中国科学: 生命科学 2023 年 第 53 卷 第 6 期: 753 ~ 762
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS

评 述

嗜肺军团菌调控宿主非折叠蛋白反应的研究进展

陈海航[1,2], 陈涛涛[1,2], 欧阳松应[1,2*]

1. 福建师范大学生命科学学院, 福州 350117;
2. 福建师范大学南方生物医学研究中心, 福州 350117
* 联系人, E-mail: ouyangsy@fjnu.edu.cn

收稿日期: 2023-01-31; 接受日期: 2023-03-13; 网络版发表日期: 2023-05-11
国家自然科学基金(批准号: 82225028, 82172287)和国家重点研发项目(批准号: 2021YFC2301403)资助

摘要 内质网作为细胞内重要的细胞器之一, 参与胞内蛋白的成熟及转运, 其稳态与细胞的存活及免疫反应密切相关. 非折叠蛋白反应是维持内质网稳态的重要机制之一, 参与细胞的天然免疫反应, 在宿主细胞抵抗病原体入侵中具有重要作用. 嗜肺军团菌是一种革兰氏阴性致病菌, 能够感染人体肺泡巨噬细胞引起严重肺炎, 即军团菌病. 在入侵宿主细胞后, 嗜肺军团菌通过其Ⅳ型分泌系统将330多个效应蛋白转运至宿主细胞中, 干扰宿主细胞的多种细胞进程, 以形成其生存和复制所需的场所——含嗜肺军团菌囊泡(*Legionella*-containing vacuole, LCV). LCV的形成与宿主细胞的内质网密切相关. 本文主要从嗜肺军团菌的致病机制、细胞非折叠蛋白反应及其与病原体的关系、军团菌对宿主细胞的非折叠蛋白反应的调控等方面进行综述, 以期为揭示病原体与内质网应激之间的关系提供参考.

关键词 非折叠蛋白反应, 嗜肺军团菌, 致病机制, 病原体感染

嗜肺军团菌作为一种条件性致病菌, 通过胞吞等途径入侵人体肺泡巨噬细胞, 引发嗜肺军团菌病. 嗜肺军团菌入侵宿主过程中通过Ⅳ型分泌系统释放330多个效应蛋白调控宿主细胞, 以完成自身的生存和复制. 非折叠蛋白反应是真核细胞内质网自我调节中一种重要机制, 能抵抗病原体入侵, 恢复内质网稳态. 尽管目前已有许多关于嗜肺军团菌效应蛋白调控宿主细胞机制的研究, 但关于嗜肺军团菌与宿主细胞内质网非折叠蛋白反应之间机制的研究仍有欠缺. 本文旨在概述目前已发现的嗜肺军团菌致病机制中与非折叠蛋白反应相关联部分的研究进展, 为之后对嗜肺军团菌与非折叠蛋白反应进行相关研究提供参考.

1 嗜肺军团菌概述

嗜肺军团菌是一种带鞭毛的革兰氏阴性胞内致病菌, 是军团菌病的主要病原体. 嗜肺军团菌是在1976年美国费城举行的退伍军人大会上暴发的非典型肺炎中首次被分离出来[1]. 嗜肺军团菌广泛存在于天然及人工水环境, 包括空调冷却塔、建筑供水系统和水疗池等[2]. 其天然宿主包括阿米巴原虫、草履虫等[3]. 人们在吸入被军团菌污染的气溶胶后会被感染, 引起严重

引用格式: 陈海航, 陈涛涛, 欧阳松应. 嗜肺军团菌调控宿主非折叠蛋白反应的研究进展. 中国科学: 生命科学, 2023, 53: 753–762
Chen H H, Chen T T, Ouyang S Y. Research progress on the regulation of host unfolded protein response by *Legionella pneumophila* (in Chinese). Sci Sin Vitae, 2023, 53: 753–762, doi: 10.1360/SSV-2022-0302

肺炎，即军团菌病. 入侵宿主细胞后，嗜肺军团菌的Ⅳ型分泌系统会分泌330多种效应蛋白，干扰宿主细胞内各种的途径，如蛋白运输、自噬、免疫反应和宿主染色质重塑等，最终导致疾病发生. 据美国疾病控制与预防中心统计，美国军团菌病发病率从1999年的0.40例/100000人上升到2018年的2.69例/100000人[4]; 根据欧洲疾病控制与预防中心发布的数据, 全年军团菌病病例从2011年的1.2例/100000人增加到2018年的1.8例/100000人，相当于年均增加0.075例/100000人[5]. 另外, 我国报道的嗜肺军团菌的感染病例也呈逐年增加的趋势[6]，如香港地区从2005年至2015年的军团菌发病率增加了近10倍[7].

嗜肺军团菌的致病机制的是嗜肺军团菌致病的关键. 本文将从嗜肺军团菌对受体细胞的识别、嗜肺军团菌ⅣB型分泌系统、含嗜肺军团菌囊泡(*Legionella*-containing vacuole, LCV)的形成三个方面概述嗜肺军团菌的致病机制.

1.1 嗜肺军团菌对受体细胞的识别

嗜肺军团菌与宿主细胞的相互作用在很大程度上取决于它们的表面结构. 嗜肺军团菌的外膜(outer membrane, OM)是由磷脂、脂蛋白(lipoproteins, LPS)和其他蛋白组成的脂质双分子层，与嗜肺军团菌对受体细胞的识别及入侵相关. 嗜肺军团菌OM上存在表面相关蛋白，在嗜肺军团菌附着于宿主细胞表面中起关键作用，如多种磷脂酶、主要外膜蛋白(main outer membrane protein, MOMP)和巨噬细胞感染增强剂蛋白(macrophage infectivity potentiator, Mip).

