Clinical Microbiology, 2012, 50(12): 3853−3861.

[69] Comas I, Borrell S, Roetzer A, et al. Whole-genome sequencing of rifampicin-resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations in RNA polymerase genes[J]. Nature Genetics, 2012, 44(1): 106−110.

[70] Ford CB, Lin PL, Chase MR, et al. Use of whole genome sequencing to estimate the mutation rate of *Mycobacterium tuberculosis* during latent infection[J]. Nature Genetics, 2011, 43(5): 482−486.

[71] Aagaard C, Govaerts M, Meng Okkels L, et al. Genomic approach to identification of *Mycobacterium bovis* diagnostic antigens in cattle[J]. Journal of Clinical Microbiology, 2003, 41(8): 3719−3728.

[72] Bannantine JP, Baechler E, Zhang Q, et al. Genome scale comparison of *Mycobacterium avium* subsp. *paratuberculosis* with *Mycobacterium avium* subsp. *avium* reveals potential diagnostic sequences[J]. Journal of Clinical Microbiology, 2002, 40(4): 1303−1310.

[73] Das S, Duggal P, Roy R, et al. Identification of hot and cold spots in genome of *Mycobacterium tuberculosis* using shewhart control charts[J]. Scientific Report, 2012, 2: 297.

[74] Biek R, O'Hare A, Wright D, et al. Whole genome sequencing reveals local transmission patterns of *Mycobacterium bovis* in sympatric cattle and badger populations[J]. PLoS Pathogens, 2012, 8(11):

e1003008.

[75] Asif SM, Asad A, Faizan A, et al. Dataset of potential targets for *Mycobacterium tuberculosis* H37Rv through comparative genome analysis[J]. Bioinformation, 2009, 4(6): 245−248.

[76] Dai J, Chen Y, Dean S, et al. Multiple-genome comparison reveals new loci for *Mycobacterium* species identification[J]. Journal of Clinical Microbiology, 2011, 49(1): 144−153.

[77] Gupta A. Killing activity and rescue function of genome-wide toxin-antitoxin loci of *Mycobacterium tuberculosis*[J]. FEMS Microbiology Letters, 2009, 290(1): 45−53.

[78] Ramage HR, Connolly LE, Cox JS. Comprehensive functional analysis of *Mycobacterium tuberculosis* toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution[J]. PLoS Genetics, 2009, 5(12): e1000767.

[79] Hartkoorn RC, Sala C, Uplekar S, et al. Genome-wide definition of the SigF regulon in *Mycobacterium tuberculosis*[J]. Journal of Bacteriology, 2012, 194(8): 2001−2009.

[80] Pellin D, Miotto P, Ambrosi A, et al. A genome-wide identification analysis of small regulatory RNAs in *Mycobacterium tuberculosis* by RNA-Seq and conservation analysis[J]. PLoS One, 2012, 7(3): e32723.

[81] Arnvig KB, Comas I, Thomson NR, et al. Sequence-based analysis uncovers an abundance of non-coding RNA in the total transcriptome of *Mycobacterium tuberculosis*[J]. PLoS Pathogens, 2011, 7(11): e1002342.

[82] Miotto P, Forti F, Ambrosi A, et al. Genome-wide discovery of small RNAs in *Mycobacterium tuberculosis*[J]. PLoS One, 2012, 7(12): e51950.

[83] Camus JC, Pryor MJ, Medigue C, et al. Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv[J]. Microbiology, 2002, 148(Pt 10): 2967−2973.

[84] Dejesus MA, Sacchettini JC, Ioerger TR. Reannotation of translational start sites in the genome of *Mycobacterium tuberculosis*[J]. Tuberculosis (Edinb), 2012, 93: 18−25.

[85] Blanco FC, Nunez-Garcia J, Garcia-Pelayo C, et al.

Differential transcriptome profiles of attenuated and hypervirulent strains of *Mycobacterium bovis*[J]. Microbes and Infection, 2009, 11(12): 956−963.

[86] Rachman H, Strong M, Ulrichs T, et al. Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis[J]. Infection and Immunity, 2006, 74(2): 1233−1242.

[87] de Knegt GJ, Bruning O, Ten Kate MT, et al. Rifampicin-induced transcriptome response in rifampicin-resistant *Mycobacterium tuberculosis*[J]. Tuberculosis (Edinb), 2012, 93: 96−101.

