生 物 工 程 学 报
Chinese Journal of Biotechnology
http://journals.im.ac.cn/cjbcn
DOI: 10.13345/j.cjb.240241

Nov. 25, 2024, 40(11): 3861-3871

·综 述·

活细菌作为抗肿瘤药物递送载体的研究进展

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华婷婷, 郑斌, 白洋. 活细菌作为抗肿瘤药物递送载体的研究进展[J]. 生物工程学报, 2024, 40(11): 3861-3871.
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摘 要：细菌可以选择性地在肿瘤部位定殖并抑制肿瘤生长，是递送抗肿瘤药物的良好载体。以活细菌为载体递送抗肿瘤药物的系统具有生物相容性好、靶向性强等特点。然而，细菌自身的免疫原性限制了其作为药物递送载体的发展。本文从底盘细菌的选择、细菌负载药物策略、抗肿瘤药物递送应用及其局限性等方面进行了详细阐述，并展望了其未来的发展方向，为活细菌作为抗肿瘤药物递送载体的研究提供了参考。

关键词：活细菌；工程细菌；递送策略；药物；肿瘤

Advances in the application of live bacteria as vehicles for delivering antitumor drugs

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Abstract: Some bacteria can selectively colonize the tumor site and inhibit tumor growth, serving as ideal vehicles for delivering antitumor drugs. The system of delivering antitumor drugs with live bacteria as vehicles is characterized by good biocompatibility and precise targeting. However, the development of bacteria as drug delivery vehicles is limited by their own immunogenicity. In this paper, the selection of chassis bacteria, bacterial loading drug strategies, antitumor drug delivery applications and their limitations are elaborated in detail, and its future development direction is envisioned, with a view to providing a reference for the study of live bacteria as antitumor drug delivery carriers.

Keywords: live bacteria; engineered bacteria; delivery strategies; drugs; tumors

资助项目：国家自然科学基金(32000999, 32271400)；天津市自然科学基金(19JCZDJC34100)
This work was supported by the National Natural Science Foundation of China (32000999, 32271400) and the Tianjin Natural Science Foundation (19JCZDJC34100).
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Received: 2024-03-19; Accepted: 2024-05-30; Published online: 2024-06-03

肿瘤是公共卫生领域面临的一项重大挑战。细菌包括双歧杆菌、大肠杆菌和沙门氏菌等已被尝试用于肿瘤治疗的研究[1]。这些细菌可以通过生物或化学方法进行改造以满足特定需求，并且很容易进行大规模培养。此外，细菌自身的肿瘤靶向性为其作为抗肿瘤药物的输送载体提供了一种新的思路。专性和兼性厌氧菌可以选择性地在肿瘤组织中定殖，与正常组织的分布比大于1 000:1[2]，通过竞争营养物质、诱导肿瘤细胞凋亡和免疫激活等多种机制发挥抗肿瘤作用。因此，细菌具有天然的优势，既可作为治疗药物，又可作为药物载体[3]。然而，细菌自身的免疫原性限制了其作为药物递送载体的发展[4-5]。本文深入探讨了底盘细菌的选择标准，以确保其在药物递送系统中的高效性和稳定性；进一步阐述了细菌介导的药物负载策略，以及这些策略在抗肿瘤药物递送中的实际应用，同时也指出了当前存在的局限性。同时，对细菌疗法在未来抗肿瘤领域的发展趋势进行了前瞻性的展望，以期为该领域的研究提供新的思路和方向(图 1)。

1 细菌选择

选择抗肿瘤药物递送细菌的策略主要有2种：一是细菌毒性低、应用安全；二是细菌靶向肿瘤区域，能够将药物高效递送到肿瘤区域。

1.1 低毒性

1997 年，Pawelek 等[6]使用野生型鼠伤寒沙门氏菌对携带黑色素瘤的小鼠进行接种，小鼠因细菌毒性而死亡。细菌的致病性已成为肿瘤治疗的主要障碍，活细菌可能会在血液中增殖、释放毒素，甚至导致严重的败血性休克和死亡[7-8]。因此，为确保安全应用，细菌的毒性必须减弱。目前，研究人员主要通过基因工程来降低细菌的毒性。与传统减毒方法相比，使用基因工程构建减毒细菌突变株不仅有效降低了细菌的致病性，还保留了细菌的肿瘤靶向能力[9-10]。