最初, 嗜肺军团菌的磷脂酶会破坏宿主细胞膜, 并可操纵宿主的信号通路[8]. 之后, 嗜肺军团菌MOMP参与宿主细胞的附着. MOMP能特异性与宿主细胞的补体受体CR1(CD35)及CR3(CD18/CD11b)结合，增加嗜肺军团菌的附着能力[9]. 巨噬细胞感染性增强因子Mip同样是嗜肺军团菌侵袭过程中必不可少的毒力因子. Mip具有肽基-脯氨酰顺式/反式异构酶活性，是嗜肺军团菌感染宿主细胞早期阶段存活所必需的. Mip能够影响宿主免疫细胞的趋化性并抑制吞噬作用[10]，但其调控机制尚不完全清楚. LPS是所有革兰氏阴性菌的主要抗原，其也参与黏附到宿主细胞的过程[11]. 另外, 嗜肺军团菌PilY1蛋白含有血管性血友病因子A(von Willebrand factor A, vWFa)结构域, 增加了嗜肺军团菌侵入非吞噬细胞的能力[12].

1.2 嗜肺军团菌ⅣB型分泌系统-T4BSS

嗜肺军团菌有多种分泌系统, 包括: Ⅰ型分泌系统(type Ⅰ secretion system, T1SS)[13]、Ⅱ型分泌系统(type Ⅱ secretion system, T2SS)[14]、ⅣA型分泌系统(type ⅣA secretion system, T4ASS)、ⅣB型分泌系统(type Ⅳ B secretion system, T4BSS)[15]及Ⅵ型分泌系统(type Ⅵ secretion system, T6SS)[16]等. 嗜肺军团菌的ⅣB型分泌系统与其毒力密切相关[17]. ⅣB型分泌系统在军团菌属中高度保守[18]，分为两个主要部分：核心跨膜复合物(core transmembrane complex, CTMC)和Dot/Icm型ⅣB偶联复合体(T4 coupling complexT4CP). CTMC形成效应蛋白转位的孔道, 由DotC, DotD, DotF, DotG, DotH和DotK组成[19]; T4CC识别效应蛋白，由DotL(IcmO), DotM, DotN(IcmJ), IcmS, IcmW, LvgA, DotY和DotZ组成. 六个由DotLMNYZ组成的异五聚体单元形成了用于传递效应蛋白的内膜通道[20]. 蛋白种类功能具体如表1所示.

嗜肺军团菌是目前已知的编码效应蛋白数量最多的胞内病原菌，其编码有330多个效应蛋白，通过T4BSS分泌至宿主细胞内发挥作用. 例如，嗜肺军团菌效应蛋白MavC通过谷氨酰胺转氨活性，将宿主细胞的泛素分子共价连接至泛素结合酶UBE2N，抑制宿主的NF-κB通路[31]; SidJ通过宿主细胞的钙调蛋白CaM激活，对SidE家族效应蛋白进行谷氨酸化修饰，抑制SidE家族效应蛋白介导的非经典泛素化修饰作用[32]; 效应蛋白Lem27具有去泛素化活性, 能够调节嗜肺军团菌LCV上的蛋白泛素化，促进LCV的生物发生和成熟[33]等. 总之，嗜肺军团菌利用各种效应蛋白调节宿主细胞内各种途径，确保嗜肺军团菌在宿主细胞内的生存和复制.

1.3 含嗜肺军团菌囊泡的形成

多数细菌在侵染宿主细胞后，一般会被转运至溶酶体进行消化清除. 但嗜肺军团菌进入宿主细胞后能够释放效应蛋白发挥功能抵抗消化，特别是在感染的早期阶段. 例如，2010年罗招庆团队[34]发现的特异性靶向宿主液泡型腺嘌呤核苷三磷酸酶(vacuolar-type ATPase, V-ATPase)的军团菌效应蛋白SidK. SidK与质子泵的关键组成蛋白VatA结合，抑制ATP水解和质子

表 1　嗜肺军团菌T4BSS的蛋白组成

Table 1　Protein components of *L. pneumophila* T4BSS

分类	蛋白	功能
核心跨膜通道蛋白	DotC DotD DotH DotG DotF	DotC和DotD是镶嵌在外膜上的脂质蛋白, DotH则是被招募到外膜上并发生寡聚化. DotC, DotD, DotH这三种蛋白会在外膜上形成复合物. 接着, DotG的C端结构域会与该复合物相互作用, 该复合物穿过内膜和外膜. 之后, 该复合物会与DotF, DotG结合, DotD发生构象变化. 最终形成核心跨膜复合物[21]
ⅣB型偶联蛋白复合物蛋白	IcmS IcmW	IcmS和IcmW形成异源二聚体, 该二聚体与DotL形成ⅣB型偶联蛋白复合物, 识别和运输不同效应蛋白到Dot/Icm分泌系统, 完成效应蛋白的分泌[22]
	DotL DotM DotN	DotL, DotM, DotN这三种蛋白会在外膜上形成复合物, 招募嗜肺军团菌中富含谷氨酸基序的效应蛋白. 此外, DotL是ATP酶, 水解ATP提供能量[23]
	LvgA	LvgA在IcmS的复合体中起作用, 也许可以作为额外的ⅣB型偶联蛋白复合物[22]
其他蛋白	DotK	DotK有结合寡聚糖的功能, 推测DotK与DotC, DotD, DotH复合物能和寡聚糖结合有关[24]
	DotU IcmF	DotU和IcmF在协助组装或维持Dot/Icm型分泌系统稳定方面发挥作用[25]
	DotI DotJ	DotI能与DotJ结合, 但目前功能还不清楚[26]
	IcmT	IcmT可能与嗜肺军团菌在感染宿主细胞的后期发生的宿主细胞裂解和嗜肺军团菌逃逸有关[27]
	DotB	DotB形成同源六聚体, 水解ATP提供能量用于转运效应蛋白[28]
	DotO	DotO具有ATP酶活性, 与DotB的六聚体相互作用, 促进DotB与军团菌的Dot/Icm T4BSS结合[28]
	IcmX	IcmX介导蛋白穿过细菌内膜进入周质内[29]
	IcmQ IcmR	IcmQ的N端结构域与IcmR结合形成两亲性四螺旋束. IcmQ的C端会与NAD^+结合[30]
	DotE DotP DotV DotA lcmV	在嗜肺军团菌中对T4BSS分泌系统的组装及其效应蛋白的分泌的作用还不清楚.

