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减毒沙门氏菌介导的肿瘤基因治疗

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摘　要：细菌介导的肿瘤治疗最早可追溯到一个多世纪前，其具有的瘤内定植增殖、靶向性高的特性，是放化疗、免疫检查点调控等传统肿瘤治疗方法难以企及的。近年来，随着合成生物学的发展，该疗法得到了极大的发展，以沙门氏菌为代表的多种抗肿瘤菌株相继涌现。本文综述了基于平衡细菌抗肿瘤疗效与毒副作用改造策略的沙门氏菌减毒菌株构建，以及减毒菌株作为药物递送载体介导肿瘤基因治疗的现状和进展。

关键词：癌症；鼠伤寒沙门氏菌；减毒菌株；载药；基因治疗

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Gene therapy mediated by attenuated *Salmonella*

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Abstract: Bacteria-mediated tumor therapy can be traced back to more than one century ago. Its characteristic of intratumoral colonization and proliferation with high targeting capacity is unachievable by traditional tumor therapies, such as radiotherapy and immune checkpoint modulation. In recent years, with the development of synthetic biology, this therapy has been greatly developed, and a variety of antitumor strains represented by *Salmonella* have been found. This paper reviews the construction of attenuated *Salmonella* strains based on a

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modification strategy that balances bacterial antitumor efficacy and virulence, as well as the current status and development of attenuated strains as drug delivery vehicles mediating oncogene therapy.

Key words: cancer; *Salmonella typhimurium*; attenuated strains; drug delivery; gene therapy

细菌介导的肿瘤治疗 (BMCT) 历久弥新，早在 17 世纪就有肿瘤患者感染细菌后肿瘤消退的记载 [1]；19 世纪末，美国骨科医生 William B. Coley 发明了 Coley 毒素，即化脓性链球菌为主、其他菌株为辅的热灭活的混合物，用于治疗无法手术的骨肉瘤患者 [2-3]。Coley 毒素在部分患者中产生疗效，使肿瘤缩小，生存期延长。这一方法直到 20 世纪 60 年代还应用于骨肉瘤患者的治疗，但存在诸多问题：治疗过程如同黑箱，机制不明；不同患者的个体情况不同，响应率与响应程度不一，给药剂量难以量化；副作用大，引起的过度免疫反应造成高烧、脏器衰竭，感染失控引起败血症，甚至休克死亡 [4-5]。这些问题为细菌介导的肿瘤治疗带来了诸多质疑，人们难以在治疗效果与安全性之间找到平衡 [6]。在其他更易量化、更普适的肿瘤治疗方法如放疗、化疗出现后，细菌介导的肿瘤治疗逐渐退出历史舞台。

然而，放化疗、免疫检查点调节剂、单克隆抗体等肿瘤治疗方法，在经历大量临床应用和实践检验后，肿瘤靶向性有限、肿瘤渗透和积累不足、剂量与副作用难以平衡等局限或不足也日益显露 [7]。实体肿瘤微环境往往具有乏氧、细胞死亡后释放的营养性内容物丰富、免疫清除程度低等特点。利用肿瘤微环境的上述特点，结合厌氧细菌靶向渗透、生长积累的特性，细菌介导的肿瘤治疗具有给药后肿瘤靶向性高、瘤内药物浓度富集等独特优势 [8-9]。随着生物技术的迅猛发展，尤其是重组 DNA、合成生物学等技术的发展，生物学家们拥有了更多的改造与检测手段；随着对细菌 - 宿主关系解读的日渐深入，科学家通过定向改造已经获得了一些具有更好抗肿瘤性能的细菌，细菌介导的肿瘤治疗重新成为热门话题。与正在成为生物药物开发热点的溶瘤病毒介导的肿瘤治疗相比，细菌介导的肿瘤治疗有着免疫原性更低、有效荷载能力强、进入体内后更易控制等优势 [10]。

在过去的三十年间，经过体内外肿瘤模型的筛选，包括大肠杆菌、沙门氏菌、双歧杆菌、李斯特菌、梭状芽胞杆菌在内的多种兼性或专性厌氧的细菌被发掘出来，用于肿瘤治疗 [11–26]。研究者对这些细菌进行各种基因工程改造以提高疗效、降低毒性；其中，鼠伤寒沙门氏菌 (*Salmonella Typhimurium*) 是被研究最广泛、最具有成药前景的细菌之一 [27]。鼠伤寒沙门氏菌是一种杆状、革兰氏阴性的兼性厌氧菌，具有易于遗传操作的特点。20 世纪 30 年代，人们首次观察到沙门氏菌在动物模型中的肿瘤治疗作用 [28]；随后的研究发现，静脉注射后，沙门氏菌可以在有氧的循环系统环境中存活，而后定植在乏氧的肿瘤微环境之中，而在循环系统和肝脾等其他组织内的细菌则会在几天内被逐渐清除，导致肿瘤与正常组织中菌的比例可达 1 000:1~10 000:1，具有高度的肿瘤靶向性 [29-30]；沙门氏菌定植到肿瘤乏氧区域后，可以侵入肿瘤细胞，启动局部与全身性的肿瘤特异性免疫反应，在动物模型上对多种人或小鼠的肿瘤具有显著的抑制效果 [31-33]。

1 减毒沙门氏菌菌株

1.1 野生沙门氏菌的局限

与 Coley 在一百多年前面临的困境相似，野生的沙门氏菌往往会引起过度的免疫反应或感染失控。1997 年的一项实验显示，使用野生型沙门氏菌 14028 治疗小鼠黑色素瘤荷瘤模型时，菌在瘤内繁殖到超过 10^9 CFU/g，导致小鼠因细菌性感染而死亡 [34]。显然，野生菌株的安全性远不能达到作为临床用药的要求。