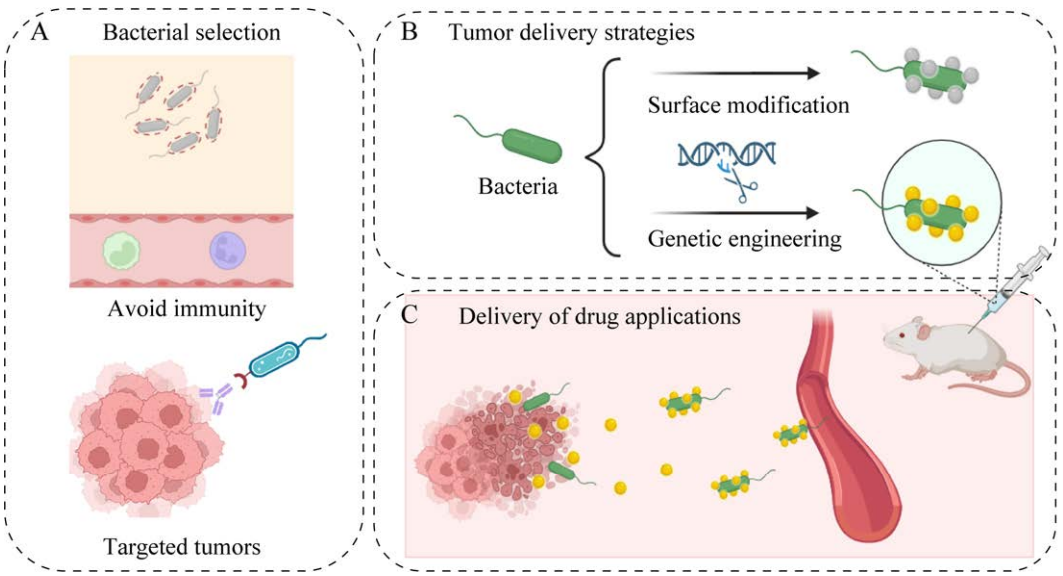

图 1 活细菌作为抗肿瘤药物递送载体的研究进展 A：细菌选择. B：细菌负载药物策略. C：抗肿瘤药物递送

Figure 1 Advances in the study of live bacteria as tumor drug delivery vehicles. A: Bacterial selection. B: Bacterial load drug strategies. C: Tumor drug delivery.

减毒细菌作为癌症治疗工具在过去十几年中引发了越来越多的关注[11]。同时，低毒性也成为肿瘤递送药物载体的一项重要选择指标。

沙门氏菌、李斯特菌、双歧杆菌、大肠杆菌等减毒株已经被广泛应用于肿瘤治疗[12]。常用的减毒方法有突变毒力基因、引入营养突变等[13]。部分沙门氏菌减毒株突变基因见表 1。值得注意的是，并不是所有致病基因都可以作为减毒基因，如删除其基因组中致病岛 2 (salmonella pathogenicity islands 2, SPI2)会使细菌丧失抗肿瘤活性[19]。在沙门氏菌减毒的研究中，Zhang 等[23]发现 Δ*htrA*-VNP20009 具有与野生型 VNP 20009 相同的高肿瘤靶向特性且安全性优异。这证明了减毒细菌突变株不仅能有效降低细菌的致病性，同时还能保留其肿瘤靶向能力。

