

# 肺炎克雷伯氏菌噬菌体解聚酶的研究进展

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**摘要:**肺炎克雷伯氏菌(*Klebsiella pneumoniae*)作为重要的临床致病菌, 可引发多种严重的感染性疾病。近年来, 高毒力多重耐药肺炎克雷伯氏菌的全球性传播给临床治疗带来极大挑战, 对公共卫生安全构成严重威胁。值得关注的是, 肺炎克雷伯氏菌噬菌体编码的解聚酶因其特异性降解细菌多糖的独特机制, 相较于传统噬菌体疗法具有规避宿主免疫识别风险、降低基因水平转移概率及提升药代动力学特性等多重优势, 现已成为应对超级耐药菌感染的创新性治疗策略研发核心方向。本文对现有解聚酶的结构与功能特性进行了系统梳理, 探讨了噬菌体解聚酶作为多重耐药肺炎克雷伯氏菌感染替代治疗策略的应用潜力, 并进一步展望了肺炎克雷伯氏菌噬菌体解聚酶的未来应用方向与改造策略。

**关键词:**耐碳青霉烯类高毒力肺炎克雷伯氏菌; 噬菌体; 解聚酶; 生物膜; 抗菌治疗

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## Advances in bacteriophage depolymerases targeting *Klebsiella pneumoniae*

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**Abstract:** *Klebsiella pneumoniae*, a major clinical pathogen, can cause various severe infectious diseases. In recent years, the emergence of multidrug-resistant hypervirulent *K. pneumoniae* strains has posed substantial challenges to clinical treatment and has become a serious threat to global public health. Depolymerases encoded by *K. pneumoniae* phages, through their unique mechanism of specifically degrading bacterial capsular polysaccharides, offer multiple advantages over conventional phage therapy. These advantages include evading host immune recognition, reducing the probability of horizontal gene transfer, and enhancing pharmacokinetic properties. Accordingly, bacteriophage depolymerases have now become a core direction for the development of innovative therapeutic strategies against multidrug-resistant bacterial infections. This article comprehensively summarizes the structural and functional characteristics of existing depolymerases, assesses their potential as alternative therapeutic strategies against multidrug-resistant *K. pneumoniae* infections, and explores the future application directions and modification strategies for *K. pneumoniae* phage depolymerases.

**Keywords:** carbapenem-resistant hypervirulent *Klebsiella pneumoniae*; bacteriophage; depolymerase; biofilm; antibacterial therapy

肺炎克雷伯氏菌(*Klebsiella pneumoniae*, Kp)是一种广泛分布于自然环境(如水、土壤、植物表面)及生物体内(动物体内、人体上呼吸道和肠道)的条件致病菌<sup>[1]</sup>。该菌可引发肺炎、尿路感染、血流感染、软组织感染、手术伤口感染及导管相关感染等疾病,涵盖社区获得性与医院获得性感染<sup>[2]</sup>。根据毒力差异,肺炎克雷伯氏菌可分为经典型肺炎克雷伯氏菌(classical *K. pneumoniae*, cKp)和高毒力肺炎克雷伯氏菌(hypervirulent *pneumoniae*, hvKp)。cKp 主要感染免疫功能低下人群,多为医院获得性感染;而 hvKp 能感染任何年龄的健康个体,主要引发社区获得性感染<sup>[3]</sup>。hvKp 感染常经血行播散,导致化脓性肝脓肿、

眼内炎、脑膜炎等多部位侵袭性病灶<sup>[4]</sup>;相较于 cKp, 病程发展更迅速、预后更差、死亡率更高。

针对肺炎克雷伯氏菌感染,其临床治疗方案遵循差异化原则。对于药物敏感菌株,二代和/或三代头孢菌素是常规治疗的首选;而对于耐药菌株,则需根据其耐药谱选择相应抗生素。用于治疗耐药菌的抗生素通常为  $\beta$ -内酰胺类/ $\beta$ 内酰胺酶抑制剂合剂、替加环素、多黏菌素、磷霉素、氨基糖苷类、碳青霉烯类、喹诺酮类和半合成四环素类抗生素等<sup>[5]</sup>。然而,由于抗生素在临床及其他多领域中的广泛使用甚至滥用,肺炎克雷伯氏菌通过移动元件等途径持续获取耐药基因<sup>[6]</sup>,上述抗生素也逐一面临失效

风险。全国细菌耐药监测网发布的《2023 年全国细菌耐药监测报告》显示,在临床分离的革兰氏阴性菌中,肺炎克雷伯氏菌检出率位列第 2 (仅次于大肠杆菌),并且对碳青霉烯类抗生素(如亚胺培南、美罗培南)的耐药率呈显著上升趋势(分别从 2005 年的 3.0%和 2.9%增至 2023 年的 24.8%和 26%)<sup>[7]</sup>。多黏菌素和替加环素常被视为对抗耐碳青霉烯类肺炎克雷伯氏菌(carbapenem-resistant *K. pneumoniae*, CRKp)的“最后手段”<sup>[8]</sup>,但临床上已经出现了对这 2 种药物耐药的病例<sup>[9-11]</sup>。世界卫生组织已将 CRKp 列为需要紧急研发新药的“关键优先级”菌株<sup>[12]</sup>。此外,兼具高毒力与耐药性双重特性的临床菌株带来了新一轮的治疗难题。据 2024 年世界卫生组织发布的报告,基因型为 ST23 型的耐碳青霉烯类高毒力肺炎克雷伯氏菌(carbapenem-resistant hypervirulent *K. pneumoniae*, CR-hvKp)已在全球出现流行趋势<sup>[13]</sup>。在中国,ST11 基因型是最常见的 CR-hvKp<sup>[14]</sup>。CR-hvKp 的出现和迅速传播导致其相关感染的发病率和死亡率显著上升,构成重大的全球公共卫生威胁和临床治疗挑战。当前,面对细菌耐药性加速进化与传统抗生素研发周期长(平均 10-15 年)、投入高(约 10-15 亿美元/品种)的技术瓶颈,开发新型抗菌策略已成为防控高毒力多重耐药菌感染的迫切临床需求<sup>[15]</sup>。

## 1 噬菌体解聚酶

噬菌体是一类能特异性感染并裂解细菌的病毒。在多重耐药菌感染加剧与新型抗生素研发滞后的双重压力下,噬菌体疗法凭借其精准抗菌特性,重新成为抗感染治疗的突破方向<sup>[16]</sup>。噬菌体侵染宿主时,可编码多种在裂解宿主细菌的过程中发挥关键作用的功能蛋白,如裂解酶(负责溶解细胞壁)、穿孔素(在细胞膜上形成孔洞)、解聚酶(降解细菌表面多糖)等。其中,解聚酶因其能特异性降解细菌表面重要的多糖成分,具备较强的临床转化潜力,成为目前的

研究焦点。解聚酶的作用靶标主要包括细菌表面的荚膜多糖(capsular polysaccharide, CPS)、胞外多糖(extracellular polymeric substances, EPS)及脂多糖(lipopolysaccharide, LPS)<sup>[17]</sup>。在肺炎克雷伯氏菌噬菌体相关解聚酶的研究中,尤以靶向 CPS 与 EPS 的解聚酶备受关注。

