微生物学报
Acta Microbiologica Sinica
2023, 63(8): 2948–2966
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20220896

Review 综 述

结核分枝杆菌抗原蛋白的研究及其应用新进展

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陈莹, 徐平, 戴二黑, 张瑶. 结核分枝杆菌抗原蛋白的研究及其应用新进展[J]. 微生物学报, 2023, 63(8): 2948-2966.
CHEN Ying, XU Ping, DAI Erhei, ZHANG Yao. Effective antigens of *Mycobacterium tuberculosis*[J]. Acta Microbiologica Sinica, 2023, 63(8): 2948-2966.

摘　要：结核病(tuberculosis, TB)是由结核分枝杆菌(*Mycobacterium tuberculosis*, MTB)感染引起的慢性传染病，是仅次于正在暴发的新型冠状病毒肺炎(COVID-19)的第二大单一感染致死病因。COVID-19 的大流行对 TB 的诊断及治疗造成了破坏性的影响，全球实现终结 TB 目标的进展偏离了轨道。因此，早诊断、早治疗依然是防控 TB 蔓延的关键。TB 精准诊断一直受 MTB 抗原特异性、检测技术特异性和灵敏度的影响，因此亟需挖掘高特异性新抗原、开发新检测技术。随着蛋白质基因组学(proteogenomics)和质谱技术的快速发展，从临床体液、组织样本中高效、精准靶向检测 MTB 特异性已知、甚至新抗原的表达，以及监测治疗过程中的抗原表达量的动态变化，是TB诊断及治疗的发展趋势。在MTB标准菌株H37Rv的4 008个注释基因中(NC_000 962.3, NCBI)，国内外报道的已注释抗原虽有 140 多个，但仅有极少的抗原应用于 TB 的筛查及辅助诊断，离世界卫生组织(World Health Organization, WHO)的诊断标准尚远。本文通过对 MTB 已报道抗原以及基于蛋白质基因组学筛选特异性新抗原的潜力进行综述，为理解已知抗原及开发新抗原提供参考。

关键词：结核分枝杆菌；结核病；诊断；抗原；血清学；蛋白质基因组学

资助项目：京津冀基础研究合作专项(J200001)；国家自然科学基金(32141003，31901037)
This work was supported by the Beijing-Tianjin-Hebei Basic Research Cooperation Project (J200001) and the National Natural Science Foundation of China (32141003, 31901037).
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Received: 2022-12-03; Accepted: 2023-02-22; Published online: 2023-03-02

Effective antigens of *Mycobacterium tuberculosis*

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Abstract: Tuberculosis (TB), the second (after COVID-19) deadliest infectious killer, is caused by *Mycobacterium tuberculosis* (MTB). COVID-19 pandemic has shown devastating effect on the diagnosis and treatment of TB, posing a huge challenge to the ending of TB. Thus, early diagnosis and treatment is still the key to prevention and control of TB spread. The accurate diagnosis of TB depends on the specificity of MTB antigen and the specificity and sensitivity of detection techniques. Therefore, it is urgent to develop highly specific antigens and detection techniques. The advanced proteogenomics and mass spectrometry make it easy to detect known or new MTB-specific antigens from clinical body fluids and tissue samples and monitor the dynamic expression of antigen during treatment. Among 4 008 annotated genes of MTB in NCBI (NC_000 962.3), more than 140 known genes have been listed as potential antigens for TB diagnosis, while only a few annotated antigens have been used in the screening and auxiliary diagnosis of TB, which are still far from the WHO diagnostic standards. In this paper, we reviewed the reported antigens of MTB and the potential of screening specific neoantigens based on proteogenomic technologies for better understanding them and developing new efficient antigens.

Keywords: *Mycobacterium tuberculosis*; tuberculosis; diagnosis; antigens; serology; proteogenomics

结核分枝杆菌复合体(*Mycobacterium tuberculosis* complex, MTBC)是导致动物和人类结核病(tuberculosis, TB)的相关物种[1]，包括结核分枝杆菌(*M. tuberculosis*, MTB)、非洲分枝杆菌(*M. africanum*)、牛分枝杆菌(*M. bovis*)、微小分枝杆菌(*M. microti*)、卡内蒂分枝杆菌(*M. canettii*)、山羊分枝杆菌(*M. caprae*)、海豹分枝杆菌(*M. pinnipedii*)、猫鼬分枝杆菌(*M. suricattae*)、獴分枝杆菌(*M. mungi*)、蹄兔分枝杆菌(*M. dassie*)和羚羊分枝杆菌(*M. oryx*)等[1-3]，其中 MTB 是人类 TB 的主要病原体。因 MTBC 中各物种间编码基因数量及基因相似性高达 99%及以上[3]，MTBC 新抗原及诊断新技术的开发将有助于提高人和动物 TB 的精准诊断及防控。

MTB 是兼性胞内菌，主要通过气溶胶进行传播，感染宿主后，感染程度因个人免疫力不同，导致感染程度有异，如(1) 被宿主免疫系统完全清除；(2) 处于潜伏感染阶段(latent tuberculosis infection, LTBI)，宿主对 MTB 的清