21 世纪初的几项 I 期临床试验显示，沙门氏菌治疗后，尽管有部分患者的肿瘤中出现细菌成功定植，但肿瘤并未缩小，因此，沙门氏菌的疗效尚需进一步提升 [16]。这一结果提示人们，单凭野生型沙门氏菌自身的作用，其肿瘤治疗的疗效有限。应进一步利用基因工程与合成生物学方法，设计和构造“基因治疗药物活体递送机器”，即以安全、可控的细菌为运输载体，将不同来源的、抗肿瘤机制各异的蛋白质和核酸药物，在可控型启动子的控制下，实现肿瘤靶向递送、肿瘤原位表达，从而发挥强劲的抗肿瘤作用 [35-36]。

此外，野生沙门氏菌毒性过强、安全性低、难以调控的特点，使其难以成为可利用的递送载体。因此，构建工程化的减毒沙门氏菌，在降低毒副作用以提高安全性、递送药物以提高疗效两方面都有重要意义。

1.2 减毒沙门氏菌的构建策略

野生沙门氏菌的毒性，来自于靶向性不足导致其在肝脾等正常脏器中的积累，对包含肿瘤与健康细胞在内的宿主细胞直接或间接的杀伤，过度激活宿主全身性免疫反应等原因。构建减毒沙门氏菌的一个重要先决条件是明确其靶向渗透并定植于肿瘤、直接或间接杀伤细胞、激活先天与获得性免疫等生物过程的机制。(1) 沙门氏菌在静脉注射或腹腔注射后，随着血液循环抵达宿主全身各器官，因其兼性厌氧的特性，细菌经过乏氧的肿瘤区域时会从血管中迁移出来，趋向缺氧的肿瘤核心 [37]。(2) 肿瘤微环境中存在大量细胞死亡后释放出的内容物，如大量的糖类、核酸与蛋白质片段，不仅可以被沙门氏菌感知 (如天冬氨酸、丝氨酸等氨基酸，核糖、嘌呤等核酸组分)，进一步提高其肿瘤趋化作用，还为细菌提供了丰富的营养条件，使其得以繁殖 [38-40]。(3) 肿瘤微环境通常为免疫抑制环境，免疫细胞浸润不足，且功能受限，因此对沙门氏菌的清除能力不足，这也是沙门氏菌能在肿瘤而非正常组织定植的重要因素。(4) 定植后的沙门氏菌可以直接或间接杀伤肿瘤细胞。最直接的方法是与肿瘤细胞竞争营养，限制其生长；还可以进入肿瘤细胞内部，诱发异源自噬、细胞凋亡来直接攻击肿瘤细胞 [41]。(5) 间接杀伤主要是诱导先天免疫与获得性免疫反应，激活免疫系统，通过免疫细胞或各种细胞因子攻击肿瘤细胞。沙门氏菌诱导的先天免疫反应如下：沙门氏菌的鞭毛蛋白与脂多糖 (LPS) 可与 Toll 样受体 4 (TLR4) 结合，激活 TLR-MyD88 信号通路，诱导树突状细胞 (DCs)、中性粒细胞或巨噬细胞迁移到肿瘤中，或直接吞噬肿瘤细胞，或促进肿瘤相关抗原的呈递，或分泌 TNF-α、IFN-γ、IL-1β 等细胞因子来杀伤肿瘤细胞、破坏肿瘤血管、招募并激活其他免疫细胞 [42-46]；沙门氏菌诱导的获得性免疫：沙门氏菌可通过 Toll 样受体 5 (TLR5) 促进 $CD8^+$ T 细胞的抗肿瘤免疫反应，并下调 $CD4^+$-$CD25^+$ 调节性 T 细胞 (Treg) 的浸润，同时招募并激活更多的自然杀伤细胞 (NK)，在多种细胞免疫的共同作用下杀伤肿瘤细胞 [22, 47]。

针对上述机制，减毒沙门氏菌的构建策略方向已然明晰：(1) 改造菌体成分，降低免疫原性 [16, 48]；(2) 敲除基因，下调毒力蛋白的表达、分泌，降低其对细胞的杀伤作用，降低宿主的系统性免疫激活 [49-50]；(3) 构造基础代谢物 (如氨基酸、嘌呤) 的营养缺陷株，使沙门氏菌仅能够在肿瘤内生存繁殖，降低全身性毒副作用 [51-53]。

1.3 常见的减毒沙门氏菌(表1)

1.3.1 VNP20009

VNP20009 携带 *ΔmsbB*、*ΔpurI* 两个基因突变；*msbB* 是毒力基因，编码 LPS 上脂质 A 的酰基转移酶，缺失导致脂质 A 缺少一个肉豆蔻酰基，对宿主的免疫激活程度降低，显著减少了 TNF-α 的产生；*purI* 是代谢相关基因，缺失导致嘌呤营养缺陷，限制菌在正常组织中的存活，进一步提高靶向性。动物实验表明，与野生型沙门氏菌相比，VNP20009 毒力显著降低，在瘤内的滴度比肝脾中高 1 000 倍，比其他正常组织中高 10 000 多倍，外周中 TNF-α 减少约 90%，对小鼠的 LD50 增加了 10 000 倍，显示出良好的安全性。VNP20009 已在多种小鼠肿瘤模型中进行了验证，包括小鼠黑色素瘤、肺癌、乳腺癌、结肠癌，人胃癌、非小细胞肺癌、乳腺癌、宫颈癌等 [19, 48, 54-67]。此外，一项研究发现，*htrA* 基因缺陷的 VNP20009 突变菌株 *ΔhtrA* VNP20009 相比于 VNP20009，在安全性、靶向性不变的情况下，丧失了抗肿瘤能力，这是首次将沙门氏菌的肿瘤靶向性与抗肿瘤能力相分离，为揭示和阐述沙门氏菌抗肿瘤机制提供了思路 [109]。