相较于完整噬菌体颗粒,解聚酶展现出显著的临床转化优势。噬菌体需识别细菌表面受体进行侵染,并借助在宿主内的增殖最终实现裂解;而解聚酶本身作为一种蛋白质,其抗菌效应不依赖于细菌活力或代谢过程,仅通过直接降解细菌表面的多糖屏障(如荚膜)来削弱细菌防护,使细菌更易被清除<sup>[18]</sup>。这种基于物理屏障破坏而非牵涉生命过程的作用机制,理论上能减少细菌因生存压力产生的耐药突变,同时由于无需完整噬菌体作为载体,也有效规避了细菌间毒力或耐药基因水平传播的风险<sup>[19]</sup>。此外,解聚酶还具有良好的化学稳定性,能够耐受不同环境条件,更易于生产、储存、运输及临床使用<sup>[20]</sup>。基于这些特性,解聚酶已成为抗感染领域极具潜力的下一代生物制剂候选分子,展现出良好的临床转化前景。

### 1.1 解聚酶研究历程

在噬菌体解聚酶的百年研究历程中,其作用机制与治疗潜力逐步被揭开。1924 年, Asheshov<sup>[21]</sup>观察到某些噬菌体的噬菌斑周围可形成随培养时间逐渐扩大的半透明光晕(halo)。1929 年, Sertic 等<sup>[22]</sup>证实此光晕源于噬菌体分泌的某类具有溶菌作用的酶类物质。1956 年, Adams 等<sup>[23]</sup>取得关键突破,成功分离出能够水解细菌 CPS 的噬菌体酶,并首次提出“解聚酶”这一概念。携带解聚酶的噬菌体通常会在其噬菌斑周围形成半透明晕圈。随着培养时间延长,解聚酶持续降解细菌表面多糖,晕圈面积不断扩大。基于这一现象,可初步判断噬菌体是否产生解聚酶<sup>[24]</sup>。随后, Stirm 等<sup>[25]</sup>证实解聚酶是噬菌体结构的组成部分。1996 年, Steinbacher 等<sup>[26]</sup>解析了噬菌体 P22 尾刺蛋白(tail spike

protein, TSP)的 $\beta$ -螺旋三聚体结构,为理解其与特定多糖底物的互作提供了结构基础。此后, Hughes 等<sup>[27]</sup>证实解聚酶可降解生物膜胞外多糖,为清除顽固生物被膜提供了新思路。Mushtaq 等<sup>[28]</sup>验证了重组解聚酶治疗全身性感染的有效性。Waseh 等<sup>[29]</sup>于2010年开发了首款可有效预防肠道细菌定殖的解聚酶口服制剂。Bansal 等<sup>[30]</sup>于2014年开创性地联用解聚酶与庆大霉素治疗全身及肺部感染,证明了解聚酶-抗生素的协

同杀菌效应。目前,探究解聚酶在不同领域的潜在应用已成为研究热点<sup>[31-33]</sup>。

解聚酶的核心特性在于其高度的底物特异性,即一种解聚酶通常仅作用于特定荚膜血清型的肺炎克雷伯氏菌<sup>[34]</sup>。基于该特性,针对不同血清型肺炎克雷伯氏菌的噬菌体解聚酶的研究已取得众多成果——迄今为止已鉴定出68种肺炎克雷伯氏菌噬菌体解聚酶,覆盖32种荚膜血清型(表1)。

表1 针对不同荚膜血清型肺炎克雷伯氏菌的解聚酶及其来源噬菌体

Table 1 Depolymerases of different capsular serotypes of *K. pneumoniae* and their source bacteriophages

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	噬菌体 Bacteriophage	参考文献 Reference
K1	K1-ORF34	NTUH-K2044-K1-1	[35-36]
	S2-4	$\Phi$ K64-1	[37]
	Kpv71_52	Kpv71	[38]
	GBH001_056	GBH001	[39]
	Depo16	vB_KpnP_ZK1	[40]
	phiA2-dep	phiA2	[41]
	K2	GBH038_054	GBH038
Depo32		GH-K3	[42]
DpK2		RAD2	[43]
K2-ORF16		1611E-K2-1	[36]
Dep1979		$\Phi$ FK1979	[44]
ORF20		Kpph1	[45]
NPatgp22		NPat	[46]
BMacgp22		BMac	[46]
ORF2		P12L	[47]
K2/13	Dep_Kpv74	KpV74	[38,48]
	ORF58	$\pi$ VLC6	[49]
	B1dep	B1	[50]
K3	ORF58	$\pi$ VLC6	[49]
	KP32gp37	KP32	[51]
	gp32	GH-K3	[52]
K5	K5-2 ORF38	K5-2	[53]
	K5-4 ORF38	K5-4	[53]
	dep1011	P1011	[54]
K7	K7dep	vB_KpnM-20	[55]
K8	K5-4 ORF37	K5-4	[53]
K11	S1-1	$\Phi$ K64-1	[37]
K19	Dpo41	SH-KP156570	[56]

(待续)

(续表 1)

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	噬菌体 Bacteriophage	参考文献 Reference
K20	K20dep	vB_KpnM-20	[55]
	K1-dep	Klyazma	[57]
K21	S1-3	ΦK64-1	[37]
	KP32gp38	KP32	[51,58]
	K5-RBP2	K5	[59]
K22/37	ORF49	πVLC5	[49]
	ORF51	πVLC6	[49]
K23	Dep622	vB_KpnP_Dlv622	[60]
	DepS8	KpS8	[60]
K25	S2-2	ΦK64-1	[37]
K27	K27dep	vB_KpnM-20	[55]
K30/K69	S2-6	ΦK64-1	[37]
	K5-2 ORF37	K5-2	[53]
K35	S2-3	ΦK64-1	[37]
K47	Dep42	SH-KP152226	[61]
	Dpo43	IME205	[62]
	Dpo42	IME205	[62]
	P560dep	P560	[63]
K51	GBH019_279	GBH019	[39]
K54	RaK2gp531	RaK2	[34]
K56	K56dep	KN3-1	[64]
K57	Dep_kpv79	KpV79	[65]
	Dep_kpv767	KpV767	[65]
	Dep_ZX1	vB_KpnP_ZX1	[66]
K62	K62-Dpo30	SH-KP2492	[67]
K63	depoKP36	KP36	[68]
	KP34gp57	KP34	[69]
K64	S2-5 (K64dep)	ΦK64-1	[37,70]
	P510dep	P510	[71]
	K64-ORF41	SH-KP152410	[72-73]
	Dep37	vB_KpnP_IME1309	[74]
KN1	KN1dep	KN1-1	[64]
	Dp42	IME321	[75]
KN2	ORF96	0507-KN2-1	[76]
KN3	KN3dep	KN3-1	[64]
KN4	S1-2	ΦK64-1	[37]
	KN4dep	KN4-1	[64]
KN5	S2-1	ΦK64-1	[37]
KN8	Dep108.1	KPPK108.1	[77]
	Dep108.2	KPPK108.2	[77]

## 1.2 解聚酶作用机制

解聚酶在噬菌体侵染细菌并将其裂解的过程中发挥着关键作用,其具体过程如下<sup>[78-80]</sup>:首先,游离的解聚酶接触并识别细菌细胞表面的初级受体(如 CPS 等),介导细菌胞外多糖的降解,从而突破感染过程中的物理屏障,启动噬菌体侵染;随后,噬菌体抵达细菌细胞表面,与细胞外膜的次级受体(如孔蛋白、铁载体受体、脂多糖核心区及 O 抗原等<sup>[46]</sup>)结合后,噬菌体尾管端携带的内溶素水解宿主菌细胞壁上的肽聚糖形成通道;噬菌体头部的核酸物质通过该通道注入宿主细胞胞质中;待核酸完全进入宿主细胞后,通道关闭以防止胞质泄露,噬菌体空衣壳则留在细胞外,至此完成入侵(图 1A-1E)。噬菌体将细菌裂解后,可在细菌溶解产物中检测到解聚酶的活性<sup>[81]</sup>。

