

· 综 述 ·

金黄色葡萄球菌生物被膜形成与耐药机制的研究进展

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摘要: 金黄色葡萄球菌(*Staphylococcus aureus*)是一种常见的致病菌, 但由于抗生素的滥用, 多重耐药金黄色葡萄球菌(multiple drug-resistant *S. aureus*, DR *S. aureus*)大量出现, 严重威胁人类健康。DR *S. aureus* 通常具有生物被膜, 它是细菌黏附于接触物表面, 生长并分泌多糖、蛋白质和脂质等大分子物质, 将其自身包裹其中而形成的具有复杂结构的聚集体, 能够有效保护细菌免受外界不良因素影响。同时生物被膜还可保护 DR *S. aureus* 躲避宿主免疫系统的攻击并减弱药物的渗透和杀伤作用, 是影响细菌耐药性的关键结构。因此深入认识 DR *S. aureus* 生物被膜的形成过程对治疗耐药菌相关感染疾病具有重要意义。本文综述了近年来关于 DR *S. aureus* 生物被膜的形成机制、耐药机理及抑制与清除策略的研究进展, 并对未来的研究方向进行展望。

关键词: 耐药金黄色葡萄球菌; 生物被膜; 耐药机制; 外排泵系统; 群体感应系统

Advances in mechanisms of biofilm formation and drug resistance of *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is a common pathogenic bacterium. However, due to the abuse

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of antibiotics, multiple drug-resistant *S. aureus* (DR *S. aureus*) has emerged in a large number, which seriously threatens human health. DR *S. aureus* usually forms biofilms by attaching on contact surfaces and secreting macromolecules including polysaccharides, proteins, and lipids, thus encasing themselves in a self-generated polymeric matrix. A biofilm provides an efficacious barrier that protects bacteria from detrimental environmental factors. Simultaneously, it protects DR *S. aureus* from the host immune system and attenuates the penetration and killing effects of drugs, serving as a key structure for the development of drug resistance. Therefore, gaining an in-depth understanding of the DR *S. aureus* biofilm is crucial for treating related infectious diseases. In this paper, we summarize recent research progress in the biofilm formation mechanism, drug resistance mechanism, and measures for inhibition and clearance of DR *S. aureus* and provide an outlook on the future research directions.

Keywords: drug-resistant *Staphylococcus aureus*; biofilm; drug resistance mechanism; efflux pump system; quorum sensing system

金黄色葡萄球菌(*Staphylococcus aureus*)作为一种常见的致病菌,可引起多种感染,包括肺炎、败血症、伤口感染和泌尿系统感染等。近年来,随着抗生素的不科学使用,耐药金黄色葡萄球菌(drug-resistant *S. aureus*, DR *S. aureus*)在医院和社区大量出现,已成为影响人类健康的重要公共卫生问题。相关研究表明,DR *S. aureus* 可能的耐药机制涉及多个方面,包括产生灭活抗菌药物的酶、形成主动外排抗菌药物的系统以及改变细胞膜的通透性和靶位结构等^[1]。近年来发现生物被膜也是 DR *S. aureus* 耐药的重要结构^[2]。生物被膜是由细菌分泌的、相互黏附和聚集而成的膜结构,能够有效保护细菌免受外界不良因素影响^[3]。生物被膜可作为屏障,为细菌活动创造稳定的内部环境,并保护细菌免受不利条件的影响,包括极端温度、营养限制和脱水,以及耐受抗菌药物。有研究表明,具有生物被膜的细菌对抗生素的耐受浓度比无被膜细菌高约 1 000 倍^[4];针对能产生生物被膜的 DR *S. aureus* 的治疗难度大、耗时长^[5]。因此,深入了解生物被膜形成机制,有望为 DR *S. aureus* 感染控制提供新的思路。本文综述了 DR *S. aureus* 生物被膜的形成过程、基质的结构、生物被膜生物合成调控及耐药

机理,以及近年来国内外对 DR *S. aureus* 生物被膜抑制和清除的研究进展,为开发更高效的治疗方法提供了新的方向与策略。

1 生物被膜的生物合成

1.1 生物被膜的结构

生物被膜是由多个细菌、胞外 DNA (extracellular DNA, eDNA)分子、多糖以及其他细菌分泌的物质形成的膜状物^[6]。在生物被膜结构中,多糖作为基质结构发挥了重要的支撑性功能,生物被膜内富含许多酶类及小分子物质,为微生物生存于生物被膜中提供必要支持。

