肿瘤微环境响应的纳米载体用于siRNA体内递送研究进展

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摘要 自从RNA干扰(RNA interference, RNAi)现象被发现以来, RNAi技术快速发展为高效的转录后基因沉默的工具, 被广泛应用于基因功能分析和多种疾病(如恶性肿瘤、乙型肝炎、肥胖症、多发性神经病变等)的临床或临床前治疗. 小分子干扰RNA(small interfering RNA, siRNA)是RNAi技术的效应分子. 然而作为负电性的生物大分子, 裸siRNA在体内半衰期短且容易被核酸酶降解和肾小球滤过, 因此其临床应用受到极大的限制. 如何构建安全高效的体内递送载体用于提高siRNA在体内的稳定性和靶细胞中基因沉默效率是RNAi技术临床转化的关键. 目前已有诸多纳米载体, 如脂质体被应用于siRNA体内递送, 并在临床或临床前疾病治疗上取得了较好的疗效. 实体瘤具有特殊的血管结构和生理微环境(如弱酸及乏氧环境、特定化学物质或酶过度表达等), 因此近年来国内外学者将研究兴趣聚焦在构建肿瘤微环境响应的纳米载体, 用于提高siRNA在体内稳定性、肿瘤部位富集量和肿瘤细胞中靶基因沉默效率. 本文综述了肿瘤微环境响应的siRNA递送载体的设计原理及功能特征, 分析了现有肿瘤微环境响应的siRNA递送载体的优势和不足, 探讨了肿瘤微环境响应的siRNA递送载体的未来发展趋势.

关键词 RNAi技术, siRNA递送, 纳米载体, 肿瘤微环境响应

自从诺贝尔生理学或医学奖获得者Andrew Fire和Craig C. Mello首次在线虫中揭示了RNAi现象以来^[1], RNAi技术已快速发展成为阐明机体生长、发病机制和衰老的有力工具, 被广泛应用于恶性肿瘤、乙型肝炎、肥胖症、多发性神经病变等多种疾病的临床或临床前治疗^[2~8]. 小分子干扰RNA(small interfering RNA, siRNA)是RNA干扰(RNA interference, RNAi)技术的效应分子, 长度为21~23 nt. RNAi技术的主要原理

是人工合成的siRNA或双链RNA(double-strand RNA, dsRNA)经Dicer酶加工成siRNA进入胞浆后, 与Ago蛋白形成RNA诱导沉默复合体(RNA-induced silencing complex, RISC). 随后正义链(sense strand)从RISC上解离, 反义链(antisense strand)通过与靶mRNA的碱基互补对其进行酶切, 实现靶基因沉默(图1)^[3,5]. 由于RISC可以重复使用, 因此使用siRNA沉默靶基因可在分裂细胞中持续几天, 而在非分裂细胞中可以持续几周,

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图 1 RNAi 技术主要原理示意图

Figure 1 Schematic illustration of the mechanism of RNAi technology

是目前特异性抑制靶基因表达的有效方法. 此外, siRNA 是抑制 mRNA 翻译而不是转录, 因此不会干扰染色体 DNA, 从而避免了基于 DNA 疗法中可能产生的基因突变. 同时, siRNA 是通过与 mRNA 相互作用发挥疗效的, 因此减少了合成前可能生成的有害蛋白. 最为重要的是, siRNA 可以靶向抑制任何与疾病相关 mRNA 的翻译, 即蛋白的表达, 因此在疾病治疗上有非常大的潜力, 尤其是对于那些已经明确与疾病相关、但无有效干预药物的蛋白(undruggable proteins)^[7,9-12].

由于具有上述的各种优势, siRNA 在新药研发中受到广泛关注. 尤其是自 2006 年 RNAi 的发现者 Andrew Fire 和 Craig C. Mello 获得诺贝尔生理学或医学奖以来, siRNA 药物研究领域快速发展, 各大医药巨头也纷纷进军 siRNA 药物的研发. 美国食品药品监督管理局(US Food and Drug Administration, FDA)分别于 2018 年 8 月和 2019 年 11 月批准了 Alnylam 医药公司研发的 siRNA 药物——Onpattro(Patisiran, 用于治疗遗传性转甲状腺素蛋白淀粉变性)和 Givlaari(Givosiran, 用于急性肝卟啉症)^[13]. 这是目前全球两个获批上市的 siRNA 药物, 更是 RNAi 技术领域的重大突破. 作为生物医药创新领域的前沿, 我国也在《“十三五”生物产业发展规划》中将 siRNA 药物列为重点发展领域. 虽然 siRNA 在很多疾病, 如恶性肿瘤治疗上有很大的应用潜力, 但真正转化为临床治疗药物还面临着诸多挑战, 其中最关键的是如何将 siRNA 高效递送至体内靶细胞中发挥基因沉默作用. 以恶性肿瘤为例, 静脉注射到体

内的裸 siRNA 体内半衰期短(<15 min), 且容易被核酸酶降解和肾小球滤过, 在肿瘤部位富集量极低^[3]. 另外, siRNA 是负电性的生物大分子(分子量约为 13000), 很难渗透到整个实体瘤组织, 而且也不能以自由扩散的方式进入肿瘤细胞. 即使有少量 siRNA 进入肿瘤细胞, 也很难从内涵体中逃逸出来发挥作用. 因此, 裸 siRNA 需要借助递送载体才能保持其体内稳定性并顺利进入靶细胞发挥治疗作用^[9,13-20]. 下文将从载体设计原理、功能特征以及应用前景等方面总结近年来 siRNA 体内递送载体, 尤其是肿瘤微环境响应体内递送载体的研究进展.