易位, 从而抑制宿主细胞液泡的酸化, 阻止嗜肺军团菌被消化. 嗜肺军团菌通过细胞的吞噬作用进入宿主细胞后, 通过与宿主细胞的内质网(endoplasmic reticulum, ER)、内质网衍生的囊泡和线粒体的密切相互作用以快速重塑吞噬体膜, 建立类似于内质网的嗜肺军团菌吞噬体. 嗜肺军团菌通过T4BSS分泌大量效应蛋白至宿主细胞中, 调控宿主细胞的细胞进程, 促进吞噬体成熟为LCV. 在感染后期, LCV成熟并表现出与内质网相似的特征, 包括内质网驻留蛋白、核糖体等存在于其表面(图1).

嗜肺军团菌可调控内质网和高尔基体之间的囊泡运输, 招募宿主细胞的内质网或高尔基体衍生囊泡至LCV[35], 促进LCV的成熟. 在此过程中, 一类被称为小GTP酶(small GTPase)的宿主蛋白起重要作用[36]. 嗜肺军团菌分泌多种效应蛋白调控宿主细胞的小GTP酶, 例如, 效应蛋白SidM募集RAB1到LCV的表面后诱导其与GTP结合, 并通过腺苷化作用修饰RAB1, 使其处于持续的激活状态(GTP·RAB1)[37]; 具有去腺苷化活性的效应蛋白SidD则对RAB1进行去腺苷化作用[38], 使GTP•RAB1与具有GTP酶激活活性的效应蛋白LepB结

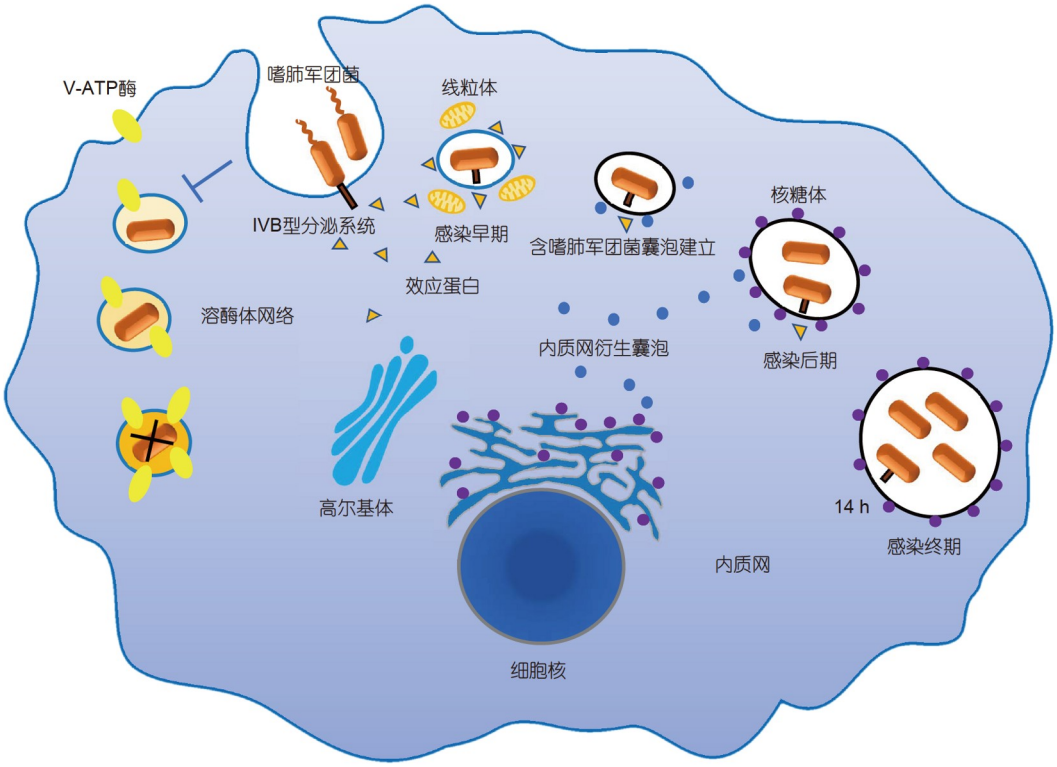

图 1 嗜肺军团菌在宿主细胞内的生命周期(左边溶酶体中黄色变深代表酸性增加, 右边膜颜色变化代表膜成分发生变化)
Figure1 Life cycle of *L. pneumophila* in host cells (the yellow color turning darker in lysosomes on the left side represents an increase in acidity, and the change in the membrane color on the right side represents a change in membrane composition)

合, 使GTP•RAB1转化为非活性的GDP•RAB1形式[39], 从而完成RAB1的激活周期; 类似地, 效应蛋白RalF作为一种鸟嘌呤核苷酸交换因子, 将小GTP酶ARF1招募至LCV表面并激活ARF1, 促进膜衣蛋白(membrane coat proteins)从LCV和高尔基体顺面再循环回ER[40]; 效应蛋白AnkX能磷酰胆碱化修饰RAB1及RAB35, 抑制其活性, 阻止内质网衍生囊泡与高尔基体顺面的融合, 从而使内质网衍生的囊泡可用于LCV[41]. 总之, 嗜肺军团菌效应蛋白对宿主细胞小GTP酶的调控, 使得宿主细胞的内质网或高尔基体衍生囊泡进行持续生物发生和分泌, 促进LCV的成熟.

2 内质网的非折叠蛋白反应

内质网是一个膜结合细胞器, 参与蛋白质、碳水化合物和脂质的生物合成过程[42]. 内质网利用易位机制和分子伴侣蛋白, 如内质网伴侣蛋白BiP(binding immunoglobulin protein)等[43]帮助蛋白完成折叠. 蛋白离开内质网需经过内质网上的质量控制位点的检测. 质量控制位点对未折叠的蛋白或错误折叠的蛋白进行识别和标记, 使此类蛋白降解, 确保蛋白的正确折叠. 然而, 在受到各种细胞内或细胞外的刺激时, 细胞的内质网稳态受到破坏会引发内质网的应激反应[44], 如非折叠蛋白反应(unfolded protein response, UPR)、内质网超负荷反应(endoplasmic reticulum overload response, EOR)等. 内质网应激是真核细胞为应对内部或外部刺激产生的一种调节作用, 能提高细胞的生存能力.