1.3.2 A1-R

A1-R 通过强诱变剂亚硝胺诱变、选育而得，具有亮氨酸、精氨酸营养缺陷，限制菌在正常组织中存活，同时靶向性提高。动物实验表明，A1-R 在肿瘤组织中比在正常器官中生长更为迅速，全身性毒副作用降低。其不仅能诱导肿瘤细胞从 G_0/G_1 期到 $S/G_2/M$ 期以增加化疗敏感性，还能抑制肿瘤细胞转移。A1-R 已在多种小鼠肿瘤模型中获得验证，包括人胶质母细胞瘤、肺癌、卵巢癌、乳腺癌、前列腺癌、胰腺癌等 [68-75]。

1.3.3 ΔppGpp/SHJ2037

ΔppGpp/SHJ2037 携带 *ΔrelA*、*ΔspoT* 两个基因突变；两个基因均为代谢相关基因，缺失导致 5′ 二磷酸 -3′ 二磷酸鸟苷 (ppGpp) 合成受阻。ppGpp 是沙门氏菌各种蛋白表达的关键中间分子，其缺失导致细菌毒力蛋白的表达大幅下调，尤其是沙门氏菌致病性岛 1 (SPI1) 上编码入侵宿主细胞和诱导细胞凋亡的必需蛋白。动物实验表明，ΔppGpp 菌全身感染对小鼠无明显毒性，其 LD50 约为野生型菌株的 $10^{5\sim6}$ 倍。其通过激活炎症小体途径发挥抑瘤效果，也可携带荧光报告基因作为临床成像剂。ΔppGpp 已在多种小鼠肿瘤模型中进行了验证，包括小鼠结

表1 减毒沙门氏菌菌株整理

菌株	敲除基因	肿瘤模型	细胞系	造模方式	文献
VNP20009	*ΔmsbB*、*ΔpurI*	小鼠黑色素瘤	B16F10	皮下注射	[19, 48, 54-56]
		小鼠肺癌	Lewis	皮下注射	[57]
		小鼠结肠癌	MC38	皮下注射	[58]
		小鼠乳腺癌	4T1	皮下注射	[59]
		人胃癌	SGC-7901	皮下注射	[60]
		人非小细胞肺癌	A549	皮下注射	[61]
		小鼠结肠癌、小鼠乳腺癌	CT26、D2F2	皮下注射	[62-65]
		人乳腺癌、人宫颈癌	MDA-MB-468、HeLa	体外实验	[66]
		人乳腺癌	MDAMB-231	体外实验	[67]
A1-R	精氨酸和亮氨酸营养缺陷型	人神经胶质母细胞瘤	U87	皮下注射	[68]
		人肺癌	XPA1	皮下注射	[69]
		人卵巢癌	SKOV、OVCAR	皮下注射	[70]
		人乳腺癌	MARY-X	注射乳腺脂肪垫	[71]
		人前列腺癌	PC-3	手术注射前列腺外侧叶	[72]
		人骨肉瘤	143B	手术注射胫骨边缘	[73]
		人神经胶质母细胞瘤	U87	开颅手术注射脑内	[74]
		人胰腺癌	XPA-1	手术注射脾脏内	[75]
ΔppGpp/ SHJ2037	*ΔrelA*、*ΔspoT*	小鼠结肠癌	CT26	皮下注射	[43, 76]
		小鼠结肠癌	MC38	皮下注射	[44]
		小鼠结肠癌、人肝癌	CT26、Hep3B	皮下注射	[77-78]
		小鼠乳腺癌、小鼠结肠癌、人胰腺癌	4T1、MC38、AsPC1	皮下注射	[79]
		小鼠结肠癌、小鼠乳腺癌、小鼠结肠癌、人结肠癌	CT26、4T-1、MC38、SW620	皮下注射	[80-81]
ΔppGpp-Δara SL3261	*ΔppGpp*、*Δara* *ΔaroA*	小鼠结肠癌	CT26	皮下注射	[82]
		小鼠乳腺癌、小鼠肺癌	4T1、 Lewis	皮下注射	[83]
		小鼠黑色素瘤	B16F1 (H-2b)	皮下注射	[84]
		小鼠黑色素瘤	B16F10	皮下注射	[85]
		大鼠结直肠癌	—	DMH诱导原位造模	[86]
SL7207	*ΔaroA*	小鼠结肠癌	CT26	皮下注射	[42, 45]
		小鼠再生前列腺癌	TRAMPC1	皮下注射	[87]
		小鼠神经母细胞瘤	NXS2	皮下注射	[88]
		人卵巢上皮细胞癌铂耐药细胞系	SKOV-3/DDP	皮下注射	[89]
		小鼠黑色素瘤、小鼠肾癌	B16F10、RenA	皮下注射、尾静脉注射	[90]
		大鼠胶质瘤、人前列腺癌、人纤维肉瘤	C6、PC-3、HT1080	皮下注射	[14]
YB1	厌氧才能存活的SL7207	人乳腺癌	MDAMB-231	体外实验	[67]