1.2 生物被膜合成过程

生物被膜的生命周期被认为是持续进化的动态过程,由不同的阶段组成,并受特定基因的调控^[7]。

金黄色葡萄球菌形成三维生物被膜是一个复杂的过程,它通常分为 4 个阶段:黏附、聚集、成熟和扩散(图 1)^[8]。最后,在扩散阶段,生物被膜内细胞从生物被膜中分离出来,回到浮游状态。

1.2.1 黏附

浮游细胞通过表面相关蛋白质附着在生物

或非生物体的表面,通常可通过范德华力、空间相互作用、静电相互作用与之可逆结合^[9]。

1.2.2 聚集

金黄色葡萄球菌在附着在细胞表面后克服了其他排斥力,在最初的附着后且存在足够营养的情况下开始进行细胞分裂,并通过为其他细菌提供黏附位点来招募其他细菌^[10]。在聚集阶段,细菌通过感知触发调节网络和细胞内信号分子来调节生物被膜的形成,进而继续增殖使得生物被膜厚度增加^[11]。

1.2.3 成熟

随着细胞分裂,逐渐形成成熟的生物被膜。成熟的生物被膜具有多样化和独特的代谢结构,使它们能够抵抗有害的环境因素和压力驱动因素。金黄色葡萄球菌生物被膜具有时空异质性的塔状三维结构^[12],在微菌落周围构建了大量管道,管道散布在这些结构中,有助于营养物质和氧气的交换、液体的渗透和毒素的清除^[13]。

1.2.4 扩散

扩散是生物被膜生命周期的最后阶段,不同种类细菌的分离和扩散机制不同,分为聚集扩

散、表面扩散、种子扩散和团块扩散^[14-15]。金黄色葡萄球菌生物被膜采用团块扩散^[8],团块不断脱落,与附着的生物被膜相似,并表现出类似的抗生素耐药性。当微菌落中的单个细菌被释放到基质中时,就会发生群体扩散。

1.3 生物被膜合成的基因调控

金黄色葡萄球菌生物被膜形成多样性取决于不同基因的协同表达,它们对环境信号敏感,且其调节作用常依赖于特定生长时期或是营养条件。目前,在金黄色葡萄球菌中发现许多不同的调控系统,如双组分信号转导系统^[16]和群体感应系统^[17]等。

1.3.1 双组分信号转导系统

双组分信号转导系统传感器组氨酸激酶 *s* 是需氧和厌氧呼吸的调节系统^[16,18],是金黄色葡萄球菌发酵生长所必需的。它可影响多种毒力基因在低氧条件下的表达,同时可与细胞间黏附基因簇 *icaADBC* 的启动子结合,刺激其转录,导致细胞间多糖黏附素(polysaccharide intercellular adhesion, PIA)的产量增加,从而导致生物被膜量的增加^[18-19]。

