

特约综述



欧阳松应教授, 福建师范大学生命科学学院院长, 博士生导师。目前的研究兴趣主要集中在病原体与宿主天然免疫系统间相互作用的分子机制等方面, 近年来特别关注细胞效应蛋白的功能机制研究。已在 *Nature*、*Cell Res*、*Advanced Science*、*Nat Commun*、*PNAS*、*EMBO J*、*PLoS Biology* 等国际知名杂志上发表学术论文70余篇。

<http://life.fjnu.edu.cn/fl/3b/c4524a127291/page.htm>

糖基化修饰在嗜肺军团菌致病过程中的作用

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摘要 糖基化修饰是由糖基转移酶催化的广泛存在于生物体内的一种蛋白质翻译后修饰, 其与致病菌的毒力密切相关。嗜肺军团菌是一种胞内致病菌, 通过其IVB型分泌系统向宿主细胞转运近300种效应蛋白, 可介导多种蛋白质翻译后修饰, 干扰宿主的细胞进程。其中, Lgt家族、SidI、SetA及LtpM等效应蛋白介导宿主蛋白糖基化修饰, 影响宿主蛋白质翻译及囊泡转运等。该文从军团菌的致病机制、糖基转移酶相关效应蛋白的结构与功能以及糖基化修饰与致病菌致病机制的关系等方面进行综述分析, 为理解糖基化修饰在嗜肺军团菌致病过程中的作用机制提供参考。

关键词 嗜肺军团菌; 致病机制; 糖基化修饰; 糖基转移酶

Role of Glycosylation in Pathogenesis of *Legionella pneumophila*

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Abstract Glycosylation found widely in various organisms is a post-translational modification catalyzed by glycosyltransferase, which is closely related to the virulence of pathogenic bacteria. *Legionella pneumophila*, an intracellular pathogenic bacteria, secretes approximately 300 protein effectors to host cells

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by its IVB secretion system. Those effectors perform various post-translational modifications of host proteins and hijack host cell processes. Effectors with glycosyltransferase activity, like Lgt family, SidI, SetA, and LtpM, etc., mediate glycosylation on host proteins, so that regulating host protein translation and vesicular trafficking. Here, pathogenesis of *L. pneumophila*, structure and function of effectors with glycosyltransferase activity, and relationship of glycosylation and pathogenesis of pathogenic bacteria are reviewed, which will provide a reference for understanding action mechanism of glycosylation in the pathogenic process of *L. pneumophila*.

Keywords *Legionella pneumophila*; pathogenesis; glycosylation; glycosyltransferase

蛋白质糖基化修饰(protein glycosylation modification)是一种普遍存在的蛋白质翻译后修饰,由糖基转移酶催化糖元通过共价键的形式连接到蛋白质氨基酸的侧链,在各种细胞功能中发挥着重要作用^[1]。蛋白质糖基化有两种主要的修饰类型:*N*-糖基化(*N*型)和*O*-糖基化(*O*型)^[2-3]。在经典的*N*型糖基化中,糖元被糖基转移酶转移至精氨酸侧链的酰胺氮(-NH₂);在*O*型糖基化中,糖元则被转移至丝氨酸或苏氨酸侧链的羟基氧(-OH)^[2-3]。蛋白质糖基化修饰一直被认为仅限于真核生物,但随着糖生物学研究不断深入,越来越多糖基化修饰系统被发现。例如,在细菌甚至古细菌中也发现有糖基转移酶活性的蛋白质,其能够通过*N*-和*O*-糖基化生成大量的糖蛋白。

近10年,致病菌的蛋白质糖基化修饰已成为国内外研究的热点:越来越多的证据表明,致病菌介导的蛋白质糖基化修饰与细菌毒力紧密相关^[4-5]。在致病性细菌如绿脓杆菌(*Pseudomonas aeruginosa*)^[6]、结核杆菌(*Mycobacterium tuberculosis*)^[7]、链球菌(*Streptococcus parasanguis*)^[8]、致病性大肠杆菌(*Enterotoxigenic escherichia coli*)^[9]及弗朗西斯菌(*Francisella*)^[10]等中,都已鉴定出大量的具有糖基转移酶活性的蛋白质。致病菌通过黏附到宿主细胞表面或黏膜上,分泌毒力因子或效应蛋白至宿主细胞,对宿主细胞的关键信号分子进行翻译后修饰,以利于其侵染、调控宿主先天免疫防御。需要注意的是,在嗜肺军团菌感染宿主期间,一些具有糖基转移酶的细菌毒素或毒力因子参与修饰宿主靶标,与细菌的致病性有着密切联系。嗜肺军团菌(*Legionella pneumophila*)作为胞内致病菌,在感染宿主细胞期间能够分泌近300种效应蛋白至宿主细胞,以利于其在宿主细胞内的生长增殖。目前在嗜肺军团菌中也发现若干具有糖基转移酶活性的效应蛋白,其能够对宿主底物进行糖基化修饰,调控宿主细胞的蛋白质翻译及囊泡转运等。本文从军团菌的致病机制及其效