不同噬菌体可编码不同解聚酶,靶向结合同一宿主菌 CPS 表面的多种解聚酶识别位点,进而实现 CPS 降解<sup>[49]</sup>。此外,单个噬菌体也可编码多种不同特异性解聚酶,分解不同宿主 CPS<sup>[37]</sup>。根据作用机制的不同,解聚酶主要分为水解酶和裂解酶:水解酶通过水解反应,利用水分子切割糖苷键中的糖-氧键,从而分解细菌细胞表面荚膜多糖或脂多糖 O 抗原侧链,已知的水解酶包括唾液酸酶、木糖苷酶、果聚糖酶、葡聚糖酶、鼠李糖苷酶及肽酶等;裂解酶则采用  $\beta$ -消除机制,定向切割单糖与糖醛酸 C4 之间的糖苷键(如 1,4-糖苷键),同时在非还原性糖醛酸的 C4 和 C5 之间引入双键,将多糖裂解为不饱和寡糖,目前已发现的裂解酶主要包括透明质酸酶、海藻酸盐裂解酶及果胶酸裂解酶等<sup>[80,82]</sup>。此外,极少数解聚酶属于脂肪酶,通过作用于三酰基甘油的羧基酯键来裂解多糖,同时释放有机酸和甘油<sup>[83]</sup>。

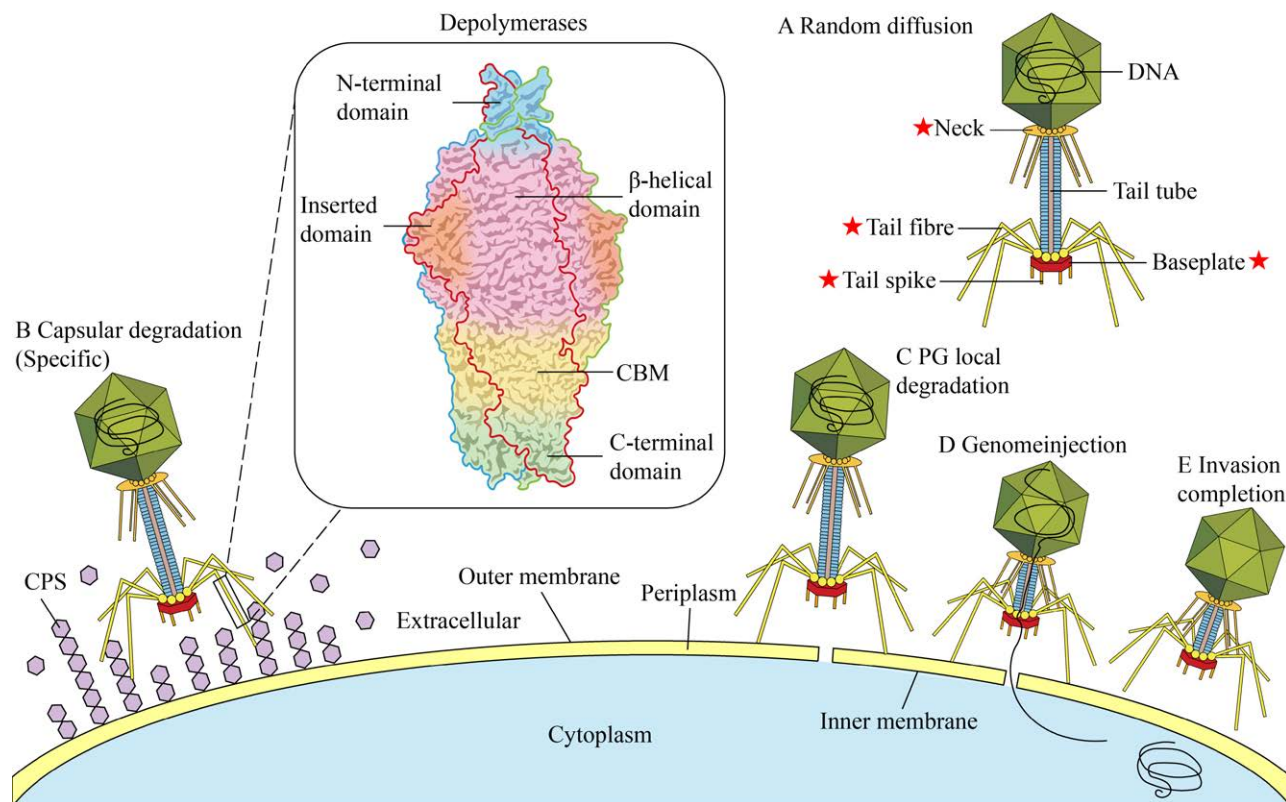
## 1.3 解聚酶分类

不同解聚酶除作用机制存在差异外,还可按其存在形式分为结构蛋白型和可溶性蛋白型<sup>[82]</sup>。前者构成噬菌体吸附装置的一部分,主要定位

在噬菌体的尾丝、尾刺或基板上(少数在颈部)(图 1A);作为包括尾丝/尾刺蛋白在内的受体结合蛋白(receptor binding protein, RBP)结构元件组成部分,介导噬菌体对细菌表面多糖的识别与降解,为后续感染奠定基础<sup>[17]</sup>;其编码基因与噬菌体结构蛋白编码基因高度相近,甚至可共用同一开放阅读框。而可溶型解聚酶则在噬菌体成熟后,伴随宿主裂解过程释放至胞外;其编码基因通常远离噬菌体结构蛋白基因位置<sup>[80]</sup>。结构蛋白型是解聚酶的主要存在形式<sup>[82]</sup>。但无论是结构型还是可溶型,解聚酶均能通过琼脂扩散并引发多糖降解,从而在噬菌斑周围呈现出典型的透明光晕<sup>[20]</sup>。不同解聚酶介导形成的晕圈大小和形态通常存在显著差异。本课题组前期研究发现,在培养 18-20 h 后,侵染 K2 型 CR-hvKp 的德雷克斯勒噬菌体科(*Drexlerviridae*)、*Webervirus* 属噬菌体 Kpph1 可形成直径 5-7 mm 的噬菌斑,其伴随的光晕宽度约 1.5 mm;而侵染相同宿主的自复制短尾噬菌体亚科(*Autographiviridae*)、*Druavisvirus* 属噬菌体 Kpph9 形成的噬菌斑直径为 3-5 mm,光晕宽度约 1 mm<sup>[45]</sup>。

## 1.4 解聚酶结构特征

在肺炎克雷伯氏菌 CPS 高频变异的选择压力下,噬菌体解聚酶通过垂直基因转移(酶活性位点突变)与水平基因转移(跨物种结构域重组)2 种策略实现进化<sup>[84]</sup>。水平基因转移是进化的主要力量,解聚酶借此快速获取新功能,实现宿主范围转换<sup>[85]</sup>,由此塑造了其模块化结构特征。不同肺炎克雷伯氏菌噬菌体的解聚酶虽在结构域组合、活性中心、空间构象及生物学功能上呈现多样性(表 2),但仍蕴含着诸多共性。在整体结构上,多以同源三聚体形式存在,每个单体至少包含 N 端结构域、 $\beta$ -螺旋中心结构域及 C 端结构域(图 1B 黑框部分)。在结构域功能上,N 端结构域具有柔性,负责将解聚酶锚定至噬菌体基板等尾部结构; $\beta$ -螺旋中心结构域包含底物结合位点和催化位点,是识别并结