1.2 靶向肿瘤区域

抗肿瘤药物在体内的非特异性分布不仅会降低疗效，而且会带来严重的副作用[24]。为了解决这个问题，可以将细菌作为抗肿瘤药物递送的有效工具。肿瘤微环境具有低氧、营养物质丰富的特点，有利于细菌增殖[25]。此外，肿瘤微环境呈免疫抑制状态，避免瘤内细菌被宿主免疫系统清除[26]。因此，专性厌氧菌和兼性厌氧菌可以靶向肿瘤区域，与正常组织的分布比大于 1 000:1[2]。细菌的靶向作用还可以通过外部能量、基因工程或表面化学修饰等方式进一步增强[27-28]。另外，细菌作为活生物体具有感知周围环境的能力并具有由鞭毛推动的运动性，可以自发地迁移，从而深入渗透到肿瘤组织中[29]。Westphal 等[30]发现沙门氏菌在感染早期在血管附近形成菌落，随后逐渐迁移到肿瘤的坏死核心并在其中增殖。值得注意的是，Thornlow 等[31]在体外实体瘤模型中研究了细菌运动性对渗透深度的影响，结果表明细菌运动性越强，渗透得会越深。细菌主要通过细胞间易位穿透肿瘤内，它们的运输性不受负载药物的阻碍，这些区域由于缺乏血管而难以通过传统的药物递送途径到达[32]。因此，可以通过细菌载体将抗肿瘤药物递送到传统制剂难以到达的部位。

表 1 沙门氏菌减毒株突变基因

Table 1 Mutated genes of *Salmonella* attenuated strains

Strain	Mutant gene	References
VNP 20009	*purI*, *msbB*	[14]
A1-R	*Leu*, *arg*	[15]
ΔppGpp	*relA*, *spot*	[16]
Ty21a	*galE*, *ilvD*, *viaB*, *rpoS*	[17]
YB1	*aroA*, *asd*	[18]
ZJ III	*Dam*	[19]
SL 7207, SL 3235, WR 4017	*aroA*	[20]
X4064, X4990	*cya*, *crp*	[21]
SF 102	*fliF*, *fliHIJ*	[22]

2 细菌负载药物策略

目前常用细菌负载药物策略主要有两种：一种是使用基因工程改造细菌，使细菌在肿瘤区域原位产生药物；另一种是使用工程化策略，在细菌表面修饰药物或让药物进入细菌内部。

2.1 基因工程策略

细菌通过基因工程改造可以在原位产生治疗物质，从而最大限度地提高药物疗效，减少对其他组织的损伤[33]。细菌通过基因工程产生的药物常与细菌载体联合作用，以激活肿瘤微环境并诱导癌细胞凋亡。通常与细菌载体结合的药物有细胞因子、趋化因子、免疫激活剂、细胞毒素、小分子药物、前体药物转化酶等(表 2)。Hyun 等[49]使用 Δ*ppGpp* 鼠伤寒沙门氏菌表达新抗原，注射这种新型工程菌可持续表达新抗原，有效延长了 CT26 荷瘤小鼠的存活时间。此外，细菌还可以产生血管生成抑制剂。He 等[44]使用工程化长双歧杆菌分泌内源性肿瘤抑素 5 (tumor suppressor 5，TUM-5)，通过诱导肿瘤血管内皮细胞凋亡，有效抑制皮下结直肠癌小鼠模型中的肿瘤进展。