1 siRNA 体内递送载体

体内稳定性对于 siRNA 发挥基因沉默作用至关重要. 病毒载体是最早用于体内递送 siRNA 的载体, 但病毒载体有潜在的免疫原性、致突变性以及无法大规模制备等缺点^[15], 目前已不是 siRNA 体内递送的首选. 由于具有生物相容性好、低免疫原性以及容易大规模制备等特点, 非病毒载体尤其是纳米载体一直备受研究者的青睐, 被广泛用于 siRNA 的体内递送. 例如上文提到的 siRNA 药物 Onpattro, 其本质上是包载有 siRNA 的纳米脂质体. 由于实体瘤内部血管结构不完整、具有高通透性和高滞留效应(enhanced permeability and retention effect, EPR effect)^[21-23], 纳米载体更是可以利用 EPR 效应“被动靶向”到肿瘤部位, 显著提高 siRNA 的瘤内富集量并增强其基因沉默效果. 虽然具有上述优势, 传统纳米载体仍不能完全克服 siRNA 体内递送时所面临的诸多生理屏障(图 2), 尤其是无法解决肿瘤组织穿透能力弱、肿瘤细胞摄取量低及内涵体逃逸能力差等难题^[3]. 因此, 理想的 siRNA 体内递送载体需要具备以下 5 个特征: (i) 生物安全性好, 不引起机体免疫反应; (ii) 体内循环时间长, 能够保护 siRNA 不被核酸酶降解; (iii) 在肿瘤部位富集量高并能深度穿透肿瘤组织; (iv) 被肿瘤细胞摄取量高; (v) 能够高效地从内涵体逃逸出来进入胞浆, 快速释放 siRNA 并发挥基因沉默作用. 为了实现这一目的, 近年来国内外研究者基于肿瘤组织特殊的生理微环境(如弱酸及乏氧环境、特定化学物质或酶过度表达等), 采用化学手段设计构建了肿瘤微环境响应纳米载体, 用于提高 siRNA 的体内递送效率和在肿瘤细胞

图2 纳米载体用于体内传递siRNA时需要克服的多重生理屏障示意图

Figure 2 Schematic illustration of the multiple physiological barriers to the systemic delivery of siRNA by nanocarriers

中基因沉默效果^[16,24~27]。

2 肿瘤微环境响应的纳米载体

恶性肿瘤是一个系统性疾病, 就像种子生长需要营养和水分充足的土壤一样, 许多肿瘤都依赖于周围的微环境才能在患者体内生长^[28]。实体瘤除了含有高异质性的肿瘤细胞外, 还包含细胞外基质、成纤维细胞、血管/淋巴管、免疫细胞等, 它们共同组成了肿瘤微环境^[29~34]。肿瘤微环境与肿瘤细胞相互作用, 在肿瘤发生、进展和转移过程中发挥重要作用。肿瘤细胞通过分泌生长因子和蛋白酶等作用于微环境; 同时被“驯化”的微环境细胞则回馈肿瘤细胞, 使肿瘤组织表现出与正常组织显著不同的生理微环境(图3)^[29,31,33], 主要包括: (i) 肿瘤细胞外及肿瘤细胞内溶酶体、内涵体的弱酸性环境; (ii) 肿瘤组织的乏氧环境; (iii) 肿瘤细胞外及肿瘤细胞内多种酶过度表达, 如基质金属蛋白酶(matrix metalloproteinase, MMP)等; (iv) 肿瘤细胞中存在高水平的反应活性氧(reactive oxygen species, ROS); (v) 肿瘤细胞内有高浓度还原性物质, 如谷胱甘肽(glutathione, GSH)等。基于以上肿瘤组织的特殊生理微环境, 设计肿瘤微环境响应的纳米载体, 可以解决传统纳米载体用于siRNA体内传递时尚未解决的难题。这些肿瘤微环境响应纳米载体能在血液循

图3 肿瘤组织表现出与正常组织显著不同的生理微环境, 包括弱酸及乏氧环境、特定酶、GSH及ROS过度表达等

Figure 3 Tumor tissues show different physiological microenvironments from normal tissues, including weakly acidic and hypoxic microenvironments and over-expression of specific enzymes, GSH, and ROS

环和正常生理组织中稳定存在; 到达肿瘤部位后, 在肿瘤组织中被肿瘤细胞摄取后快速释放出siRNA, 能显著提高siRNA的生物利用度, 增强抗肿瘤效果。目前已报道的肿瘤微环境响应纳米载体主要包括酸响应、乏氧响应、酶响应、ROS响应、GSH响应等纳米载体^[35~41]。下文将逐一展开这些肿瘤微环境响应纳米载体在siRNA体内传递上的应用。

2.1 酸响应纳米载体用于siRNA体内递送

正常生理组织pH值为7.4左右, 而肿瘤细胞外pH值约为6.5~6.8。这是由于肿瘤生长十分迅速, 肿瘤血管往往无法提供充足养料和氧气来供应肿瘤细胞增殖, 肿瘤内部缺氧状态使肿瘤细胞无氧糖酵解产生乳酸, 细胞膜表面质子泵活性的增加促进了酸性分泌物释放至细胞外, 而肿瘤内部血流速度减慢使得肿瘤组织难以恢复至中性而维持酸性环境^[42~46]。此外, 除了肿瘤组织自身的弱酸环境, 肿瘤细胞中内涵体(pH 5.0~6.0)和溶酶体(pH 4.0~5.0)均处于酸性环境^[47]。酸响应纳米载体主要是利用肿瘤组织与正常组织生理pH值的差异或内涵体中酸性pH的特征, 将siRNA体内递送至肿瘤部位并快速定位释放于肿瘤组织或肿瘤细胞中, 是一个具有良好发展前景的纳米载体系统。目前, 酸响应纳米载体的构建主要有两种方式: (i) 利用含有酸响应基团的材料构建纳米载体; (ii) 利用酸响应的化学键构建纳米载体^[16,24,37]。

图4 肿瘤组织弱酸微环境响应纳米载体用于siBRD4体内递送. A: 两亲性高分子材料Meo-PEG-*b*-PHMEMA; B: 具有肿瘤细胞靶向和膜穿透功能的两亲性多肽TCPA结构式; C: 使用这两种材料构建纳米载体用于siBRD4体内递送时利用其肿瘤组织弱酸微环境响应性促进基因沉默和抑制肿瘤生长示意图^[48]

Figure 4 TME pH-responsive nanocarriers for *in vivo* delivery of siBRD4. A: Chemical structure of the amphiphilic polymer Meo-PEG-*b*-PHMEMA; B: chemical structure of the amphiphilic peptide TCPA with tumor-targeting and membrane-penetrating functions; C: the schematic illustration of the siBRD4-loaded nanocarriers made with the above polymer and peptide to utilize the tumor microenvironment pH-responsive characteristic to improve gene silencing and inhibition of tumor growth^[48]