**图 1 噬菌体解聚酶结构及噬菌体借助解聚酶感染细菌示意图** A: 目前研究认为, 噬菌体可能通过随机扩散接触到宿主表面随后进行感染。噬菌体可通过颈部、尾丝、尾刺及基板等结构接触细菌表面并结合识别受体。B: 噬菌体解聚酶通过降解细菌表面的荚膜多糖突破感染的第一道物理屏障, 启动噬菌体感染。以位于尾丝上的解聚酶为例, 其主要由 N 端结构域、 $\beta$ -螺旋中心结构域以及 C 端结构域组成, 部分解聚酶还含有碳水化合物结合模块或插入结构域。C-E: 噬菌体与次级受体结合后, 将核酸物质注入到宿主细胞后完成入侵。图片由 Adobe Illustrator 2024 (28.0) 进行绘制。

**Figure 1 Schematic Diagram of bacteriophage depolymerase structure and depolymerase-mediated phage infection mechanism.** A: Current research indicates that phages typically reach host surfaces through random diffusion before initiating infection. Phages recognize and bind to bacterial surface receptors via structures including the neck, tail fibres, tail spikes, and baseplate. B: Phage depolymerases degrade bacterial capsular polysaccharides to penetrate the primary physical barrier of infection, triggering phage invasion. For instance, depolymerases located on tail fibres are primarily composed of an N-terminal domain, a  $\beta$ -helical central domain, and a C-terminal domain. Some also harbor a carbohydrate-binding module (CBM) or insertion domain. C-E: Following binding to secondary receptors, phages inject their nucleic acid into the host cell, thereby completing the invasion. The figure was illustrated by Adobe Illustrator 2024 (28.0).

合 CPS 从而发挥催化活性的核心区域; C 端结构域则主要负责解聚酶三聚体结构的形成与稳定性维持<sup>[20,85]</sup>; 各结构域间紧密协作, 共同保障酶行使高效催化功能<sup>[58]</sup>。在催化剂机制上, 催化位点多位于三聚体相邻单体界面的  $\beta$ -螺旋

结构域内, 通常为酸性/碱性氨基酸残基, 通过酸碱催化机制降解多糖。因此一般认为三聚体构象对于维持催化口袋的几何形态至关重要。但最新研究表明, 解聚酶 C 端融合 His 标签形成的四聚体 K2-2C-His 对 K2 型 CPS 也具有显

表 2 肺炎克雷伯氏菌噬菌体解聚酶的结构解析

Table 2 Structural analysis of *K. pneumoniae* bacteriophage depolymerases

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	结构域组成 Domain composition	催化位点 Catalytic site	结构域特性 Domain property	参考文献 Reference
K2	DpK2	N 端结构域、 β-螺旋结构域、 C 端结构域 N-terminal domain, β-helix domain, C-terminal domain	位于相邻单体界面 β-螺旋结构域; 带负电荷的酸性氨基酸残基分别构成 D543/E545/D546 核心催化三联体和 D399-E423 与 D619-E620 核心催化残基对 Situated at β-helix interfaces. Acidic residues constitute: Core catalytic triad: D543/E545/D546, Core catalytic residue pairs: D399-E423 and D619-E620	C 端结构域 β-三明治折叠介导酶与 CPS 初始结合 β-sandwich fold of C-terminal domain mediates initial CPS binding	[43]
	Depo32	N 端结构域、颈螺旋与连接结构域、β-螺旋结构域、连接螺旋结构域、碳水化合物结合模块 (CBM)、C 端结构域 N-terminal domain, Neck helix & linker domain, β-helix domain, Linker helix domain, CBM, C-terminal domain	位于相邻单体界面 β-螺旋结构域; 带负电荷的酸性氨基酸残基 D546/E545/E423 构成核心催化三联体; D497 在催化过程中起辅助作用 Situated at β-helix interfaces. Acidic residues constitute: Core catalytic triad: D546/E545/E423, Auxiliary catalytic residue: D497	/	[42]
K21	KP32gp38	N 端结构域、β-螺旋结构域、CBM、C 端结构域 N-terminal domain, β-helix domain, CBM, C-terminal domain	位于相邻单体界面 β-螺旋结构域; 带负电荷的酸性氨基酸残基构成 D241-E170 核心催化残基对和 E239-D229 辅助催化残基对; D167 具有弱催化活性 Situated at β-helix interfaces. Acidic residues constitute: Core catalytic residue pair: D241-E170, Auxiliary catalytic residue pair: E239-D229, Weak catalytic residue: D167	KP32gp38 通过 N 端 29 个残基的柔性区域, 以纳摩尔级亲和力结合 KP32gp37, 实现对 K3 与 K21 型肺炎克雷伯氏菌协同靶向; 含有芳香族残基的 CBM 将 CPS 锚定在催化位点进而促进催化; C 端呈 H 型凝集素样折叠结构, 促进 CPS 结合 KP32gp38 utilizes a 29-residue flexible region at its N-terminal domain to bind KP32gp37 with nanomolar affinity, enabling cooperative targeting of <i>K. pneumoniae</i> capsular types K3 and K21. The aromatic residues of CBM anchor CPS at the catalytic site, facilitating catalysis. H-type lectin-like fold of C-terminal domain enhances CPS binding	[58]

(待续)

(续表 2)

荚膜血清型	解聚酶	结构域组成	催化位点	结构域特性	参考文献
Capsular serotype	Depolymerase	Domain composition	Catalytic site	Domain property	Reference
K63	KP34gp57	N 端结构域、 $\beta$ -螺旋结构域、插入结构域、C 端结构域 N-terminal domain, $\beta$ -helix domain, Insertion domain, C-terminal domain	位于单体内 $\beta$ -螺旋结构域和 $\beta$ -桶状插入域共同形成催化口袋; 带负电荷的酸性氨基酸残基 E66 和 E330 为核心催化残基; 位于插入域的 D151 辅助 CPS 结合 Formed within monomer by $\beta$ -helix and $\beta$ -barrel insertion domains. Acidic residues constitute: Core catalytic residues: E66/E330, Auxiliary catalytic residue (associated with CPS binding): D151 (located in insertion domain)	含延伸环(extended loop, EL)以稳定插入域; C 端结构域的构象类似于 CBM, 表现出高度抗蛋白酶解特性 Extended loop (EL) stabilizes insertion domain. C-terminal domain exhibits a CBM-like conformation, demonstrating marked resistance to proteolytic degradation	[69]
K64	K64-ORF1	N 端结构域、 $\beta$ -螺旋结构域、插入结构域、C 端结构域 N-terminal domain, $\beta$ -helix domain, Insertion domain, C-terminal domain	位于单体内 $\beta$ -螺旋结构域和 $\beta$ -桶状插入域共同形成催化口袋; 带正电荷的碱性氨基酸残基 Y528/H574/R628 构成核心催化三联体 Formed within monomer by $\beta$ -helix and $\beta$ -barrel insertion domains. Acidic residues constitute: Core catalytic triad: Y528/H574/R628	含 EL 以稳定插入域, EL 通过塑造表面沟槽影响底物特异性 EL shapes substrate-binding groove, modulating specificity	[73]

