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吴锦慧, 南京大学医学院教授、博士生导师。吴锦慧实验室通过工程化活性蛋白或者活菌作为载体构建新型靶向递送系统, 期望对肿瘤免疫和组织再生实现精准调控。实验室在前沿研究领域及成果转化方面均取得了一定的成绩, 在 *Nat Biomed Eng*、*PNAS*、*Nat Comm*、*Sci Adv*、*Adv Sci* 等 SCI 期刊上发表相关论文 100 余篇, 获得授权发明专利 10 余项, 成果转化 2 项。

活菌作为智能递送载体用于肿瘤治疗的研究进展

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摘要: 细菌经常被用作药物的载体实现被装载药物的肿瘤靶向、深部组织渗透等。近年来, 通过合成生物学技术对细菌的基因进行改造, 赋予了细菌环境感知和响应的功能, 实现了细菌负荷药物的时空调控, 促进细菌作为递送载体向更加智能化的方向发展。为此, 本文综述了近年来利用细菌作为药物载体, 以及基于环境感知和响应控制药物释放的细菌智能递送载体应用于癌症治疗的研究进展, 最后对未来智能化的细菌载体应用于癌症治疗进行展望。

关键词: 细菌载体; 药物递送; 肿瘤治疗

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Research advances of living bacterial intelligent delivery carriers for cancer therapy

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Abstract: Living bacteria is often used as drug carriers to achieve drug tumor targeting and deep tissue penetration. In recent years, gene modification by synthetic biology technology gave bacteria the function of environmental sensing and response, which realized the spatio-temporal regulation of bacterial drug loading, and promoted the development of bacteria as delivery carriers to a more intelligent direction. To this end, this paper reviews the recent progress on the application of bacteria as drug carriers and bacterial intelligent delivery carrier based on environmental awareness and response for the control of drug release in cancer therapy. Finally, the future application of intelligent bacterial carriers in cancer therapy is prospected.

Key words: living bacterial carrier; drug delivery; tumor treatment

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当前抗肿瘤治疗策略面临的特异性差、深层组织渗透弱等挑战,促进了新的替代肿瘤治疗方法的发展。自1891年以来 William Coley 应用链球菌和“Coley”毒素治疗无法手术的癌症患者取得了积极的疗效,但是由于20世纪以来放射治疗和化疗的发展,利用细菌治疗癌症的进一步临床应用及研究受到限制^[1]。近年来免疫学与生物技术领域的最新进展再次激起了人们对“Coley”毒素抗肿瘤活性机制的兴趣,使细菌应用于癌症治疗重新成为研究的重要方向。

兼性或专性厌氧菌,如梭状芽孢杆菌、李斯特菌、沙门氏菌、大肠杆菌和双歧杆菌,具有固有的肿瘤靶向性和肿瘤杀伤活性。这些活细菌定植肿瘤后可以通过多种机制发挥直接的抗肿瘤活性。除了细菌固有的肿瘤抑制作用,利用肿瘤靶向细菌作为药物载体,可以将药物递送至深层肿瘤组织,最大限度地发挥药物的抗肿瘤活性并降低对宿主的全身毒性。细菌负载的药物包括化学毒剂、基因指导的蛋白质或DNA和RNA药物等。根据细菌装载药物的位置可概括为表面装载、细菌外分泌。此外,研究人员将多种基因调控元件植入细菌,赋予细菌环境感知和响应的功能,实现对细菌负载药物的合成及释放的时空调控。本文将重点关注研究较多的沙门氏菌、梭状芽孢杆菌、李斯特菌,讨论活菌发挥内在抗肿瘤活性的独特方式及负载药物后作为药物递送载体在肿瘤治疗中的应用;最后,也总结了被应用于“智能化”细菌药物载体的多种调控元件,并对未来智能化的细菌载体应用于癌症治疗进行展望。

1 活菌直接的肿瘤抑制作用

活细菌感染肿瘤后可以导致肿瘤消退,发挥直接的肿瘤抑制作用。细菌发挥内在抗肿瘤活性的生物学机制是多样化的,不同菌株可能采用不同的机制导致肿瘤消退。本文从细菌肿瘤靶向、不同细菌引起肿瘤杀伤的不同机制的角度讨论活菌直接的肿瘤抑制作用。

1.1 活菌肿瘤靶向

活菌能够通过独特的机制靶向肿瘤是细菌肿瘤治疗策略具有优势的基础。在细菌注射后不久,肿瘤中蓄积的细菌量通常与正常组织相当。但在数小时和数天后,正常组织中的细菌被免疫系统识别并清除,而免疫抑制的实体肿瘤中的细菌继续增殖^[2-6]。肿瘤中的低氧环境吸引专性厌氧菌和兼性