应蛋白中糖基转移酶的结构与功能等方面进行综述分析,为理解糖基化修饰在嗜肺军团菌致病过程中的作用机制提供参考。

1 军团菌概述

军团菌是革兰氏阴性菌,直到1976年费城爆发军团菌肺炎(军团菌病)才被人们注意到,至今已经有超过60种军团菌被分离鉴定出。军团菌广泛存在于淡水环境、潮湿的土壤和堆肥材料中,并以阿米巴虫作为其天然宿主^[11]。军团菌病又称现代病,随着人造水环境(如淋浴、冷却塔和喷泉)的发展,军团菌的生活范围被扩大,而人造水环境的气雾化使人类更容易暴露在军团菌污染水源的环境中。军团菌通过被其污染的气溶胶被人类吸入到肺部,进而感染肺泡巨噬细胞,最后导致“军团菌病”的发生。研究表明,夏季和初秋是一年中更容易感染军团菌病的时候,易感人群包括:50岁以上的男性、吸烟者和患有糖尿病、癌症或免疫抑制等潜在疾病的人^[12]。根据欧洲疾病预防控制中心数据显示,2019年欧洲联盟共报告患“军团菌病”人数为11 298人,其中患者主要分布在法国、德国、意大利、西班牙四个国家,占欧洲联盟所有报告病例的71%^[13]。与2015年、2016年、2017年相比呈现出上升的趋势,与2018年报告的病例数量相近。根据美国疾病预防控制中心数据显示,从2000年至2018年期间,患军团菌病人数在逐年增加^[14]。其中2018年一年中,美国共报告患“军团菌病”人数为10 000人左右。然而可能由于诊断不足的因素,真实的“军团菌病”患病人数会高于报告的人数。

2 嗜肺军团菌致病机制

嗜肺军团菌通过宿主细胞的胞吞作用进入细胞,通过IVB型分泌系统向宿主细胞转运效应蛋白,干扰宿主的细胞进程,完成在宿主内生存所需的吞

噬体LCV(*Legionella*-containing vacuoles)的生物发生和成熟,使军团菌在宿主细胞内大量复制增殖^[15-16]。

2.1 嗜肺军团菌IV型分泌系统的组成

细菌已经进化出至少7种不同的分泌系统^[17-18]。其中I型分泌系统(T1SS)和IX型分泌系统(T9SS)与细菌的营养摄取和毒性有关;II型分泌系统(T2SS)位于内膜上,参与底物运输;III型分泌系统(T3SS)、IV分泌系统(T4SS)以及VI型分泌系统(T6SS)主要参与效应蛋白的转运;V型分泌系统(T5SS)参与细菌的黏附和生物膜的形成;而嗜肺军团菌的致病性则完全依赖于其IVB(Dot/Icm)分泌系统^[19]。该分泌系统由20多种蛋白组成,可分为核心跨膜单位和IV型蛋白偶合(type IV coupling protein, T4CP)单元。其中跨膜单元由DotC、DotD、DotF、DotG和DotH等5种蛋白质组成,形成一个环状结构。DotF和DotG为内膜蛋白,以二聚体的形式构成复合体的核心,还可以独立地与外膜上的DotH、DotC以及DotD相互作用^[20]。DotH是外膜蛋白,而DotC/DotD是定位在外膜上的脂蛋白, DotH在外膜上的定位需要DotC/DotD辅助^[20]。IV分泌系统的IV型蛋白偶合单元包括DotL、DotM、DotN、IcmS和IcmW等5种蛋白^[21]。其中DotL是内膜蛋白,含有一个ATPase结构域和C末端延伸,其C末端延伸可与DotN、IcmS和LvgA相互作用^[22]。DotM是内膜蛋白,可通过识别一个C-端富含酸性谷氨酸的区域与独立于IcmS-IcmW转运的效应器结合^[23]。在嗜肺军团菌中缺失DotL、DotM中的任何一个,都不能使其在培养基中存活。DotN富含半胱氨酸,能和DotM一起与DotL相互作用以稳定DotL; IcmS与IcmW能形成异源二聚体,从而调控效应蛋白的分泌;此外, IcmS-IcmW复合物将LvgA招募至DotL,并组装成独特的T4CP^[24-25]。