表1 减毒沙门氏菌菌株整理(续表)

菌株	敲除基因	肿瘤模型	细胞系	造模方式	文献
LH430	*ΔphoP*、*ΔphoQ*	小鼠前列腺癌	RM-1	皮下注射	[91]
		人喉癌	Hep-2	皮下注射	[92]
		人宫颈癌	SiHa	皮下注射	[93]
		人黑色素瘤	SK-MEL-37	皮下注射	[94]
RE88	*ΔaroA*、*Δdam*	小鼠乳腺癌、小鼠乳腺癌	4T1、D2F2	皮下注射	[95]
		小鼠乳腺癌	4T1	注射乳腺脂肪垫	[96]
		小鼠乳腺癌	D2F2	皮下注射	[97]
BRD509	*ΔaroA*、*ΔaroD*	小鼠黑色素瘤	B16F1 (H-2b)	皮下注射	[84]
LVR01	*ΔaroC*	小鼠B细胞淋巴瘤	A20	皮下注射	[98]
SA186	*ΔznuABC*	小鼠乳腺癌	4T1	皮下注射	[99]
SB824	*ΔaroA*、*ΔsptP*	小鼠纤维肉瘤	WEHI164	皮下注射	[100]
SF200	*ΔlpxR9*、*ΔpagL7*、*ΔpagP8*、*ΔaroA*、*ΔydiV*、*ΔfliF*	小鼠结肠癌	CT26	皮下注射	[101]
MvP664	*ΔgalE::aph*	小鼠结肠癌、小鼠胶质母细胞瘤、小鼠黑色素瘤、小鼠胶质母细胞瘤	CT26、DBT、B16、GL261	体外实验	[102]
MvP679	*ΔgalE*				
MvP680	*ΔpurD*				
MvP728	*ΔpurD*、*ΔhtrA*		GL261	开颅手术注射脑内	
MvP729	*ΔgalE*、*ΔhtrA*				
MvP740	*ΔpurD::aph*、*ΔgalE*				
S378	*Δrfc48*	小鼠结肠癌、小鼠黑色素瘤	CT26、B16F10	皮下注射	[103]
S633	*ΔpagL7*、*ΔpagP8*、*ΔlpxR9*、*ΔarnT2*、*ΔeptA3*、*ΔlpxT4*				
S634	*ΔpagL7*、*ΔpagP8*、*ΔlpxR9*、*ΔarnT2*、*ΔeptA3*、*ΔlpxT4*、*ΔaroA8*				
S636	*ΔpagL7*、*ΔpagP8*、*ΔlpxR9*、*ΔarnT2*、*ΔeptA3*、*ΔlpxT4*、*ΔaroA8*、*Δasd-66*				
χ4550	*Δcya*、*Δcrp*	人转移性胃肠道肿瘤	—	口服	[104]
TAPET-CD	表达*E. coli*胞嘧啶脱氨酶	小鼠黑色素瘤、小鼠结肠癌细胞	B16F10、C38	皮下注射	[105]
ST8	—	小鼠结肠癌	CT26	注射乳腺脂肪垫	[106]
SC36	Purine-auxotrophic (*his G aro A cys pur I*)	小鼠肺癌、小鼠黑色素瘤	Lewis、B16F10	皮下注射	[107]
		小鼠乳腺癌	4T1	皮下注射	[108]

肠癌、乳腺癌，人肝癌、结肠癌、胰腺癌等[43, 44, 76–82]。

1.3.4　SL3261、SL7207、YB1

SL3261、SL7207 携带 *ΔaroA* 突变；*aroA* 编码 3-磷酸莽草酸 1- 羧基乙烯基转移酶，缺失导致对氨基苯甲酸酯、2,3- 二羟基苯甲酸酯合成的下调，芳香族氨基酸合成受阻。动物实验表明，该系列菌株在肿瘤中的增殖能力减弱，毒力明显减弱。其可以作为安全的口服 DNA 肿瘤疫苗载体，或携带生物发光探针用于肿瘤诊断。SL3261、SL7207 已在多种小鼠肿瘤模型中进行了验证，包括小鼠黑色素瘤、肺癌、结肠癌、乳腺癌，人前列腺癌、卵巢癌铂耐药株等[14, 42, 45, 83-90]。

YB1 是工程化的、只能在乏氧环境存活的 SL7207。其构建方法为，将细菌生存必需的、编码天冬氨酸半醛脱氢酶的基因 *asd* 敲除，再将由厌氧启动子 PpepT 起始转录的 *asd* 基因和由有氧启动子 PsodA 起始转录的 *asd* 反义核酸一起重新整合入细菌基因组。YB1 只能在低氧条件下生存，大大降低了它们对正常组织的细胞毒性，并增强了它们在肿瘤组织的特异性增殖。动物实验表明，其对正常组织的毒力降至极低[67]。