厌氧菌在肿瘤中定植^[7-8],但专性厌氧菌和兼性厌氧菌的实验结果表明,厌氧菌不会选择性定植在非肿瘤区域的缺氧或病变的环境^[3,9-10]。专性厌氧菌如梭状芽孢杆菌由于无法在富氧环境中生存,这进一步增强了它们的肿瘤靶向性。因此,细菌的肿瘤选择性定植可能是实体瘤中免疫微环境和独特生化环境(如缺氧)共同作用的结果^[11-13]。李斯特菌可通过一种利用宿主免疫细胞靶向肿瘤的独特机制定植肿瘤:李斯特菌感染骨髓来源的抑制细胞(myeloid-derived suppressor cells, MDSCs), MDSCs可以选择性地将细菌递送至肿瘤微环境中。装载李斯特菌的MDSCs不仅可作为细菌递送至肿瘤的载体,还可以防止其被免疫系统清除^[5,14]。类似地,巨噬细胞也被报道可作为沙门氏菌肿瘤靶向的载体,介导沙门氏菌在肿瘤中的靶向输送^[15]。

1.2 活菌内在的抗肿瘤活性

沙门氏菌入侵至肿瘤细胞后会诱导凋亡或自噬相关信号通路,诱导凋亡与自噬介导的肿瘤消退^[16-18]。细胞内不断增殖的沙门氏菌也会引起细胞破裂,最终导致细胞死亡^[19]。细菌定植肿瘤后会激活宿主的先天免疫和自适应免疫反应来对抗肿瘤。鞭毛蛋白是组成细菌鞭毛的亚单位,沙门氏菌鞭毛蛋白作为Toll样受体(Toll-like receptors, TLR) 5激动剂可促进NK细胞分泌穿孔素杀伤肿瘤细胞,激活NK依赖的先天免疫^[20]。鞭毛蛋白还被证明可以通过激活细胞表面的TLR5受体来提高抗原疫苗引起的CD8⁺T细胞依赖性的自适应免疫应答,并减少CD4⁺CD25⁺调节T细胞的比例^[21-22]。此外,除了调节宿主的免疫活性,鞭毛还能抑制TLR5受体表达的肿瘤细胞的增殖,从而直接抑制肿瘤生长^[23]。与鞭毛蛋白类似,细菌感染肿瘤后,沙门氏菌中的脂多糖(lipopolysaccharide, LPS)成分同样作为病原体相关分子模式通过TLR4-MYD88信号促进巨噬细胞和树突状细胞分泌促炎因子IL-1 β ^[24],而在沙门氏菌定植的肿瘤中,巨噬细胞和树突状细胞分泌的IL-1 β 是产生抗肿瘤活性的主要因素^[25]。沙门氏菌LPS激活TLR4后也会提高肿瘤坏死因子TNF- α 的表达^[26-27],除了通过已报道的免疫调节功能促进肿瘤消退外,TNF- α 也会引起肿瘤血管的破裂^[28]。沙门氏菌对肿瘤血管的破坏还可以表现在下调血管生长因子(VEGF),抑制新的肿瘤血管生成^[29];VEGF表达减少可能与沙门氏菌诱导的间隙连接蛋白Cx43表达上调有关^[30-31]。有趣的是,经沙门氏菌处理后,黑色素瘤细胞的Cx43显著上调,导致

肿瘤细胞与树突状细胞之间形成间隙连接,从而促进肿瘤抗原肽的转移和交叉提呈,最终导致抗原依赖性自适应性免疫应答增强^[30]。

细菌可通过多种方式直接引起细胞凋亡,包括抑制宿主蛋白质合成、激活宿主细胞内源性死亡通路等^[32]。与此不同的是,李斯特菌可以直接感染细胞,并通过一种活性氧成分(reactive oxygen species, ROS)依赖性的机制诱导肿瘤细胞死亡。实验数据表明,李斯特菌通过激活NADPH氧化酶产生更多的ROS,诱导4T1和MCF-7细胞死亡^[33]。高浓度ROS诱导肿瘤细胞免疫原性死亡的同时激活肿瘤抗原特异性的T细胞免疫,清除了原发及转移后的肿瘤^[33-34]。李斯特菌侵入细胞质后分泌的细菌毒力因子也成为产生CD8⁺T细胞特异性免疫的外源抗原肽来源。细菌来源的抗原经泛素-溶酶体途径降解并由MHC-1分子提呈在肿瘤细胞表面,供特异性T细胞识别^[35]。李斯特菌治疗减少了小鼠血液和原发肿瘤中MDSCs的数量,并抑制了MDSCs和Treg在肿瘤微环境中的免疫抑制能力,导致这些细胞抑制T细胞的能力丧失,这与MDSCs中精氨酸酶I和Treg中IL-10的表达减少有关^[14,36]。