2.2 LCV的形成

军团菌通过胞吞作用进入宿主细胞后,会产生一层由类内质网膜组成的吞噬体LCV。吞噬体LCV的形成是一个复杂的过程,依赖于IVB系统分泌的效应蛋白。在LCV形成前期,效应蛋白SidM(DrrA)将小G蛋白Rab1招募至LCV,腺苷酸化Rab1,从而阻断由GTPase激活蛋白引起的Rab1失活^[26];效应蛋白Ra1F具有类似激活ADP核糖基化因子1(ADP ribosylation factor-1, ARF1)的结构域,能够激活ARF1,促进宿主囊泡与LCV的融合,使LCV进一步成熟^[27-28];在LCV形成的过程中, SdhA与小G蛋白Rab11b、Rab8b

和Rab5相互作用,能够避免其被降解从而能够保持LCV的完整性^[29-30];效应蛋白AnkX具有磷酸胆碱转移酶活性,能够共价修饰宿主Rab1和Rab35,抑制LVC与宿主溶酶体融合^[31]。效应蛋白RavD能够结合磷脂酰肌醇-3-磷酸(phosphatidylinositol-3-phosphate, PI3P)并去除Rab5b的泛素化修饰,从而抑制含有LVC的溶酶体成熟^[32]。除此之外,嗜肺军团菌效应蛋白AnkB^[33]、SidC^[34]等泛素连接酶能够招募泛素化蛋白至LCV上,从而提高细菌内氨基酸水平,为细菌复制提供能量;效应蛋白SidF^[35]、VipD^[36]和LepB^[37]等具有磷酸酰肌醇激酶、磷脂酶活性,对LCV膜的脂质组成进行调控,从而促进LCV的生物合成和成熟。

此外,嗜肺军团菌的致病性还与其表面结构组成有关,如内毒素、鞭毛、菌毛、外膜孔蛋白、巨噬细胞感染增强蛋白(macrophage-increased protein, Mip)等^[38]。其中位于外膜的Mip与FK506连接蛋白酶家族相似,其C-端具有PPIase活性,与军团菌穿透上皮细胞屏障和宿主内传播的能力有关,可促进军团菌在宿主的体内传播,增强军团菌的感染性^[39-40]。军团菌的鞭毛则能促进其进入宿主细胞,增强感染巨噬细胞的能力^[41]。

3 嗜肺军团菌效应蛋白介导的翻译后修饰

嗜肺军团菌分泌的近300种效应蛋白能够介导多种蛋白质翻译后修饰,进而能够调控宿主囊泡运输、细胞凋亡、脂质代谢和蛋白质合成等。例如:介导甲基化修饰的甲基化酶LegAS4(Lpg1718)和RomA(Lpg1683)都含有一个SET结构域^[42-43],其中LegAS4能够对宿主细胞核仁中组蛋白H3进行甲基化修饰,还能结合异染色质蛋白1(heterochromatin protein 1, HP1)行使组蛋白赖氨酸甲基转移酶活性,从而促进rDNA的转录^[44]; RomA(Lpg1683)能够对组蛋白H3的K13位点进行甲基化修饰,从而降低K13位点的乙酰化水平,独特地修饰宿主染色质以抑制基因表达和促进细胞内细菌的复制^[43];参与磷酸化修饰的磷酸激酶LegK1(Lpg1483)能够磷酸化IκB抑制剂家族来激活NF-κB通路^[45]; LegK2(Lpg2137)能够磷酸化修饰肌动蛋白相关蛋白2/3复合亚基1B(actin-related protein 2/3 complex subunit 1B, ARPC1B)和肌动蛋白相关蛋白3(actin-related protein 3, ARP3),从而使LCV上的肌动蛋白聚合,并干扰晚期宿主体内溶酶体向LCV的转运^[46]; LegK7(Lpg1924)