2.1 非折叠蛋白反应的信号通路

非折叠蛋白反应是由于大量的未折叠蛋白或错误折叠蛋白在内质网上积累而引发的一种内质网应激反应, 对恢复细胞内蛋白平衡起关键作用[45]. 铁失衡、钙过量、脂质类合成过量及病毒细菌感染等因素, 都可能引起细胞的非折叠蛋白反应[46]. 目前, 非折叠蛋白反应在真核细胞中高度保守, 其激活主要依赖于与内质网相关的三个膜受体, 包括RNA活化蛋白激酶

(protein kinase, PKR)样ER激酶(protein kinase RNA-like ER kinase, PERK)、激活转录因子6(activating transcription factor 6, ATF6)和需要肌醇酶1(inositol requiring enzyme-1, IRE1)[47]. 在静息状态时, 受体PERK, IRE1, ATF6与BiP结合, 其活性受到抑制. 当细胞受到刺激时, 因大量未折叠蛋白或错误折叠蛋白在内质网内堆积, 内质网发生应激, 使内质网分子伴侣BiP从膜受体上脱落, 进而激活非折叠蛋白反应通路. 非折叠蛋白反应使相关内质网应激响应因子表达上调, 如BiP、促凋亡因子CCAAT增强蛋白结合蛋白同源蛋白(CCAAT enhancer binding protein homologous Protein, CHOP)及激活转录因子4(activating transcription factor 4, ATF4)[47]等.

发生非折叠蛋白反应时, 三个膜受体分别被激活. 受体PERK与BiP分离后发生二聚化并自磷酸化激活, 将其下游的真核翻译起始因子2α亚基(eukaryotic initiation factor 2, eIF2α)磷酸化. 磷酸化的eIF2α促进其下游ATF4蛋白的表达. ATF4蛋白转移至细胞核内促使内质网应激相关蛋白CHOP及DNA损伤诱导蛋白(DNA damage-inducible protein 34, GADD34)等转录翻译. CHOP在内质网稳态无法恢复时诱导细胞凋亡[48]. GADD34可对磷酸化eIF2α进行负反馈调控, 使其去磷酸化[49]; 受体IRE1具有丝氨酸/苏氨酸激酶及核酸酶活性, 与BiP分离后, 发生二聚化并自磷酸化激活. 激活的IRE1通过其核酸酶功能对X-box结合蛋白1(X-box binding protein 1, XBP1)的mRNA进行剪接, 使XBP1mRNA转变为XBP1smRNA, 翻译产生XBP1s蛋白. XBP1s蛋白转移至细胞核内上调相关蛋白转录表达以响应内质网应激[50]; 受体ATF6与BiP解离后, 转位至高尔基体, 在蛋白酶S1P和S2P的切割作用下产生ATF6的N末端蛋白(ATF6 N-terminal protein, ATF6-N). ATF6-N蛋白进入细胞核上调相关基因的转录翻译[45](图2).

2.2　非折叠蛋白反应参与细胞的天然免疫反应

在内质网应激状态下, 活化的IRE1与肿瘤坏死因子受体(tumor necrosis factor receptor 2, TRAF2)互相作用形成复合物. 该复合物激活c-Jun氨基末端激酶(c-Jun amino-terminal kinases, JNK)诱导炎症反应[51]. IRE1-XBP1通路产生的内源性mRNA片段能激活细胞先天免疫受体视黄酸诱导基因蛋白I(retinoic acid-inducible gene I, RIG-I)进而激活干扰素调节因子3(interferon regulatory factor 3, IRF-3), 上调干扰素β(interferon β, IFNβ)的表达, 促进机体的抗病毒反

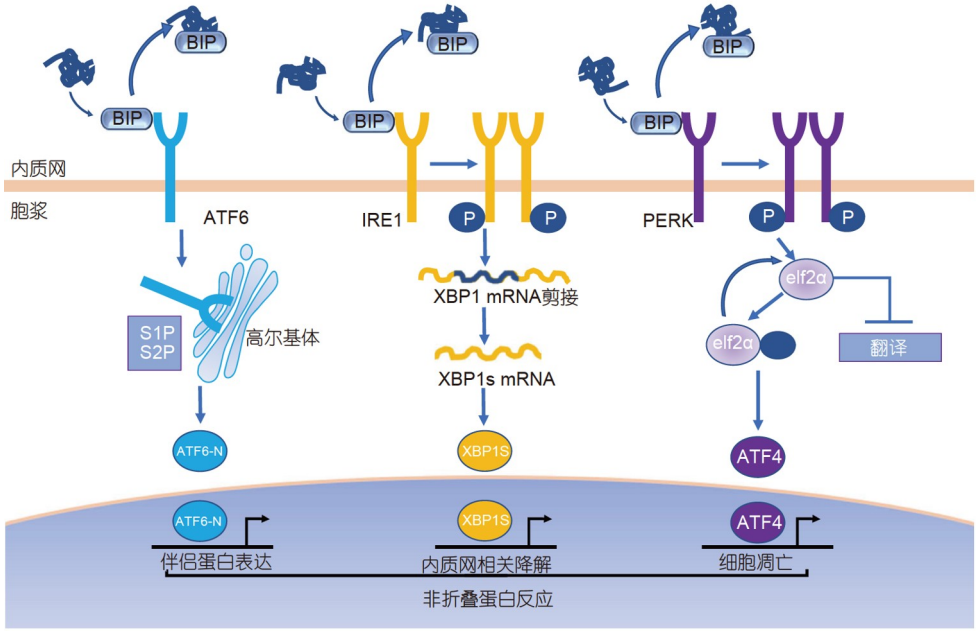

图 2　非折叠蛋白反应的信号通路
Figure 2　Signaling pathway of unfolded protein response

应[52]. 受体PERK直接结合Janus激酶1(Janus kinase-1, JAK1), 促进信号转导子和转录激活子3(signal transducer and activator of transcription 3, STAT3)的磷酸化, 激活JAK1-STAT3通路, 促进白介素-6(interleukin- 6, IL-6)的产生, 导致炎症反应[53]. 此外, 受体PERK及IRE1能与干扰素基因刺激蛋白(stimulator of interferon genes, STING)直接互作, 促进环状GMP-AMP合成酶(cyclic GMP-AMP synthetase, cGAS)与STING的cGAS-STING通路的激活, 上调IFNβ的表达[54]. 总之, 非折叠蛋白反应参与细胞的天然免疫反应.