除作用与 MTB 对宿主的抵抗作用处于相对平衡状态，WHO 定义为“对 MTB 的持久免疫反应状态，但无活动性结核病(active tuberculosis, ATB)的临床证据”；(3) MTB 在宿主体内迅速增殖，宿主的抵抗能力失衡[4-5]，发展为 ATB。最新世界卫生组织(World Health Organization, WHO)报告显示全球约有四分之一的人，即 20 亿人感染了 MTB，其中 5%–10%的潜伏感染者会在生命过程中罹患 TB[6]。因此，对 TB 尤其是高危潜伏感染者的早发现、早诊断、早治疗，可阻止 TB 的进一步发展及传播。临床体液样本中特异性抗原的快速检测技术一直备受青睐，其中研究最多的是基于血清学的诊断方法，研究者们通过筛查临床 TB 患者和健康人血清的抗体反应，或基于有效抗原的特异性酶切肽段进行磁珠富集，评估 MTB 抗原的免疫原性。本文就 MTB 血清学诊断中有效已知抗原、检测技术及新抗原的筛选研究进展进行综述。

1 与 TB 诊断相关的抗原

1.1 RD 区抗原

预防 TB 的 BCG 疫苗，是 *M. bovis* 经连续传代获得的减毒株，在传代过程中缺失了十多个基因簇，这些基因簇被称为差异区域(region of difference, RD)[7]。已报道的 RD 区有 16 个，即 RD 1–16，由 129 个开放阅读框(open reading frame, ORF)组成，其中 91 个 ORF 属于 MTB 特异性的，另 38 个缺失的 ORF 仅存在于 BCG 基因组中[7]。基于血清学样本，国内外已报道的具有抗原性的 RD 区蛋白有 38 个，其中 RD1 和 RD2 区的抗原居多(表 1)，研究发现 RD1 区基因编码的蛋白还与 MTB 的毒力相关[27]，可能参与感染及致病过程。

目前研究及应用最多的两个抗原 CFP-10 (10 kDa culture filtrate antigen CFP-10)和 ESAT-6 (6 kDa early secretory antigenic target)均来自于 RD1 区，其中 CFP-10 由 *Rv3874* 编码，ESAT-6 则由 *Rv3875* 编码，可诱导 T 淋巴细胞产生较高 INF-γ[28]，这两个抗原或与其他抗原的组合已应用于国内外多种 TB 诊断商用试剂盒中。在疫苗研制方面，在目前正在进行临床试验的减毒活疫苗 MTBVAC 中，保留了 ESAT-6 和 CFP-10 这两种可激发 T 淋巴细胞反应的表位，具有更好的免疫潜力。RD2 区中研究应用较为广泛的是 *Rv1980c* 编码的 MPT-64 (major protein antigen 64, MPT-64)与 *Rv1984c* 编码的 CFP-21。MPT-64 能诱发机体产生体液免疫应答和细胞免疫应答，是 RD2 区较理想的抗原[29]。CFP-21 是 MTB 早期培养滤液蛋白的重要组分之一，具有酯酶活性，可诱导 Th1 和 Th2 反应[30]，具有诊断潜力。此外，除 RD3 区和 RD15 区的抗原研究较少，其他 RD 区的抗原均有报道。

1.2 PE/PPE 蛋白家族

PE/PPE 家族蛋白 N-末端富含脯氨酸-谷氨酸(Pro-Glu, PE)或脯氨酸-脯氨酸-谷氨酸(Pro-Pro-Glu, PPE)基序，是 MTB 特有的蛋白家族，约占 MTB 总注释蛋白的 10% [2]，可以调控 MTB 的毒力[31]。PE/PPE 家族共有 168 个蛋白，包括 99 个 PE 蛋白和 69 个 PPE 蛋白[32]。PE 蛋白分 2 个亚类，包括 PE-PGRS (polymorphic GC-rich-repetitive sequence)亚家族和具有低同源性 C 末端结构域的 PE 亚家族；PPE 蛋白家族分 4 个亚家族，包括 PPE-SVP (含有 Gly-X-X-Ser-Val-Pro-X-X-Trp 基序)、PPE-MPTR (含有 Asn-X-Gly-X-Gly-Asn-X-Gly 基序)、PPE-PPW (含有 Gly-Phe-X-Gly-Thr or Pro-X-X-Pro-X-X-Trp 基序)和具有低同源性 C 末端结构域的 PPE 亚家族[32]。PE/PPE 家族具有抗原性的蛋白有 17 个，约占 PE/PPE 家族蛋白总数的 10% (表 2)。其中

表 1 RD 区抗原在 TB 血清学诊断中的灵敏度和特异度

Table 1 Sensitivities and specificities of RD region antigens in the serological diagnosis of TB