具有Hippo激酶的活性,能够模拟宿主Hippo激酶来影响Hippo信号通路,主要体现在其激酶结构域能够磷酸化修饰人MOB激酶激活因子1A(MOB kinase activator 1A, MOB1A),这导致Hippo信号通路下游底物相关蛋白的降解^[47-48]。此外,效应蛋白SidE家族(SidE、SdeA、SdeB及SdeC)以及MavC(Lpg2147)可以对多种宿主蛋白进行非典型的泛素化修饰。其中SdeA(Lpg2157)通过其单ADP核糖基转移酶结构域(mono-ADP-ribosyltransferase, mART)和磷酸二酯酶结构域(phosphodiesterase, PDE)对泛素(ubiquitin, Ub)进行连续修饰,从而介导独特的磷酸核糖基连接的泛素化^[49]。而效应蛋白SidJ(Lpg2155)能够谷氨酰化修饰SidE家族,从而抑制SidE家族的泛素化活性^[50]; MavC(Lpg2147)通过转谷氨酰胺酶活性完成对宿主E2泛素结合酶(UBE2N)的非典型泛素化过程,从而抑制NF- κ B信号通路的激活^[51],而MvcA(Lpg2148)可以特异性逆转由MavC催化的泛素化过程^[52]。

4 嗜肺军团菌效应蛋白介导的蛋白质糖基化修饰

致病菌的毒力与糖基化修饰有关,体现在致病菌的黏附、增殖、信号转导以及免疫逃逸等生物学过程中。糖基化修饰能够调节蛋白质构象、活性和稳定性等^[53]。嗜肺军团菌分泌的糖基转移酶不仅参与了感染最初的调控,还可通过直接糖基化修饰宿主蛋白来调控宿主体内的反应^[54]。目前在嗜肺军团菌分泌的近300种效应蛋白中,已发现多个效应蛋白参与糖基化修饰(表1),如: Lgt1(Lpg1368)、Lgt2(Lpg2862)、Lgt3(Lpg1488)、SetA(Lpg1978)、SidI(Lpg2504)和LtpM(Lpp0356)。它们通过介导不同类型的糖基化修饰,在军团菌致病过程中行使重要功能。其中Lgt1、Lgt2、Lgt3属于Lgt家族能够葡萄糖基化修饰宿主的转录延伸因子eEF1A^[55-56]。敲除军团菌的糖基化转移酶基因如Lgt家族、SidI及SetA基因均可导致军团菌在宿主细胞内的生长受到抑制^[57-60]。

4.1 糖基转移酶Lgt家族

Lgt1是最先被鉴定为葡萄糖基转移酶的军团菌效应蛋白。通过序列对比,在嗜肺军团菌效应蛋白中又鉴定出2个与Lgt1具有序列同源性的基因Lgt2和Lgt3^[56]。在临床菌株中Lgt2基因军团菌的致病率

要高于未含Lgt2基因的军团菌株^[61]。而在小鼠实验中,Lgt2编码基因的功能丧失会导致受感染小鼠肺中嗜肺军团菌活性的增强,但在宿主细胞中却没有,表明Lgt2可能通过宿主免疫系统提高对细菌的清除率^[62]。此外,在嗜肺军团菌感染阿米巴虫的过程中,Lgt1、Lgt2以及Lgt3的表达也受到严格控制。军团菌在阿米巴虫体内处于细菌生产稳定期时,效应蛋白Lgt1和Lgt2的表达量大大增加,而在对数生长期之前的整个过程中,均可检测到Lgt3的表达;编码Lgt1的mRNA水平在细菌-阿米巴共感染的晚期达到最高,而Lgt3主要在细菌-阿米巴共感染的初始阶段表达^[56]。这些结果表明,效应蛋白Lgt1、Lgt2以及Lgt3在军团菌感染宿主过程中存在差异性调节,Lgt3可能与启动感染周期有关,而Lgt1和Lgt2可能与军团菌从宿主细胞的排出有关。研究表明,Lgt家族对宿主转录延伸因子eEF1A的苏氨酸Thr53位点进行葡萄糖糖基化修饰,从而抑制宿主蛋白质的合成^[55-56]。另外,Lgt家族对蛋白质合成的抑制可激活雷帕霉素复合物1(mechanistic target of rapamycin complex-1, mTORC1),有趣的是,SidE家族对小G蛋白的泛素化作用则抑制mTORC1的表达^[63],这暗示着Lgt家族可能和SidE家族共同调控宿主促进宿主氨基酸的释放,以利于军团菌在宿主内的生长复制。