1.3.5　其他菌株

LH430 携带 *ΔphoP*、*ΔphoQ* 两个突变，*phoP* 编码细胞质转录调节蛋白，*phoQ* 编码膜相关传感蛋白激酶，缺失导致毒力蛋白表达下降，菌株繁殖能力下降[91-94]。RE88 携带 *ΔaroA*、*Δdam* 两个突变，*aroA* 影响芳香族氨基酸合成，*dam* 影响碱基 A 的甲基化，缺失导致无法分泌蛋白质，菌株对哺乳动物细胞无害[95-97]。BRD509 携带 *ΔaroA*、*ΔaroD* 两个突变，均影响芳香族氨基酸合成[84]。LVR01 携带 *ΔaroC* 突变，导致部分芳香族化合物的营养缺乏[98]。SA186 携带 *ΔznuABC* 突变，该基因编码高亲和力锌转运蛋白的整个操纵子，其缺失导致毒力蛋白表达下降[99]。SB824 携带 *ΔaroA*、*ΔsptP* 两个突变，*aroA* 影响芳香族氨基酸合成，*sptP* 编码致病性岛 1 效应蛋白，基因缺失导致毒力下降[100]。SF200 携带多个基因突变，包括 *ΔlpxR9*、*ΔpagL7*、*ΔpagP8*、*ΔaroA*、*ΔydiV*、*ΔfliF*，它们都是脂质 A 与鞭毛合成相关的基因，缺失导致细菌免疫原性降低，对宿主免疫激活下降[101]。MvP728 等携带嘌呤合成、转运相关突变，基因缺失导致嘌呤营养缺陷，不能在巨噬细胞中存活[102]。S636 等携带 *ΔaroA* 及脂质 A 修饰相关突变，基因缺失导致芳香族氨基酸合成受阻，免疫原性降低[103]。

2　减毒菌株介导的基因治疗

减毒沙门氏菌可以携带利用基因工程与合成生物学方法构造的质粒，根据需要将蛋白质、DNA 或 RNA 作为有效载荷，靶向运输至肿瘤微环境。已经有大量的研究从不同的角度 (图 1)、以不同的策略实现减毒沙门氏菌介导的肿瘤基因治疗，在多种小鼠肿瘤模型中得到验证，详见表 2。