与其他细菌类似,定植在肿瘤中的梭状芽孢杆菌可以通过分泌各种毒素杀死肿瘤细胞,如磷脂酶、溶血素等可以破坏细胞膜结构或干扰细胞内功能^[37-40]。梭状芽孢杆菌还可以诱导中性粒细胞合成分泌TNF相关凋亡诱导配体,通过促进细胞凋亡来杀死肿瘤细胞。除了直接导致细胞死亡,梭状芽孢杆菌在感染肿瘤后,招募粒细胞和细胞毒性淋巴细胞至肿瘤微环境,随后感染部位一些细胞因子和趋化因子浓度显著升高,促进了肿瘤消除^[12,41]。

定植肿瘤后,细菌除了利用自身成分或分泌毒力因子等调节肿瘤细胞死亡发挥固有的抗肿瘤作用外,还可以诱导针对细菌本身和肿瘤细胞的固有免疫和适应性免疫反应。发挥抗肿瘤活性可能的机制取决于细菌种类、肿瘤类型以及细菌和

宿主之间的相互作用。更重要的是,可以通过基因工程或表面化学修饰等方式使细菌负载其他具有抗肿瘤活性的成分。这进一步扩大了细菌在肿瘤治疗中的应用,使其成为基于临床需求递送有效药物的多功能平台。

2 细菌装载药物对抗肿瘤

仅靠细菌固有属性往往无法完全根除实体瘤。通过肿瘤靶向细菌递送抗肿瘤药物来增强治疗效果最早在20世纪90年代中期就被提出,并一直在积极探索。已报道的各种细菌载药系统归纳为表1。这里,我们从细菌装载方式和装载药物类型的角度描述不同的细菌载药系统。

2.1 表面装载

细菌细胞壁成分中含有的多种活性反应基团,如游离的巯基和氨基等可被用来进行表面化学修饰并与化疗药物或纳米颗粒包裹的药物偶联,实现化疗药的表面装载^[71]。另外,利用基因工程技术将具有肿瘤抑制活性的蛋白药物或抗体与细菌跨膜蛋白融合,指导生物药物在细菌表面表达同样可以实现表面装载。

2.1.1 表面装载化疗药物

传统化疗缺乏对肿瘤组织的选择性而引起全身性毒性,限制了其临床应用。基于大肠杆菌、沙门氏菌等肿瘤靶向细菌设计化疗药物递送载体增强了药物的选择性^[42,72]。细菌表面多种化学活性基团被用于化疗药物在细菌表面的装载。利用大肠杆菌(*Escherichia coli* Nissle 1917, EcN)表面的游离氨基,将阿霉素(doxorubicin, DOX)分子偶联在EcN表面,实现了肿瘤靶向细菌介导DOX在肿瘤中的特异性蓄积和酸响应释放。静脉注射后,EcN介导的DOX在每克肿瘤组织中的蓄积量占总注射剂量的比例高达12.9%^[42]。将减毒沙门氏菌表面氨基作为锚定位点,利用基于生物素-链霉亲和素的两步法反应将紫杉醇(paclitaxel, PTX)脂质体负载在细菌表面,

表1 细菌载体负载的各类药物分子

载药方式	药物类型	药物或效应分子	参考文献
表面装载	化疗药物	阿霉素、紫杉醇	[42-46]
	生物药物	癌胚抗原单链抗体、核酸适配体、Endoglin、TRAIL	[47-50]
	肿瘤抗原	肿瘤新抗原、肿瘤相关抗原	[51-53]
表达分泌	前药转化酶	胞嘧啶脱氨酶、硝基还原酶	[54-57]
	细胞毒素	溶细胞素A、肿瘤坏死因子 α 、死亡受体配体(FasL、TRAL)	[58-63]
	免疫调节因子	细胞因子及趋化因子(IL-2、IL-12、IL-18等)、	[64-68]
		PD-L1/PD-1/CTLA-4纳米抗体	[69-70]

实现将紫杉醇脂质体高效递送至肿瘤微环境^[44]。此外,基于叠氮化物-炔基的点击化学反应的两步法偶联也被用于将 DOX 装载在大肠杆菌表面,用于向肿瘤中靶向递送化疗药物^[43]。