在使用含酸响应基团的材料构建纳米载体研究上, 本团队^[48]设计合成如图4A所示的肿瘤组织弱酸微环境响应的两亲性高分子材料Meo-PEG-*b*-PHMEMA, 其 pK_a 为6.9, 接近肿瘤组织弱酸微环境pH (6.5~6.8). 该高分子材料在水溶液可自组装形成以PEG链为亲水性外壳、PHMEMA链为疏水性内核的纳米载体. 其中, 疏水性内核可以用于包载siRNA与两亲性功能肽TCPA形成的复合物(图4B). 如图4C所示, 使用上述纳米载体包载bromodomain-containing protein 4 siRNA(siBRD4)并注射到荷瘤小鼠体内后, 其表面的PEG外壳可以大大延长siRNA的体内循环时间^[49-51]. 在纳米载体利用EPR效应富集到肿瘤部位后, 肿瘤组织弱酸微环境会引起PHMEMA链的质子化^[52], 导致纳米载体结构的解散和siRNA/TCPA复合物的快速释放. 随后, TCPA分别利用其结构中具有肿瘤细胞靶向功能的RGD肽序列^[53]和膜穿透功能的寡聚精氨酸序列^[54], 携带siRNA靶向肿瘤细胞并增强其

内涵体逃逸能力, 进入胞浆沉默BRD4表达并抑制肿瘤生长.

在上一工作基础上, 本团队^[55]最近基于抗肿瘤药物米托蒽醌(Mitoxantrone, MTO)的阳离子特性, 设计合成了如图5A所示的基于MTO的两亲性阳离子前药SA-MTO. 类似于上文提到的两亲性功能肽TCPA, SA-MTO也可通过静电作用与siRNA形成复合物^[56], 而且这些siRNA/SA-MTO复合物可被包裹在由肿瘤组织弱酸微环境响应的两亲性高分子材料Meo-PEG-*b*-PHMEMA(pK_a ~6.89)形成的纳米粒子的疏水性内核中. 如图5B所示, 该纳米载体用于siRNA的体内递送时具有“多级逐步递送”(multistaged delivery)的特性. 首先纳米载体通过血液循环并利用肿瘤EPR效应, 富集在荷瘤小鼠的肿瘤部位; 随后肿瘤组织弱酸微环境会引起PHMEMA链的质子化^[52], 导致纳米载体结构的解散和siRNA/SA-MTO复合物的快速释放; 然后siRNA/SA-MTO复合物利用其尺寸较小的特性, 深度穿透肿

图 5 肿瘤组织弱酸微环境响应纳米载体用于siRNA和化疗药物体内共递送. A: 两性亲性阳离子前药SA-MTO和高分子材料Meo-PEG-*b*-PPMEMA结构式; B: 构建的纳米载体用于siRNA体内递送时利用其肿瘤组织弱酸微环境响应性促进基因沉默和联合抑制肿瘤生长示意图^[55]

Figure 5 TME pH-responsive nanocarriers for *in vivo* co-delivery of siRNA and chemotherapeutic drug. A: Chemical structures of the amphiphilic cationic prodrug SA-MTO and polymer Meo-PEG-*b*-PPMEMA; B: the schematic illustration of the siRNA-loaded nanocarriers made with the above prodrug and polymer to utilize the tumor microenvironment pH-responsive characteristic to improve gene silencing and synergistically inhibit tumor growth^[55]

瘤组织并进入肿瘤细胞; 最后肿瘤细胞中高表达的脂酶降解SA-MTO^[57], 导致siRNA和MTO在胞浆内快速释放, 分别用于沉默癌基因和抑制DNA链复制, 实现基于RNAi疗法和化学疗法的肿瘤联合治疗。

除了利用肿瘤组织弱酸微环境, 国内外研究者还基于内涵体酸性pH的特性, 设计出内涵体pH响应的纳米载体, 用于增强siRNA的内涵体逃逸能力和基因沉默效率^[16,24]。例如本团队^[58]综合考虑siRNA体内传递所面临的多重生理屏障, 设计制备了如图6所示的具有体内长循环、肿瘤组织穿透能力强和内涵体pH响应的多功能纳米载体, 用于siRNA的体内递送和抗肿瘤研究。该纳米载体是由一个新合成的两性亲性高分子材料iRGD-PEG-*b*-PDPA和Meo-PEG-*b*-P(DPA-co-GMA-TEPA-C14)共同自组装形成(图6A)。这两种高

分子材料结构中疏水性PDPA链段具有超快内涵体pH响应的特性, 其 pK_a (~6.31)在内涵体的酸性pH范围内(pH 6.0~6.5)^[52,59]。如图6B所示, 使用上述多功能纳米载体进行siRNA的体内递送具有如下4个性能: (i) 由iRGD肽提供的肿瘤组织穿透能力和肿瘤靶向能力^[60]; (ii) PEG链段可以保证纳米载体的体内长循环能力^[49]; (iii) 少量嵌入在疏水性PDPA链段内的阳离子脂可与siRNA相互作用, 并将其包裹在纳米载体的疏水性内核中; (iv) PDPA高分子链段的内涵体pH响应的特性通过“质子海绵”效应引起内涵体的溶胀^[61,62], 同时PDPA链上阳离子脂结构中烷基链能够插入内涵体膜中造成内涵体的不稳定^[63], 两种作用相互协同增加了siRNA的内涵体逃逸能力, 因此提高了肿瘤细胞内靶基因的沉默效率。使用上述多功能载体包载Survi-

图 6 内体酸性pH响应纳米载体用于siSurv体内递送. A: 内体pH响应的高分子材料Meo-PEG-*b*-P(DPA-co-GMA-TEPA-C14)和iRGD-PEG-*b*-PDPA结构式; B: 构建的多功能纳米载体用于siSurv体内传递时利用其肿瘤组织穿透功能、肿瘤细胞靶向功能和高效内体逃逸功能提高siRNA的基因沉默效率和抑制肿瘤生长效果示意图^[58]

Figure 6 Endosomal pH-responsive nanocarriers for *in vivo* delivery of siSurv. A: Chemical structures of the endosomal pH-responsive polymer Meo-PEG-*b*-P(DPA-co-GMA-TEPA-C14) and iRGD-PEG-*b*-PDPA; B: the schematic illustration of the siSurv-loaded nanocarriers made with these two polymers to utilize the tumor tissue-penetrating, tumor cell-targeting, and efficient endosomal escape functions to improve gene silencing and inhibition of tumor growth^[58]

vin siRNA(siSurv)并注射到荷瘤小鼠体内后, siSurv可以显著下调肿瘤细胞Survivin的表达并抑制肿瘤的生长.