2.1　细胞毒性蛋白&抗血管生成

杀伤肿瘤细胞最直接的方法就是递送细胞毒性蛋白。具有更高靶向性的减毒沙门氏菌，再加上肿瘤微环境特异性诱导型启动子，这两者的共同作用

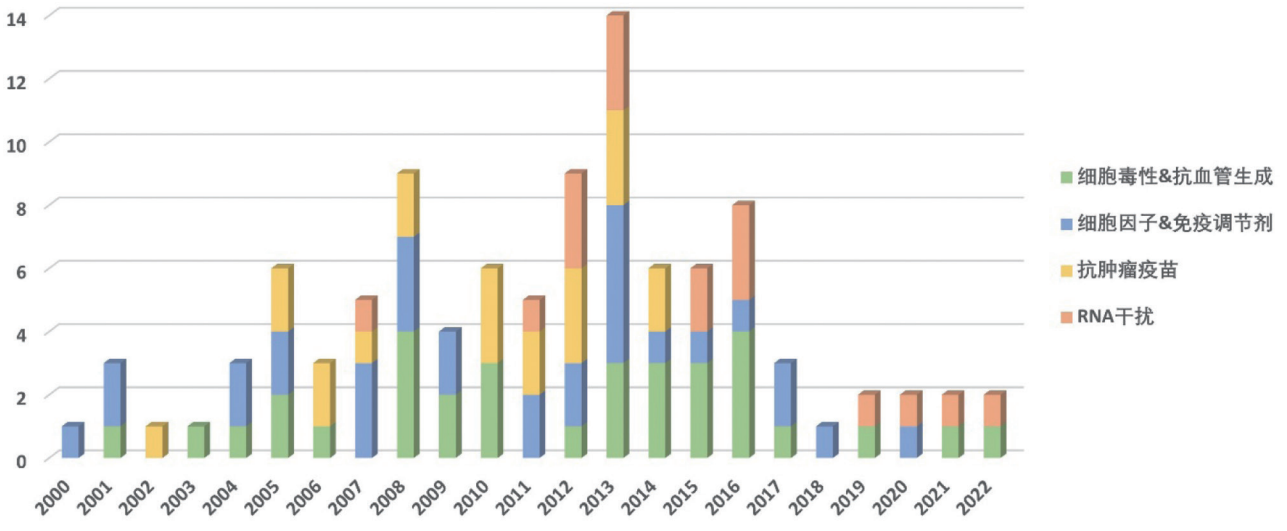

图1　近年减毒沙门氏菌介导的基因治疗相关研究论文统计

表2 基因治疗载荷

分类	载药靶点	靶点全称	文献
细胞毒性&抗血管生成	*clyA/hlyE*	溶细胞素A/溶血素E	[77-78, 82, 110-111]
	Apoptin	凋亡素	[92]
	DTA	白喉毒素A链	[53]
	FasL	促凋亡细胞因子*Fas*配体	[62]
	TRAIL	肿瘤坏死因子相关的细胞凋亡诱导配体	[60, 112-113]
	FADD	具有死亡结构域的*Fas*相关蛋白	[56]
	L-ASNase	左旋天冬酰胺酶	[79]
	ePNP	嘌呤核苷磷酸化酶	[55, 107-108]
	AFP	小鼠甲胎蛋白	[114]
	RGD	RGD肽序列(ACDCRGDCFCG)	[81]
	VEGFR2	血管内皮生长因子受体2	[59]
	PEA	假单胞菌外毒素A	[66]
	DNase I	DNA酶1	[54]
	CD	胞嘧啶脱氨酶，将5-FC转换为5-FU	[115-117]
	TSP-1	血栓反应蛋白-1	[118]
	CPG2	羧肽酶G2	[119]
	TGF-α-PE38	肿瘤生长因子α-假单胞菌外毒素38融合蛋白	[80]
	HPV16-E7	人乳头瘤病毒16型E7抗体	[120]
	HSV1-tk	单纯疱疹病毒胸苷激酶	[121-122]
	Tumstatin	肿瘤抑素	[123]
	HPRG	组氨酸富含脯氨酸糖蛋白	[124]
细胞因子&免疫调节剂	*IL-2*	白介素2	[84, 104, 125-128]
	IL-4	白介素4	[129]
	IL-12	白介素12	[83, 130]
	IL-18	白介素18	[129]
	IL-21	白介素21	[19]
	CCL21	细胞趋化因子21	[65, 131]
	CD20	CD20	[132]
	CD40L	CD40配体	[133]
	Endostatin	内皮抑素	[103, 106, 134-135]
	LIGHT/TNFSF14	肿瘤坏死因子配体超家族14	[63]
	FlaB	鞭毛蛋白B	[22]
	TNF-α	肿瘤坏死因子α	[136]
	IFN-γ	干扰素γ	[137]
	hGM-CSF	人粒细胞/巨噬细胞集落刺激因子	[83]
	SPYR	Sprouty蛋白	[138]
	p53	p53蛋白	[93, 139]
	GRIM-19	维甲酸和干扰素诱导的死亡相关基因19	[140]
	RBM5	RNA 结合基序蛋白5	[141]
	MTD	*Noxa*的线粒体靶向结构域	[76]
抗肿瘤疫苗	*Survivin*	生存素	[88, 102, 131, 142]
	4-1BBL	*4-1BB*配体	[86]
	Endoglin/CD105	内皮糖蛋白	[90, 97, 134]
	MTDH/AEG-1	金属黏附素/星形胶质细胞上调基因-1	[96]
	TAAs	肿瘤相关抗原	[94, 142-144]
	mtHSP70	结核分枝杆菌热休克蛋白70	[145]
	VEGFR2/flk1	血管内皮生长因子受体2/胎肝激酶1	[146-147]
	gp100	糖蛋白100	[148]
	CEA	癌胚抗原/抗体特异链	[58, 149]

表2 基因治疗载荷(续表)

分类	载药靶点	靶点全称	文献
	PCSA	前列腺干细胞抗原	[87]
	Legumain	天冬酰胺内肽酶	[95]
RNA干扰	*Stat3*	信号转导和转录激活因子	[91, 150-152]
	IDO	吲哚胺2,3-双加氧酶	[153-154]
	Survivin	生存素	[140, 155]
	MDR1	多药耐药基因1	[89]
	Mdm2	鼠双微体2，*p53*的负性调节因子	[139]
	PD-1	程序性死亡受体1	[156-159]
	Sox2	性别决定区Y盒2	[61]
	PLK1	polo 样激酶1	[53]
	ABCB5	ATP结合盒亚家族B成员5	[160]

使得被表达的细胞毒性蛋白可严格作用于肿瘤细胞而不伤害正常组织。溶细胞素A，又称溶血素E (*clyA/hlyE*)，是多种伤寒菌产生的天然细菌毒素，可造成细胞膜穿孔，导致细胞内容物释放进而发生细胞凋亡。已有多个研究报道，使用不同的减毒沙门氏菌递送 *clyA/hlyE*，在 4T1、CT26、Hep3B 等多种小鼠肿瘤模型中均能有效抑制肿瘤生长 [77-78, 82, 110, 111]；在 Hep-2 喉癌模型中，沙门氏菌 LH430 递送凋亡素 (*Aoptin*)，在全身毒性有限的情况下，有效引起细胞凋亡增加，导致肿瘤消退 [92]；使用减毒沙门氏菌 ST8 递送白喉毒素 (*DTA*) 治疗侵袭性肿瘤模型小鼠的一项研究显示，手术去除肿瘤后，在细菌治疗作用下，26% 的小鼠出现肿瘤完全消退 [53]；也有研究向肿瘤微环境有效递送左旋天冬酰胺酶 (*L-ASNase*)，限制肿瘤生长必需的氨基酸合成，造成肿瘤饥饿，以达到治疗目的 [79]；其他毒性蛋白，如 DNA 酶 1 (*DNase I*)[54]、小鼠甲胎蛋白 (*AFP*)[114]、假单胞菌外毒素 A (*PEA*)[66] 等，经过减毒沙门氏菌的递送，也有一定的肿瘤杀伤效果。

诱导肿瘤细胞凋亡是一种强大的细胞杀伤方法。数个研究将不同类型的凋亡诱导配体递送至肿瘤细胞中，如促凋亡细胞因子 Fas 配体 (*FasL*)、肿瘤坏死因子相关的细胞凋亡诱导配体 (*TRAIL*)、具有死亡结构域的 Fas 相关蛋白 (*FADD*) 等，均显示出较强的肿瘤杀伤能力：静脉注射携带 *FasL* 表达质粒的沙门氏菌后，D2F2、CT26 小鼠肿瘤模型的肿瘤生长被显著抑制，抑制率分别为 59%、82%[62]；携带 *TRAIL* 表达质粒的沙门氏菌在体内外均可造成显著的肿瘤消退，caspase-3 和 caspase-9 的表达水平显著提高，在另一项研究中沙门氏菌递送 *TRAIL* 的治疗将 4T1 荷瘤鼠的 30 天生存率提高到 100%[60, 112-113]；而沙门氏菌递送 *FADD* 的 C 端截短体的治疗，在 B16F10 小鼠肿瘤模型中显示出极强的细胞毒性，显著抑制肿瘤生长 [56]。