2.1.2 表面装载生物药物

利用细菌外膜蛋白融合表达系统可以指导基因编码的生物药物有效表达在细菌表面,实现在细菌表面装载生物药物。细菌表面展示的生物药物发挥多样化的功能,包括增强细菌的肿瘤靶向、破坏肿瘤血管或增强细菌介导的细胞凋亡等,最终达到促进肿瘤消退的目的。癌胚抗原(carcinoembryonic antigen, CEA)在胃肠道、胰腺癌等人类癌症中大量表达。将癌胚抗原单链抗体以 Lpp-OmpA-scFv 融合蛋白方式展示于大肠杆菌和沙门氏菌表面增强了细菌在肿瘤部位的蓄积,被应用于癌胚抗原相关的快速诊断和细菌介导的肿瘤治疗^[47]。与细菌表面氨基偶联的核酸适配体作为“化学抗体”同样促进了细菌对肿瘤的靶向^[48]。Ag43 是在大肠杆菌中发现的一种膜蛋白, Huang 等^[49]构建了一种新的 Ag43 表面展示系统,在大肠杆菌表面表达了 Ag43-Endoglin 融合蛋白并将其作为肿瘤疫苗应用于小鼠实体瘤模型的治疗;研究数据表明,Ag43-Endoglin 融合蛋白可以破坏免疫系统对自身 Endoglin 的耐受,并诱导抗 Endoglin 自身抗体的产生,抑制肿瘤血管生成,从而抑制肿瘤的生长和转移。大肠杆菌 MG1655 外膜蛋白 YiaT 介导了肿瘤坏死因子相关的凋亡诱导配体(TRAIL)的表面表达,进一步提高了大肠杆菌的抗肿瘤疗效^[50]。细菌表面展示技术利用锚定蛋白将异源蛋白药物以融合蛋白的形式展示于宿主细胞外膜,其优势在于被展示的蛋白质能够保持原有的生物活性,利用细菌“蛋白质工厂”和“递送载体”的双重功能,有助于最大化细菌表面装载的生物药物的抗肿瘤活性。

2.1.3 表面装载肿瘤抗原

在细菌表面表达异种蛋白首次在大肠杆菌中被报道^[73]。随着肿瘤免疫治疗的发展,在细菌表面展示肿瘤抗原以研制细菌活疫苗受到越来越多的关注。与其他生物药物类似,肿瘤抗原一般通过细菌的跨膜蛋白展示在细菌表面。在最新的两项研究中,分别来源于小鼠黑色素瘤和结肠癌细胞的新抗原被以抗原-AIDA(减毒沙门氏菌的外膜蛋白)和抗原-Lpp-OmpA(大肠杆菌的脂蛋白外膜蛋白 A)融合蛋白的方式展示在沙门氏菌和大肠杆菌表面。新抗原具有肿瘤特异性,能够引起抗原特异性免疫

反应。以细菌合成新抗原并作为载体显著提高了抗原的免疫原性,引起更强烈的自适应免疫应答^[51-52]。除了以融合蛋白的形式将抗原装载在细菌表面之外,我们团队通过表面化学修饰,将带正电荷的纳米颗粒修饰在沙门氏菌表面,原位注射至肿瘤部位后将肿瘤抗原原位装载在细菌表面,利用细菌主动运动的特性转运抗原,促进瘤周 APC 对抗原的识别以增强抗肿瘤免疫^[53]。

2.2 装载分泌型药物

细菌具备的转录-翻译机器允许其作为“蛋白质工厂”表达具有抗肿瘤活性的蛋白质。可将携带治疗基因的表达质粒转化至细菌中指导蛋白表达,并从细菌中分泌出来发挥药物功效;也可作为载体,将 DNA 和 RNA 分泌至宿主细胞内进行表达。细菌生产递送的治疗药物根据作用方式和原理可分为以下几类:(1)前药转化酶;(2)细胞毒素;(3)免疫调节因子(表 1)。

2.2.1 前药转化酶

前药转化酶(prodrug-converting enzymes, PCE)在肿瘤部位的特异性表达可只将肿瘤内的无毒前体药物转化为细胞毒性药物。这一策略可以在提高肿瘤疗效的同时减少全身给药相关的副作用,肿瘤靶向细菌作为 PCE 的有效合成、递送的载体实现了 PCE 的肿瘤靶向递送,使前体药物向毒性药物的转化只局限在肿瘤区域内。胞嘧啶脱氨酶可将无毒的 5-氟胞嘧啶(5-FC)转化为 5-氟尿嘧啶(5-FU)。表达大肠杆菌胞嘧啶脱氨酶的沙门氏菌系统性给药后在小鼠肿瘤中实现了高效的 5-FC 至 5-FU 的转化,而在正常组织中未检测到 5-FU,显示良好的抗肿瘤活性^[54]。