[88] Fu LM, Shinnick TM. Genome-wide exploration of the drug action of capreomycin on *Mycobacterium tuberculosis* using Affymetrix oligonucleotide GeneChips[J]. Journal of Infection, 2007, 54(3): 277−284.

[89] Liang J, Tang X, Guo N, et al. Genome-wide expression profiling of the response to linezolid in *Mycobacterium tuberculosis*[J]. Current Microbiology, 2012, 64(6): 530−538.

[90] Liang J, Zeng F, Guo A, et al. Microarray analysis of the chelerythrine-induced transcriptome of *Mycobacterium tuberculosis*[J]. Current Microbiology, 2011, 62(4): 1200−1208.

[91] Michele TM, Ko C, Bishai WR. Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis* sigF gene: implications for chemotherapy against mycobacterial persistors[J]. Antimicrobial Agents and Chemotherapy, 1999, 43(2): 218−225.

[92] Keren I, Minami S, Rubin E, et al. Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters[J]. MBio, 2011, 2(3): e00100−00111.

[93] Rohde KH, Veiga DF, Caldwell S, et al. Linking the transcriptional profiles and the physiological states of *Mycobacterium tuberculosis* during an extended intracellular infection[J]. PLoS Pathogens, 2012, 8(6): e1002769.

[94] Uplekar S, Rougemont J, Cole ST, et al. High-resolution transcriptome and genome-wide dynamics of RNA polymerase and NusA in *Mycobacterium tuberculosis*[J]. Nucleic Acids Research, 2013, 41(2): 961−977.

[95] Rengarajan J, Bloom BR, Rubin EJ. Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(23): 8327−8332.

[96] Beaulieu AM, Rath P, Imhof M, et al. Genome-wide screen for *Mycobacterium tuberculosis* genes that regulate host immunity[J]. PLoS One, 2010, 5(12): e15120.

[97] Braunstein M, Griffin TI, Kriakov JI, et al. Identification of genes encoding exported *Mycobacterium tuberculosis* proteins using a Tn552'phoA *in vitro* transposition system[J]. Journal of Bacteriology, 2000, 182(10): 2732−2740.

[98] Commandeur S, van Meijgaarden KE, Prins C, et al. An unbiased genome-wide *Mycobacterium tuberculosis* gene expression approach to discover antigens targeted by human T cells expressed during pulmonary infection[J]. The Journal of Immunology, 2013, 190: 1659−1671.

[99] McCann JR, McDonough JA, Sullivan JT, et al. Genome-wide identification of *Mycobacterium tuberculosis* exported proteins with roles in intracellular growth[J]. Journal of Bacteriology, 2011, 193(4): 854−861.

[100] Mazandu GK, Opap K, Mulder NJ. Contribution of microarray data to the advancement of knowledge on the *Mycobacterium tuberculosis* interactome: use of the random partial least squares approach[J]. Infection, Genetics and Evolution, 2011, 11(4): 725−733.

[101] Sanz J, Navarro J, Arbues A, et al. The transcriptional regulatory network of *Mycobacterium tuberculosis*[J]. PLoS One, 2011, 6(7): e22178.

[102] Bell C, Smith GT, Sweredoski MJ, et al. Characterization of the *Mycobacterium tuberculosis* proteome by liquid chromatography mass spectrometry-based proteomics techniques: a comprehensive resource for tuberculosis research[J]. Journal of Proteome Research, 2012, 11(1): 119−130.

[103] Kelkar DS, Kumar D, Kumar P, et al. Proteogenomic analysis of *Mycobacterium tuberculosis* by high

resolution mass spectrometry[J]. Molecular & Cellular Proteomics, 2011, 10(12): M111.011627.

[104] Rosenkrands I, Slayden RA, Crawford J, et al. Hypoxic response of *Mycobacterium tuberculosis* studied by metabolic labeling and proteome analysis of cellular and extracellular proteins[J]. Journal of Bacteriology, 2002, 184(13): 3485−3491.

[105] Betts JC, Dodson P, Quan S, et al. Comparison of the proteome of *Mycobacterium tuberculosis* strain H37Rv with clinical isolate CDC 1551[J]. Microbiology, 2000, 146(Pt 12): 3205−3216.