内质网是真核细胞内重要的细胞器, 是许多病原体的靶标之一. 病原体感染宿主细胞后会激活宿主细胞内质网的非折叠蛋白反应. 非折叠蛋白反应的激活有利于宿主细胞启动天然免疫反应, 清除入侵的病原体. 例如, 产志贺毒素大肠杆菌(Shiga-toxigenic *E. coli*)分泌的毒素枯草酶(Subtilase)会切割宿主细胞的BiP, 导致DNA片段化和非折叠蛋白反应介导的细胞凋亡[55]; 肠道病原体痢疾志贺氏菌血清型和肠出血性大肠杆菌分泌的志贺毒素诱导内质网钙离子释放和上调PERK-CHOP通路, 引起由内质网应激反应介导的细胞凋亡[56]; 此外, 猪细小病毒(porcine parvovirus virus, PPV)[57]及西尼罗病毒(West Nile virus, WNV)[58]等病原体感染宿主细胞后会激活宿主细胞的PERK-eIF2α-ATF4-CHOP通路, 引发宿主细胞凋亡, 达到清除病原体的目的. 因此, 非折叠蛋白反应通过诱导被感染细胞的凋亡以抑制病原体在宿主细胞内的复制和扩散, 是宿主细胞抵御病原体感染的有效防御机制之一.

2.3 病原体对宿主细胞内质网应激的调控作用

为完成感染生命周期, 许多病原体感染宿主细胞后会干扰宿主细胞内质网的功能, 从而诱发宿主细胞的非折叠蛋白反应. 然而, 入侵的病原体可以选择性地调节宿主细胞的非折叠蛋白反应途径, 促进自身的生存.

病毒的复制需要利用宿主内质网产生病毒的结构蛋白及非结构蛋白. 同时, 病毒的复制也会干扰宿主细胞的蛋白合成, 进而诱发宿主细胞的非折叠蛋白反应. 因此, 许多病毒进化出不同的策略抑制宿主细胞内质网的非折叠蛋白反应, 促进自身的生存和复制. 例如, 丙型肝炎病毒(hepatitis C virus, HCV)的囊膜糖蛋白1(envelope glycoprotein1, E1)、囊膜糖蛋白2(envelope glycoprotein2, E2)能与内质网上的PERK结合, 抑制PERK的激活及eIF2α的磷酸化, 进而抑制非折叠蛋白反应PERK-eIF2α-ATF4-CHOP通路的激活[59]; 单纯疱疹病毒(herpes simplex virus, HSV)感染宿主细胞后, 其糖蛋白(glycoprotein B, gB)与PERK结合并抑制激酶活性, 抑制非折叠蛋白反应, 调节各种病毒蛋白的积累, 确保病毒在宿主细胞内的正常复制[60]; 人类巨细胞病毒(human cytomegalovirus, HCMV)会抑制PERK下游的eIF2α磷酸化, 并通过PERK激活固醇调节元件结合蛋白1裂解来诱导脂肪生成等多种途径, 促进病毒的复制[61].

病毒外的其他病原体, 即细菌、寄生虫等也可调控宿主的非折叠蛋白反应. 例如, 布鲁氏杆菌(*Brucella*)[62]、弓形虫等. 布鲁氏杆菌感染宿主细胞后会对宿主内质网进行重组, 激活非折叠蛋白反应, 促使IRE1表达上调, 激活该非折叠蛋白反应通路. 这促使含布鲁氏杆菌囊泡(*Brucella*-containing vacuole, BCV)成熟[63]. 此外, 布鲁氏杆菌的诸多效应蛋白能抑制非折叠蛋白反应中CHOP引发细胞凋亡[64], 如VceC[65]等. 细胞内寄生性原虫弓形虫感染宿主细胞后, 通过调节宿主细胞内微小核糖核酸(microRNA, miRNA)表达, 调控宿主细胞的非折叠蛋白反应[66].

3 嗜肺军团菌对宿主细胞非折叠蛋白反应的调控

大量研究表明, 嗜肺军团菌LCV的形成与宿主细胞内质网存在密切联系[67]. 嗜肺军团菌通过其分泌的效应蛋白调控内质网. 例如, 嗜肺军团菌效应蛋白MavQ是一种磷脂酰肌醇3-激酶, 能靶向内质网并使内质网上的磷脂酰肌醇转化为磷脂酰肌醇3-磷酸, 诱导囊泡和小管出芽, 重塑内质网[68]; 军团菌效应蛋白SdeA靶向并泛素化内质网网状体-4(reticulon-4, RTN4), 重塑内质网[69]. 嗜肺军团菌还有多种蛋白靶向宿主细胞内质网的非折叠蛋白反应. 嗜肺军团菌在感染宿主细胞后招募内质网分子伴侣蛋白BiP至LCV, 导致BiP与非折叠蛋白反应相关的受体PERK, IRE1及ATF6分离, 激活内质网的非折叠蛋白反应的相关通路[70]. 军团菌效应蛋白LegK4通过磷酸化修饰宿主细胞蛋白Hsp70(the 70-kD heat shock proteins, Hsp70), 抑制宿主细胞的蛋白质翻译, 进而抑制非折叠蛋白反