梭状芽孢杆菌同样也被用于表达递送前药转化酶用于肿瘤治疗。硝基还原酶可催化单功能 DNA 烷基化试剂 CB1954 转化为双功能 DNA 烷基化衍生物,诱导 DNA 损伤和细胞凋亡^[55]。多项研究显示,表达流感嗜血杆菌硝基还原酶的梭状芽孢杆菌显示出非常显著的抗肿瘤效果^[56-57, 74]。在人 HCT116 结肠癌皮下移植瘤模型中,硝酸还原酶表达菌株和 CB1954 组合的多次给药实现了持续的肿瘤抑制^[56]。最近报道的来源于脑膜炎奈瑟菌的硝基还原酶显示出更强的 CB1954 催化活性,并对另一 DNA 交联剂前药 PR-104 也具有催化活性,催化产物导致 DNA 链间交联及细胞周期阻滞^[57, 75]。前药转化酶策略的有效性依赖于细菌载体显著和持续的肿瘤定植,确保了前药转化酶在肿瘤部位的持续高水平

表达。

2.2.2 细胞毒素

以肿瘤细菌为载体携带的细胞毒素具有直接的抗肿瘤活性。例如,携带溶细胞素 A 表达载体的沙门氏菌显著定植在皮下移植瘤和原位移植瘤小鼠模型的肿瘤部位,注射 L-阿拉伯糖能够启动溶细胞素 A 在肿瘤中的表达,抑制肿瘤生长^[58]。另外,诱导肿瘤细胞凋亡同样是具有前景的一种癌症治疗思路。直接全身性注射 TNF、死亡配体 FasL 的方法由于毒副作用及较短的循环半衰期限制了治疗效果^[76-77]。为了降低凋亡诱导因子的毒副作用并提高循环半衰期,研究人员对偏好在肿瘤定植的菌株,如沙门氏菌、大肠杆菌进行工程化改造,实现了 TNF- α 、FasL、TRAIL 的靶向递送^[59-63],显著抑制了肿瘤生长,延长了荷瘤小鼠的生存期。

2.2.3 免疫调节因子

细胞因子通过调节免疫细胞的增殖、分化和激活促进其对肿瘤细胞的杀伤,最终达到抗肿瘤效果,在肿瘤免疫应答中具有重要作用。集落刺激因子 GM-SF、IL-12 和 IL-18 等已作为抗肿瘤药物进入临床试验或临床应用^[78]。利用肿瘤靶向细菌作为载体,将细胞因子表达序列克隆至肿瘤靶向细菌中并诱导表达,以细菌作为活体载体递送至肿瘤部位,能够加强细胞因子的抗肿瘤效果^[64-68]。与直接注射 IL-18 相比,以减毒沙门氏菌作为载体递送表达 IL-18 在小鼠结肠癌和乳腺癌模型中呈现出更显著的肿瘤抑制效果^[64]。除了用于递送经典的细胞因子之外,表达 PD-1 抗体的工程化沙门氏菌也被用于肿瘤治疗。工程化沙门氏菌靶向肿瘤后在肿瘤中持续表达并分泌 PD-1 抗体,这种持久的治疗策略显著抑制了黑色素瘤模型的生长^[70]。

3 控制细菌药物递送系统的智能调控元件

使用多种调控元件控制药物的表达时间及释放位置往往可以最大限度地发挥细菌载药系统的治疗效果并降低毒性,与组成型的表达和无序释放相比具有明显优势。借助合成生物学和基因工程技术,多种基因表达调控元件和基因回路被设计并导入细菌,赋予其更高级别的“智能化”。“智能化”的微生物载体对环境中的特殊因素具有感知能力并做出响应,在体内实现对负载药物表达和释放的时空特异性控制。为赋予细菌“感知”能力,多种环境感知元件和基因回路等“智能化器件”被应用于研制新型“智能化”细菌载体(图 1)。