上述提到的酸响应纳米载体主要是基于含pH响应基团的高分子材料构建的. 这些pH响应基团在正常生理条件下处于电中性, 能够维持纳米载体的稳定结构; 而在肿瘤组织或内涵体的酸性微环境刺激下, pH响应基团会进行质子化而产生静电排斥作用, 导致纳米载体的解散和siRNA的快速释放, 因此能显著提高siRNA的靶基因沉默效率. 事实上, 除了使用含pH响应基团的高分子材料, 酸响应化学键断裂也是一种广泛应用的方法, 用于调控纳米载体的稳定性或表面性质而实现高效基因沉默的目的. 常用的酸响应化学键包括胺键、酯键、缩醛键、酰胺键和脲键等^[16,24,37]. 例如, Sun等人^[64]利用2,3-二甲基马来酸形成的酰胺键酸响应性, 将聚乙二醇(polyethylene glycol, PEG)通过1,2-二甲基马来酸连接到细胞穿膜肽(cell-penetrating peptide, CPP)修饰的聚己内酯(polycaprolactone, PCL)上, 制备对肿瘤组织弱酸微环境响应的两亲性高分子材料PEG-PEG-Dlinkm-R9-PCL. 如图7所示, 该高分子材料可与siRNA共组装形成稳定的纳米粒子, 而其表面的PEG链段能够显著延长siRNA的体内循环时间.

在纳米粒子利用EPR效应富集在肿瘤部位后, 肿瘤组织弱酸微环境会引起2,3-二甲基马来酸断裂^[26], 导致PEG链段从纳米粒子表面脱落, 随之裸露出来的CPP可增强siRNA的细胞内摄取和内体逃逸能力^[54], 显著提高siRNA的靶基因沉默效率. 使用上述纳米载体包载polo-like kinase 1 siRNA(siPLK1)并注射到荷瘤小鼠体内后, siPLK1能显著敲低肿瘤细胞中PLK1的表达和抑制肿瘤的生长.

同样利用2,3-二甲基马来酰胺键酸响应性, Yang等人^[65]设计制备了如图8A所示的、基于聚磷酸酯的纳米载体用于siRNA的体内递送和抗肿瘤研究. 通过在聚磷酸酯的侧链上引入2,3-二甲基马来酸基团, 该纳米载体在正常生理条件下呈现负电性. 如图8B所示, 使用该纳米载体包载siPLK1并注射到荷瘤小鼠体内后, 其电负性表面能有效避免血液对siRNA的快速清除, 大大延长siRNA的体内循环时间. 在纳米载体利用EPR效应富集在肿瘤部位后, 肿瘤组织弱酸微环境会导致1,2-二甲基马来酸从聚磷酸酯侧链上脱落, 纳米载体的表面因此由负电性反转为正电性. 这一表面电荷反转特性显著提高siPLK1的细胞摄取量, 增强其敲低肿瘤细胞中PLK1表达的效率 and 抑制肿瘤生长的效果.

图7 两亲性高分子材料PEG-PEG-Dlinkm-R9-PCL结构式, 及其构建的纳米载体用于siRNA体内传递时利用其肿瘤组织弱酸微环境响应的化学键断裂增强siRNA的靶基因沉默效率和抑制肿瘤生长效果示意图^[64]

Figure 7 Chemical structure of the amphiphilic polymer PEG-PEG-Dlinkm-R9-PCL, and the schematic illustration of the siRNA-loaded nanocarriers made with this polymer to utilize the tumor microenvironment pH-responsive chemical bond breakage to improve gene silencing and inhibition of tumor growth^[64]

2.2 乏氧响应纳米载体用于siRNA体内递送

相对于正常组织, 由于肿瘤生长非常旺盛, 肿瘤血管往往无法提供充足养料和氧气来维持肿瘤细胞增殖, 因此造成了肿瘤组织的乏氧微环境^[66-68]。由于乏氧环境会导致一些还原酶(如硝基还原酶和偶氮还原酶等)在肿瘤细胞中高表达并分泌到肿瘤组织中, 因此含硝基或偶氮基团的高分子材料被广泛应用于构建乏氧响应的纳米载体和siRNA的体内递送^[16,24,37]。

2-硝基咪唑是一种最为常见的乏氧响应性基团, 具有高灵敏性。在正常生理环境下, 2-硝基咪唑为疏水性分子; 但在乏氧环境中, 2-硝基咪唑在硝基还原酶的作用下变为亲水性2-氨基咪唑, 呈现正电性^[69-71]。正是由于2-硝基咪唑具有高灵敏的乏氧响应性, 该基团被引入到很多高分子材料的结构中, 用于构建乏氧响应的纳米载体。例如, Liu等人^[72]构建了一种乏氧和酸双重响应的纳米载体用于siRNA的体内递送和神经胶质瘤的治疗。该纳米载体是由新设计合成的脂类分子(如图9A所示, 叔胺作为亲水头部, 连有硝基咪唑基团的十六烷基链为疏水尾部)、胆固醇和二硬脂酰基磷脂酰乙醇胺-聚乙二醇(distearoyl phosphoethanolamine-

polyethylene glycol, DSPE-PEG)共同组装而成。使用这一纳米载体体内传递siPLK1时, 载体表面的PEG链段能显著延长siRNA的体内循环时间。当纳米载体到达肿瘤部位后, 肿瘤组织弱酸微环境会导致叔胺基团质子化并呈现正电性, 同时肿瘤组织的乏氧环境也会使得疏水性硝基咪唑基团转化为正电性的氨基咪唑。这种乏氧和酸双重响应性可以大幅度提高肿瘤细胞对siPLK1的摄取量, 显著增强siPLK1的基因沉默效率和抑制肿瘤生长效果。