[106] Jiang X, Zhang W, Gao F, et al. Comparison of the proteome of isoniazid-resistant and -susceptible strains of *Mycobacterium tuberculosis*[J]. Microbial Drug Resistance, 2006, 12(4): 231−238.

[107] Jungblut PR, Schaible UE, Mollenkopf HJ, et al. Comparative proteome analysis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG strains: towards functional genomics of microbial pathogens[J]. Molecular Microbiology, 1999, 33(6): 1103−1117.

[108] Mattow J, Schaible UE, Schmidt F, et al. Comparative proteome analysis of culture supernatant proteins from virulent *Mycobacterium tuberculosis* H37Rv and attenuated *M. bovis* BCG Copenhagen[J]. Electrophoresis, 2003, 24(19/20): 3405−3420.

[109] Starck J, Kallenius G, Marklund BI, et al. Comparative proteome analysis of *Mycobacterium tuberculosis* grown under aerobic and anaerobic conditions[J]. Microbiology, 2004, 150(Pt 11): 3821−3829.

[110] Cho YS, Dobos KM, Prenni J, et al. Deciphering the proteome of the *in vivo* diagnostic reagent "purified protein derivative" from *Mycobacterium tuberculosis*[J]. Proteomics, 2012, 12(7): 979−991.

[111] Schmidt F, Donahoe S, Hagens K, et al. Complementary analysis of the *Mycobacterium tuberculosis* proteome by two-dimensional electrophoresis and isotope-coded affinity tag technology[J]. Molecular & Cellular Proteomics, 2004, 3(1): 24−42.

[112] Argyrou A, Jin L, Siconilfi-Baez L, et al. Proteome-wide profiling of isoniazid targets in *Mycobacterium tuberculosis*[J]. Biochemistry, 2006, 45(47):

13947−13953.

[113] Rhee KY, Erdjument-Bromage H, Tempst P, et al. S-nitroso proteome of *Mycobacterium tuberculosis*: Enzymes of intermediary metabolism and antioxidant defense[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(2): 467−472.

[114] Badejo AC, Choi CW, Badejo AO, et al. A global proteome study of *Mycobacterium gilvum* PYR-GCK grown on pyrene and glucose reveals the activation of glyoxylate, shikimate and gluconeogenetic pathways through the central carbon metabolism highway[J]. Biodegradation, 2013.

[115] Kruh NA, Troudt J, Izzo A, et al. Portrait of a pathogen: the *Mycobacterium tuberculosis* proteome *in vivo*[J]. PLoS One, 2010, 5(11): e13938.

[116] de Souza GA, Softeland T, Koehler CJ, et al. Validating divergent ORF annotation of the *Mycobacterium leprae* genome through a full translation data set and peptide identification by tandem mass spectrometry[J]. Proteomics, 2009, 9(12): 3233−3243.

[117] Rison SC, Mattow J, Jungblut PR, et al. Experimental determination of translational starts using peptide mass mapping and tandem mass spectrometry within the proteome of *Mycobacterium tuberculosis*[J]. Microbiology, 2007, 153(Pt 2): 521−528.

[118] Smollett KL, Fivian-Hughes AS, Smith JE, et al. Experimental determination of translational start sites resolves uncertainties in genomic open reading frame predictions - application to *Mycobacterium tuberculosis*[J]. Microbiology, 2009, 155(Pt 1): 186−197.

[119] Goulding CW, Perry LJ, Anderson D, et al. Structural genomics of *Mycobacterium tuberculosis*: a preliminary report of progress at UCLA[J]. Biophysical Chemistry, 2003, 105(2/3): 361−370.

[120] Ehebauer MT, Wilmanns M. The progress made in determining the *Mycobacterium tuberculosis* structural proteome[J]. Proteomics, 2011, 11(15): 3128−3133.

[121] Jena L, Wankhade G, Kumar S, et al. MTB-PCDB: *Mycobacterium tuberculosis* proteome comparison

database[J]. Bioinformation, 2011, 6(3): 131−133.

[122] Strong M, Graeber TG, Beeby M, et al. Visualization and interpretation of protein networks in *Mycobacterium tuberculosis* based on hierarchical clustering of genome-wide functional linkage maps[J]. Nucleic Acids Research, 2003, 31(24): 7099−7109.