肿瘤生长是依赖血管生成的过程。针对肿瘤血管的治疗方案也已经被研究者用于与减毒沙门氏菌介导的基因治疗联合使用。血栓反应蛋白 -1 (*TSP-1*) 是一种内源性的血管生成抑制剂，经过减毒沙门氏菌的递送，在 B16F10 小鼠肿瘤模型中显著降低瘤内微血管密度、抑制肿瘤生长 [118]。肿瘤抑素 (*Tumstatin*) 是一种肿瘤来源的特异性血管生成抑制剂，可抑制肿瘤血管内皮细胞的增殖并诱导其细胞凋亡，减毒沙门氏菌递送厌氧表达的 Tumstatin，可有效抑制肿瘤生长并延长模型小鼠生存期 [123]。富含组氨酸脯氨酸的糖蛋白 (*HPRG*) 是脊椎动物的血浆蛋白，可抗血管生成、促进肿瘤血管正常化。减毒沙门氏菌介导的 HPRG 厌氧表达，在原发性及转移性肿瘤中均可延迟肿瘤生长，延长荷瘤小鼠生存时间 [124]。

2.2 细胞因子&免疫调节剂

肿瘤细胞往往处于免疫细胞浸润少、功能降低的免疫抑制环境，增强免疫系统以治疗肿瘤是一种常用的肿瘤治疗思路。减毒沙门氏菌已被研究者用来递送各种不同的细胞因子或免疫调节剂，如多种白介素、细胞趋化因子、免疫检查点抑制剂与激动剂、异源鞭毛蛋白等。白介素 2 (*IL-2*) 是关键的淋巴细胞激活信号分子，其可以增加 T 细胞激活程度，造成肿瘤坏死增加，抑制瘤内血管生成 [84, 104, 125-128]；白介素 4 (*IL-4*) 是典型的 2 型细胞因子，已被证明是多种肿瘤的生长抑制剂 [129]；白介素 12 (*IL-12*) 主要由抗原呈递细胞响应感染因子而产生，可刺激活化的 NK 和 T 细胞增殖，并诱导其产生 IFN-γ，增

强 T 细胞免疫毒性 [83, 130]；白介素 18 (*IL-18*) 也称为 IFN-γ 诱导因子，可诱导 NK 和 T 细胞分泌 IFN-γ，已有许多研究报道其在体内对各类型肿瘤的抗肿瘤作用 [129]；白介素 21 (*IL-21*) 由活化的 T 细胞和自然杀伤 T 细胞 (NKT) 产生，可以增加细胞毒性 $CD8^+$ T、NK 以及 NKT 细胞的浸润 [19]；细胞趋化因子 21 (*CCL21*) 调控淋巴细胞、树突状细胞和 NK 细胞的迁移，已有研究表明，免疫系统的抗肿瘤能力与 CCL21 诱导产生的多种细胞因子 / 趋化因子的表达水平正相关 [65, 131]；LIGHT (也称为 *TNFSF14*)，是肿瘤坏死因子配体超家族的一员，可招募 DC、T、B、NK 等多种免疫细胞，并提高 DC 细胞参与抗原呈递与免疫反应的程度 [63]。上述 7 种细胞因子，已全部被用于减毒沙门氏菌介导的基因治疗，分别在包含多药耐药肿瘤模型在内的多种肿瘤动物模型上显示出显著的抗肿瘤效果。

用于免疫增强、细胞周期调控的多种免疫调节剂，也被用于减毒沙门氏菌递送。创伤弧菌鞭毛蛋白 B (*FlaB*) 是一种免疫原性极强的异源鞭毛蛋白，可在肿瘤区域产生强烈的免疫招募信号，通过 TLR4、TLR5 途径招募并激活巨噬细胞等免疫细胞，减毒沙门氏菌递送 *FlaB* 的疗法在小鼠黑色素瘤模型中表现出良好的抑瘤效果 [22, 161]；*p53* 是关键的抑癌基因，其在癌症发生发展进程中的作用至关重要，可诱导细胞周期停滞相关的下游基因的表达，减毒沙门氏菌递送 *p53* 在体内外实验中显示抑瘤效果 [93, 139]；Sprouty 蛋白 (*SPRY*) 是 RTK 途径的内源性负调节因子，这一途径的激活与癌细胞增殖、血管生成有关，通过 VNP20009 递送 *SPRY* 显著抑制了小鼠黑色素瘤的生长 [138]；维甲酸和干扰素诱导的死亡相关基因 19 (*GRIM-19*) 是干扰素和维甲酸诱导的细胞死亡途径的抑制物，现有数据表明其在几种人类癌症中表达下调，减毒沙门氏菌介导的 *GRIM-19* 基因治疗显示出优越的抗肿瘤活性 [140]。