RD	Gene	Description	Number of sample	Sensitivity (%)	Specificity (%)	Verification*	References
RD1 (8/9)	*Rv3871*	ESX-1 secretion system protein EccCb1	393	81	75	No	[8]
	Rv3872	PE family immunomodulator PE35	542	91	95	No	[9]
	Rv3873	PPE family immunomodulator PPE68	137	28	95	No	[10]
	Rv3874	10 kDa culture filtrate antigen CFP-10	143	63	95	No	[8]
	Rv3875	6 kDa early secretory antigenic target	143	32	87	No	[8]
	Rv3876	ESX-1 secretion-associated protein EspI	393	62	85	No	[8]
	Rv3878	ESX-1 secretion-associated protein EspJ	542	85	91	No	[9]
	Rv3879c	ESX-1 secretion-associated protein EspK	393	84	83	No	[8]
RD2 (5/11)	*Rv1978*	Conserved protein	105	67	90	No	[11]
	Rv1980c	Immunogenic protein MPT64	106	84	76	No	[11]
	Rv1981c	Ribonucleoside-diphosphate reductase subunit beta nrdF1	109	57	94	No	[11]
	Rv1984c	Carboxylesterase Culp1	108	53	93	No	[11]
	Rv1985c	HTH-type transcriptional regulator LysG	106	58	97	No	[11]
RD4 (1/3)	*Rv0222*	Probable enoyl-CoA hydratase EchA1	156	87	80	No	[12]
RD5 (5/5)	*Rv3117*	Putative thiosulfate sulfurtransferase	92	25	97	No	[13]
	Rv3118	Uncharacterized protein Rv3118	92	12	91	No	[13]
	Rv3119	Molybdopterin synthase catalytic subunit 1	92	12	97	No	[13]
	Rv3120	Methyltransferase domain-containing protein	92	32	97	No	[13]
	Rv3121	Putative cytochrome P450 141	92	5	88	No	[13]
RD6 (3/11)	*Rv1508c*	Probable membrane protein	200	93	72	No	[14]
	Rv1512	GDP-L-fucose synthase	–	–	–	No	[15]
	Rv1516c	Probable sugar transferase	200	66	90	No	[14]
RD7 (2/8)	*Rv2351c*	Phospholipase C A	159	61	91	No	[16]
	Rv2352c	Uncharacterized PPE family protein PPE38	–	–	–	No	[17]
RD8 (2/4)	*Rv0309*	Possible conserved exported protein	–	–	–	No	[18]
	Rv0310c	Conserved protein	256	70	86	No	[19]
RD9 (2/7)	*Rv3618*	Possible monooxygenase	489	76	83	No	[20]
	Rv3621c	Uncharacterized PPE family protein PPE65	489	52	81	No	[20]
RD10 (1/3)	*Rv1255c*	Uncharacterized HTH-type transcriptional regulator *Rv1255c*	408	74	83	No	[19]
RD11 (3/5)	*Rv3425*	PPE family protein PPE57	111	56	100	No	[11]
	Rv3428c	Putative transposase *Rv3428c*	472	68	88	No	[21]
	Rv3429	Uncharacterized PPE family protein PPE59	111	47	93	No	[11]
RD12 (1/4)	*Rv2073c*	Uncharacterized oxidoreductase *Rv2073c*	–	–	–	No	[22]
RD13 (1/16)	*Rv2645*	Uncharacterized protein *Rv2645*	107	90	98	No	[23]
RD14 (1/8)	*Rv1768*	PE-PGRS family protein PE_PGRS31	121	91	97	No	[24]
RD16 (3/6)	*Rv3400*	Uncharacterized protein *Rv3400*	–	–	–	No	[25]
	Rv3403c	Uncharacterized protein *Rv3403c*	156	87	80	No	[12]
	Rv3405c	HTH-type transcriptional repressor *Rv3405c*	285	62	68	No	[26]

*: Verification means whether there is a validation cohort in the study. The number in parentheses are reported/annotated genes. –: Not mentioned in the study.

表 2　PE/PPE 蛋白在 TB 血清学诊断中的灵敏度和特异度

Table 2　Sensitivities and specificities of PE/PPE protein in the serological diagnosis of TB

PE/PPE	No. of PE/PPE	Gene	Description	Number of sample	Sensitivity (%)	Specificity (%)	Verification*	References
PE	PE35	*Rv3872*	PE family immunomodulator PE35	90	78	83	No	[33]
	PE_PGRS26	*Rv1441c*	PE-PGRS family protein PE_PGRS26	240	58	84	No	[34]
	PE_PGRS48	*Rv2853*	PE-PGRS family protein PE_PGRS48	285	31	41	No	[27]
	PE_PGRS52	*Rv3388*	PE-PGRS family protein PE_PGRS52	176	97	61	No	[35]
PPE	PPE3	*Rv0280*	Uncharacterized PPE family protein PPE3	240	–	–	No	[34]
	PPE17	*Rv1168c*	PPE family protein PPE17	90	63	83	No	[36]
	PPE18	*Rv1196*	PPE family protein PPE18	290	77	78	No	[37]
	PPE19	*Rv1361c*	Uncharacterized PPE family protein PPE19	290	59	92	No	[37]
	PPE36	*Rv2108*	Uncharacterized PPE family protein PPE36	90	67	87	No	[36]
	PPE37	*Rv2123*	Uncharacterized PPE family protein PPE37	90	65	99	No	[36]
	PPE41	*Rv2430c*	PPE family protein PPE41	90	68	87	No	[36]
	PPE57	*Rv3425*	PPE family protein PPE57	90	73	80	No	[36]
	PPE58	*Rv3426*	Uncharacterized PPE family protein PPE58	90	65	93	No	[36]
	PPE59	*Rv3429*	Uncharacterized PPE family protein PPE59	90	80	83	No	[36]
	PPE64	*Rv3558*	PPE family protein PPE64	90	63	90	No	[36]
	PPE68	*Rv3873*	PPE family immunomodulator PPE68	90	81	71	No	[33]
	PPE69	*Rv3892c*	Uncharacterized PPE family protein PPE69	90	73	83	No	[36]

*: Verification means whether there is a validation cohort in the study. The number in parentheses are reported/annotated genes. –: Not mentioned in the study.