著降解活性<sup>[86]</sup>; 部分含插入结构域的解聚酶催化残基位于单体内, 其中解聚酶 KP34gp57 已被证明可以单体形式存在并保留完整催化活性<sup>[73]</sup>, 挑战了三聚体为酶活性必要条件的传统认知。在稳定性方面, 得益于其复杂的空间结构, 解聚酶通常展现出高稳定性, 能在较宽的温度和 pH 值范围内保持活性, 并能耐受蛋白酶和洗涤剂外界因素干扰<sup>[20]</sup>。

## 2 噬菌体解聚酶的临床功能应用

### 2.1 抗生物膜

常规抗菌方法难以去除生物膜, 显著加剧了抗感染治疗的复杂性<sup>[87]</sup>。而某些噬菌体编码的解聚酶可降解生物膜的主要成分 EPS<sup>[17]</sup>。Wu 等<sup>[61]</sup>发现解聚酶 Dep42 对 K47 型肺炎克雷伯氏菌生物膜兼具抑制其形成和降解已形成生物膜的双重作用; Latka 等<sup>[88]</sup>证实噬菌体解聚酶 KP34p57、噬菌体 KP15 与环丙沙星联合使用的

抗生物膜效果显著优于单独使用抗生素; Chai 等<sup>[89]</sup>的研究表明, 肺炎克雷伯氏菌噬菌体解聚酶能显著提升二氧化氯的消毒效能, 实现对生物膜的高效清除。由此可见, 解聚酶在生物膜的预防和清除方面极具应用潜力。

### 2.2 治疗细菌性感染

CPS 是肺炎克雷伯氏菌的重要毒力因子<sup>[90]</sup>。多项体外试验证实, 解聚酶能够通过特异性地识别并降解 CPS, 降低细菌毒力并破坏其防御体系, 从而增强血清补体、巨噬细胞及中性粒细胞对细菌的杀伤作用<sup>[42-43,68,70,72]</sup>。尽管细菌可能通过群体进化产生解聚酶耐受突变, 但 Kaszowska 等<sup>[91]</sup>发现这种突变体对宿主先天免疫反应的敏感性显著高于原始菌株, 表现出适应性代偿现象。多个研究团队已建立不同动物模型模拟肺炎克雷伯氏菌感染(如小鼠或大蜡螟的全身性感染模型); 通过直接向健康小鼠体内注射细菌及在接种细菌前用环磷酸胺等免疫

抑制剂处理健康小鼠的方式构建社区感染模型和医院感染模型,并利用解聚酶进行感染治疗;这些体内试验结果进一步验证了解聚酶的治疗价值(表 3)。此外, Pan 等<sup>[70]</sup>还对解聚酶 K64dep 进行了急性毒性研究,比较治疗组(酶使用剂量高达 150  $\mu\text{g}$ )与对照组之间的血清生化指标、组织病理学损伤、体重、异常行为、外观变化及不良反应,未发现显著差异。上述研究结果充分证实了解聚酶在抗感染治疗中兼具高效性和安全性。

另外,研究结果表明解聚酶的疗效与具体给药方案密切相关(表 3)。给药时机是影响疗效的首要因素,感染早期给药可实现最佳治疗效果<sup>[42,55-56,70,74]</sup>,预防性给药也能有效降低感染风险<sup>[51,63,66,68,75]</sup>。给药模式上,低剂量、多次频繁给药可持续维持血药浓度,因此能获得优于早期单次给药的疗效<sup>[42,48]</sup>。给药剂量同样关键,较高剂量能显著增强解聚酶在体内的治疗效果<sup>[40,42,44,48]</sup>。同时,感染严重程度(受菌株类型及菌量影响)和酶的活性也会影响解聚酶的疗效<sup>[44,51,55,60,65]</sup>。

### 2.3 解聚酶与其他抗菌药物联合治疗

解聚酶通过降解细菌表面的多糖结构破坏细菌防御屏障,显著增强抗菌药物向细菌胞内的渗透能力,在联合治疗中发挥协同增效作用。在体外试验中,解聚酶 Dep\_ZX1 可通过抑制生物膜形成或降解成熟生物膜,提高生物膜内细菌对卡那霉素、庆大霉素和链霉素等抗生素的敏感性<sup>[66]</sup>;解聚酶 Dep42 与多黏菌素联用时同样表现出显著的协同效应<sup>[61]</sup>。这种协同性在体内模型中也得到进一步证实:利用解聚酶 Depo32 联合庆大霉素治疗肺炎克雷伯氏菌感染小鼠时,其疗效显著优于单用解聚酶或单用抗生素<sup>[42]</sup>。此外, Smug 等<sup>[92]</sup>尝试联合使用解聚酶 KP34p57 与噬菌体 KP15、KP27 处理肺炎克雷伯氏菌,结果表明发现解聚酶与噬菌体联用的策略能够有效抑制肺炎克雷伯氏菌群体中噬菌体抗性突变菌株的产生。

### 2.4 疫苗开发

细菌的重要毒力因子 CPS 是研发细菌感染预防疫苗的核心抗原成分。疫苗研发通常采用多糖-蛋白质结合的抗原形式,如糖结合疫苗(glycoconjugate vaccine)<sup>[93]</sup>。然而,肺炎克雷伯氏菌(尤其是高毒力 K1/K2 血清型)的 CPS 具有高分子量和高黏性特征,极大增加了疫苗制备难度。尽管化学解聚处理(如使用三氟乙酸、氢氧化铵、乙酸等) CPS 可提高其与蛋白质结合的效率,却易破坏 CPS 的乙酰化/丙酮酸化修饰,进而削弱免疫原性<sup>[94]</sup>。因此,目前临床上仍缺乏有效靶向肺炎克雷伯氏菌的疫苗。在此背景下,解聚酶展现出了突破性潜力。Lin 等<sup>[36]</sup>利用解聚酶 K1-ORF34 和 K2-ORF16 对 K1/K2 型肺炎克雷伯氏菌 CPS 进行特异性降解,获得了长度均一并保留重要翻译后修饰的寡糖片段,与载体蛋白 CRM197 偶联后,成功构建靶向性 CPS-蛋白结合疫苗。体内试验验证了肌肉注射该疫苗的小鼠,其体内产生了高效且持久的杀菌免疫反应,与未接种组相比,其抗 CPS 抗体的杀菌活性分别提高了 128 倍(K1 型)和 64 倍(K2 型),有效抵御了相应血清型肺炎克雷伯氏菌的感染<sup>[36]</sup>。

### 2.5 荚膜分型

解聚酶凭其高度的底物特异性,在细菌菌株鉴定和荚膜分型中展现出重要价值。例如, Hsu 等<sup>[76]</sup>发现,源自噬菌体 0507-KN2-1 的解聚酶 ORF96 仅能裂解 KN2 型肺炎克雷伯氏菌,对 KN2 型荚膜缺失突变株及 78 种其他血清型肺炎克雷伯氏菌菌株均无作用,印证了 ORF96 作为 KN2 型分型工具的可靠性。此外,广宿主谱噬菌体  $\Phi\text{K64-1}$  虽能感染多种血清型的肺炎克雷伯氏菌,但它编码的 9 种不同的解聚酶分别靶向不同血清型,进一步肯定了解聚酶的精准分型能力<sup>[37]</sup>。不仅如此, Lin 等<sup>[35]</sup>的研究还揭示了解聚酶在菌株分型中的稳定性优势;他们发现,尽管噬菌体 NTUH-K2044-K1-1 本身对 K1 型菌株也具有特异性,但对同属于 K1 型的