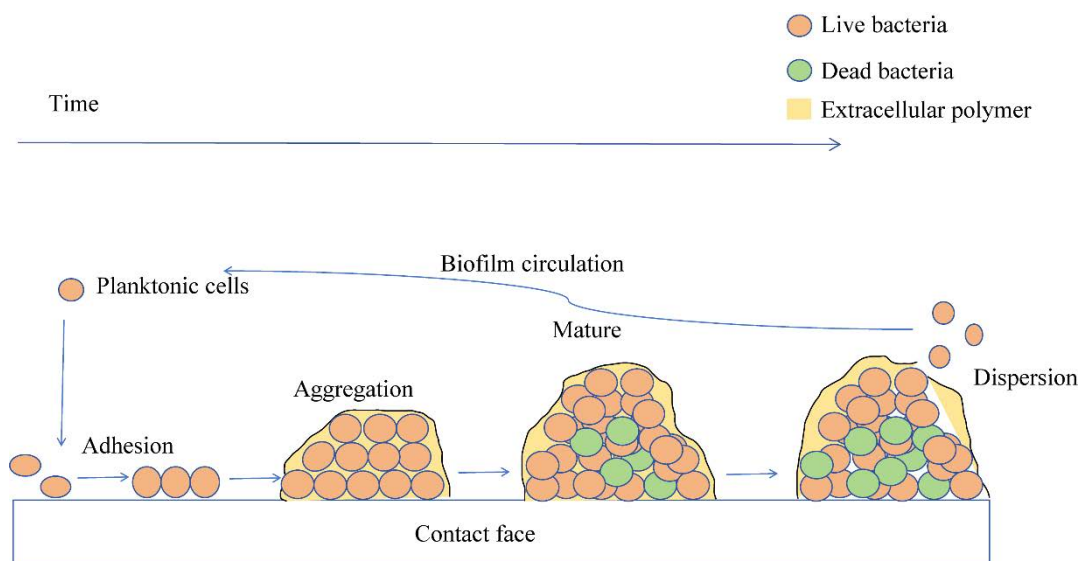


图1 金黄色葡萄球菌生物被膜形成的过程^[8]

Figure 1 The process of biofilm formation in *Staphylococcus aureus*^[8].

PIA 是金黄色葡萄球菌生物被膜形成的重要因素,也是细菌间黏附和细菌外表面黏附的媒介,以及金黄色葡萄球菌生物被膜基质中起结构性作用的重要组分^[20-21]。在金黄色葡萄球菌中,生物被膜形成的机制是通过 *ica* 位点中 *icaADBC* 操纵子编码的蛋白质产生 PIA 来控制的。*Ica* 操纵子是由 4 个功能基因 *icaA*、*icaB*、*icaC* 和 *icaD* 和 1 个调节基因 *icaR* 组成^[22]。*Ica* 编码的蛋白质中含有 PIA 糖基转移酶的结构域,其中 *icaA*、*icaD* 基因编码的蛋白协同作用^[23],可增强糖基转移酶活性,*icaB* 编码去乙酰化酶,*icaB* 的缺失会导致生物被膜无法形成^[24],*icaC* 编码蛋白主要参与多糖黏附因子的修饰作用,其大量表达促进了长链多糖的联结^[25],*icaR* 作为调节基因可以阻遏 *ica* 操纵子的转录与翻译,若 *icaR* 缺失,则直接促进 *icaA* 的表达并加速胞外多糖的产生^[26]。

另外,双组分信号转导调控 *walK* 基因在 DR *S. aureus* 的细胞壁代谢中起着关键作用^[27]。有研究证明,*walK* 基因上的单核苷酸突变可以影响细菌毒力并减少生物被膜的形成^[28-29]。

1.3.2 群体感应系统

DR *S. aureus* 的群体感应(quorum sensing, QS)系统包括附属基因调节(accessory gene regulator, Agr)系统和 LuxS/AI-2 系统^[17],这 2 个系统以不同的方式调控生物被膜的形成。Agr 系统通过上调 RNAIII 的转录来解离细菌生物被膜,而 LuxS/AI-2 系统则通过降低 PIA 的表达,抑制生物被膜的形成^[30]。

金黄色葡萄球菌 Agr 系统由 RNAII 和 RNAIII 两个相邻的转录本组成,分别由 P₂ 和 P₃ 两个启动子操纵转录调控^[31]。Agr 系统作为全局性的调控因子可负性调节生物被膜的形成及细胞壁相关黏附因子的表达,Agr 系统的激活也可以诱导 DR *S. aureus* 从已形成的生物被膜中解散^[32]。Agr 调控系统是一个复杂的多基因系统,它对 DR *S. aureus* 生物被膜的调节是多方面的,