Rv3425 (PPE57)、*Rv3429* (PPE59)、*Rv3872* (PE35)和 *Rv3873* (PPE68)也属于 RD 区抗原。

1.3　LTBI 诊断相关抗原

LTBI 和 ATB 属于 MTB 感染的不同阶段，若能对 LTBI、尤其是高危 LTBI 进行提前预警，将会有效阻止 TB 的进展。有研究者通过体外模拟 MTB 潜伏感染环境，来鉴定 LTBI 阶段病原菌和宿主差异表达的基因、蛋白质等[38-39]，进而探索 LTBI 的相关抗原，来寻找能表征 LTBI 的标志物。目前国内外已报道的可用于 LTBI 筛查的抗原共有 48 个(表 3)。

MTB 能够在营养匮乏的平台期或宿主体内持留，主要是由休眠生存调节基因 *Rv3133c* (dormancy-related, DosR)调节的[58]，常被视为 LTBI 较好的潜在生物标志物。*Rv0475* 编码的肝素结合血凝素(heparin-binding haemagglutinin adhesin, HBHA)可以凝集红细胞并聚集 MTB，TB 患者对纯化的甲基化 HBHA 能产生强烈的

表 3 LTBI 相关抗原在 TB 血清学诊断中的灵敏度和特异度

Table 3 Sensitivities and specificities of LTBI-related antigens in the serological diagnosis of TB

Gene	Description	Number of sample	Sensitivity (%)	Specificity (%)	Verification*	References
Rv0009	Peptidyl-prolyl cis-trans isomerase A	47	–	–	No	[40]
Rv0079	Dormancy associated translation inhibitor	–	–	–	No	[41]
Rv0140	Conserved protein	–	–	–	No	[42]
Rv0251c	Heat shock protein Hsp (Heat-stress-induced ribosome-binding protein A)	109	88	63	No	[43]
Rv0351	Protein GrpE	240	–	–	No	[34]
Rv0475	Heparin-binding hemagglutinin	235	92	94	No	[44]
Rv0494	Uncharacterized HTH-type transcriptional regulator *Rv0494*	240	64	71	No	[34]
Rv0569	Uncharacterized protein *Rv0569*	–	–	–	No	[45]
Rv0570	Vitamin B12-dependent ribonucleoside-diphosphate reductase	–	–	–	No	[46]
Rv0753c	Probable methylmalonate-semialdehyde dehydrogenase MmsA (Methylmalonic acid semialdehyde dehydrogenase, MMSDH)	47	–	–	No	[47]
Rv0867c	Resuscitation-promoting factor RpfA	–	–	–	No	[48]
Rv1009	Resuscitation-promoting factor RpfB	–	–	–	No	[42]
Rv1733c	Probable membrane protein *Rv1733c*	–	–	–	No	[45]
Rv1734	–	–	–	–	No	[42]
Rv1737	–	175	88	84	No	[49]
Rv1738	Uncharacterized protein *Rv1738*	–	–	–	No	[45]
Rv1813c	Uncharacterized protein *Rv1813c*	–	–	–	No	[45]
Rv1821	Protein translocase subunit SecA 2	240	–	–	No	[34]
Rv1860	Alanine and proline-rich secreted protein Apa	180	53	95	No	[40]
Rv1884c	Resuscitation-promoting factor RpfC	–	–	–	No	[42]
Rv1932	Thiol peroxidase	109	81	67	No	[43]
Rv1996	Universal stress protein *Rv1996*	–	–	–	No	[45]
Rv2003	–	–	–	–	No	[42]
Rv2004c	Uncharacterized protein *Rv2004c*	–	–	–	No	[46]
Rv2005c	Universal stress protein *Rv2005c*	–	–	–	No	[42]
Rv2006	Uncharacterized glycosyl hydrolase *Rv2006*	–	–	–	No	[42]
Rv2028c	Universal stress protein *Rv2028c*	–	85	87	No	[50]
Rv2029c	ATP-dependent 6-phosphofructokinase isozyme 2	–	84	80	No	[51]
Rv2030c	Uncharacterized protein *Rv2030c*	–	–	–	No	[45]
Rv2031c	Alpha-crystallin	279	75	85	No	[52]
Rv2032	Putative NAD(P)H nitroreductase acg	–	–	–	No	[45]
Rv2204c	Protein *Rv2204c*	47	–	–	No	[47]
Rv2389c	Resuscitation-promoting factor RpfD	–	–	–	No	[48]
Rv2450c	Resuscitation-promoting factor RpfE	–	–	–	No	[42]
Rv2626c	Hypoxic response protein 1	244	77	85	No	[53]
Rv2627c	Uncharacterized protein *Rv2627c*	–	–	–	No	[45]
Rv2628	Putative uncharacterized protein *Rv2628*	258	75	64	No	[54]