表 3 肺炎克雷伯氏菌噬菌体解聚酶在动物感染模型中的治疗效果

Table 3 The efficacy of *K. pneumoniae* bacteriophage depolymerases in animal infection models

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	动物感染模型/菌量 Animal infection model/Bacterial load	给药方案 Administration regimen	结果 Result	参考文献 Reference
K1	K1-ORF34	健康小鼠/ $3.3 \times 10^3$ CFU; 免疫缺陷小鼠/ $1.6 \times 10^3$ CFU Healthy mice/ $3.3 \times 10^3$ CFU; immunodeficient mice/ $1.6 \times 10^3$ CFU	(1) 不注射 K1-ORF34。感染 30 min 后, 单次腹腔注射 K1-ORF34 (25 $\mu$ g); (2) 健康小鼠; (3) 免疫缺陷小鼠 (1) No injection of K1-ORF34. A single intraperitoneal (i.p.) injection of K1-ORF34 (25 $\mu$ g) was administered 30 min post-infection to: (2) healthy mice; and (3) immunodeficient mice	30 d 存活率: (1) 12.5%; (2) 100%; (3) 100% 30-day survival: (1) 12.5%; (2) 100%; (3) 100%	[35]
	Depo16	小鼠菌血症模型/ $3 \times 10^5$ CFU Murine bacteremia model/ $3 \times 10^5$ CFU	(1) 不注射 Depo16。感染 1 h 后, 单次腹腔注射 Depo16: (2) 10 $\mu$ g; (3) 25 $\mu$ g (1) No injection of Depo16. A single i.p. injection of Depo16 was administered 1 h post-infection at the following doses: (2) 10 $\mu$ g; (3) 25 $\mu$ g	7 d 存活率: (1) 0%; (2) 80%; (3) 100% 7-day survival: (1) 0%; (2) 80%; (3) 100%	[40]
K2	Depo32	小鼠急性肺炎模型/ $10^7$ CFU Murine acute pneumonia model/ $10^7$ CFU	(1) 不注射 Depo32。(2) 连续 3 d 鼻内给予 Depo32 (20 $\mu$ g)。感染后单次鼻内给予 Depo32 (200 $\mu$ g): (3) 1 h; (4) 2 h; (5) 12 h。感染后单次鼻内给予 Depo32 (20 $\mu$ g): (6) 1 h; (7) 2 h; (8) 12 h (1) No injection of Depo32. (2) Intranasal administration of Depo32 (20 $\mu$ g) once daily for 3 consecutive days before infection. Post-infection single intranasal administration of Depo32 (200 $\mu$ g): (3) 1 h; (4) 2 h; (5) 12 h. Post-infection single intranasal administration of Depo32 (20 $\mu$ g): (6) 1 h; (7) 2 h; (8) 12 h	7 d 存活率: (1) 0%; (2) 100%; (3) 100%; (4) 40%; (5) 20%; (6) 40%; (7-8) 20%	[42]
	Dep1979	健康小鼠和免疫缺陷小鼠败血症感染模型 Sepsis model in healthy/ immunodeficient mice	(1) 不注射 Dep1979。健康小鼠感染菌量 $10^6$ CFU 后 1 h, 单次尾静脉注射 Dep1979: (2) 1 mg/kg; (3) 0.1 mg/kg; (4) 0.01 mg/kg; (5) 0.001 mg/kg。健康小鼠感染 1 h 后, 单次尾静脉注射 Dep1979 (0.1 mg/kg), 感染菌量为: (6) $10^7$ CFU; (7) $10^6$ CFU; (8) $10^5$ CFU。免疫缺陷小鼠感染 1 h 后, 单次尾静脉注射 Dep1979 (0.1 mg/kg), 感染菌量为: (9) $10^7$ CFU; (10) $10^6$ CFU; (11) $10^5$ CFU; (12) $10^4$ CFU; (13) $10^3$ CFU (1) No injection of Dep1979. A single dose of Dep1979 was administered via tail vein injection to healthy mice 1 h after infection with a bacterial load of $10^6$ CFU, at the following doses: (2) 1 mg/kg; (3) 0.1 mg/kg; (4) 0.01 mg/kg; (5) 0.001 mg/kg. A single dose of Dep1979 (0.1 mg/kg) was administered via tail vein injection to healthy mice 1 h after infection, the infection dose varied among groups as follows: (6) $10^7$ CFU; (7) $10^6$ CFU; (8) $10^5$ CFU. A single dose of Dep1979	7 d 存活率: (1) 0%; (2) 100%; (3) 100%; (4) 60%; (5) 0%; (6) 100%; (7) 100%; (8) 100%; (9) 0%; (10) 0%; (11) 0%; (12) 30%; (13) 60% 7-day survival: (1) 0%; (2-3) 100%; (4) 60%; (5) 0%; (6-8) 100%; (9-11) 0%; (12) 30%; (13) 60%	[44]

(待续)

(续表 3)

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	动物感染模型/菌量 Animal infection model/Bacterial load	给药方案 Administration regimen	结果 Result	参考文献 Reference
			(0.1 mg/kg) was administered via tail vein injection to immunodeficient mice 1 h after infection, the infection dose varied among groups as follows: (9) $10^7$ CFU; (10) $10^6$ CFU; (11) $10^5$ CFU; (12) $10^4$ CFU; (13) $10^3$ CFU		
	Dep_Kpv74	小鼠大腿软组织感染模型/ $10^4$ CFU Murine thigh soft tissue infection model/ $10^4$ CFU	(1) 不注射 Dep_Kpv74。感染 30 min 后, 单次腹腔注射 Dep_Kpv74; (2) 10 $\mu$ g; (3) 20 $\mu$ g; (4) 40 $\mu$ g。连续 5 d 腹腔注射(40 $\mu$ g); (5) 感染 3 h 后; (6) 感染 24 h 后 (1) No injection of Dep_Kpv74. A single i.p. injection of Dep_Kpv74 was administered 30 min post-infection at the following doses: (2) 10 $\mu$ g; (3) 20 $\mu$ g; (4) 40 $\mu$ g. A 5-day consecutive i.p. injection regimen (40 $\mu$ g/d) was initiated: (5) 3 h post-infection; (6) 24 h post-infection	14 d 存活率: (1) 0; (2) 80%; (3) 80%; (4) 90%; (5) 60%; (6) 60% 14-day survival: (1) 0; (2-3) 80%; (4) 90%; (5-6) 60%	[48]
K3	KP32gp37	大蜡螟感染模型/ $10^7$ CFU <i>Galleria mellonella</i> infection model/ $10^7$ CFU	(1) 不注射 KP32gp37。(2) 感染前 KP32gp37 (200 $\mu$ g/mL) 预孵育 2 h 细菌。(3) 接种细菌的同时注射 KP32gp37 (200 $\mu$ g/mL) (1) No injection of KP32gp37. (2) Bacterial pre-incubation with KP32gp37 (200 $\mu$ g/mL) for 2 h. (3) Co-injection with KP32gp37 (200 $\mu$ g/mL)	感染 24 h 后, (2) 和(3)幼虫的存活率较组(1)至少提高 13% At 24 h post-infection, survival rates in groups (2) and (3) were at least 13% higher than in group (1)	[51]
K5	dep1011	小鼠模型/ $10^8$ CFU Murine model/ $10^8$ CFU	感染 K5 型菌株 B16/KP181 后 2 h: (1) 不注射 dep1011; (2) 单次腹腔注射(50 $\mu$ g) At 2 h post-infection with the K5-type strain B16/KP181: (1) No injection of dep1011; (2) A single i.p. injection of dep1011 (50 $\mu$ g)	3 d 存活率: (1) 0; (2) 100% 3-day survival: (1) 0; (2) 100%	[54]
K19	Dpo41	大蜡螟感染模型/ $5 \times 10^4$ CFU <i>G. mellonella</i> infection model/ $5 \times 10^4$ CFU	(1) 不注射 Dpo41。感染后单次腹腔 Dpo41 (2 $\mu$ g); (2) 5 min; (3) 30 min (1) No injection of Dpo41. A single i.p. injection of Dpo41 (2 $\mu$ g) was administered post-infection at: (2) 5 min; (3) 30 min	5 d 存活率: (1) 0; (2) 70%; (3) 50% 5-day survival: (1) 0; (2) 70%; (3) 50%	[56]
K20	K20dep	小鼠模型 Murine model	(1) 不注射 K20dep。感染高毒力 K20 型 A13 菌株( $3 \times 10^3$ CFU)后, 单次腹腔注射 K20dep (35 $\mu$ g); (2) 1 h; (3) 8 h。感染碳青霉烯耐药 K20 型 KP440 菌株( $3 \times 10^7$ CFU)后, 单次腹腔注射 K20dep (35 $\mu$ g); (4) 1 h; (5) 8 h (1) No injection of K20dep. Following infection with the hypervirulent K20-type strain A13	30 d 存活率: (1) 0; (2) 100%; (3) 100%; (4) 100%; (5) 0 30-day survival: (1) 0; (2-4) 100%; (5) 0	[55]