[123] Vashisht R, Mondal AK, Jain A, et al. Crowd sourcing a new paradigm for interactome driven drug target identification in *Mycobacterium tuberculosis*[J]. PLoS One, 2012, 7(7): e39808.

[124] Beste DJ, Hooper T, Stewart G, et al. GSMN-TB: a web-based genome-scale network model of *Mycobacterium tuberculosis* metabolism[J]. Genome Biology, 2007, 8(5): R89.

[125] Beste DJ, McFadden J. System-level strategies for studying the metabolism of *Mycobacterium tuberculosis*[J]. Molecular BioSystems, 2010, 6(12): 2363−2372.

[126] Beste DJ, Peters J, Hooper T, et al. Compiling a molecular inventory for *Mycobacterium bovis* BCG at two growth rates: evidence for growth rate-mediated regulation of ribosome biosynthesis and lipid metabolism[J]. Journal of Bacteriology, 2005, 187(5): 1677−1684.

[127] de Carvalho LP, Fischer SM, Marrero J, et al. Metabolomics of *Mycobacterium tuberculosis* reveals compartmentalized co-catabolism of carbon substrates[J]. Chemistry & Biology, 2010, 17(10): 1122−1131.

[128] Rhee KY, de Carvalho LP, Bryk R, et al. Central carbon metabolism in *Mycobacterium tuberculosis*: an unexpected frontier[J]. Trends in Microbiology, 2011, 19(7): 307−314.

[129] Marrero J, Trujillo C, Rhee KY, et al. Glucose phosphorylation is required for *Mycobacterium tuberculosis* persistence in mice[J]. PLoS Pathogens, 2013, 9(1): e1003116.

[130] Shin JH, Yang JY, Jeon BY, et al. (1)H NMR-based metabolomic profiling in mice infected with *Mycobacterium tuberculosis*[J]. Journal of Proteome Research, 2011, 10(5): 2238−2247.

[131] Somashekar BS, Amin AG, Tripathi P, et al. Metabolomic signatures in guinea pigs infected with epidemic-associated W-Beijing strains of *Mycobacterium tuberculosis*[J]. Journal of Proteome Research, 2012, 11(10): 4873−4884.

[132] Olivier I, Loots du T. A metabolomics approach to characterise and identify various *Mycobacterium* species[J]. Journal of Microbiological Methods, 2012, 88(3): 419−426.

[133] Bhat AG, Vashisht R, Chandra N. Modeling metabolic adjustment in *Mycobacterium tuberculosis* upon treatment with isoniazid[J]. Systems and Synthetic Biology, 2010, 4(4): 299−309.

[134] Fang X, Wallqvist A, Reifman J. A systems biology framework for modeling metabolic enzyme inhibition of *Mycobacterium tuberculosis*[J]. BMC Systems Biology, 2009, 3: 92.

[135] Fang X, Wallqvist A, Reifman J. Modeling synergistic drug inhibition of *Mycobacterium tuberculosis* growth in murine macrophages[J]. Molecular BioSystems, 2011, 7(9): 2622−2636.

[136] Raman K, Vashisht R, Chandra N. Strategies for efficient disruption of metabolism in *Mycobacterium tuberculosis* from network analysis[J]. Molecular BioSystems, 2009, 5(12): 1740−1751.

[137] Fang X, Wallqvist A, Reifman J. Development and analysis of an *in vivo*-compatible metabolic network of *Mycobacterium tuberculosis*[J]. BMC Systems Biology, 2010, 4: 160.

[138] Kim SJ, Kweon O, Jones RC, et al. Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology[J]. Journal of Bacteriology, 2007, 189(2): 464−472.

[139] Chindelevitch L, Stanley S, Hung D, et al. MetaMerge: scaling up genome-scale metabolic reconstructions with application to *Mycobacterium tuberculosis*[J]. Genome Biology, 2012, 13(1): r6.

[140] Hestvik AL, Hmama Z, Av-Gay Y. Kinome analysis of host response to mycobacterial infection: a novel technique in proteomics[J]. Infection and Immunity, 2003, 71(10): 5514−5522.

[141] Kuijl C, Savage ND, Marsman M, et al. Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1[J]. Nature, 2007, 450(7170): 725−730.