3.1 肿瘤环境响应元件

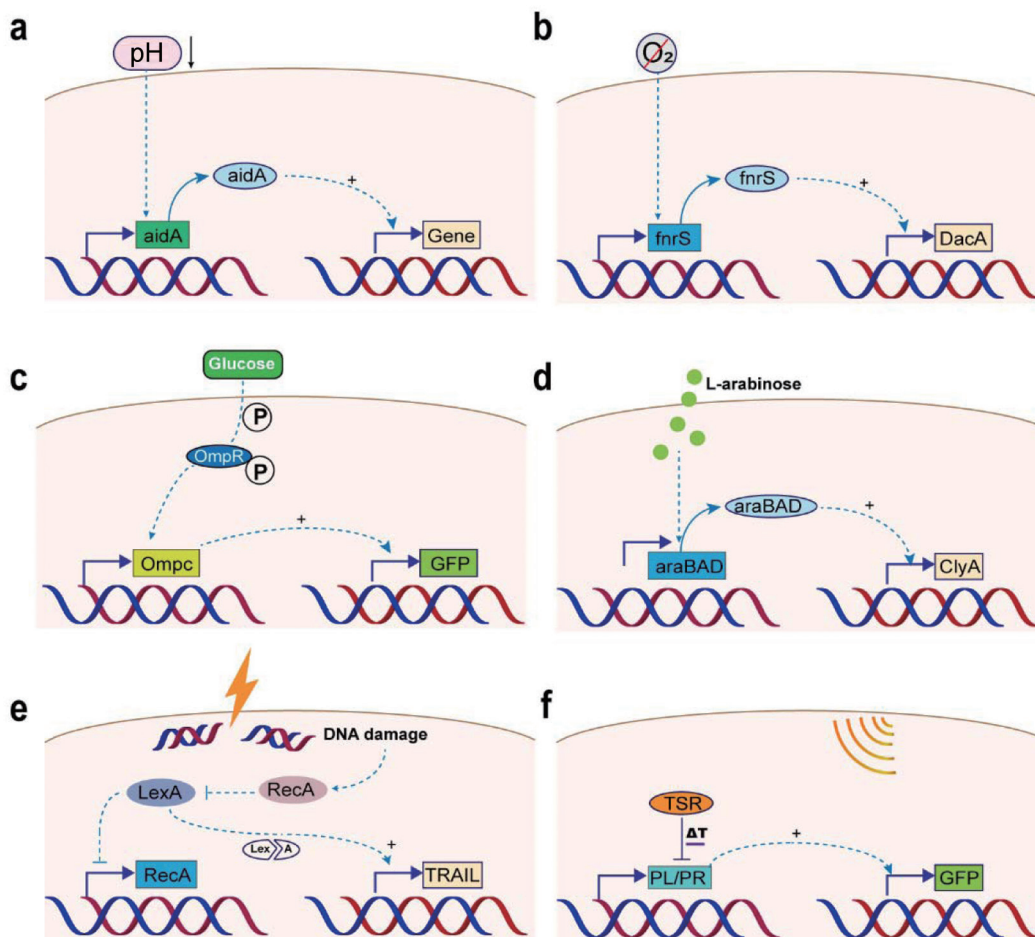
肿瘤在生长过程中形成低 pH 值、低葡萄糖浓度及缺氧的独特微环境,利用这些特征可设计调节元件控制细菌载体。

过度的增殖需求导致肿瘤细胞优先利用糖酵解作为能量代谢途径,产生的大量乳酸在肿瘤微环境中导致了酸性环境的形成^[79]。表达溶细胞素 A 的工程化大肠杆菌靶向肿瘤后,肿瘤酸性环境启动了酸响应型启动子控制的溶细胞素 A 的表达(图 1a),该策略对肿瘤生长抑制程度达到 79% 且没有引起明显的宿主毒性。Flentie 等^[80]筛选出沙门氏菌中 pH 响应表达的 STM1787 基因,并用于控制志贺毒素在肿瘤微环境中的 pH 响应表达,该系统在体外和体内实验中都显示出特异性的抗肿瘤活性。肿瘤细胞有氧糖酵解的另一个结果是肿瘤间质中的葡萄糖浓度大幅降低^[81]。具有感知葡萄糖浓度能力的肿瘤定植细菌被设计用来在肿瘤微环境中选择性递送药物。Panteli 等^[82]将 OmpC 启动子与葡萄糖感应受体 Trz1 受体结合(图 1c),构建了葡萄糖浓度敏感型基因表达载体;将载体导入大肠杆菌中,赋予大肠杆菌感知葡萄糖浓度的能力,提出了一种利用肿瘤微环境中葡萄糖浓度启动药物表达的方法。

肿瘤细胞高度耗氧,且肿瘤中功能失调的血管系统氧气输送效率下降导致肿瘤环境中高度缺氧。将缺氧启动子导入细菌载药系统中有助于控制细菌负载药物在肿瘤部位的选择性表达释放。缺氧诱导启动子,如 Pfnrs 等(图 1b)为细菌载体提供了缺氧环境的感知能力,实现抗肿瘤活性药物在肿瘤部位选择性表达^[83-86]。缺氧响应型启动子利用肿瘤中的缺氧环境,无需再往体内注射其他外源诱导剂即可实现基因表达的控制,避免外源诱导剂对宿主代谢带来的影响。