分析Lgt1的结构发现,Lgt1由N-端结构域(Domain I)、糖基转移酶结构域(Domain II)和C-端结构域(Domain III)等3个结构域组成(图1)。其中,Domain I由7个功能未知的 α 螺旋组成;Domain II构成典型的糖基转移酶GT-A核心组装体,中心 β 片被 α 螺旋包围构成Rossmann-like折叠结构,形成糖基供体底物UDP-葡萄糖(UDP-glucose)结合位点及介导糖基转移反应催化保守氨基酸基序DXD;Domain III主要由 α 螺旋结构构成,参与受体-底物结合的结构域。Lgt1在C-端还具有一个柔性区域(flexible loop),这个区域不仅对于底物结合具有重要作用,还对糖基转移酶结构域内UDP-葡萄糖在催化中心的结合以及反应产物的释放具有重要作用^[64-65]。此外,Lgt1的D₂₄₆XD₂₄₈序列对于其与二价的金属镁离子结合有着重要的作用,其中天冬氨酸(Asp248)直接参与镁离子的配位,天冬氨酸(Asp246)通过一分子水与镁离子配位,Lgt1还可以借助D₂₄₆XD₂₄₈序列中的2个关键的天冬氨酸来稳定催化过程中的过渡态^[66]。D₂₄₆XD₂₄₈基序提供的金属离子与供体UDP-葡萄糖

底物的结合位点也是诱导C-端柔性环区构象变化的先决条件。

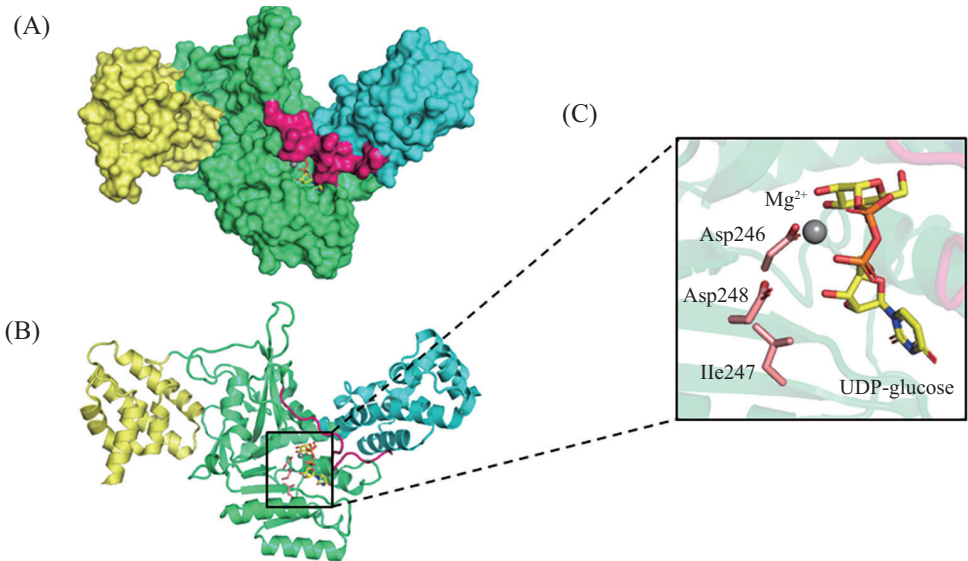
4.2 糖基转移酶SetA

SetA是一个分子量约为75 kDa的效应蛋白, 能够识别底物S/T-X-L-P/G的基序, 介导O-葡萄糖基修饰, 参与对宿主细胞骨架、基因转录和囊泡转运的调控^[67]。与Lgt家族类似, SetA的序列中也包含一个GT-A型糖基转移酶的DXD序列^[68]。但与Lgt家族不同的是, SetA除了具有葡萄糖糖基转移酶活性和自糖基化活性外, 还具有葡萄糖水解酶活性。SetA蛋白发挥糖基转移酶和水解酶的功能区域位于N-端, 其C-端是PI3P结构域, 它主要识别宿主细胞内的PI3P, 介