应[71]. 此外, 嗜肺军团菌的葡萄糖基转移酶Lgt家族效应蛋白Lgt1, Lgt2及Lgt3作用于宿主细胞的IRE1途径, 抑制IRE1下游的XBP1蛋白的mRNA转录剪接[72]. 这种抑制可能与Lgt家族葡萄糖基化修饰延长因子eEF1A[73]而抑制宿主蛋白的翻译有关[72,74]. 嗜肺军团菌阻止XBP1 mRNA转录剪接产生XBP1s蛋白并转位至细胞核内上调相关基因表达, 抑制宿主细胞的非折叠蛋白反应[72], 表明嗜肺军团菌能通过自身效应蛋白在不诱发宿主细胞内质网非折叠蛋白反应的情况下利用内质网, 避免了宿主细胞凋亡. Ibe等人[75]发现, 嗜肺军团菌的多个效应蛋白如lpg0519, lpg2131能够选择性激活ATF6通路, 而这种激活方式不依赖于高尔基体的转运及蛋白酶S1P/S2P的切割. 同时, 敲除Lgt家族基因的嗜肺军团菌菌株在感染细胞后, 仍能抑制非折叠蛋白反应相关相应蛋白因子BiP, CHOP及ATF4等基因的表达, 阻止宿主细胞发生凋亡. 这说明嗜肺军团菌存在多种效应蛋白能够作用于宿主细胞的非折叠蛋白反应, 但这些效应蛋白具体信息还不清楚[72]. 总之, 嗜肺军团菌通过不同机制调控宿主内质网非折叠蛋白反应, 利用宿主内质网完成自身生存和复制.

4　总结与展望

内质网的未折叠蛋白反应不仅对维持细胞和组织稳态至关重要, 而且在调节细胞的免疫和炎症反应中也起重要作用. 病原体感染宿主细胞后会激活宿主细胞的非折叠蛋白反应, 这有利于宿主细胞启动天然免疫反应, 清除入侵的病原体. 同时, 入侵的病原体可以选择性地调节宿主细胞的非折叠蛋白反应途径, 促进自身的生存. 尽管目前研究表明, 嗜肺军团菌通过不同的机制调控宿主细胞的非折叠蛋白反应, 但许多问题仍不清楚, 例如, 宿主细胞在嗜肺军团菌感染后如何调节这种平衡以进行适当的免疫反应和恢复稳态; 嗜肺军团菌如何调控宿主细胞的未折叠蛋白反应以利于自身的生存等. 对以上问题的解答将有利于了解嗜肺军团菌感染与宿主非折叠蛋白反应、天然免疫反应相关的机制, 这也将是未来研究的重要方向. 探究嗜肺军团菌致病性与非折叠蛋白反应的关系, 将有利于揭示内质网应激与机体天然免疫的关系, 及病原体在宿主细胞内感染复制的新机制, 为治疗相关疾病提供理论基础.

参考文献

1 Qin T, Zhao D, Zhu L, et al. *Legionella pneumophila* risk from cooling tower systems in China. Appl Environ Microbiol, 2022, 88: e0192121
2 Bassett M T, Balter S. Regulating cooling towers to prevent outbreaks of Legionnaires' disease. Public Health Rep, 2017, 132: 133–135
3 Watanabe K, Nakao R, Fujishima M, et al. Ciliate *Paramecium* is a natural reservoir of *Legionella pneumophila*. Sci Rep, 2016, 6: 24322
4 Han X Y. Effects of climate changes and road exposure on the rapidly rising legionellosis incidence rates in the United States. PLoS ONE, 2021, 16: e0250364
5 Cassell K, Thomas-Lopez D, Kjelsø C, et al. Provincial trends in Legionnaires' disease are not explained by population structure in Denmark, 2015 to 2018. Euro Surveill, 2021, 26
6 Qin T, Ren H, Chen D, et al. National surveillance of Legionnaires' disease, China, 2014–2016. Emerg Infect Dis, 2019, 25: 1218–1219
7 Leung Y H, Lam C K, Cheung Y Y, et al. Epidemiology of Legionnaires' disease, Hong Kong, China, 2005−2015. Emerg Infect Dis, 2020, 26: 1695–1702
8 Kuhle K, Flieger A. *Legionella* phospholipases implicated in virulence. In: Hilbi H, ed. Molecular Mechanisms in *Legionella* Pathogenesis. Current Topics in Microbiology and Immunology. Berlin, Heidelberg: Springer. 2013. 175–209
9 Weissgerber P, Faigle M, Northoff H, et al. Investigation of mechanisms involved in phagocytosis of *Legionella pneumophila* by human cells. FEMS Microbiol Lett, 2003, 219: 173–179
10 Shen Y, Xu J, Zhi S, et al. MIP from *Legionella pneumophila* influences the phagocytosis and chemotaxis of RAW264.7 macrophages by regulating the lncRNA GAS5/miR-21/SOCS6 axis. Front Cell Infect Microbiol, 2022, 12: 810865
11 Palusinska-Szysz M, Luchowski R, Gruszecki W I, et al. The role of *Legionella pneumophila* serogroup 1 lipopolysaccharide in host-pathogen interaction. Front Microbiol, 2019, 10: 2890
12 Hoppe J, Ünal C M, Thiem S, et al. PilY1 promotes *Legionella pneumophila* infection of human lung tissue explants and contributes to bacterial adhesion, host cell invasion, and twitching motility. Front Cell Infect Microbiol, 2017, 7: 63