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(续表 3)

荚膜血清型	解聚酶	动物感染模型/菌量	给药方案	结果	参考文献
Capsular serotype	Depolymerase	Animal infection model/Bacterial load	Administration regimen	Result	Reference
K21	KP32gp38	大蜡螟感染模型/ 10 <sup>7</sup> CFU <i>G. mellonella</i> infection model/10 <sup>7</sup> CFU	(3×10 <sup>3</sup> CFU), a single i.p. injection of K20dep (35 μg) was administered post-infection at: (2) 1 h; (3) 8 h. Following infection with the carbapenem-resistant K20-type strain KP440 (3×10 <sup>7</sup> CFU), a single i.p. injection of K20dep (35 μg) was administered post-infection at: (4) 1 h; (5) 8 h	3 d 存活率: (1) 0; (2) 大于 50%; (3) 大于 70%; (4) 0; (5) 大于 70%; (6) 大于 70%; (7) 0	[51]
K23	Dep622 DepS8	大蜡螟感染模型 <i>G. mellonella</i> infection model	(1) 不注射 KP32gp38。感染前 KP32gp38 (终浓度 200 μg/mL) 预孵育 2 h 细菌: (2) K21 型菌株 358; (3) K21 型菌株 968; (4) K21 型菌株 45。接种细菌的同时注射 KP32gp38 (终浓度 200 μg/mL): (5) K21 型菌株 358; (6) K21 型菌株 968; (7) K21 型菌株 45 (1) No injection of KP32gp38. Bacterial pre-incubation with KP32gp38 (final concentration 200 μg/mL) for 2 h prior to infection was performed for the following strains: (2) K21 type strain 358; (3) K21 type strain 968; (4) K21 type strain 45. Co-injection of KP32gp38 (final concentration 200 μg/mL) with bacterial inoculation was performed for the following strains: (5) K21 type strain 358; (6) K21 type strain 968; (7) K21 type strain 45	3-day survival: (1) 0; (2) >50%; (3) >70%; (4) 0; (5) >50%; (6) >70%; (7) 0	[60]
K47	P560dep	小鼠菌血症模型/ 2.5×10 <sup>7</sup> CFU Murine bacteremia model/2.5×10 <sup>7</sup> CFU	感染 3×10 <sup>5</sup> CFU: (1) 不注射 Dep622/DepS8; 接种同时注射 (2 μg): (2) Dep622; (3) DepS8。感染 3×10 <sup>6</sup> CFU: (4) 不注射 Dep622/DepS8; 接种同时注射 (2 μg): (5) Dep622; (6) DepS8 With an inoculum size of 3×10 <sup>5</sup> CFU, (1) No injection of Dep622/DepS8; Co-injection (2 μg) with bacterial inoculation: (2) Dep622; (3) DepS8. With an inoculum size of 3×10 <sup>6</sup> CFU: (4) No injection of Dep622/DepS8; Co-injection (2 μg) with bacterial inoculation: (5) Dep622; (6) DepS8	5 d 存活率: (1) 30%; (2) 70%–90%; (3) 70%–90%; (4) 0; (5) 70%–90%; (6) 70%–90%; (7) 70%–90%	[63]
K57	Dep_kpv79 Dep_kpv767	小鼠败血症和大腿软组织感染模型/2×10 <sup>8</sup> CFU Murine models of sepsis/thigh soft tissue infection/2×10 <sup>8</sup> CFU	(1) 不注射 P560dep。单次腹腔注射 P560dep (50 μg): (2) 感染前 1 h; (3) 感染 2 h 后 (1) No injection of P560dep. A single i.p. injection of P560dep (50 μg) was administered: (2) 1 h before infection; (3) 2 h after infection	7 d 存活率: (1) 10%; (2) 100%; (3) 90%	[63]
			败血症: A. 感染 K57 型菌株 550 后 30 min: (1) 不注射; 单次腹腔注射 (50 μg): (2) Dep_kpv79; (3) Dep_kpv767。B. 感染 K57 型菌株 156 (毒力较弱) 后 30 min: (4) 不注射; 单次腹腔注射 (50 μg): (5) Dep_kpv79;	15 d 存活率: (1) 10%; (2) 90%; (3) 80%; (4) 40%; (5) 100%; (6) 90%; (7) 20%;	

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(续表 3)