(待续)

(续表 3)

Gene	Description	Number of sample	Sensitivity (%)	Specificity (%)	Verification*	References
Rv2659c	Putative prophage phiRv2 integrase	–	55	81	No	[51]
Rv2660	–	–	–	–	No	[55]
Rv2986c	DNA-binding protein HU homolog	–	–	–	No	[56]
Rv3132c	Oxygen sensor histidine kinase response regulator DevS/DosS	–	–	–	No	[45]
Rv3133c	DNA-binding transcriptional activator DevR/DosR	–	–	–	No	[45]
Rv3301c	Phosphate-specific transport system accessory protein PhoU homolog 1	240	–	–	No	[34]
Rv3407	Antitoxin VapB47	–	–	–	No	[42]
Rv3126c	Uncharacterized protein *Rv3126c*	–	–	–	No	[45]
Rv3129	Uncharacterized protein *Rv3129*	–	–	–	No	[45]
Rv3130c	Probable diacyglycerol O-acyltransferase tgs1	–	–	–	No	[45]
Rv3716c	Nucleoid-associated protein *Rv3716c*	–	–	–	No	[57]

*: Verification means whether there is a validation cohort in the study. The number in parentheses are reported/annotated genes. –: Not mentioned in the study.

体液反应，使得 HBHA 蛋白的 T 细胞抗原成为 ATB 和 LTBI 的诊断标志物[59]。而 *Rv2031c* (16 kDa) 含有能够被 T 细胞特异性识别的抗原表位，在 MTB 缺氧时被显著激活，对潜伏期 MTB 的生存维持起重要作用，同样重要的是该蛋白在机体感染 MTB 早期就能被检测到，是一个潜在的潜伏感染标志物[60]。此外，*Rv2986c* 编码的 DNA 结合蛋白 1 (mycobacterial DNA binding protein 1, MDP1)也在 MTB 的休眠和潜伏适应过程中起作用，具有作为潜伏感染标志物的潜力[61]。

1.4 其他抗原

目前研究较深以及应用较广泛的诊断 TB 的潜在标志物还包括 *Rv0934c* (38 kDa)、*Rv1837c* (Mtb81)、Ag85 复合物和脂阿拉伯甘露聚糖(lipoarabinomannan, LAM)等。国内外已报道的此类抗原共 42 个(表 4)。

Rv0934c (38 kDa/PstS-1) 编码的蛋白为 MTB 特异抗原，属于磷酸盐转运相关的膜脂蛋白，也是目前最常用的 TB 血清学诊断标志物之一，可诱导 $CD4^+$ T 细胞增殖，并诱导高水平的干扰素和免疫球蛋白(Ig) G2a 的表达[74]，常与其他抗原联合使用，在不降低其他抗原特异度的前提下，能够提高抗原组合检测 TB 的灵敏度。*Rv1837c* (Mtb81)抗原是一种苹果酸盐合酶蛋白，能在 TB 患者体内产生强烈的免疫应答[70]。Ag85 复合物是 MTB 中期培养过程中主要的分泌蛋白家族之一，主要由 *Rv3804c* (Ag85A)、*Rv1886c* (Ag85B)和 *Rv0129c* (Ag85C) 这 3 个抗原组成，在诱导机体产生免疫反应的过程中发挥着重要作用。Ag85B 是一种免疫优势抗原，可引发宿主对 MTB 产生强烈的免疫应答，并在宿主体液中产生大量的 IgG 抗体。LAM 是从 MTB 细胞壁中分离出的一种血清活性多糖，可抑制巨噬细胞对 MTB 的杀伤作用，可以刺激人体产生 LAM 抗体，在 MTB 感染机体期间，LAM 可以作为一种潜在的生物标志物来识别机体的感染状态，除血液外，尿液等其他体液中的 LAM 抗原也在临床研究之中[75-76]。

表 4　其他抗原在 TB 血清学诊断中的灵敏度和特异度

Table 4　Sensitivities and specificities of other protein in the serological diagnosis of TB