13 Brown C L, Garner E, Jospin G, et al. Whole genome sequence analysis reveals the broad distribution of the RtxA type 1 secretion system and four novel putative type 1 secretion systems throughout the *Legionella* genus. PLoS ONE, 2020, 15: e0223033
14 Grigoryeva L, Rehman S, White R, et al. Assay for assessing mucin binding to bacteria and bacterial proteins. Bio Protoc, 2021, 11: e3933
15 Kitao T, Kubori T, Nagai H. Recent advances in structural studies of the *Legionella pneumophila* Dot/Icm type IV secretion system. Microbiol Immunol, 2022, 66: 67–74
16 de Pace F, Boldrin de Paiva J, Nakazato G, et al. Characterization of IcmF of the type VI secretion system in an avian pathogenic *Escherichia coli* (APEC) strain. Microbiology, 2011, 157: 2954–2962
17 He L, Lin Y, Ge Z H, et al. The *Legionella pneumophila* effector WipA disrupts host F-actin polymerisation by hijacking phosphotyrosine signalling. Cell Microbiol, 2019, 21: e13014
18 Burstein D, Amaro F, Zusman T, et al. Genomic analysis of 38 *Legionella* species identifies large and diverse effector repertoires. Nat Genet, 2016, 48: 167–175
19 Vincent C D, Friedman J R, Jeong K C, et al. Identification of the core transmembrane complex of the *Legionella* Dot/Icm type IV secretion system. Mol Microbiol, 2006, 62: 1278–1291
20 Meir A, Macé K, Lukoyanova N, et al. Mechanism of effector capture and delivery by the type IV secretion system from *Legionella pneumophila*. Nat Commun, 2020, 11: 2864
21 Kubori T, Koike M, Bui X T, et al. Native structure of a type IV secretion system core complex essential for *Legionella* pathogenesis. Proc Natl Acad Sci USA, 2014, 111: 11804–11809
22 Kwak M J, Kim J D, Kim H, et al. Architecture of the type IV coupling protein complex of *Legionella pneumophila*. Nat Microbiol, 2017, 2: 17114
23 Meir A, Chetrit D, Liu L, et al. *Legionella* DotM structure reveals a role in effector recruiting to the Type 4B secretion system. Nat Commun, 2018, 9: 507
24 Morozova I. Comparative sequence analysis of the *icm/dot* genes in *Legionella*. Plasmid, 2004, 51: 127–147
25 VanRheenen S M, Dumenil G, Isberg R R. IcmF and DotU are required for optimal effector translocation and trafficking of the *Legionella pneumophila* vacuole. Infect Immun, 2004, 72: 5972–5982
26 Kuroda T, Kubori T, Thanh Bui X, et al. Molecular and structural analysis of *Legionella* DotI gives insights into an inner membrane complex essential for type IV secretion. Sci Rep, 2015, 5: 10912
27 Molmeret M, Alli O A T, Zink S, et al. *icmT* is essential for pore formation-mediated egress of *Legionella pneumophila* from mammalian and protozoan cells. Infect Immun, 2002, 70: 69–78
28 Chetrit D, Hu B, Christie P J, et al. A unique cytoplasmic ATPase complex defines the *Legionella pneumophila* type IV secretion channel. Nat Microbiol, 2018, 3: 678–686
29 Matthews M, Roy C R. Identification and subcellular localization of the *Legionella pneumophila* IcmX protein: a factor essential for establishment of a replicative organelle in eukaryotic host cells. Infect Immun, 2000, 68: 3971–3982
30 Farelli J D, Gumbart J C, Akey I V, et al. IcmQ in the Type 4b secretion system contains an NAD^+ binding domain. Structure, 2013, 21: 1361–1373
31 Guan H, Fu J, Yu T, et al. Molecular basis of ubiquitination catalyzed by the bacterial transglutaminase MavC. Adv Sci, 2020, 7: 2000871
32 Gan N, Zhen X, Liu Y, et al. Regulation of phosphoribosyl ubiquitination by a calmodulin-dependent glutamylase. Nature, 2019, 572: 387–391
33 Liu S, Luo J, Zhen X, et al. Interplay between bacterial deubiquitinase and ubiquitin E3 ligase regulates ubiquitin dynamics on *Legionella* phagosomes. eLife, 2020, 9: e58114
34 Xu L, Shen X, Bryan A, et al. Inhibition of host vacuolar H^+-ATPase activity by a *Legionella pneumophila* effector. PLoS Pathog, 2010, 6: e1000822
35 Hardiman C A, McDonough J A, Newton H J, et al. The role of Rab GTPases in the transport of vacuoles containing *Legionella pneumophila* and *Coxiella burnetii*. Biochem Soc Trans, 2012, 40: 1353–1359
36 Peurois F, Peyroche G, Cherfils J. Small GTPase peripheral binding to membranes: molecular determinants and supramolecular organization. Biochem Soc Trans, 2019, 47: 13–22
37 Brombacher E, Urwyler S, Ragaz C, et al. Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of *Legionella pneumophila*. J Biol Chem, 2009, 284: 4846–4856

38 Neunuebel M R, Chen Y, Gaspar A H, et al. De-AMPylation of the small GTPase Rab1 by The Pathogen *Legionella pneumophila*. Science, 2011, 333: 453–456

39 Ingmundson A, Delprato A, Lambright D G, et al. *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. Nature, 2007, 450: 365–369

40 Mizuno-Yamasaki E, Rivera-Molina F, Novick P. GTPase networks in membrane traffic. Annu Rev Biochem, 2012, 81: 637–659

41 Kouranti I, Sachse M, Arouche N, et al. Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. Curr Biol, 2006, 16: 1719–1725

42 Wiseman R L, Mesgarzadeh J S, Hendershot L M. Reshaping endoplasmic reticulum quality control through the unfolded protein response. Mol Cell, 2022, 82: 1477–1491

43 Kepp O, Bezu L, Kroemer G. The endoplasmic reticulum chaperone BiP: a target for immunogenic cell death inducers? OncoImmunology, 2022, 11: 2092328

44 Celli J, Tsolis R M. Bacteria, the endoplasmic reticulum and the unfolded protein response: friends or foes? Nat Rev Microbiol, 2015, 13: 71–82

45 So J S. Erratum to: roles of endoplasmic reticulum stress in immune responses. Mol Cells, 2019, 42: 501

46 Kaufman R J, Scheuner D, Schröder M, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol, 2002, 3: 411–421

47 Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science, 2011, 334: 1081–1086

48 Endo M, Oyadomari S, Suga M, et al. The ER stress pathway involving CHOP is activated in the lungs of LPS-treated mice. J Biochem, 2005, 138: 501–507

49 Harding H P, Zhang Y, Scheuner D, et al. Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2α) dephosphorylation in mammalian development. Proc Natl Acad Sci USA, 2009, 106: 1832–1837

50 Calfon M, Zeng H, Urano F, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature, 2002, 415: 92–96

51 Urano F, Wang X Z, Bertolotti A, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science, 2000, 287: 664–666

52 Lencer W I, DeLuca H, Grey M J, et al. Innate immunity at mucosal surfaces: the IRE1-RIDD-RIG-I pathway. Trends Immunol, 2015, 36: 401–409

53 Meares G P, Liu Y, Rajbhandari R, et al. PERK-dependent activation of JAK1 and STAT3 contributes to endoplasmic reticulum stress-induced inflammation. Mol Cell Biol, 2014, 34: 3911–3925