荚膜血清型 Capsular serotype	解聚酶 Depolymerase	动物感染模型/菌量 Animal infection model/Bacterial load	给药方案 Administration regimen	结果 Result	参考文献 Reference
			(6) Dep_kpv767。大腿软组织感染(K57 型菌株 550); (7) 不注射; 单次腹腔注射(50 μg); (8) Dep_kpv79; (9) Dep_kpv767	(8) 90%; (9) 80% [65] 15-day survival: (1) 10%; (2) 90%; (3) 80%; (4) 40%; (5) 100%; (6) 90%; (7) 20%; (8) 90%; (9) 80%	
			Sepsis model: A. Infection with K57 550 for 30 min: (1) No injection. A single i.p. injection (50 μg); (2) Dep_kpv79; (3) Dep_kpv767. B. Infection with K57 type strain 156 (with lower virulence) for 30 min: (4) No injection. A single i.p. injection (50 μg); (5) Dep_kpv79; (6) Dep_kpv767. Thigh soft tissue infection (K57 type strain 550): (7) No injection. A single i.p. injection (50 μg); (8) Dep_kpv79; (9) Dep_kpv767		
	Dep_ZX1	小鼠菌血症模型/ 10 <sup>7</sup> CFU Murine bacteremia model/10 <sup>7</sup> CFU	(1) 不注射 Dep_ZX1。单次腹腔注射 Dep_ZX1 (50 μg); (2) 感染前 1 h; (3) 感染 1 h 后	7 d 存活率: (1) 0; (2) 100%; (3) 100%	[66]
K63	depoKP36	大蜡螟感染模型/ 10 <sup>7</sup> CFU <i>G. mellonella</i> infection model/10 <sup>7</sup> CFU	(1) 不注射 depoKP36。(2) 感染前 2 h, 单次注射(280 μg/mL)	3 d 存活率:(1) 0; [68] (2) 43%; (3) 20%	[68]
			后, 单次注射(280 μg/mL)	3-day survival: (1) 0; (2) 43%; (3) 20%	
K64	S2-5(K64dep)	免疫缺陷小鼠菌血症模型/6×10 <sup>6</sup> CFU Bacteremia model in immunodeficient mice/6×10 <sup>6</sup> CFU	(1) 不注射 K64dep。(2) 感染 1 h 后, 单次腹腔注射 K64dep (18.75/37.5/150 μg)。(3) 感染 8 h 后, 单次腹腔注射 K64dep (18.75/37.5/150 μg)。(4) 感染 24 h 后, 单次腹腔注射 K64dep (18.75/37.5/150 μg)	(1) 2 d 存活率 25%。(2) 30 d 存活率均为 100%。 (3) 无效。 (4) 无效。	[70]
			(1) No injection of K64dep. (2) A single i.p. injection of K64dep (18.75/37.5/150 μg) at 1 h post-infection. (3) Single i.p. injection of K64dep (18.75/37.5/150 μg) at 8 h post-infection. (4) Single i.p. injection of K64dep (18.75/37.5/150 μg) at 24 h post-infection	(1) 2-day survival: 25%; (2) 30-day survival: 100% in all treated groups; (3-4) No effect	
	Dep37	大蜡螟感染模型/ 1.5×10 <sup>4</sup> CFU/mL <i>G. mellonella</i> infection model/1.5×10 <sup>4</sup> CFU	(1) 不注射 Dep37。感染后单次注射 Dep37 (10 μL); (2) 5 min; (3) 2 h	5 d 存活率:(1) 0; [74] (2) 73%; (3) 53%	[74]
			(1) No injection of Dep37. A single injection of Dep37 (10 μL) was administered post-infection at: (2) 5 min; (3) 2 h	5-day survival: (1) 0; (2) 73%; (3) 53%	
KN1	Dp42	小鼠菌血症模型/ 2×10 <sup>7</sup> CFU Murine bacteremia model/2×10 <sup>7</sup> CFU	(1) 不注射 Dp42。单次腹腔注射 Dp42 (50 μg); (2) 感染前 6 h; (3) 感染 30 min 后	4 d 存活率: (1) 0; (2) 100%; (3) 100%	[75]
			(1) No injection of Dp42. A single i.p. injection of Dp42 (50 μg) was administered: (2) 6 h before infection; (3) 30 mins after infection	4-day survival: (1) 0; (2-3) 100%	

21 种菌株感染效力存在显著差异；然而，其编码的解聚酶 K1-ORF34 却对这些菌株具有一致的裂解效果。以上研究结果充分表明，解聚酶在菌株分型应用中兼具高特异性与稳定性，是更具发展潜能的分型技术手段。

### 3 解聚酶的工程改造

解聚酶的结构特征为其目的性改造提供了结构生物学理论基础，对催化位点关键氨基酸进行定点突变可增强酶的活性，而通过突变底物识别位点则可改变其裂解谱范围。例如，Squeglia 等<sup>[58]</sup>揭示了解聚酶 KP32gp38 催化位点的不同关键氨基酸残基对酶活性影响差异显著且存在多残基协同效应。此外，研究表明可通过序列截短的方式获取性能优化的微型酶。一般而言，较小分子量的酶通常更易制备，并表现出更好的药代动力学和储存稳定性。例如，截短解聚酶 KP34gp37 的 N 端和 C 端所获得的突变体 NC-A17-G581 和 NC-A17-T522，在催化活性、稳定性及组织渗透能力上均显著优于原始酶<sup>[69]</sup>。进一步可基于解聚酶的模块化结构特征重组或融合不同功能域赋予其新功能。例如，将可提升催化效率的 CBM 域、具备底物结合力的 C 端结构域及能将不同血清特异性解聚酶相互结合的柔性 N 端结构域等进行组合<sup>[58]</sup>。综合运用上述基因工程手段，有望构建出靶向目标宿主的高效嵌合解聚酶，显著拓展其应用潜能。

### 4 总结与展望

肺炎克雷伯氏菌作为一种临床重要的条件致病菌，其耐药问题日趋严峻，给临床治疗带来了巨大挑战。噬菌体编码的解聚酶作为一种新型抗菌策略，较完整噬菌体颗粒具有稳定性高、特异性强及不易诱导细菌产生抗性等优势，在抗耐药菌感染领域展现丰富的应用潜力。然而，在推进临床转化的进程中，仍有若干关键科学问题亟待突破。首先，在安全性与有效性方面，当前关于解聚酶防治感染的研究

局限于体外试验和小鼠动物模型，与人体复杂感染微环境存在较大差异。未来需借助类器官模型等，动态解析解聚酶在真实感染场景中的作用效果。同时，需建立标准化安全评价体系，重点关注解聚酶的致敏性、免疫原性(如是否诱导中和抗体)及内毒素污染[美国食品药品监督管理局(Food and Drug Administration, FDA)]规定，内毒素的控制量通常以 K/M 表示。其中 K 为致热阈值，普通注射 K=5 EU/kg、鞘内注射 K=0.2 EU/kg；M 为最大给药剂量<sup>[95]</sup>。其次，高度特异性是解聚酶的显著优势，却也成为广谱治疗的瓶颈。为突破这一局限，可通过以下策略进行优化：(1) 建立基于解聚酶的快速荚膜分型技术，实现感染菌株的床旁鉴定与解聚酶的个性化匹配；(2) 基因工程定向改造解聚酶的底物结合域，拓展其识别范围；(3) 构建“解聚酶鸡尾酒”制剂，联合使用针对不同荚膜型的解聚酶。另外，可添加表面活性剂、蛋白酶抑制剂等稳定辅料制成配方，确保其在储存及给药过程中的理化稳定性；可采用冻干解聚酶技术、设计水凝胶或纳米递送系统等实现其在体内的稳定转运与高效靶向递送<sup>[96]</sup>。在给药方案上，需结合药代动力学优化给药剂量与途径，探索解聚酶与抗生素等多种抗菌剂的协同作用效果。尤为关键的是，需通过多中心 III 期临床试验夯实解聚酶的临床证据基础。可设计随机双盲试验，在不同地区的三甲医院招募 CR-hvKp 感染患者，以标准抗生素治疗为对照，评估解聚酶单药或联合用药的有效性(如临床治愈率、细菌清除率等)与安全性(如药物相关不良反应发生率、肝肾功能影响)。此外，需建立跨中心耐药监测网络，长期追踪解聚酶对细菌荚膜表型及宿主免疫应答的影响。综上所述，肺炎克雷伯氏菌解聚酶为解决肺炎克雷伯氏菌感染和耐药问题提供了全新思路。尽管目前面临诸多挑战，但随着研究的不断深入，这一新型生物制剂有望成为对抗耐药菌的有效手段，为公共卫生健康做出重要贡献。

## 作者贡献声明

刘伊晨：数据收集，提供材料，撰写文章；黄焯：提出概念，方案设计，监督指导，稿件润色修改，获取基金；徐新平：项目管理，审阅，稿件润色修改，获取基金。

## 作者利益冲突公开声明

作者声明绝无任何可能会影响本文所报告工作的已知经济利益或个人关系。

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