偶氮苯基团是另外一种常见的乏氧响应性基团, 也常被引入到材料结构中, 用于构建乏氧响应的纳米载体^[37,73-75]。例如, Perche等人^[75]使用偶氮苯将PEG和聚乙烯亚胺(poly(ethylene imine), PEI)偶联在一起, 同时将二油酰基磷脂酰乙醇胺(dioleoyl phosphoethanolamine, DOPE)连接到PEI另外一端, 构建了如图10A所示的新型脂类分子(PEG-Azo-PEI-DOPE)。使用该脂类分子构建纳米载体并用于siRNA体内传递时, 载体表面的PEG链段可以显著延长siRNA的体内循环时间。到达肿瘤部位后, 肿瘤组织的乏氧环境会导致偶氮苯基团的断裂和PEG链的脱去, 裸露出来的PEI与siRNA复合物可被肿瘤细胞大量摄取, 因此可显著提高siR-

图 8 酸响应电荷反转纳米载体用于siRNA体内递送。A: 侧链为酸敏的2,3-二甲基马来酰胺基团的聚磷酸酯PPC-DA的结构式; B: 构建的纳米载体用于siRNA体内传递时利用载体表面电荷反转增强siRNA的靶基因沉默效率和抑制肿瘤生长效果示意图^[65]

Figure 8 Nanocarriers with the characteristic of acid-responsive charge reversal for *in vivo* siRNA delivery. A: Chemical structure of the polyphosphoric ester PPC-DA containing acid-responsive 2,3-dimethylmaleamide group in the side chain; B: the schematic illustration of the siRNA-loaded nanocarriers made with this polymer to utilize the charge reversal characteristic to improve gene silencing and inhibition of tumor growth^[65]

NA的基因沉默效率。

2.3 酶响应纳米载体用于siRNA体内递送

肿瘤的发生发展以及相关基因的过表达, 会导致肿瘤细胞内外环境中某些酶的表达和活性发生改变^[33]。利用这一特性, 可以构建酶响应的纳米载体来实现siRNA在肿瘤组织或肿瘤细胞内的快速释放。在众多酶响应的纳米载体中, 基质金属蛋白酶(matrix metalloproteases, MMPs)的研究最为活跃^[76]。MMPs是一个蛋白水解酶大家族, 可降解多种胞外基质成分。MMPs分为跨膜和胞外分泌两种类型, 包括26种亚型, 不同亚型在不同组织中表达量不同, MMP被认为与肿瘤侵袭转移和血管新生有关^[77]。其中跨膜蛋白MT1-MMP以及胞外分泌MMP-2和MMP-9在多种肿瘤中高

表达, 包括乳腺癌、宫颈癌、结肠癌、前列腺癌、肺癌和卵巢癌等^[78-80]。因此, 构建MMP-2/9响应的纳米载体用于siRNA的体内传递在恶性肿瘤治疗上有广阔的应用前景。

Wang等人^[81]设计合成了一种MMP-2/9响应的两亲性高分子聚乙二醇-聚己内酯(PEG-PCL), 其中PEG和PCL通过MMP-2/9响应的多肽序列XLALGPGR9相互连接(图11)。该高分子材料可通过其结构中具有膜穿透功能的寡聚精氨酸与siPLK1间静电作用组装形成稳定的纳米载体。注射到荷瘤小鼠体内后, 纳米载体具有长的血液循环时间和高的肿瘤富集量。在肿瘤组织内, 高表达的MMP-2/9能够水解多肽序列XLALGPGR9^[76], 导致PEG从纳米载体表面脱落, 裸露出来的寡聚精氨酸序列会增强siRNA的细胞摄取和内

图 9 乏氧和酸双重响应纳米载体用于siRNA体内递送。A: 以叔胺作为亲水头部、连有硝基咪唑基团的烷基链为疏水尾部的脂类分子MDH结构式; B: 构建的纳米载体用于siPLK1体内递送时利用其乏氧和酸双重响应性提高siRNA的细胞摄取和增强基因沉默效率示意图^[72]

Figure 9 Hypoxia and acid dual-responsive nanocarriers for *in vivo* siRNA delivery. A: Chemical structure of lipid-like MDH containing tertiary amine as the hydrophilic head and nitroimidazole group as the hydrophobic tail; B: the schematic illustration of the siPLK1-loaded nanocarriers made with this lipid-like molecule to utilize the hypoxia and acid dual-responsive characteristic to improve cellular uptake and gene silencing^[72]

涵体逃逸能力^[54], 显著提高基因沉默效率和抑制肿瘤生长的效果。最近, Tang等人^[82]基于肿瘤组织的酸性微环境和MMP过度表达的特性, 设计制备了MMP-2/9和酸双重响应的纳米载体用于紫杉醇(paclitaxel, PTX)和Twist siRNA(siTwist)的共传递和乳腺癌的联合治疗。该纳米载体到达肿瘤部位后, MMP-2/9会降解其结构中PLGLAG多肽序列, 导致PEG链从载体表面脱落, 显著增强乳腺癌细胞的摄取及PTX和siRNA的内涵体逃逸能力(图12)。动物实验结果显示, 相对于只包载PTX或siTwist的纳米载体, PTX和siTwist共传递纳米载体具有

更好的肿瘤抑制效果。

2.4 ROS响应纳米载体用于siRNA体内递送

ROS主要包括过氧化氢(H_2O_2)、单线态氧(1O_2)、羟基自由基($\cdot OH$)和超氧化物等, 在许多生理过程中(如细胞信号和先天免疫)起关键性作用^[83,84]。然而高浓度ROS会破坏生物大分子, 引发疾病。在很多疾病中都发现ROS升高的现象, 包括肿瘤、糖尿病、心血管疾病和神经退行性疾病等^[83,85,86]。例如, 肿瘤组织中ROS水平明显比正常组织高, 正常组织中ROS浓度约

图 10 乏氧响应纳米载体用于siRNA体内递送。A: 以偶氮苯为偶联剂的新型脂类分子PEG-Azo-PEI-DOPE结构式; B: 构建的纳米载体用于siRNA体内递送时利用其乏氧响应性提高siRNA的细胞摄取和基因沉默效率示意图^[75]

Figure 10 Hypoxia-responsive nanocarriers for *in vivo* siRNA delivery. A: Chemical structure of the new lipid PEG-Azo-PEI-DOPE containing Azo linker; B: the schematic illustration of the siRNA-loaded nanocarriers made with this lipid to utilize the hypoxia-responsive characteristic to improve cellular uptake and gene silencing^[75]