导SetA在宿主细胞内的定位。PI3P也作为SetA的辅因子能够诱导SetA的构象变化以利于其与底物的结合, 进而提高SetA的葡萄糖基转移酶活性, 但对其葡萄糖水解酶活性和自糖基化反应则无影响^[69]。SetA可以糖基化修饰肌动蛋白、波形蛋白、伴侣蛋白CCT5、Rab1及转录因子TFEB(transcription factor EB, TFEB)^[70]。其中SetA对Rab1的第75位苏氨酸(Thr75)的糖基化修饰抑制Rab1的GTPase活性, 对TFEB多个位点(Ser38、Ser195、Ser196、Thr201、Ser203以及Thr208)的糖基化修饰不仅破坏TFEB与14-3-3的相互作用, 而且抑制SetA核输出, 促进SetA核定位^[58], 但对肌动蛋白、波形蛋白和伴侣蛋白

表1 嗜肺军团菌糖基转移酶
Table 1 Glycosyltransferases of *L. pneumophila*

基因 Gene	效应蛋白 Effector	功能 Function	底物 Substrate	参考文献 Reference
Lpg1368	Lgt1	Glucosyltransferase	eEF1A	[55-56]
Lpg2862	Lgt2	Glucosyltransferase	eEF1A	[55-56]
Lpg1488	Lgt3	Glucosyltransferase	eEF1A	[55-56]
Lpg1978	SetA	Glucoside hydrolase Glucosyltransferase	TFEB	[58]
Lpg2504	SidI	Mannose hydrolase Glucoside hydrolase	eEF1A eEF1A γ	[59]
Lpp0356	LtpM	Glucoside hydrolase	Unknown	[60]



A、B: Lgt1的整体结构图, 黄色代表N-端结构域, 绿色代表糖基转移酶结构域, 蓝色代表C-端结构域, 紫色代表flexible loop; C: Lgt1中D₂₄₆X D₂₄₈基序与UDP-葡萄糖的位置。

A,B: overall structure of Lgt1, N-terminal domain is indicated by yellow, glycosyltransferase domain is shown by green, and C-terminal domain is colored in blue, and the flexible C-tail is represented by purple; C: the position of D₂₄₆X D₂₄₈ motif and UDP-glucose in Lgt1.

图1 嗜肺军团菌糖基转移酶Lgt1的结构(PDB_ID: 3JSZ)(根据参考文献[65]修改)

Fig.1 Structure of glycosyltransferase Lgt1 from *L. pneumophila* (PDB_ID: 3JSZ) (modified from reference [65])

CCT5的位点的作用暂不清楚。

4.3 甘露糖水解酶SidI和葡萄糖水解酶LtpM

效应蛋白SidI是一个具有多个结构域的蛋白,分子量大小为110 kDa左右,具有细胞毒性。通过序列分析发现,SidI与GT-B型糖基转移酶在序列上相似。实验表明,SidI具有水解GDP-甘露糖和微弱水解UDP-葡萄糖的能力。SidI通过其C-端结构域识别并结合eEF1A和eEF1B γ ,进而抑制蛋白质合成。SidI基因的缺失会影响eEF1A介导的热休克调节蛋白HSF1以毒力依赖的方式激活,这影响嗜肺军团菌感染细胞^[59]。而效应蛋白MesI(Lpg2505)能够直接与SidI结合,减弱SidI对宿主蛋白质合成抑制作用^[71]。

LtpM是最近被鉴定为具有葡萄糖水解酶活性的军团菌的效应蛋白^[60]。LtpM的C-端结构域为PI3P结合域,N-端为GT-A型的糖基转移酶结构域。在LtpM的糖基转移酶结构域中存在一个DXN序列,对其发挥葡萄糖水解酶活性至关重要。但与Lgt家族不同的是,LtpM的活性并不依赖金属离子,而与SetA类似,与PI3P的结合则能够激活LtpM的活性^[60]。

5 蛋白糖基化修饰在致病菌与宿主相互作用中的作用

蛋白质是生物体不可或缺的组成部分,在生物体中发挥着广泛的功能。蛋白质需要经过多种类型的翻译后修饰才能成熟和发挥功能,其中糖基化修饰起着重要作用。经过糖基化修饰的蛋白能够参与病原体感染和宿主对病原体的防御。致病菌分泌的糖基转移酶对宿主蛋白质进行糖基化修饰,能够增强致病菌在宿主细胞表面的附着能力和毒力,使其侵染宿主细胞的能力大大提高,同时还有利于其在宿主体内的增殖^[72]。