表 2 基于细菌载体的肿瘤药物递送

Table 2 Bacterial vehicles based on tumor drug delivery

Type of drug	Therapeutic drug	References
Cytokines	Interferon α (IFN-α)	[34]
Cytokines	Tum or necrosis factor superfamily member 14 (TNFSF 14)	[34]
Cytokines	Interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 12 (IL-12)	[35]
Chemokines	C-C motif chemokine ligand 21 (CCL 21)	[36]
Immune activators	Bacterial flagellin	[37]
Immune activators	Immune checkpoint nanobodies	[38]
Cytotoxins	TNF-related apoptosis-inducing ligand (TRAIL)	[39]
Cytotoxins	Fas associated with the structural domain of death (FADD)	[40]
Cytotoxins	Bacterial toxins	[41]
Angiogenesis inhibitors	Endothelial repressors	[42]
Angiogenesis inhibitors	Histidine-rich glycoprotein (HPRG)	[43]
Angiogenesis inhibitors	Tumor suppressor 5 (TUM-5)	[44]
Small molecule drug	Azurin	[45]
Small molecule drug	Glucose dehydrogenase (GDH)	[46]
Precursor drug-converting enzyme	Cytosine deaminase	[47]
Tumor antigen	Tumor antigen (Ag) and mouse immunoglobulin G (IgG)	[48]
Tumor antigen	Neoantigen	[49]
Small interfering RNA (siRNA)	shRNA targeting indoleamine 2,3-dioxygenase	[50]
Small interfering RNA (siRNA)	STAT3-specific siRNA	[51]

2.2 工程化策略

细菌负载药物的工程化策略包括物理包封、化学包封和细菌内化(图 2)。

2.2.1 物理包封

物理包封是细菌作为药物载体的常用方法。物理包封主要通过金属有机框架(metal-organic frameworks, MOFs)包裹、外膜包裹、静电吸附等方式将药物负载在细菌表面上。通过这种方式，药物稳定地与细菌结合，形成一个紧密的复合物，从而实现药物的有效递送。其中，MOFs 以其独特的性质在物理包封中发挥着关键作用。MOFs 具有大孔体积、高药物负载能力、可调组成和易于官能化的特点，使用其封装细菌能够改善细菌的生物稳定性和靶向性[52]。Yan 等[53]将光敏剂和化学药物负载到沸石咪唑酯骨架-8 (zeolitic imidazolate frameworks, ZIF-8)上，并在 ZIF-8 形成期间原位包封细菌，实现了肿瘤靶向治疗。此外，生物大分子包括核酸和蛋白质也可以通过细菌传递。细菌的表面带负电，可以通过其他带正电荷的材料将带负电荷生物大分子负载到细菌的表面上[54]。Hu 等[55]将编码血管内皮生长因子受体 2 (vascular endothelial growth factor receptor 2, VEGFR2)的质粒与阳离子聚合物组装以形成阳离子纳米颗粒，然后将它们吸附到细菌上作为涂层，所获得的杂交系统可以有效激活 T 细胞并抑制肿瘤生长。

2.2.2 化学包封

细菌表面存在高水平的固有官能团，如氨基、羧基、羟基和磷酸基等[56]，这些官能团为药物与细菌之间的化学反应提供了可能。因此，除物理包封的方式外，小分子药物还可以通过化学反应连接到细菌表面。通过化学反应，药物能够牢固地负载在细菌表面，减少药物在递送过程中脱落。在众多化学反应中，共价键反应凭借其

稳定性，成为细菌负载小分子药物的一种常用方法。Chen 等[57]将光敏剂吲哚菁绿(indocyanine green，ICG)加载到固体脂质纳米颗粒中，通过酰胺键将纳米颗粒共价连接到鼠伤寒沙门氏菌 YB1 上，在近红外(near infrared, NIR)激光照射下实现了高效光热治疗。此外，Cu(I)催化的叠氮-炔基加成反应，俗称“点击反应”，因其条件温和、效率高、选择性好等优点，在分子连接中得到了广泛应用[58]。Gao 等[59]通过点击反应将紫杉醇叠氮分子锚定在大肠杆菌表面，在耐药肿瘤细胞中富集治疗药物，选择性诱导肿瘤细胞凋亡。