54 Zhang D, Liu Y, Zhu Y, et al. A non-canonical cGAS-STING-PERK pathway facilitates the translational program critical for senescence and organ fibrosis. Nat Cell Biol, 2022, 24: 766–782

55 Morinaga N, Yahiro K, Matsuura G, et al. Subtilase cytotoxin, produced by Shiga-toxigenic *Escherichia coli*, transiently inhibits protein synthesis of Vero cells via degradation of BiP and induces cell cycle arrest at G1 by downregulation of cyclin D1. Cell Microbiol, 2008, 10: 921–929

56 Bernales S, Papa F R, Walter P. Intracellular signaling by the unfolded protein response. Annu Rev Cell Dev Biol, 2006, 22: 487–508

57 Cao L, Xue M, Chen J, et al. Porcine parvovirus replication is suppressed by activation of the PERK signaling pathway and endoplasmic reticulum stress-mediated apoptosis. Virology, 2020, 539: 1–10

58 Ambrose R L, Mackenzie J M. West Nile virus differentially modulates the unfolded protein response to facilitate replication and immune evasion. J Virol, 2011, 85: 2723–2732

59 Egan P A, Sobkowiak M, Chan S W. Hepatitis C virus envelope protein E1 binds PERK and represses the unfolded protein response. Open Virol J, 2013, 7: 37–40

60 Mulvey M, Arias C, Mohr I. Maintenance of endoplasmic reticulum (ER) homeostasis in herpes simplex virus type 1-infected cells through the association of a viral glycoprotein with PERK, a cellular ER stress sensor. J Virol, 2007, 81: 3377–3390

61 Xi Y, Lindenmayer L, Kline I, et al. Human cytomegalovirus uses a host stress response to balance the elongation of saturated/monounsaturated and polyunsaturated very-long-chain fatty acids. mBio, 2021, 12

62 Luizet J B, Raymond J, Lacerda T L S, et al. The *Brucella* effector BspL targets the ER-associated degradation (ERAD) pathway and delays bacterial egress from infected cells. Proc Natl Acad Sci USA, 2021, 118: e2105324118

63 Pandey A, Lin F, Cabello A L, et al. Activation of host IRE1α-dependent signaling axis contributes the intracellular parasitism of *Brucella*

melitensis. Front Cell Infect Microbiol, 2018, 8: 103
64 de Jong M F, Starr T, Winter M G, et al. Sensing of bacterial type IV secretion via the unfolded protein response. mBio, 2013, 4: e00418-12
65 Li C, Wang J, Sun W, et al. The *Brucella* effector BspI suppresses inflammation via inhibition of IRE1 kinase activity during *Brucella* infection. J Immunol, 2022, 209: 488–497
66 Hou Z, Wang L, Su D, et al. Global microRNAs expression profile analysis reveals possible regulatory mechanisms of brain injury induced by *Toxoplasma gondii* infection. Front Neurosci, 2022, 16: 827570
67 Kawabata M, Matsuo H, Koito T, et al. *Legionella* hijacks the host Golgi-to-ER retrograde pathway for the association of *Legionella*-containing vacuole with the ER. PLoS Pathog, 2021, 17: e1009437
68 Hsieh T S, Lopez V A, Black M H, et al. Dynamic remodeling of host membranes by self-organizing bacterial effectors. Science, 2021, 372: 935–941
69 Nixon-Abell J, Obara C J, Weigel A V, et al. Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER. Science, 2016, 354: aaf3928
70 Takayanagi S, Fukuda R, Takeuchi Y, et al. Gene regulatory network of unfolded protein response genes in endoplasmic reticulum stress. Cell Stress Chaperones, 2013, 18: 11–23
71 Moss S M, Taylor I R, Ruggero D, et al. A *Legionella pneumophila* kinase phosphorylates the Hsp70 chaperone family to inhibit eukaryotic protein synthesis. Cell Host Microbe, 2019, 25: 454–462.e6
72 Treacy-Abarca S, Mukherjee S. *Legionella* suppresses the host unfolded protein response via multiple mechanisms. Nat Commun, 2015, 6: 7887
73 Belyi Y, Niggeweg R, Opitz B, et al. *Legionella pneumophila* glucosyltransferase inhibits host elongation factor 1A. Proc Natl Acad Sci USA, 2006, 103: 16953–16958
74 Hempstead A D, Isberg R R. Inhibition of host cell translation elongation by *Legionella pneumophila* blocks the host cell unfolded protein response. Proc Natl Acad Sci USA, 2015, 112: E6790–7
75 Ibe N U, Subramanian A, Mukherjee S. Non-canonical activation of the ER stress sensor ATF6 by *Legionella pneumophila* effectors. Life Sci Alliance, 2021, 4: e202101247

Research progress on the regulation of host unfolded protein response by *Legionella pneumophila*

CHEN HaiHang[1,2], CHEN TaoTao[1,2] & OUYANG SongYing[1,2]

1 College of life Science, Fujian Normal University, Fuzhou 350117, China;
2 FJNU Biomedical Research Center of South, Fuzhou 350117, China

Endoplasmic reticulum (ER) is one of the important intracellular organelles implicated in the maturation and transport of intracellular proteins, and its homeostasis is closely relevant to cell survival and immune responses. The unfolded protein response is one of the critical mechanisms to maintain ER homeostasis, it participates in natural immune responses of cells and plays an important role in host cell resistance to pathogen invasion. *Legionella pneumophila* is a gram-negative bacterial pathogen that infects the human alveolar macrophages, thus causing severe pneumonia. After invading host cells, *L. pneumophila* transports more than 330 effector proteins into the host cell through its type IV secretory system, which interfer with various cellular processes of the host cell, so as to form a niche for its survival and replication—*Legionella*-containing vacuole (LCV). Moreover, the formation of LCV is closely relevant to the ER of host cells. In this paper, we mainly reviewed the *L. pneumophila* pathogenesis, host unfolded protein response (UPR) and its relationship with pathogens, and regulation of host UPR by *L. pneumophila*, in order to provide a reference for understanding the relationship between pathogens and ER stress.

unfolded protein response, *L. pneumophila*, pathogenesis, pathogen infection

doi: 10.1360/SSV-2022-0302