5.1 糖基化修饰增强军团菌侵染及在宿主内繁殖的能力

在嗜肺军团菌中,糖基化修饰主要影响宿主的转录和蛋白质翻译,以利于嗜肺军团菌侵染宿主以及其在宿主中的繁殖。嗜肺军团菌糖基转移酶SetA能够糖基化修饰宿主转录因子TFEB抑制其核输出,抑制宿主细胞的转录^[58];糖基转移酶SidI能够影响宿主的转录延伸因子eEF1A和eEF1B γ 介导的热休克调节蛋白HSF1的激活,SidI基因的缺失会影响军团菌侵染能力和在宿主内的繁殖^[59];糖基转移酶Lgt家族不仅能够糖基化修饰转录延伸因子eEF1A抑制宿

主细胞的蛋白质合成,而且能够与SidE家族共同调节mTORC1的表达,从而共同调控宿主氨基酸的释放,以利于军团菌在宿主内的繁殖^[56,63]。此外,Lgt家族在军团菌感染宿主的过程存在差异性调节,这与军团菌感染及感染后期军团菌的释放紧密相关。

5.2 糖基化蛋白增强致病菌在宿主细胞表面的黏附力

致病菌表面的蛋白经过糖基化修饰后,能够提高致病菌的吸附能力,这对于致病菌感染宿主细胞的启动至关重要^[73]。流感嗜血杆菌(*Haemophilus influenza*)细胞表面蛋白质HMW1能够通过与人细胞表面的特定受体相互作用来介导黏附的过程^[74]。HMW1的活性依赖于HMW1B和HMW1C两种蛋白,其中HMW1B插入到细菌外膜,介导HMW1定位在细胞表面^[75],而HMW1C具有糖基转移酶活性,能够在细胞质中糖基化修饰HMW1,使其连接到致病菌表面^[76]。在没有糖基化修饰的情况下,HMW1被降解并从致病菌表面掉落,导致其黏附能力减弱。糖基化修饰能够稳定HMW1的活性,从而增强致病菌对宿主细胞膜的黏附作用^[77]。Fap1是一种在副血链球菌(*Streptococcus parasanguinis*)中被鉴定并分离出的富含丝氨酸的黏附素,对致病菌的黏附和生物膜形成至关重要^[78]。糖基转移酶Gtf1能够糖基化修饰黏附素Fap1,增强致病菌对宿主的黏附能力^[79]。而Gtf2作为Gtf1分子伴侣与Gtf1互作,稳定Gtf1,增强Gtf1对黏附素Fap1的糖基化修饰,进而增强副血链球菌对宿主的吸附能力。

5.3 糖基化蛋白增强致病菌的毒性

致病菌能够糖基化修饰自身蛋白和宿主蛋白以增强其毒性。毒素A(TcdA)和毒素B(TcdB)是梭状芽孢杆菌的两种主要毒力因子,它们在N-端都含有葡萄糖基转移酶结构域,通过O-葡萄糖基化修饰宿主的Rho GTPase蛋白,导致其失活,从而阻断所有依赖Rho的信号通路^[4-5,80]。Rho蛋白是多种致病菌毒力因子的主要靶标和信号通路中的关键成员,宿主细胞的病变、细胞毒性效应以及免疫反应都是由Rho蛋白的失活造成^[81]。毒素B能够上调宿主的SLC11A1(solute carrier family 11 member 1)基因,进而促进其对Rho蛋白的糖基化修饰,最后导致其毒力提高^[82]。庚糖转移酶WaaC是一种在革兰氏阴性菌中高度保守的糖基转移酶,它能够将一个庚糖残基转移到外膜的内毒素上。敲除WaaC基因发现,降