[142] Arsenault RJ, Li Y, Bell K, et al. *Mycobacterium avium* subsp. *paratuberculosis* inhibits gamma interferon-induced signaling in bovine monocytes: insights into the cellular mechanisms of Johne's disease[J]. Infection and Immunity, 2012, 80(9): 3039−3048.

[143] Arsenault RJ, Li Y, Maattanen P, et al. Altered Toll-like receptor 9 signaling in *Mycobacterium avium* subsp. *paratuberculosis*-infected bovine monocytes reveals potential therapeutic targets[J]. Infection and Immunity, 2013, 81(1): 226−237.

[144] Shepelkova G, Pommerenke C, Alberts R, et al. Analysis of the lung transcriptome in *Mycobacterium tuberculosis*-infected mice reveals major differences in immune response pathways between TB-susceptible and resistant hosts[J]. Tuberculosis (Edinb), 2012, 93(2): 263−269.

[145] Kunnath-Velayudhan S, Salamon H, Wang HY, et al. Dynamic antibody responses to the *Mycobacterium tuberculosis* proteome[J]. Proceedings of the National Academy of Sciences of the United States of America, 2010, 107(33): 14703−14708.

[146] Kunnath-Velayudhan S, Davidow AL, Wang HY, et al. Proteome-scale antibody responses and outcome of *Mycobacterium tuberculosis* infection in nonhuman primates and in tuberculosis patients[J]. The Journal of Infectious Diseases, 2012, 206(5): 697−705.

[147] Axelsson-Robertson R, Magalhaes I, Parida SK, et al. The immunological footprint of *Mycobacterium tuberculosis* T-cell epitope recognition[J]. The Journal of Infectious Diseases, 2012, 205(Suppl 2): S301−315.

[148] Aranday Cortes E, Kaveh D, Nunez-Garcia J, et al. *Mycobacterium bovis*-BCG vaccination induces specific pulmonary transcriptome biosignatures in mice[J]. PLoS One, 2010, 5(6): e11319.

[149] Hammond AS, Klein MR, Corrah T, et al. *Mycobacterium tuberculosis* genome-wide screen exposes multiple CD8 T cell epitopes[J]. Clinical & Experimental Immunolog, 2005, 140(1): 109−116.

[150] Lee BY, Jethwaney D, Schilling B, et al. The *Mycobacterium bovis* bacille Calmette-Guerin phagosome proteome[J]. Molecular & Cellular Proteomics, 2010, 9(1): 32−53.

[151] Hegedus Z, Zakrzewska A, Agoston VC, et al. Deep sequencing of the zebrafish transcriptome response to mycobacterium infection[J]. Molecular Immunology, 2009, 46(15): 2918−2930.

[152] Carvalho RV, Kleijn J, Meijer AH, et al. Modeling innate immune response to early mycobacterium infection[J]. Computational and Mathematical Methods in Medicine, 2012, 2012: 790482.

[153] Chandrasekaran S, Price ND. Probabilistic integrative modeling of genome-scale metabolic and regulatory networks in *Escherichia coli* and *Mycobacterium tuberculosis*[J]. Proceedings of the National Academy of Sciences of the United States of America, 2010, 107(41): 17845−17850.

[154] Raman K, Yeturu K, Chandra N. targetTB: a target identification pipeline for *Mycobacterium tuberculosis* through an interactome, reactome and genome-scale structural analysis[J]. BMC Systems Biology, 2008, 2: 109−130.

[155] Zheng H, Lu L, Wang B, et al. Genetic basis of virulence attenuation revealed by comparative genomic analysis of *Mycobacterium tuberculosis* strain H37Ra versus H37Rv[J]. PLoS One, 2008, 3(6): e2375.

[156] Zhao Y, Xu S, Wang L, et al. National survey of drug-resistant tuberculosis in China[J]. The New England Journal of Medicine, 2012, 366(23): 2161−2170.

[157] Sun G, Luo T, Yang C, et al. Dynamic population changes in *Mycobacterium tuberculosis* during acquisition and fixation of drug resistance in patients[J]. The Journal of Infectious Diseases, 2012, 206(11): 1724−1733.

[158] Wang Y, Cui T, Zhang C, et al. Global protein-protein interaction network in the human pathogen *Mycobacterium tuberculosis* H37Rv[J]. Journal of Proteome Research, 2010, 9(12): 6665−6677.