2.2.3 细菌内化

通过物理包封和化学包封将药物负载在细菌表面，需要考虑药物对细菌的潜在毒性。部分药物能够破坏细菌细胞壁和细胞膜，导致细菌迅速死亡。小分子药物的内化策略可以替代物理包封和化学包封。该策略不仅避免了药物对细菌产生毒性，还能够使细菌具有更高的负载量和更好的负载稳定性，从而实现药物的高效靶向递送[60]。药物内化可以通过脂质相似相溶进行，脂质类药物与细菌细胞膜相互作用，使得药物进入细菌内部[61]。Huo 等[62]用双酰胺封端的聚乙二醇(polyethylene glycol, PEG)修饰了阴离子光敏剂，包括二氢卟酚 e6 (chlorin e6, Ce6)和原卟啉(protoporphyrin IX, PpIX)，使蓝藻能够有效内化离子光敏剂。此外，药物内化还可以通过细菌自身的转运蛋白进行。细菌转运蛋白能够识别并主动转运特定的药物分子，从而实现药物的高效内化[63]。Chu 等[64]发现装载在麦芽糊精(maltodextrin, MD)缀合纳米颗粒中的 ICG 可通过细菌特异性转运蛋白(MD 蛋白)内化到细菌中。药物内化充分利用了细菌的生物特性，能够实现更为精准和高效的药物递送，减少药物在递送过程中的损失和副作用，为药物递送提供了新的方向。

3 细菌载体在肿瘤递送药物中的应用

3.1 递送药物

由于细菌在肿瘤组织中的深度和选择性递送，细菌可以将药物递送到肿瘤中。阿霉素(doxorubicin, DOX)是目前临床上常用的一种广谱抗肿瘤药物[65]。近年来，细菌递送 DOX 的研究层出不穷，展示了细菌作为抗肿瘤药物递送载体的蓬勃生机与广阔前景。Xie 等[66]将 DOX 偶联到大肠杆菌 *Nissle* 1917 上，在不影响细菌运动和活力的情况下，实现肿瘤靶向药物递送。Zoaby 等[67]将脂质体 DOX (doxorubicin liposome，Doxil)通过电穿孔加载到沙门氏菌中，细菌到达肿瘤内部后，DOX 从 Doxil 中释放出来，破坏细菌并进行肿瘤治疗。此外，将细菌与放射治疗药物相结合，已成功地在减少副作用的同时，达到了显著的治疗效果。Quispe-tintaya 等[68]用抗

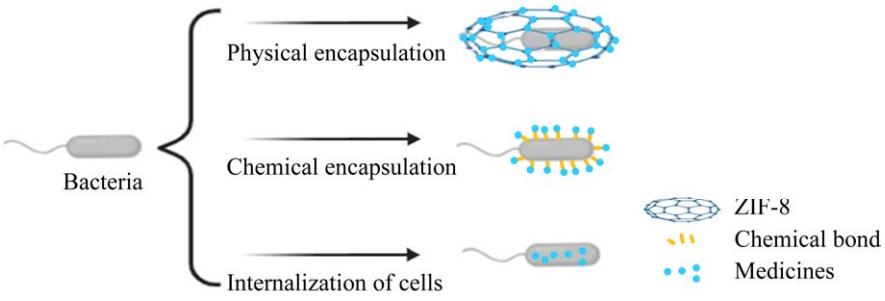

图 2 抗肿瘤药物递送的工程化策略

Figure 2 Chemical engineering strategies for tumor drug delivery.

癌放射性同位素 188-铼修饰的减毒李斯特菌治疗小鼠的转移瘤，在体内高效杀死肿瘤细胞且不伤害正常细胞。Chandra 等[69]把放射治疗药物 32-磷掺入李斯特菌中，以杀死肿瘤细胞。还有一些研究表明，工程菌与放射治疗联合使用时，能够改善放射治疗结果并克服放射治疗的抵抗力[70]。