为20 nmol/L, 而肿瘤组织由于大量ROS的产生和累积, 其浓度高达50~100 $\mu\text{mol/L}$ ^[87-89], 而且ROS水平在肿瘤转移期间会进一步升高。利用病变组织高浓度ROS的特性, 国内外研究者开发出许多ROS响应的纳米载体用于siRNA的体内递送和疾病治疗^[90-92]。用于构建ROS响应纳米载体的高分子材料主要包括含硫聚合物、含硒聚合物、含碲聚合物、含草酸酯聚合物和含苯硼酸或苯硼酸酯聚合物等^[90,93]。其中, 在siRNA体内递送研究领域内使用比较多的是含苯硼酸或苯硼酸酯的高分子材料。

Li等人^[94]设计制备了一种新型ROS响应的纳米载体用于vascular endothelial growth factor siRNA(si-VEGF)的体内递送和结肠癌治疗。如图13所示, 该纳米载体由苯硼酸修饰的聚N,N-二乙基丙烯酸氨基乙酯(PDP)、二油酰基卵磷脂(dioleoyl phosphatidylcholine, DOPC)、二油酰基磷脂酸(dioleoyl phosphatidic acid, DOPA)和二硬脂酰基磷脂酰乙醇胺-聚乙二醇(DSPE-PEG)在水溶液中共同组装而成。其中聚阳离子高分子材料PDP可通过静电作用将siRNA包载在纳米载体中, 而DSPE-PEG上的PEG链段能够延长siRNA的体内循

环时间^[49]。为了增强上述纳米载体的肿瘤细胞靶向能力和内涵体逃逸性能, 该团队进一步将具有肿瘤细胞靶向和膜穿透功能的TAT肽修饰到纳米载体表面^[54]。将其注射到荷瘤小鼠体内后, 纳米载体具有长的血液循环时间和高的肿瘤富集量。在肿瘤组织中, 纳米载体利用其表面修饰的TAT肽靶向肿瘤细胞并携带siRNA进入胞浆。最后肿瘤细胞内高浓度ROS会导致聚阳离子材料PDP的分解^[95], 显著提高siVEGF在胞浆内的释放和沉默靶基因的效率。

同样是利用苯硼酸或苯硼酸酯基团的ROS响应性, Zheng等人^[96]设计合成了如图14A所示的两亲性高分子材料PEG-*b*-P(Gu/Hb), 用于构建纳米载体, 实现siVEGF和siPLK1两种siRNA共传递和脑胶质瘤的联合治疗。在该高分子材料的结构中, 侧链上胍基具有正电性, 可通过静电作用将siRNA包载在纳米载体中; 而侧链上苯硼酸酯为疏水性基团, 在ROS作用下可从侧链上脱落, 导致高分子材料PEG-*b*-P(Gu/Hb)由两亲性变为亲水性, 最终引起纳米载体的解散和siRNA的快速释放。同时, 由于血-脑屏障(blood-brain barrier, BBB)极大限制脑胶质瘤的治疗效果^[97-99], 该团队进一步将具有穿过BBB功能的Angiopep-2多肽修饰上述高分子材料, 用于增强纳米子载体穿过BBB的能力(图14B)^[99]。将上述纳米载体注射到荷瘤小鼠体内后, 表面修饰的Angiopep-2多肽会与BBB上高表达的低密度脂蛋白受体相关蛋白(low-density lipoprotein-receptor-related protein, LRP)特异性结合^[100,101], 介导纳米载体穿过BBB进入脑组织。随后, 纳米载体进一步利用其表面修饰的Angiopep-2多肽与胶质瘤细胞表面高表达的LRP间特异性识别作用靶向胶质瘤细胞, 提高siRNA的细胞摄取量。进入胶质瘤细胞后, 胞内高浓度ROS会导致疏水性苯硼酸酯基团的脱落和纳米载体的解散, 因此可显著提高siVEGF和siPLK1在胞浆内的释放及其协同抑制胶质瘤生长的效果(图14C和D)。

2.5 GSH响应纳米载体用于siRNA体内递送

如上文所述, 相对于正常组织, 肿瘤组织内部处于乏氧状态, 造成了肿瘤组织的乏氧微环境和ROS水平升高。正常情况下, ROS水平升高会导致DNA链损伤和细胞凋亡。但是肿瘤细胞会适应性的高表达一些还原性酶(如硫氧还原蛋白还原酶、溶酶体硫醇还原酶等)和抗氧化物质(如GSH等), 用于抵消胞内高浓度

图 11 酶响应纳米载体用于siRNA体内递送. A: 高分子材料PEG-XLALGPGR9-PCL结构式; B: 使用该高分子材料构建的纳米载体用于siRNA体内递送时利用其MMP-2/9响应性促进siRNA的细胞内吞、内涵体逃逸和基因沉默效率示意图^[81]

Figure 11 Enzyme-responsive nanocarriers for *in vivo* siRNA delivery. A: Chemical structure of the polymer EG-XLALGPGR9-PCL; B: the schematic illustration of the siRNA-loaded nanocarriers made with this polymer to utilize the MMP2/9-responsive characteristic to improve cellular uptake, endosomal escape, and gene silencing^[81]

ROS, 维持其生存和增殖^[84]. GSH是细胞内主要的抗氧化剂, 在正常生理活动中发挥重要作用. 细胞内GSH在NADPH和谷胱甘肽还原酶作用下维持还原态, 正常细胞胞内GSH浓度(约2~10 mmol/L)是胞外浓度(2~20 μmol/L)的100~1000倍^[102~104]. 肿瘤细胞内GSH浓度更高, 至少是正常细胞的4倍^[105], 尤其在一些耐药肿瘤细胞中, GSH甚至要高出10倍^[106]. 因此, 使用GSH响应的高分子材料构建纳米载体可利用肿瘤细胞内外GSH浓度的显著差异来促进siRNA的胞内释放及基因沉默效率的提高. 常见GSH响应的高分子材料主要包括含二硫键^[104]、二硒键^[107]、琥珀酰亚胺-硫醚键的聚合物^[108], 其中使用最多的是含二硫键的高分子材料. 例如, 本团队^[109]选取脂肪二酸和胱氨酸二甲酯(结构中含有二硫键)作为单体, 制备了结构中含有多个二硫键