主要涉及黏附、成熟和分散阶段^[33],对细菌密度进行调控,并控制黏附和细胞外蛋白的表达^[34]。Agr 系统使用自诱导肽(autoinducible peptides, AIP)作为细胞密度的信号分子^[35],该系统中 P₂ 启动 RNAII 的转录^[27],RNAII 转录本包含 4 个开放的阅读框,包括 *agrA*、*agrB*、*agrC* 和 *agrD*,它们编码 AIP 生物合成^[36]、转运^[33]、信号传导和靶基因调控^[37]的蛋白质。AgrC 是一种组氨酸蛋白激酶^[38],AgrA 是一个响应调节器^[33,36],AgrD 是 AIP 的前身,AgrB 是一种多功能内肽酶和伴侣蛋白^[39]。一方面,AgrB 作为蛋白酶参与 AgrD 的加工,使其成为成熟的 AIP^[37]。另一方面,AgrB 还可以充当寡肽转运蛋白,有助于将成熟的 AIP 分泌至胞外^[40]。当环境中 AIP 的浓度达到一定阈值时,它与组氨酸激酶 AgrC 结合并激活,从而导致磷酸化并初始化信号转导^[40-41]。磷酸化的 AgrC 进一步激活 AgrA,进而诱导 P₃ 启动子转录表达群体感应系统的主要效应分子 RNAIII^[42]。RNAIII 通过阻止毒素阻遏因子的翻译,促进毒素基因的表达^[43],并减少不利于生物被膜形成的几种多糖黏附素的表达^[44]。同时,AIP 水平升高可通过增加细胞外蛋白酶的分泌来促进金黄色葡萄球菌生物被膜的解聚^[10,45]。研究表明,尼古丁促进生物被膜形成可能与 Agr 系统的下调相关^[46]。

LuxS/AI-2 群体感应系统由 *luxS* 基因(AI-2 合酶)合成的信号分子呋喃硼酸二酯(furanosyl borate diester, AI-2)介导,它是金黄色葡萄球菌生物被膜形成的负调控因子^[47]。Ju 等^[48]通过敲除 *luxS*,发现生物被膜和 PIA 产生增加,这证明 *luxS* 基因在生物被膜形成中具有调控作用,LuxS/AI-2 系统通过抑制金黄色葡萄球菌中 *rbf* 促进 *icaADBC* 的转录,进而通过抑制聚-N-乙酰氨基葡萄糖(poly-N-acetylglucosamine, PANG)表达调控及生物被膜形成^[49]。Martínez 等^[50]研究发现,在 *luxS* 的突变体中,*icaR* 的转录水平显著

降低, 而 *icaA* 表达显著增加, 表明 AI-2 通过诱导 *icaR* 表达, 抑制 *icaADBC* 转录。

1.3.3 转录因子

葡萄球菌辅助调节因子 (*Staphylococcal accessory regulator A*, SarA) 是一种由 SarA 编码的 DNA 结合蛋白^[51]。它是许多毒力决定基因的主要调节因子^[52], 并直接调节 *hla* 等毒力基因的表达^[53]。SarA 位点是 *ica* 操纵子转录、PIA/PNAG 产生和 DR *S. aureus* 生物被膜形成所必需的^[51]。SarA 通过 Agr 依赖性途径调节生物被膜形成^[54], 与 Agr 启动子结合以刺激 RNAIII 的转录, 并级联和调节下游靶基因。SarA 也可以不依赖 Agr 的途径直接与靶基因启动子相互作用来控制基因表达^[55], 纯化的 SarA 以高亲和力与该序列结合, 以上调 *ica* 操纵子表达并促进生物被膜形成。SarA 不仅诱导 *ica* 操纵子的转录, 还诱导其抑制因子 *icaR* 的转录, 表明 SarA 可以防止 PIA 的过量产生。SarA 在生物被膜形成中的另一个作用是抑制细胞外蛋白酶的产生^[56]。SarA 突变体对 DR *S. aureus* 转录本的丰度具有广泛影响^[55-56], 产生

高水平的蛋白酶, 如金属蛋白酶 (aureolysin, Aur)、丝氨酸蛋白酶 (*Staphylococcal serine protease A*, SspA)、半胱氨酸蛋白酶 (*Staphylococcal cysteine protease B*, ScpB) 和 (*Staphylococcal cysteine protease A*, ScpA), 导致无法使其形成生物被膜^[56]。只有同时消除这些细胞外蛋白酶, 才能完全恢复 SarA 突变体的生物被膜形成和毒力因子产生的调节作用。

在 DR *S. aureus* 中, 转录因子 σ^B 可增强 122 个基因的表达, 涉及毒力因子、生物被膜形成等多种耐药调控系统^[57]。另有研究表明, 在氨基糖苷类抗生素亚抑菌浓度的作用下, σ^B 因子会诱导金黄色葡萄球菌小菌落突变株的出现和生物被膜形成^[58]。