3.2 细菌与递送药物协同治疗

肿瘤递送药物的工作大多集中在协同治疗上[71]，因为单一疗法的效率往往是有限的[5]。细菌递送药物可以与常规的癌症疗法联合使用，以获得更好的治疗效果。He 等[44]设计了 3 种表达抗肿瘤药物的沙门氏菌，在小鼠实验中发现，与单一菌株的相比，这些工程菌在抑制肿瘤生长方面具有更强的效力。Liu 等[26]对小鼠联合使用表达 ClyA 的鼠伤寒沙门氏菌和放射疗法能更好地抑制肿瘤生长。除了化学疗法和放射疗法之外，细菌介导的光热疗法是治疗各种类型癌症的另一种重要且有效的策略。Guo 等[72]经过生物工程改造细菌表达天然黑色素，可产生天然黑色素的生物工程细菌与双合成 ICG 和纳米多巴胺(polydopamine, PDA)共同沉积，以实现图像引导的缺氧靶向癌症光热治疗。基因工程细菌引导的新光热和免疫疗法在癌症治疗中展现出广泛的潜在应用价值。Wu 等[73]设计了一种新的生物杂交系统，该系统在大肠杆菌上负载聚集诱导发射光敏剂纳米颗粒，光暴露后光敏剂产生的活性氧(reactive oxygen species, ROS)杀死周围的癌细胞，同时细菌释放药物。这项工作开创了光调控药物递送的新方法。细菌与递送药物的协同治疗为肿瘤治疗带来了新的可能性和机遇。

4 总结与展望

目前，以细菌为基础的肿瘤治疗是一种有前途的治疗策略。专性和兼性厌氧菌展现出其固有的优势，特别是在靶向肿瘤区域以及发挥肿瘤治疗作用方面。此外，通过简单的工程化改造，细菌就能够成为抗肿瘤药物的递送载体。细菌可以将药物递送到传统方法难到达的位置。尽管细菌肿瘤治疗领域已取得一定进步，但是细菌递送抗肿瘤药物仍存在局限性。

在细菌作为肿瘤药物递送载体的应用中，细菌的免疫原性发挥着双重作用。一方面，细菌的低免疫原性减少机体免疫系统对细菌的识别，降低潜在的炎症因子风暴风险，使得细菌能够高效抵达肿瘤区域，实现药物的有效递送。另一方面，细菌在肿瘤区域的免疫识别则能够触发局部的抗肿瘤免疫反应，促进肿瘤免疫和细菌的清除，这展现了其治疗潜力，但活细菌介导的药物的设计和开发对研究人员和监管机构都提出了一些挑战。虽然在研究中使用减毒活细菌以及采取各种措施能够减少细菌引起的免疫反应，但其临床应用障碍依然存在，如巨噬细胞迅速清除、被动分布导致的治疗效果不理想以及免疫原性引起的不良反应。因此，在细菌药物递送载体的研发过程中，平衡细菌的免疫原性尤为重要，旨在实现治疗效率与安全性之间的平衡，克服临床应用中的诸多挑战。

更重要的是，细菌的活性在细菌作为肿瘤药物递送载体的应用中也具有两面性。一方面，活细菌因其独特的靶向性和免疫激活等功能，在肿瘤治疗中展现出巨大潜力；另一方面，由于细菌具有活性，其药代动力学不同于传统药物。与常规药物相比，活细菌的生长和复制大大增加了建立剂量-反应关系的难度。这使得确定细菌对不同恶性肿瘤的最佳治疗剂量、给药时间及途径成为一个重要挑战。此外，目前用于评估细菌抗肿瘤作用的动物肿瘤模型不能充分模拟临床患者的情况。动物肿瘤模型可在短期内建立，导致形成高渗透性血管，并且缺乏模仿真实的疾病状况的病理学体征。在今后的研究中，应考虑建立更

可靠的动物肿瘤模型，如患者来源的异种移植瘤(patient-derived tumor xenograft, PDX)模型。

尽管存在局限性，细菌作为抗肿瘤药物载体仍值得进一步探索。随着对抗肿瘤机制的不断深入理解，细菌疗法已成为现代科学研究中临床试验的热点，展现出巨大的未来发展潜力和空间。综上所述，细菌作为抗肿瘤药物递送载体是一种具有极大潜力的治疗方式。

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