2.3 肿瘤疫苗

相比于原核细胞，真核细胞拥有更强大的膜与蛋白修饰系统。许多研究者通过细菌将 DNA 递送至哺乳动物细胞中，在哺乳动物细胞原位表达构象正确的重组蛋白，从而提高重组蛋白的表达水平及免疫原性，这就是细菌介导的 DNA 疫苗。减毒沙门氏菌被广泛用作 DNA 疫苗载体，能递送肿瘤特异性抗原等多种直接或间接激活免疫系统的蛋白。生存素 (*Survivin*) 在细胞 G_2/M 期高表达，是重要的抗凋亡蛋白，携带 *Survivin* 的减毒沙门氏菌疫苗在动物实验中显著诱导 $CD8^+$ T 细胞介导的抗肿瘤活性 [88, 102, 131, 142]；4-1BB 配体 (*4-1BBL*) 一般表达于抗原呈递细胞，是重要的 T 细胞激活共刺激信号，递送其表达质粒的减毒沙门氏菌疫苗成功抑制了大鼠自发性结肠癌的发展 [86]；内皮糖蛋白 (*Endoglin/CD105*) 是 TGF-β 的共受体复合物之一，在增殖的内皮细胞上高度表达，因此是抗血管生成的有效靶点，基于减毒沙门氏菌的 *Endoglin* 口服 DNA 疫苗，减少了肿瘤血管生成，并一定程度缓解了肿瘤免疫抑制状态 [90, 97, 134]；金属黏附素 / 星形胶质细胞上调基因 -1 (*MTDH/AEG-1*) 在超过 40% 的乳腺癌患者中过度表达，还可促进乳腺癌细胞的肺转移并增强化疗耐药性，减毒沙门氏菌菌株 RE88 递送的相关 DNA 疫苗显著诱导细胞毒性 T 细胞介导的细胞免疫 [96]；利用沙门氏菌的 III 型分泌系统 (T3SS) 构建肿瘤相关抗原 (*TAAs*) 的口服肿瘤疫苗，递送至抗原呈递细胞后能诱导肿瘤特异性细胞毒性 T 淋巴细胞 (CTL) 增殖，造成肿瘤消退 [94, 142-144]；减毒沙门氏菌递送表达结核分枝杆菌热休克蛋白 70 (*mtHSP70*)[145]、血管内皮生长因子受体 2/ 胎肝激酶 1 (*VEGFR2/flk1*)[146, 147]、糖蛋白 100 (*gp100*)[148]、前列腺干细胞抗原 (*PCSA*)[87] 等蛋白的多种 DNA 疫苗，均能诱导宿主产生保护性免疫反应，控制肿瘤发展。

2.4 RNA干扰

沙门氏菌可在哺乳动物细胞内生存，该特性使得其可以作为 shRNA 的递送载体，已有许多课题组报道利用减毒沙门氏菌介导的 RNA 干扰沉默肿瘤蛋白的研究工作。信号转导和转录激活因子 3 (*STAT3*) 是一种抑制细胞凋亡、促进细胞生长的因子，已被证明在多种癌症中过度表达，从而成为 shRNA 介导的基因沉默的热门靶点，减毒沙门氏菌递送的 shSATA3 在小鼠前列腺癌模型中显著抑制了肿瘤生长，并延长模型小鼠的生存时间 [91, 150-152]；吲哚胺 2,3- 双加氧酶 1 (*IDO*) 能调节 T 细胞激活过程，是一种免疫抑制因子，VNP20009 携带 *IDO* 特异性 shRNA 质粒，可显著控制小鼠 B16F10 黑色素瘤，在小鼠胰腺癌模型中也可延缓肿瘤生长 [153-154]；程序性死亡受体 1 (*PD-1*) 作为众所周知的免疫检查点抑制剂，在肿瘤抵抗 T 细胞介导的杀伤中扮演重要角色，减毒沙门氏菌递送干扰其表达的 siRNA，在小鼠黑色素瘤模型中增强了肿瘤特异性免疫，抑制肿瘤进展，其与 STAT3 抑制剂 nifuroxazide 联用进一步增强了抗肿瘤效果 [156–159]；减毒沙门氏菌介导的靶向生存素及多药耐药基因 (*MDR1*) 的 RNA

干扰，可显著抑制肿瘤生长并增加化疗敏感性[89]；靶向 *p53* 的负性调节因子鼠双微体 2 (*Mdm2*) 的 RNA 干扰，恢复了 *p53* 调节细胞周期的活性，抑制肿瘤生长[139]；靶向 ATP 结合盒亚家族 B 成员 5 (*ABCB5*) 的 RNA 干扰，与化疗药物联合使用，显著延缓肿瘤生长并延长模型小鼠存活时间[160]。

3 总结

凭藉快速发展的基因工程与合成生物学的助力，由来已久的细菌介导的肿瘤治疗跃上了新的台阶，多种厌氧菌已被用于各种动物与临床试验。因其具有的靶向渗透和定植、生长简便、易于遗传操作等特性，沙门氏菌成为最为热门的肿瘤治疗菌种。野生型沙门氏菌仍具有较强的毒副作用，通过编辑和修改细菌成分，敲除或下调毒力蛋白的表达与分泌，构建基础代谢底物营养缺陷型菌株等多种策略，科研工作者已构建出二十余种减毒沙门氏菌菌株。这些菌株毒力显著减弱，安全性显著提高。同时，以减毒沙门氏菌为递送载体进行基因治疗，即递送细胞毒性蛋白、抗血管生成蛋白、细胞因子、免疫调节蛋白、肿瘤疫苗等的表达基因至肿瘤微环境，以及递送 shRNA 或 siRNA 质粒通过 RNA 干扰以沉默促癌基因表达，均产生了显著疗效。减毒沙门氏菌介导的基因治疗，在治疗的安全性与有效性间成功取得平衡，在多种同源或异源肿瘤动物模型中，显著抑制肿瘤进展，提高荷瘤动物的存活率。

值得注意的是，减毒沙门氏菌介导的肿瘤基因治疗方案尚未取得临床成功，仍然面临着许多挑战，如沙门氏菌对肿瘤微环境的影响尚不完全清楚；菌、宿主、炎症介质，三者间复杂的相互作用关系仍未明晰。随着技术的发展，生物疗法尤其是细菌介导的基因治疗，有望成为最具前景的癌症预防与治疗方法。随着更深入的机制研究、更精准的调控技术、更强大的联合治疗方案的持续发展，减毒沙门氏菌介导的基因治疗将有望发展出治疗肿瘤的颠覆性技术与产品。

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