3.2 诱导剂响应元件

1961 年, Jacob 和 Monod 在法国巴斯德研究所研究乳糖代谢所需酶的表达时提出著名的操纵子学说,从而开创了在分子水平研究基因表达调控机制的新领域^[87-88]。在细菌载体中插入外源诱导剂可控的操纵系统可实现负载药物表达的时空控制,促进细菌载药系统更加智能。常用的诱导剂包括 L-阿拉伯糖^[25, 89-92]、异丙基硫代半乳糖苷(IPTG)^[93]、四环素^[94-95]、水杨酸^[96-97]等。例如, Nguyen 等^[90]构建了表达细胞毒蛋白 ClyA 的 pBAD 载体(图 1d),并将其导入沙门氏菌,在体外实验中施用 L-阿拉伯糖后, ClyA 的表达量得到大幅提高。



a, pH感知型: 酸性敏感启动子 $aidA$ 感知pH较低的肿瘤微环境, 启动下游基因 $ClyA$ 或其他治疗基因的表达; **b**, 缺氧环境感知型: 借助缺氧启动子 P_{fnrS} , 肿瘤内缺氧微环境可被用来控制STING激动剂环状二核苷酸(cyclic di-AMP, CDA)合成酶 $dacA$ 的表达。 **c**, 葡萄糖感知型: 环境中的葡萄糖浓度梯度被受体蛋白感知后启动下游信号磷酸化OmpR蛋白, 磷酸化的OmpR作为转录激活因子结合Ompc启动子激活下游基因GFP的表达。 **d**, L-阿拉伯糖诱导型: 在缺少L-阿拉伯糖的情况下, 调节蛋白AraC结合在 $araBAD$ 启动子区发挥阻遏作用, 当加入L-阿拉伯糖后, AraC构象改变, 阻遏作用消失, 下游基因表达启动。 **e**, 电离辐射诱导型: 辐射引起的DNA损伤激活RecA蛋白, 随后激活阻遏蛋白LexA自身蛋白水解酶活性而分解, 解除对RecA启动子的抑制作用, 启动下游TNF相关凋亡配体(TRAIL)基因表达。 **f**, 超声介导型: 在该类基因表达控制系统中, 通过超声调节温度敏感抑制因子TSR如Tci42对PL/PR启动子的抑制活性, 启动下游基因表达。

图1 实现细菌载体智能感应的基本元件

除了上述若干化学诱导剂, 电离辐射^[98-101]和超声响应型^[102-103]的细菌载体也已被广泛开发。与化学诱导剂不同, 无需考虑诱导剂的生物代谢对机体造成的影响。在基于电离辐射响应的细菌智能药物载体中, RecA启动子是其中最常应用的元件(图1e)。在电离辐射造成DNA损伤后, RecA启动子启动下游基因转录。将RecA启动子控制表达的TNF相关凋亡配体导入沙门氏菌后, 施以2 Gy剂量的 γ -射线或5 J/m²剂量的UV辐射即能成功诱导效应蛋白的表达, 从而杀伤肿瘤细胞^[98]。另外, 2 Gy γ -射线辐射也成功诱导了携带TNF- α 基因的重组梭状芽孢杆菌表达效应蛋白TNF- α ^[101]。

由于聚焦超声(focused ultrasound, FUS)在组织穿透深度上的优势, 利用超声可以在深部组织中精确地将温度升高到可耐受的范围^[104]。设计温度响应型的转录调控元件可以结合超声对蛋白表达实现深度的时空控制(图1f)。Abedi等^[102]报道了FUS调控CTLA-4和PDL1表达的益生菌*E. coli* Nissle 1917应用于肿瘤免疫治疗。同时, Chen等^[103]也报道了一种产生细胞因子干扰素- γ (IFN- γ)的超声响应细菌, 该细菌可以以超声可控的方式诱导外源基因IFN- γ 的表达以促进肿瘤消退。

3.3 基因回路

基因回路通常是由多组分条件反应元件并联或

串联而构建的,可以执行逻辑运算的遗传网络。研究人员在细菌中导入组装生物分子模块,感知环境中某些条件的改变,以控制细菌载药系统的药物表达和释放。在这里我们介绍了响应不同条件的基因回路在细菌肿瘤治疗中的应用。