Gene	Description	Number of sample	Sensitivity (%)	Specificity (%)	Verification*	References
Rv0054	Single-stranded DNA-binding protein	285	52	57	No	[27]
Rv0057	Uncharacterized protein *Rv0057*	129	–	–	No	[62]
Rv0129c	Diacylglycerol acyltransferase/mycolyltransferase Ag85C	366	42	64	No	[63]
Rv0183	Monoacylglycerol lipase	519	88	73	No	[64]
Rv0288	ESAT-6-like protein EsxH	258	70	69	No	[54]
Rv0350	Chaperone protein DnaK	258	57	81	No	[54]
Rv0432	Superoxide dismutase [Cu-Zn]	258	88	66	No	[54]
Rv0446c	Possible conserved transmembrane protein	258	74	41	No	[54]
Rv0566c	UPF0234 protein *Rv0566c*	200	43	84	No	[65]
Rv0652	50S ribosomal protein L7/L12	339	18	97	No	[66]
Rv0658c	Probable conserved integral membrane protein	258	78	44	No	[54]
Rv0674	PaaX domain-containing protein, C-domain protein	258	64	75	No	[54]
Rv0819	Mycothiol acetyltransferase	103	65	100	No	[67]
Rv0831c	Conserved protein	285	77	72	No	[27]
Rv0865	Probable molybdopterin biosynthesis Mog protein	258	72	64	No	[54]
Rv0934	Phosphate-binding protein PstS 1	489	82	67	No	[21]
Rv0948c	Intracellular chorismate mutase	285	69	28	No	[27]
Rv1352	Uncharacterized protein *Rv1352*	129	–	–	No	[62]
Rv1419	Uncharacterized protein *Rv1419*	–	–	–	No	[68]
Rv1547	DNA polymerase III subunit alpha	258	62	74	No	[54]
Rv1566c	Possible Inv protein	258	47	79	No	[54]
Rv1636	Universal stress protein *Rv1636*	339	31	97	No	[66]
Rv1827	Glycogen accumulation regulator GarA	339	13	97	No	[66]
Rv1837c	Malate synthase G	196	56	100	No	[69]
Rv1886c	Diacylglycerol acyltransferase/mycolyltransferase Ag85B	366	70	78	No	[63]
Rv1926c	Immunogenic protein MPT63	555	83	93	No	[70]
Rv2041c	Probable sugar-binding lipoprotein	103	70	90	No	[22]
Rv2185c	Conserved protein TB16.3	339	55	100	No	[66]
Rv2376c	Low molecular weight antigen MTB12	418	53	95	No	[71]
Rv2318	Probable periplasmic sugar-binding lipoprotein UspC	258	40	88	No	[54]
Rv2873	Cell surface glycolipoprotein MPT83	258	55	69	No	[54]
Rv3097c	Triacylglycerol lipase	155	–	–	No	[72]
Rv3221c	Biotinylated protein TB7.3	339	10	100	No	[66]
Rv3354	DUF732 domain-containing protein	339	34	100	No	[66]
Rv3369	Conserved protein	200	60	96	No	[65]
Rv3418c	Co-chaperonin GroES	258	54	74	No	[54]
Rv3452	Phospholipase Culp4	155	–	–	No	[72]
Rv3544c	Acyl-CoA dehydrogenase FadE28	285	54	13	No	[27]
Rv3803c	MPT51/MPB51 antigen	244	69	76	No	[53]
Rv3804c	Diacylglycerol acyltransferase/mycolyltransferase Ag85A	366	65	75	No	[63]
Rv3914	Thioredoxin	69	–	–	No	[73]
LAM	Lipoarabinomannan	109	71	87	No	[43]

*: Verification means whether there is a validation cohort in the study. The number in parentheses are reported/annotated genes. –: Not mentioned in the study.

2 抗原用于血清学诊断的方法或优化

2.1 多抗原组合物

MTB 编码蛋白数高达四千多个,部分编码蛋白在不同的宿主体内呈现出不同程度的抗原性,因此使用单一抗原在诊断中会有偏差,而融合抗原或组合抗原的使用,可以彼此互补,有效提高检测的特异度和灵敏度,进而提高 TB 的诊断效率[13]。目前已报道的 MTB 组合抗原有 2–8 种不等[9-10,20-21,24-25,34-35,40,63,68,71,77-85],诊断 TB 的灵敏度为 44.4%–95%,特异度为 29.7%–100%,对不同组合抗原的灵敏度和特异度比较后发现,灵敏度和特异度并非随着组合抗原数的递增而升高,但是这些数据有待实验验证。

目前国内外报道的具有诊断潜力的 MTB 抗原较多,样本量在 47 至 1 069 例不等,单抗原和组合抗原的总体灵敏度为 5%至 96.8%,总体特异度为 13%至 100%,然而这些抗原目前均未被纳入 WHO 诊断标准中。

2.2 基于 TB 有效抗原的诊断体系优化

基于 MTB 有效抗原开发的新型检测技术,有助于提高 TB 的诊断效率。Liu 等[86]使用 NanoDisk-MS 技术提高了血清样品中 2 个常用抗原 CFP-10 和 ESAT-6 的代表性酶切肽段检出率,实现血液中抗原的量化检测。Soo 等[87]用 RD9 区的 *Rv3618* 为检测靶标,开发了一种多重嵌套 PCR-ICT (免疫色谱测试)测定法,不仅可以同时直接检测 MTBC,还可以检测临床痰标本中的 MTB,之后,其团队又开发出金纳米颗粒探针来检测 MTBC 和 MTB[88],检测 MTBC 的灵敏度为 96.6%,特异度为 98.9%,检测 MTB 的灵敏度为 94.7%,特异度为 99.6%。Yari 等[89]在大分子抗原复合物(如 A60)基础上进行优化、纯化出新型 A60 抗原(PA60-Ag),这些抗原的抗体在 TB 诊断时呈现较高的敏感度和特异度。