综上, DR *S. aureus* 生物被膜形成是由多因子调控完成的。其中最广泛的为 Agr 系统和转录因子 SarA (图 2)。因此, 可以考虑将这些调控因子和胞内信号转导分子作为 DR *S. aureus* 生物被膜感染治疗药物靶点, 阻断其生物被膜的形成, 降低其致病性。

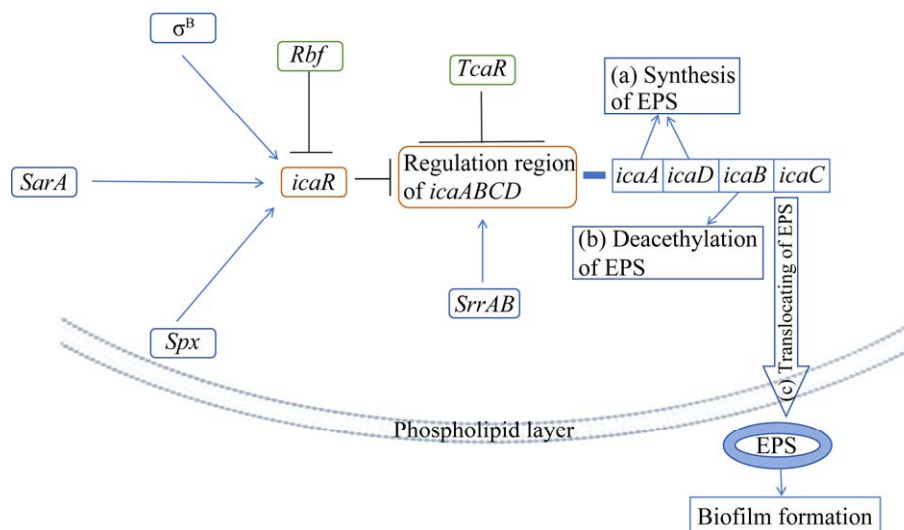


图 2 金黄色葡萄球菌胞外聚合物(extracellular polymeric substances, EPS)合成、生物被膜形成及 *icaADBC* 基因簇调控示意图^[30]

Figure 2 Schematic diagram of EPS synthesis, biofilm formation and *icaADBC* locus regulation of *Staphylococcus aureus*^[30].

2 生物被膜主要耐药机理

目前,人们认为生物被膜中 DR *S. aureus* 的高耐药性是多种机制共同作用的结果。与这种耐药性现象相关的机制,除了上述提到的群体感应外,还有物理屏障和外排泵的作用。由此可见,DR *S. aureus* 的耐药机制是以生物被膜多细胞结构为基础的,生物被膜结构一旦被破坏或丧失,其耐药性也随之减弱甚至消失^[59]。

2.1 生物被膜屏障作用

生物被膜是限制抗菌药物接近细胞的天然屏障,它能够有效地阻止一些大分子蛋白酶和补体进入,从而保护膜内细胞不受侵害^[60],因此这些病原体能够长时间抵抗宿主免疫应答^[61]。同样地,由于抗生素在复杂基质下的扩散受限,药物很难到达靶点,从而产生很强的耐药性^[62]。此外,由于缺乏营养或氧气,生物被膜内的细菌,特别是基质深处的细菌,往往会变成休眠状态持续存在,有限的生长会降低靶向活性细胞的抗生素的功效。休眠细胞代谢受限生长缓慢,导致细胞内 ATP 浓度降低,使细菌对抗生素的敏感性降低。更重要的是,经过多次抗生素治疗,处于休眠状态的细胞仍然活着,并将继续形成生物被膜,使耐药性进一步增强。研究发现,去除 DR *S. aureus* 生物被膜的 EPS 能使该菌对季铵化合物的耐药性显著下降^[63]。

2.2 外排泵系统

抗生素的主动外排是金黄色葡萄球菌耐药的主要机制之一。虽然这些外排泵本身不产生耐药性,但在阻碍抗生素在治疗中的有效性起着重要作用^[64]。

多项研究发现,外排泵在生物被膜形成中也发挥着重要作用,并证明了外排泵相关基因失活和外排泵的抑制都会导致生物被膜的形成受到影响^[65]。DR *S. aureus* 是诱导外排泵表达最强的