[159] He Y, Li W, Liao G, et al. *Mycobacterium tuberculosis*-specific phagosome proteome and underlying signaling pathways[J]. Journal of Proteome Research, 2012, 11(5): 2635−2643.

[160] Zheng J, Liu L, Wei C, et al. A comprehensive proteomic analysis of *Mycobacterium bovis* bacillus

Calmette-Guerin using high resolution Fourier transform mass spectrometry[J]. Journal of Proteomics, 2012, 77: 357−371.

[161] Wang X, Wang H, Xie J. Genes and regulatory networks involved in persistence of *Mycobacterium tuberculosis*[J]. Science China Life Sciences, 2011, 54(4): 300−310.

[162] Tao J, Han J, Wu H, et al. *Mycobacterium fluoroquinolone* resistance protein B, a novel small GTPase, is involved in the regulation of DNA gyrase and drug resistance[J]. Nucleic Acids Research, 2013, 41(4): 2370−238.

[163] Wu J, Zhang Z, Mitchenall LA, et al. The dimer state of GyrB is an active form: implications for the initial complex assembly and processive strand passage[J]. Nucleic Acids Research, 2011, 39(19): 8488−8502.

[164] Fu G, Wu J, Liu W, et al. Crystal structure of DNA gyrase B′ domain sheds lights on the mechanism for T-segment navigation[J]. Nucleic Acids Research, 2009, 37(17): 5908−5916.

[165] Long Q, Li W, Du Q, et al. *gyrA/B* fluoroquinolone resistance allele profiles amongst *Mycobacterium tuberculosis* isolates from mainland China[J]. International Journal of Antimicrobial Agents, 2012, 39(6): 486−489.

[166] Shiloh MU, DiGiuseppe Champion PA. To catch a killer. What can mycobacterial models teach us about *Mycobacterium tuberculosis* pathogenesis?[J]. Current Opinion in Microbiology, 2010, 13(1): 86−92.

[167] Temmerman S, Pethe K, Parra M, et al. Methylation-dependent T cell immunity to *Mycobacterium tuberculosis* heparin-binding hemagglutinin[J]. Nature Medicine, 2004, 10(9): 935−941.

[168] Veyron-Churlet R, Zanella-Cleon I, Cohen-Gonsaud M, et al. Phosphorylation of the *Mycobacterium tuberculosis* beta-ketoacyl-acyl carrier protein reductase MabA regulates mycolic acid biosynthesis[J]. The Journal of Biological Chemistry, 2010, 285(17): 12714−12725.

[169] Wilkinson KA, Newton SM, Stewart GR, et al. Genetic determination of the effect of post-translational modification on the innate immune response to the 19 kDa lipoprotein of *Mycobacterium tuberculosis*[J]. BMC Microbiology, 2009, 9: 93−103.

[170] Xu H, Hegde SS, Blanchard JS. Reversible acetylation and inactivation of *Mycobacterium tuberculosis* acetyl-CoA synthetase is dependent on cAMP[J]. Biochemistry, 2011, 50(26): 5883−5892.

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稿件书写规范

专论与综述论文的撰写要点

专论与综述是本刊重要栏目之一，主要反映国内外微生物学及相关领域学科研究最新成果和进展，其内容要求新颖丰富，观点明确，论述恰当，应包含作者自己的工作内容和见解。因此，作者在动笔之前必须明确选题，一般原则上应选择在理论和实践中具有重要意义的学科专题进行论述。围绕专题所涉及的各个方面，在综合分析和评价已有资料基础上提出其演变规律和趋势，即掌握其内在的精髓，深入到专题研究的本质，论述其发展前景。作者通过回顾、观察和展望，提出合乎逻辑并具有启迪性的看法和建议。另外，作者也可以采用以汇集文献资料为主的写作方法，辅以注释，客观而有少量评述，使读者对该专题的过去、现在和将来有一个全面、足够的认识。

需要特别说明的是: (1) 我刊要求作者投稿时在正文前写上主要作者的简介，并指出自己的工作(已发表的文章)在综述中的体现。(2) 在专论与综述中引用的文献应该主要是近 5 年国内外正式发表的研究论文，引用文献数量不限。