2.3 TB 抗原诊断试剂盒

MTB 有效新抗原已被应用到临床试剂盒的开发及应用中,仅我国获批的 TB 检测商业试剂盒就多达几十种。其检测方法有:胶体金法、酶联免疫吸附法、免疫层析法、免疫荧光法、固相酶联免疫斑点法、化学发光法等,这些试剂盒的优势在于操作简便、结果显示快速、易于在基层医院推广。抗体检测也存在一定的局限性,原因在于:(1) MTB 与非结核分枝杆菌(non-tuberculous *Mycobacteria*, NTM)同源性较高,导致难以精准区分这两类分枝杆菌,检测结果的假阳性也较高;(2) 由于机体中特异性免疫复合物的形成或者机体免疫功能异常,也会导致检测结果的假阴性;(3) 目前尚无结核相关抗体能准确区分 ATB 与 LTBI;(4) 目前尚无抗体能对 MTB 感染进程及抗结核治疗疗效进行有效评估。

然而,目前销售的试剂盒虽多,但暂时尚无满足 WHO 指南要求的商业化 TB 临床诊断试剂盒面世,因此,亟需开发特异、高效的标志物以及新技术来达到 WHO 的标准。

3 已知 MTB 抗原特征性肽段与 NTM 抗原肽段存在高同源性

随着质谱技术的快速发展,在临床体液样本中靶向检测 TB 抗原或 TB 抗原特征性肽段的技术逐渐成熟,尽管 Liu 等[86]基于 CFP-10 和 ESAT-6 的特征性酶切肽段 TDAATLAQEAGNFER 和 WDATATELNNALQNLAR,开发了 NanoDisk-MS 技术,但这两条肽段在多个 NTM 中的序列完全一致(表 5),在临床筛查中可能会导致误诊,回避不了 MTB 有效抗原特异性不够这个关键性问题。因此,MTB 特异性新抗原的挖掘是

表 5 CFP-10 和 ESAT-6 特征性肽段在不同分枝杆菌中的序列比较

Table 5 Sequence comparison of characteristic peptides of CFP-10 and ESAT-6 in different *Mycobacterium*

Antigen	No.	Species	Tryptic peptides*
CFP-10	1	*M. tuberculosis H37Rv*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	2	*M. attenuatum*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	3	*M. basiliense*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	4	*M. bovis*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	5	*M. decipiens*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	6	*M. innocens*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	7	*M. kansasii*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
			MAEM**K**TDAATLAQEAGNFE**R**ISGDM
	8	*M. lacus*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	9	*M. liflandii*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	10	*M. marinum*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	11	*M. novocastrense*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	12	*M. orygis*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	13	*M. persicum*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	14	*M. pinnipedii*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	15	*M. pseudokansasii*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	16	*M. riyadhense*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	17	*M. shinjukuense*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	18	*M. simiae*	MAAM**K**TDAATLAQEAGNFE**R**ISGDL
	19	*M. simulans*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	20	*M. shottsii*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	21	*M. spongiae*	MAEM**R**TDAATLAQEAGNFE**R**ISGDL
	22	*M. ulcerans*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	23	*M. riyadhense*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
	24	*M. ulcerans*	MAEM**K**TDAATLAQEAGNFE**R**ISGDL
ESAT-6	1	*M. tuberculosis*	GVQQ**K**WDATATELNNALQNLA**R**TISEA
	2	*M. bovis*	GVQQ**K**WDATATELNNALQNLA**R**TISEA
	3	*M. orygis*	GVQQ**K**WDATATELNNALQNLA**R**TISEA
	4	*M. simiae*	GVQY**K**WDATATELNNALQNLA**R**KISEA
	5	*M. shinjukuense*	GVQQ**K**WDATATELNNALQNLA**R**TISEA
	6	*Macacine betaherpesvirus*	GVQQ**K**WDATATELNNALQNLA**R**TISEA

*: The bolded letters (K and R) in the aligned sequence indicates potential tryptic cleavage sites.

业界亟待解决的核心难题，任何进步都将为 TB 临床诊断技术的发展和试剂盒的研制提供原始创新性成果和技术支撑。

4 TB 病人体液外泌体中 MTB 抗原的研究

外泌体是一种由多泡体产生的纳米级细胞外囊泡，从大多数有核细胞中释放出来，富含蛋白质、脂质、DNA 和 RNA 等成分，被 MTB 感染的巨噬细胞释放的外泌体可以携带 MTB 成分，包括 LAM、19 kDa 脂蛋白和几十种细菌蛋白等，其中许多是已知的免疫显性抗原，具有作为 TB 生物标志物的潜能[90-91]。Kruh-Garcia 等[92]分析了 ATB 患者血清样品中分离的外泌

体，发现了 Ag85B、Ag85C 等 33 种代表 MTB 的蛋白和 76 种肽段。Dahiya 等[93]在 TB 患者的尿液外泌体中发现了来自 MTB 的抗原 LAM 和 CFP-10。这些研究表明，外泌体中鉴定到的 MTB 蛋白成分可作为检测 TB 的靶标，辅助临床诊断 TB。