细菌之一。迄今为止,在 DR *S. aureus* 中已经报道了 10 多种由基因组编码或质粒编码的耐药性外排泵^[66-67]。多个全局调节器 A (multiple global regulator A, Mgr)通过控制多重外排泵的表达,如诺氟沙星耐药蛋白 A (norfloxacin resistance protein A, NorA)、诺氟沙星耐药蛋白 B (norfloxacin resistance protein B, NorB)、诺氟沙星耐药蛋白 C (norfloxacin resistance protein C, NorC)和四环素耐药蛋白 38 (tetracycline resistance protein 38, Tet38)等^[66,68],来影响细菌对多个抗生素酮康唑和四环素等的耐药性^[69]。研究表明,NorA 作为金黄色葡萄球菌的一种多药转运体^[70],它与多种抗生素具有协同作用^[67],并能提高其对野生型和过表达 NorA 的金黄色葡萄球菌分离株的活性。此外,Amiri 等^[71]发现丁香酚及其衍生物能够通过抑制金黄色葡萄球菌的外排泵 NorA 来增强其对金黄色葡萄球菌的抗菌活性。

3 生物被膜形成的抑制与清除作用研究

由于生物被膜内细菌耐药性极强、生物被膜结构难以清除,导致耐药菌感染持续存在,这些因素导致 DR *S. aureus* 生物被膜预防和治疗成为临床的一大挑战。

临床上使用的抗生素绝大部分来源于天然产物。这主要因为天然产物具有独特的化学结构、良好的细胞渗透性、靶向性和高效低毒的特性。随着新药开发的发展,天然产物将继续发挥其不可替代的作用。

研究发现从药用植物中分离得到糖苷类、黄酮类、萜类、生物碱等天然产物,微生物来源的代谢产物、噬菌体以及从动植物和微生物中分离的抗菌肽,均具有良好的抗菌活性^[72]。此外,抗菌光动力疗法 (antimicrobial photodynamic

therapy, aPDT)是目前治疗部分耐药菌感染的有效方法,其抑制机制主要包括:改变细菌形态、破坏细菌壁和细胞膜结构、干扰能量系统和群体感应系统、抑制生物被膜合成和清除成熟的生物被膜^[73-75]。

生物被膜作为金黄色葡萄球菌耐药的主要原因,研发针对生物被膜的新抗菌药物,是控制 DR *S. aureus* 感染的有效策略,目前最受关注的是抗菌光动力疗法。

3.1 植物源天然产物

许多研究表明,植物源性活性物质可以有效减少 DR *S. aureus* 的生物被膜形成,破坏其生物被膜结构。从植物中提取的莽草酸可以通过抑制 *sarA* 转录来抑制生物被膜的生物合成^[76]。Wang 等^[77]研究发现来源于黄芩根的黄芩苷通过调节腐生葡萄球菌的外排泵 MsrA 抑制群体感应和生物被膜的形成。精油(essential oils, EO)是一种具有抗菌性能的植物提取物, Tang 等^[78]利用蛋白质组学方法研究 EO 对耐甲氧西林金黄色葡萄球菌(methicillin-resistant *S. aureus*, MRSA)的抑菌机理,发现 EO 可以破坏细胞膜的完整性,使得胞内大分子物质发生渗漏,抑制了部分蛋白质以及生物被膜的合成。用从谷粒和茎秆中提取的活性组分对 DR *S. aureus* 进行处理,通过蛋白质组和代谢组分析,发现其仅抑制了 75%的生物被膜的形成,对细胞的生长没有抑制作用。这项研究还表明,大豆加工残留物也含有抗金黄色葡萄球菌生物被膜活性的物质^[79]。Cui 等^[80]发现从秸秆提取的酚酸对金黄色葡萄球菌生物被膜的形成及在静态培养中成熟生物被膜的清除均具有作用。这为金黄色葡萄球菌生物被膜处理提供了新的可选方案。