3.3.1 细菌群体感应

群体感应现象是细菌对信号分子的一种应答过程。这种应答呈现酰基高丝氨酸内酯(acyl homoserine lactone, AHL)剂量依赖模式,是细菌之间信号传递的重要机制。将群体感应系统引入智能载药细菌中,可以实现特定蛋白在细菌定植部位的表达与释放。例如,群体感应可实现沙门氏菌负载的GFP在肿瘤部位的高水平诱导,而在肝脏中的低密度的沙门氏菌不足以激活GFP的表达^[105]。在随后的研究中,研究人员构建了LuxR/I系统控制的噬菌体裂解基因 ϕ X174 E表达载体,通过群体感应控制细菌的自发裂解^[106]。细菌增殖到一定密度后即启动自裂解程序,导致细菌携带的药物在靶向部位的释放,进而实现利用细菌进行肿瘤免疫治疗药物的智能递送^[69,107]。

3.3.2 事件感应

沙门氏菌能够主动侵入细胞并原位合成药物分子,是将蛋白质药物递送至肿瘤细胞内的理想载体。沙门氏菌在入侵细胞后立刻激活II型毒力岛基因的表达促进其在细胞内存活。利用这一机制,Raman等^[108]将噬菌体裂解基因的表达与细菌进入细胞这一事件偶联,构建了PsseJ-LysE基因回路,实现细菌入侵肿瘤细胞后的自裂解,控制沙门氏菌携带的治疗性药物NIPP1-CD及CT Casp-3在细胞内的释放。

3.3.3 温度感应

单个温度响应型启动子的应用可通过超声简单地启动目的基因的表达,但基因表达只在超声期间被短暂地激活。Abedi等^[102]设计了基于温度响应启动子的基因回路,实现了短暂热激后目的基因的持久表达释放;在小鼠B细胞淋巴瘤模型中,超声激活了细菌载体装载的CTLA4纳米抗体在肿瘤中的表达,并显著抑制肿瘤生长。

4 总结与展望

肿瘤靶向细菌是理想的药物载体,能够有效地装载活性药物至肿瘤组织,这归因于细菌的肿瘤选择性、自主运动性和强大的基因改造能力。这种无限的基因改造潜能不仅可以使细菌成为“药物工厂”表达多种治疗性蛋白质,还可以构建基因回路控制

细菌的药物表达、释放和进一步提高细菌的靶向性,使它们执行更加复杂的抗肿瘤治疗药物递送任务。另外,不可忽视的一点是细菌表面多种修饰基因为化学药物的装载提供了便利条件,这在一定程度上丰富了细菌装载的药物库。

然而,在工程化菌被批准应用于临床之前,仍然存在许多挑战,包括细菌固有的毒性、有限的药物表达、肿瘤靶向效率等。梭状芽孢杆菌和沙门氏菌的减毒菌株已在多种动物物种^[109-110]和人体试验^[111-113]中被证明是非致病的,但保留的任何毒力因子对免疫受损的晚期癌症患者都可能具有致病性。为了在病灶内达到足够的药物浓度以产生治疗效果,对细菌表达分泌药物分子的能力进行调整是非常重要的环节。另外,细菌不确定的肿瘤靶向效率可能会由于在肿瘤灶中细菌蓄积不足,导致局部药物浓度不足而难以产生治疗效果,并且伴随靶向效率低下而来的是全身毒性的升高。降低毒性将增加最大耐受剂量,高效的药物表达分泌将有效促进肿瘤消退,增加靶向效率将增加肿瘤定植,解决这些挑战将克服目前细菌疗法在临床上的限制。这些挑战都有望通过合成生物学技术来解决,例如蛋白药物的产量可通过调节基因拷贝数、启动子强度、密码子优化等要素来实现;研究筛选细菌中发挥不必要毒性的功能蛋白并对其适度敲除以降低细菌毒力;精准调控的基因回路和表达调控元件可增加细菌载体的智能化属性,增强对肿瘤的选择性和病灶的药物浓度。

目前初步的概念验证实验发掘了细菌治疗癌症的巨大潜力,并阐明了细菌被改造为“智能化”药物载体的多种有效手段。尽管“智能化”的细菌载体具有巨大的癌症治疗潜能,但在不久的将来,其癌症治疗的成功应用仍可能需要联合现有的治疗方法以产生协同效应。目前,已有多项利用细菌或基于细菌的联合疗法治疗癌症的临床试验正在进行^[114-115],尽管这些临床试验处于早期阶段,但随着越来越多以合理设计的工程细菌为载体的药物智能递送的癌症疗法进入临床试验阶段,工程细菌成功治疗肿瘤将会成为人类对抗癌症的武器库中另一项强大武器。

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