5 基于蛋白质基因组学的 MTB 特异性新抗原研究

随着蛋白质组学及其他测序技术的日渐完善，蛋白质基因组学(proteogenomics)技术快速发展，并成为一门新的交叉学科，不仅可解密数据库中尚未注释、甚至数据库不存在的新突变，近年来还被广泛地应用至多种疾病研究中，包括疾病的新分型、疾病进程评估、新标志物、潜在治疗靶点等等[94-96]。作者所在实验室在蛋白质基因组学方面已有多年的工作积累，和中国科学院计算技术研究所贺思敏研究员合作建立了精准蛋白质基因组学技术流程[97]，基于课题组建立的小蛋白质富集技术[98]和开发的镜像酶[99]进一步优化了假阳性控制体系。围绕“MTB 菌株中存在诸多翻译错误、N 端起始密码子错误”的前言问题，本课题组基于 N 末端蛋白质组学技术，矫正了 H37Rv 中 43 个注释错误的 N 端，鉴定并发现了 1 个遗漏注释基因[100]。此外，本课题组基于深度覆盖技术体系，对 H37Rv 菌株系统开展了蛋白质基因组学研究，矫正了 102 个 N 端注释错误，补充完善了 49 个全新编码基因；进一步的比较基因组学研究发现了十多个新基因是 MTBC 特异性基因，进一步 PCR 湿实验发现，这些新基因仅仅在 MTBC 样本的理论大小处扩增到特异性条带，而其他亲缘关系非常近的 44 株 NTM 菌株及其他呼吸道感染性病原菌中均显示阴性结果，意味着这些特异性新基因不仅补充、完善了 MTB 数据库，还可用于 MTBC 与其他病原菌的精准鉴别；部分特异性新基因还有较好的抗原性，可补充诊断现有试剂盒、38 kDa 抗原无法正确诊断的 TB，避免误诊，提高 TB 的诊断效率，为此，本课题组逐个申请了国家发明专利，并获得了授权。

国内外报道的 NTM 感染病例越来越多，但因不少 NTM 物种存在较高同源性，基于 16S rRNA 或其他标志物难以鉴别，会影响临床对症用药，错过最佳治疗时期。因此，对于 NTM 的精准、快速鉴定也尤为重要。蛋白质基因组学技术可以快速、高通量的筛选到非同源性、高丰度、且有编码特征的新肽段或新蛋白质，基于高特异性的新肽段或新蛋白质，可以进一步发展基于质谱的新型技术，用于临床样本检测。脓肿和龟分枝杆菌的精准鉴定一直是业界难题，课题组前期基于蛋白质基因组学技术发现了一个新的标志物，可用于脓肿菌的快速筛选，已申请国家发明专利。

6 展望

MTB 侵袭宿主后，易“欺骗”宿主免疫系统，使得免疫体统攻击体内健康组织，进而发展为不同感染阶段的 LTBI 或 ATB。在 TB 的进展过程中，机体合并有其他疾病或个体免疫力发生变化等因素时，易对细胞因子、宿主蛋白质的表达产生影响，因此，迫切需要高特异性、强灵敏度的标志物以及新型技术体系来实现 LTBI 和 ATB 快速、精准的鉴别及诊断。TB 诊断的金标准是菌体培养，但所需时间较长；涂片抗酸染色法检出率低；分子生物学检测法对检测仪器和操作人员的要求较高；免疫学诊断方法相对较简单、快速、价格便宜，在 TB 的辅助诊断中虽一直备受青睐，尤其是基于血清学的 B 细胞检测方法，但国内外尚无较理想的

血清学检测的高效新抗原，试剂盒的开发也几乎离不开 RD1 区的 2 个常用分泌性抗原。由于测定的方法、使用的抗原不同等，导致检测结果的差异较大，WHO 也对来自不同国家的 19 种试剂盒产品进行评估，结果表明，与痰培养相比，这些试剂盒的检测灵敏度为 1%–60%，特异度为 53%–99%[101]。WHO 认为现有的血清学诊断试剂需进一步提高灵敏度和特异度，且具有较高的假阳性和假阴性，不推荐血清学检测方法诊断 ATB[102]。

自 1998 年 MTB H37Rv 菌株完成了全基因测序[2]，其注释信息被 TubercuList 数据库收录，2008 年，de Souza 等[103]比较了英国的 Sanger 研究所和美国的基因组研究所 TIGR 两家单位对 H37Rv 的重注释结果，发现高达 12%的注释基因数目不同；即使对共同注释的基因，有高达 46%基因的翻译起始位点不同，从侧面反映了 MTB 典型菌株 H37Rv 的基因组注释中存在诸多错误及不完善现象。近年来，蛋白质基因组学被用于 MTB 的重新认识[104-105]、临床分离株的突变位点检测[106]、新型免疫肽的探索[107]等。He 等[108]基于 MTB 蛋白质组微阵列 SOPHIE (systematic unlOcking of pathogen and host interacting effectors)，发明并利用 MTB 蛋白质组芯片，筛选人巨噬细胞裂解液，高通量反向鉴定 MTB 潜在的效应因子。本课题组也基于蛋白质基因组学技术筛选到一系列 MTB 及典型 NTM 菌株特异性全新编码基因，这些新成果为进一步筛选 MTB 特异性新标志物、开发新的 TB 诊断试剂盒及新的 TB 临床诊断技术提供了原始创新性成果和技术支撑。

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