3.2 微生物源天然产物

目前临床上使用的很多抗生素均来自微生物的代谢产物,其中放线菌、细菌、真菌和微藻

等在内的微生物均是抗菌物质的重要来源^[81]。

本课题组研究发现,作为微生物次级代谢产物的灵菌红素^[82]在低浓度下对临床上分离的 41 株 MRSA 均具有显著的抑菌活性。通过扫描电镜、透射电镜、共聚焦显微镜及结晶紫染色分析显示 0.31 mg/L (1/8 MIC)灵菌红素破坏了细胞壁的结构和细胞膜的完整性,对 MRSA 生物被膜的形成也有显著的抑制作用,且具有浓度依赖性;2.5 mg/L 灵菌红素对生物被膜内细菌活性有显著的清除作用。通过转录组分析显示涉及细胞壁主要成分肽聚糖合成的多个基因如 N-乙酰壁酸 6-磷酸醚酶(N-acetylmuramic acid 6-phosphate etherase, MurQ)、N-乙酰葡糖胺-1-磷酸尿苷基转移酶(N-acetylglucosamine-1-phosphate uridyltransferase, GlmU)以及细胞壁表面成分识别蛋白,包括丝氨酸天冬氨酸重复序列蛋白 C (serine-aspartate repeat-containing protein C, SdrC)、丝氨酸天冬氨酸重复蛋白 D (serine-aspartate repeat-containing protein D, SdrD)、表面蛋白 A (*Staphylococcal protein A*, Spa)、免疫显性抗原 B (immunodominant *Staphylococcal antigen B*, IsaB)、纤维蛋白原结合蛋白(extracellular fibrinogen-binding protein, Efb)等的编码基因均显著下调。而多个荚膜多糖生物合成蛋白均显著上调。同时分子对接结果显示灵菌红素可能通过靶向 MurQ、GlmU、Spa、铁硫簇修复蛋白 A (sulfur cluster defect protein A, ScdA)和氧传感器组氨酸激酶 A (nitrate regulatory element A, NreA),抑制细胞壁合成与细胞壁表面蛋白的共价附着,同时影响铁硫簇蛋白修复及抑制硝酸盐传感器的作用,此研究为开发新型 MRSA 抑制剂提供了新的潜在靶点。

3.3 噬菌体

噬菌体是特异性感染和裂解细菌的天然抗菌剂,可用作针对生物被膜中细菌的生物防治

剂。噬菌体可通过刺激宿主细菌产生 EPS 降解酶,分解细胞外基质中的蛋白质和多糖^[83],且噬菌体在生物被膜去除方面比抗生素更有效^[84]。

Song 等^[85]发现用噬菌体 vB_SauM_SDQ (SDQ)处理后,生物被膜内的细菌数量显著减少,生物被膜结构的完整性被破坏。目前,噬菌体-抗生素联用的方法已被报道为一种有前途的清除生物被膜策略。Wang 等^[86]和 Tkhalishvili 等^[87]发现金黄色葡萄球菌特异性噬菌体 Sb-1 能抑制其抗生素敏感菌株和抗生素耐药菌株的生长,且当 Sb-1 与其他抗生素联用时,通过降解细胞外基质,起到清除生物被膜的作用。作为生物被膜抑制剂,噬菌体与传统抗生素相比具有高特异性、自我复制、自限性、低固有毒性和易于分离等优点^[88],与临床抗生素联用时,具有协同作用可有效降低临床抗生素的用量^[89]。

3.4 抗菌肽

抗菌肽(antimicrobial peptides, AMPs)是先天免疫系统的关键成分,广泛存在于微生物、植物、动物体内。随着抗生素耐药性危机的日益严重,抗菌肽被认为是解决这一问题的潜在候选药物,并得到了广泛的研究。

Yang 等^[90]利用生物信息学,从大黄鱼乳清酸性蛋白(whey acidic protein, WAP)中筛选并鉴定出一种新型大黄鱼(*Larimichthys crocea*)乳清酸性蛋白衍生肽抗菌肽(*Larimichthys crocea* whey acidic protein-derived peptide, LCWAP),它通过聚集在细胞表面,破坏细菌膜的完整性,导致细胞内物质的泄漏,抑制金黄色葡萄球菌生物被膜的形成,杀灭细菌。Demirci 等^[91]研究抗菌肽 LL-37 对从慢性伤口感染中分离的甲氧西林敏感金黄色葡萄球菌(methicillin-sensitive *S. aureus*, MSSA)和 MRSA 菌株的抗菌效果,结果发现,LL-37 通过影响金黄色葡萄球菌群体感应系统从而抑制生物被膜的合成。Colagiorgi 等^[92]发现