的疏水性高分子材料Cys-PDSA(图15A). 通过调控脂肪二酸的烷基链长度, 成功得到一种超快GSH响应的Cys-PDSA. 该高分子材料可与DSPE-PEG、烷基链修饰的聚乙二醇树枝状聚合物(G0-C14)共同自组装形成稳定的纳米载体, 用于MYC siRNA(siMYC)或Kinesin family member 11 siRNA(siKIF11)的体内递送和前列腺癌治疗^[110]. 如图15B所示, 该纳米载体的亲水性外层由PEG链组成, 疏水性内核由Cys-PDSA组成, 而siRNA则通过静电作用与G0-C14形成复合物并被包载在纳米载体的疏水性内核中. 将该纳米载体注射到荷瘤小鼠体内后, 其表面PEG链段可以显著延长siRNA的体内循环时间^[49]. 当纳米载体到达肿瘤部位并被肿瘤细胞内吞后, 胞浆内高浓度的GSH会导致Cys-PDSA结构中二硫键的快速断裂, 引起纳米载体的解散和siR-

图 12 MMP和酸双重响应的纳米载体用于PTX和siTwi的体内共传递和乳腺癌的联合治疗示意图^[82]

Figure 12 Schematic illustration of the MMP and acid dual-responsive nanocarriers for systemic co-delivery of PTX and siTwi for synergistic breast cancer therapy^[82]

图 13 反应活性氧响应纳米载体用于siVEGF体内递送。A: 聚阳离子高分子材料PDP结构式及其ROS响应机理; B: 使用该高分子材料构建的纳米载体用于siVEGF体内递送时, 利用其ROS响应性促进siRNA在胞浆中释放和基因沉默效率示意图^[94]

Figure 13 ROS-responsive nanocarriers for *in vivo* siVEGF delivery. A: Chemical structure of the polycationic polymer PDP and its ROS-responsive mechanism; B: the schematic illustration of the siVEGF-loaded nanocarriers made with this polymer to utilize the ROS-responsive characteristic to improve the intracellular siRNA release and gene silencing^[94]

NA的快速释放, 显著提高siRNA的基因沉默效率和抑制肿瘤生长的效果。

同样是利用二硫键的GSH响应性, Zou等人^[111]将其用于控制siRNA传递载体的松散度, 以达到提高siRNA基因沉默效率和抗肿瘤的作用。如图16所示, 该团队设计合成了新型两亲性高分子材料PEG-*b*-P(TMC-DTC)-PEI和cNGQ-PEG-*b*-P(TMC-DTC), 用于构建纳米载体实现siPLK1体内递送和肺癌治疗。在上述高分子材料结构中, PEI通过静电作用将siRNA包载在纳米载体中; 环状DTC基团相互之间能形成二硫键^[112], 通过二硫键的GSH响应性控制纳米载体的松散度; 而环状功能肽cNGQ则通过与肺癌细胞表面高表达的 $\alpha_3\beta_1$ 整合素相互作用, 提高纳米载体的肿瘤细胞靶向性和siRNA的细胞摄取量。将上述纳米载体注射到携有原位肺肿瘤的小鼠体内后, 载体表面的PEG链可以有效

图 14 反应活性氧响应纳米载体用于siVEGF和siPLK1体内共递送。A: ROS响应的两亲性高分子材料PEG-b-P(Gu/Hb)结构式; B~D: 构建的纳米载体用于siVEGF和siPLK1体内共递送时利用其ROS响应性促进siRNA在脑胶质瘤细胞内释放和协同抑制肿瘤生长示意图^[96]

Figure 14 ROS-responsive nanocarriers for *in vivo* co-delivery of siVEGF and siPLK1. A: Chemical structure of the ROS-responsive amphiphilic polymer PEG-b-P(Gu/Hb); B~D: the schematic illustration of the siVEGF- and siPLK1-loaded nanocarriers made with this polymer to utilize the ROS-responsive characteristic to improve the siRNA release in glioma cells and synergistic inhibition of tumor growth^[96]

避免血液对siRNA的清除作用, 显著延长其体内循环时间. 当纳米载体通过EPR效应富集到肿瘤部位后, 其表面修饰的cNGQ能识别肺癌细胞表面高表达的 $\alpha_3\beta_1$ 整合素^[113], 提高纳米载体的肿瘤细胞靶向性和siRNA

的细胞摄取量. 进入肿瘤细胞后, 胞浆内高浓度GSH会打断纳米载体结构中的二硫键, 导致纳米载体的溶胀和siRNA的快速释放, 显著提高siRNA的基因沉默效率和抑制肿瘤生长的效果.

图 15 谷胱甘肽响应纳米载体用于 siRNA 体内递送。A: GSH 响应的高分子材料 Cys-PDSA 结构式; B: 构建的纳米载体用于 siKIF11 或 siMYC 体内递送时利用其 GSH 响应性促进 siRNA 在肿瘤细胞内释放和抑制肿瘤生长的示意图^[110]

Figure 15 GSH-responsive nanocarriers for *in vivo* siRNA delivery. A: Chemical structure of the GSH-responsive polymer Cys-PDSA; B: the schematic illustration of the siKIF11- or siMYC-loaded nanocarriers made with this polymer to utilize the GSH-responsive characteristic to improve the intracellular siRNA release and inhibition of tumor growth^[110]

2.6 多重肿瘤微环境响应纳米载体用于 siRNA 体内递送

除了上文所述的单一肿瘤微环境响应纳米载体,不少国内外研究者还基于肿瘤组织/细胞不同的生理微环境,构建了多重肿瘤微环境响应型纳米载体用于提高 siRNA 的体内递送效率和肿瘤治疗效果。其中最为常见的是酸和还原双重响应纳米载体,这种载体可首先利用其酸响应性增强 siRNA 的内涵体逃逸能力,随后利用还原性提高 siRNA 在胞浆中释放效率^[114~117]。例如,最近 Gao 等人^[117]设计合成了两亲性高分子材料 mPEG-*b*-PLA-PHis-ssOEI 并用于构建酸和还原双重响应纳米载体。在该纳米载体结构中,聚组氨酸(PHis)链