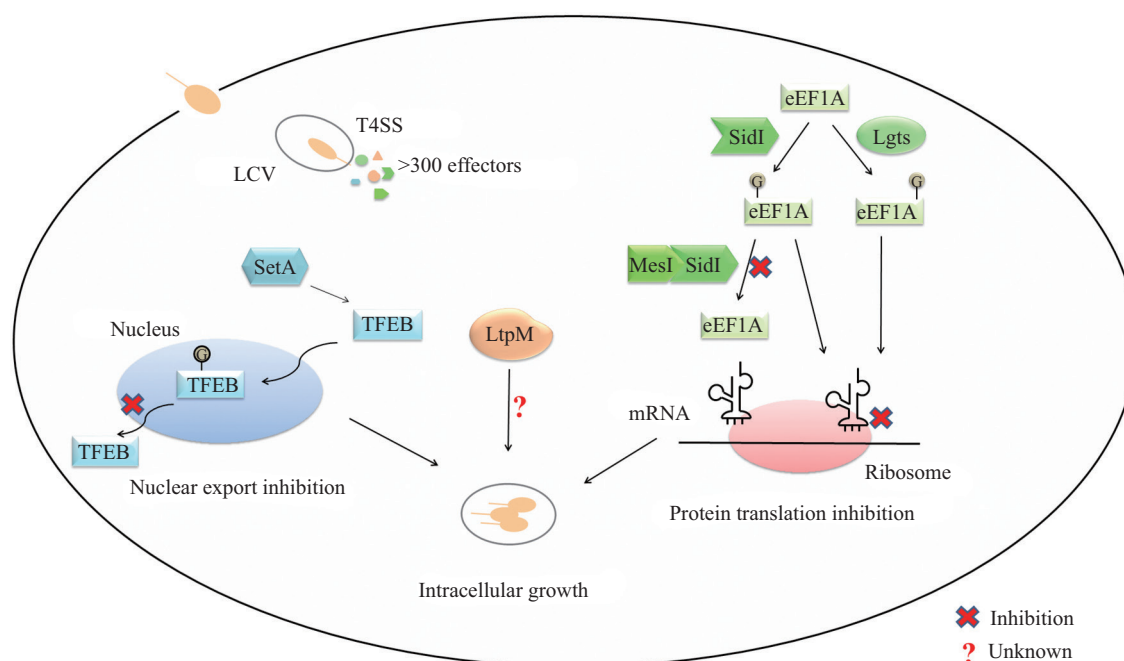


图2 效应蛋白介导糖基化修饰对嗜肺军团菌在细胞内生长的影响

Fig.2 Effect of effectors-mediated glycosylation on intracellular growth of *L. pneumophila*

低致病菌的毒力,可增加其对抗生素的敏感性^[83]。寡糖转移酶pglL是广泛存在于致病菌的一种糖基转移酶。使用类鼻疽杆菌(*Burkholderia pseudomallei*) *pglL*突变体感染急性类鼻疽的小鼠模型,发现小鼠体内的毒力显著减弱,说明类鼻疽杆菌毒力依赖于其寡糖转移酶pglL^[84]。

6 总结与展望

蛋白质糖基化修饰是一种常见的蛋白质翻译后修饰,调控许多重要的生物过程,参与病原体感染和宿主对病原体的防御。目前,嗜肺军团菌被鉴定为具有糖基转移酶活性或与糖基化修饰有关的效应因子的数目仅十个左右。尽管已被证实Lgt家族直接靶向并糖基化宿主翻译延长因子eEF1A,从而抑制蛋白质的合成,但嗜肺军团菌在其感染周期过程中如何调控Lgt家族(Lgt1、Lgt2和Lgt3)的机制并不清楚。效应蛋白SidI被报道能够直接与eEF1A及eEF1A γ 互作发挥抑制蛋白质合成的作用,而且其抑制作用能够被效应蛋白MesI所抑制,但其底物及MesI对其抑制机制也暂不清楚。效应蛋白LtpM的生物学功能也并不清楚。目前,嗜肺军团菌中发挥糖基转移酶功能的效应蛋白只有Lgt1的结构被解析,但其与底物复合物的结构及其他糖基转移酶效

应蛋白的结构仍未得到解析,至今肺军团菌糖基转移酶的催化机制暂未得到阐明。此外,嗜肺军团菌参与糖基化修饰相关的效应蛋白存在功能多样性,如效应蛋白SidI具有甘露糖水解酶活性,而LtpM则具有葡萄糖水解酶活性;同时也存在着功能的冗余性,如效应蛋白SetA和Lgt家族都具有葡萄糖基转移酶活性,但这些参与糖基化修饰相关的效应蛋白的功能多样性和冗余性如何在军团菌的致病过程中发挥作用,其机理仍存在诸多疑问。因此,本文从军团菌的致病机制及其效应蛋白中糖基转移酶的结构与功能等方面进行综述分析,为理解糖基化修饰在嗜肺军团菌致病过程中的作用机制提供参考,也为了解和探究糖基化蛋白如何参与病原体与宿主互作提供一定的理论借鉴。

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