抗菌肽 1018-K6 能够在 15 min 内快速完全杀死金黄色葡萄球菌生物被膜内的细胞,并完全阻止生物被膜的形成,对浮游细胞也显示出强大的杀菌活性。总之,抗菌肽有望作为预防或治疗耐药菌新的候选抗菌药物,用于解决耐药菌引起的严重感染问题。

3.5 光动力学疗法

APDT 是一种利用光敏剂和特定波长的光线和氧气,通过产生活性氧(reactive oxygen species, ROS)来破坏细菌的细胞结构和功能的细菌感染的替代疗法,近年来备受瞩目^[93]。Sharma 等^[94]使用微流控技术获得的海藻酸盐微纤维作为姜黄素的载体,在蓝光(450 nm)照射下,姜黄素通过产生 ROS 对 DR *S. aureus* 形成的生物被膜表现出良好的清除作用。纳米颗粒作为光敏剂的递送载体,可有效提高光敏剂的生物利用度,增强生物被膜的清除效果^[95]。为了进一步增强光动力疗法的效果,Bu 等^[96]合成了负载光敏剂 PNIR-II 且具有谷胱甘肽(glutathione, GSH)触发 NO 产生的纳米聚合物颗粒(nanopolymer particles, NPs),在近红外 1 064 nm 激光照射下,NPs 在生物被膜微环境中释放出 NO,NO 与 ROS 形成的杀菌活性氮,有效地清除深层组织内 DR *S. aureus* 形成的生物被膜。Chen 等^[97]利用负载了疏水性氯 e6 (chlorin e6, Ce6)和亲水性 S-亚硝基谷胱甘肽(S-nitrosoglutathione, GSNO)的两性纳米颗粒,一方面增强其生物被膜穿透能力,产生杀菌活性很强的 NO 和活性氮;另一方面通过有效降低生物被膜中的 GSH 水平,进一步增强了光动力疗法清除生物被膜的效果。

另外将 aPDT 与噬菌体疗法^[98]、超声照射法^[99]以及释放氧气的纳米酶增强的可注射水凝胶^[100]等相结合的策略,显著增强了 aPDT 对 DR *S. aureus* 生物被膜清除效果。此外, Pourhajbagher 等^[101]

还报道了声敏剂纳米大黄素介导的声动力学疗法对多细菌形成的混合生物被膜的清除作用,结果显示这种声动力学疗法对已形成的混合生物被膜的清除率高达 85%。

生物被膜作为金黄色葡萄球菌耐药的主要原因,针对抑制其形成及清除作用的研究,对预防和治疗 DR *S. aureus* 导致的持续感染具有重要的临床意义。

4 结论与展望

生物被膜是 DR *S. aureus* 的一种生存策略,由于其固有的免疫反应和抗生素耐药性,给医学界带来巨大的挑战。因此,有必要深入研究 DR *S. aureus* 生物被膜的形成和调控机制,用于研究和开发抑制生物被膜形成的抗菌药物。DR *S. aureus* 生物被膜的形成依赖于复杂的调节系统网络,以互补或相反的方式共同调控生物被膜的形成。

目前,虽然 DR *S. aureus* 生物被膜形成的调控机制研究已经取得一些进展,但以下涉及 DR *S. aureus* 生物被膜调控机制及清除的方面还需进一步研究:(1) 生物被膜合成的不同调控网络之间的相关作用;(2) 不同时空下生物被膜的动态调控机制;(3) 体内外的生物被膜生物合成调控机制的差异;(4) MSSA 菌株与临床分离的 DR *S. aureus* 的生物被膜调控机制差异性;(5) 在临床感染中,DR *S. aureus* 与其他种属的微生物形成混合物种生物被膜的调控机制;(6) 成熟生物被膜的高效清除。在这些方面进行深入研究,将为 DR *S. aureus* 感染提供新的治疗思路和方案。

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