段在内涵体酸性 pH 下会发生质子化,通过“质子海绵”效应增强 siRNA 的内涵体逃逸能力;而与 siRNA 相互作用的寡聚乙烯亚胺(OEI)则是通过二硫键与 PHis 链段相互连接。使用上述纳米载体包载 multidrug resistance (MDR) siRNA (siMDR) 和抗肿瘤药物阿霉素并注射到阿霉素耐药的荷瘤小鼠体内后,载体表面的 PEG 链可以有效避免血液对 siRNA 的清除作用,显著延长其体内循环时间。当纳米载体通过 EPR 效应富集到肿瘤部位并被肿瘤细胞摄取后,纳米载体利用其 PHis 链段的酸响应性增强 siRNA 的内涵体逃逸能力;进入胞浆后会利用二硫键的还原响应性,显著提高 siMDR 和阿霉素的释放效率,最终起到逆转阿霉素耐

图 16 GSH响应的高分子材料PEG-*b*-P(TMC-DTC)-PEI和cNGQ-PEG-*b*-P(TMC-DTC)结构式及其构建的纳米载体用于siRNA体内递送时利用其GSH响应性促进siRNA在肺癌细胞内释放和抑制肿瘤生长的示意图^[11]

Figure 16 Chemical structures of the GSH-responsive polymer PEG-*b*-P(TMC-DTC)-PEI and cNGQ-PEG-*b*-P(TMC-DTC), and the schematic illustration of the siRNA-loaded nanocarriers made with these two polymers to utilize the GSH-responsive characteristic to improve the intracellular siRNA release and inhibition of tumor growth^[11]

药和增强肿瘤治疗效果的作用。

3 总结与展望

肿瘤微环境响应纳米载体利用了肿瘤组织特殊的生理微环境,有效解决了siRNA体内递送时需克服的稳定性差、肿瘤富集量低、内涵体逃逸能力弱以及基因沉默效率低等困难,提高了siRNA的抗肿瘤效果。但是,肿瘤尤其是实体瘤的发生发展是一个非常复杂的过程,其内部成分也相当复杂,存在多重生理屏障。而目前报道的肿瘤微环境响应纳米载体还没有兼具长血液循环、高肿瘤富集量、肿瘤细胞靶向内吞、高效内涵体逃逸、快速siRNA释放等功能,因此很难完全克服siRNA体内递送时面临的多重生理屏障。

此外,肿瘤的发生发展伴随着多种基因突变,因此使用单一功能的siRNA很难完全抑制肿瘤的进展。所以除了考虑ROS、还原物质、酶等肿瘤微环境内在刺激因子,诸多外部刺激因子,如离子、磁场、超声、光等也需要引入到纳米载体设计中,用于构建多重刺激-响应型纳米载体。这些多重刺激响应型载体能够充分发挥不同环境响应性间的协同效应,对siRNA进行多级逐步递送,有望克服siRNA体内传递时遇到的多重生理屏障。但在构建和使用多重刺激-响应型纳米载体时,还要兼顾如下3个因素:(i)制备方法的优化和生物安全性。由于引入不同的刺激响应性,必然会增加载体材料的合成难度和引起潜在的毒副作用,最终将影响多重刺激-响应型纳米载体的转化应用。因此必须从材料学角度优化载体的制备方

法, 避免因复杂合成方法引起的毒副作用. (ii) 肿瘤治疗靶点的选择. 肿瘤具有高度异质性, 因此治疗靶点的选择上也需要结合测序技术和大规模临床样本进行验证. (iii) 肿瘤患者的个体差异. 纳米载体用于 siRNA 体内传递的最主要优势是可以利用实体瘤特有的 EPR 效应在肿瘤部位富集. 但肿瘤患者个体存在差异, 有些患者的 EPR 效应强, 但有些患者的 EPR 效应弱甚至几乎无 EPR 效应. 所以需要借助纳米造影剂(如磁性纳米材料)对患者的 EPR 效应进行评价, 筛选出 EPR 效应强的患者用于开展纳米载体用于肿瘤治

疗的临床转化研究. 因此, 对于未来 siRNA 体内递送的基础及转化研究, 首先需要考虑肿瘤的异质性并结合测序技术和大规模临床样本寻找出调控肿瘤恶性进展的关键靶点^[118,119]; 其次需要评价肿瘤患者的 EPR 效应, 通过联合使用纳米造影剂筛选出 EPR 效应较强的患者; 最后在保证生物安全性的基础上, 使用多重刺激-响应型纳米载体联合递送不同功能的 siRNA 或其他形式的治疗药物(如小分子抑制剂、mRNA、化疗药物、单抗等)^[120~122], 采取联合治疗的方式实现恶性肿瘤的有效治疗.

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Progress in the tumor environment (TME)-responsive nanocarriers for *in vivo* siRNA delivery

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Since the discovery of RNA interference (RNAi), RNAi technology has rapidly developed into an efficient tool for post-transcriptional gene silencing, which has been widely used for gene function analysis and clinical or preclinical treatment of various diseases (e.g., malignant tumor, Hepatitis B, obesity, and multiple neuropathy). Small interfering RNA (siRNA) is the effector molecule of RNAi technology. As a biological macromolecule with negative charges, naked siRNA has a short half-life *in vivo*, and is easily attacked by nucleases and filtered by glomeruli. Therefore, the clinical application of naked siRNA is significantly limited. The key challenge for clinical translation of RNAi technology is to construct safe and efficient delivery carriers to improve the siRNA stability *in vivo* and gene silencing efficacy in target cells. Currently, numerous nanocarriers such as liposomes have been employed for *in vivo* siRNA delivery, which have achieved favorable therapeutic outcomes in clinical or preclinical disease treatment. It has been demonstrated that solid tumors show specific vascular structures and physiological microenvironment (e.g., weakly acidic environment, hypoxia, over-expression of specific chemicals and enzymes). Based on this fact, researches have recently paid great attention to designing and constructing tumor microenvironment (TME)-responsive nanocarriers to improve the accumulation of siRNA in tumor sites and enhance gene silencing in tumor cells. In this review, we summarize the design principles and functional characteristics of recently developed TME-responsive siRNA delivery nanocarriers, analyze their advantages and disadvantages, and explore the development trend of TME-responsive siRNA delivery nanocarriers in the future.

RNAi technology, siRNA delivery, nanocarriers, TME-responsive

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