纳米医学策略在口服肿瘤疫苗中的应用: 从屏障工程到免疫激活

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摘要: 胃肠道具有最大的黏膜免疫系统, 肿瘤疫苗可借助口服给药来充分利用这一优势, 以实现更好的免疫应答。然而疫苗经口服后可能会面临多重生理学屏障, 阻碍疫苗作用的充分发挥, 借助纳米载体可克服这些屏障。本文介绍了当前口服疫苗所面临的生理学屏障及纳米技术为突破这些屏障所做出的努力; 同时, 介绍了纳米载体递送口服肿瘤疫苗的相关研究进展和临床转化现状, 旨在为口服纳米疫苗治疗肿瘤相关疾病提供研究思路。

关键词: 口服; 疫苗; 黏膜免疫; 纳米载体; 癌症

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Application of nanomedicine strategies in oral mucosal tumor vaccines: From barrier engineering to immune activation

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Abstract: The gastrointestinal tract harbors the largest mucosal immune system, and tumor vaccines can leverage this advantage through oral administration to achieve enhanced immune responses. However, vaccines administered orally may encounter multiple physiological barriers that hinder their full efficacy. Nanocarriers can overcome these barriers. This paper discusses the physiological barriers currently faced by oral vaccines and the efforts made by nanotechnology to overcome these barriers. It also reviews the latest research progress and clinical translation status of nanoparticle-based delivery systems for oral tumor vaccines, aiming to provide research insights for the development of oral nanovaccines for the treatment of tumor-related diseases.

Key words: oral administration; vaccine; mucosal immunity; nanocarrier; cancer

肿瘤免疫治疗可通过唤醒机体的免疫应答以实现肿瘤控制, 其中肿瘤疫苗作为主动免疫策略, 因能诱导特异性、长效免疫[1]而成为研究热点。与传统注射疫苗相比, 口服治疗性肿瘤疫苗具有无创、便捷的突出优势, 可极大提高患者依从性, 尤其适用于需长期接种疫苗的肿瘤患者。肠道作为人体最大的黏膜免疫器官, 为免疫应答的启动提供了理想的环境, 口服疫苗可通过黏膜免疫途径同时激活局部黏膜免疫与全身系统免疫[2], 继而更高效地抑制肿瘤。然而, 疫苗经口服后会面临多重屏障: 包括胃肠道中的酸性环境、酶解作用及肠上皮屏障等, 这些屏障不仅导致大量抗原降解, 还严重阻碍其与肠道免疫细胞的相互作用。

纳米医学的兴起为突破上述瓶颈提供了解决方案。首先, 纳米载体可以保护疫苗免受降解或过早释放[3], 通过设计 pH 敏感、酶响应型纳米材料, 能在胃肠道恶劣环境中保护抗原的完整性; 借助黏膜黏附材料的表面修饰, 可调控载体在黏液层的滞留与渗透, 高效穿越肠上皮屏障; 其次, 纳米载体还可改善疫苗的免疫应答[3]。纳米颗粒和病原体的大小相似, 可被免疫系统有效识别, 从而触发细胞和体液免疫反应[4]。纳米颗粒还可通过淋巴转运/组织分布机制将疫苗特异性

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地输送到淋巴结, 促进其在肿瘤组织中的分布, 激发特异性免疫反应[5]。纳米平台不仅可以同时递送抗原与免疫佐剂[6], 某些纳米粒本身还可充当免疫佐剂来增强免疫反应[7]。本文综述了近年来纳米技术在口服递送肿瘤疫苗的研究进展, 旨在为口服肿瘤疫苗的发展及临床研究提供理论依据 (图 1)。

1 口服黏膜免疫的优势与障碍

1.1 口服免疫机制 口服肿瘤疫苗作为一种无创、高依从性的免疫干预手段, 其可借助黏膜免疫系统实现抗原的高效递送、呈递及免疫激活, 从而实现抗肿瘤免疫应答。黏膜免疫系统是人体免疫系统的重要组成部分, 广泛分布于胃肠道、呼吸道、泌尿生殖道及眼腔等部位, 覆盖人体 30～40 m^2 的表面积, 肠道因具有最大的黏膜表面积成为口服肿瘤疫苗发挥作用的核心靶标[8]。

黏膜免疫系统的层级结构为口服肿瘤疫苗的抗原处理与免疫激活提供了天然平台, 其从外到内依次为: 覆盖上皮的黏液层、上皮层、固有层和肠道相关淋巴组织 (gut-associated lymphoid tissue, GALT)[1]。黏液层作为生物体生理屏障的第一道防线, 不仅能物理阻隔病原体侵袭, 还能通过黏蛋白等成分捕获抗原并参与免疫信号传递[9], 在启动黏膜免疫应答中发挥关键作用。上皮层作为肠黏膜免疫系统的第二道物理屏障, 其特化细胞群直接参与肿瘤疫苗的识别与转运: 肠上皮细胞维持屏障完整性, 杯状细胞分泌黏蛋白以维持黏液层结构, 潘氏细胞释放抗菌因子营造免疫微环境, 微折叠细胞 (M 细胞) 则可直接摄取抗原并转运至下方的树突状细胞 (dendritic cells, DC)、T 细胞等免疫细胞, 为启动免疫应答传递信号[10]。固有层中的淋巴管则承担“运输通道”功能, 将可溶性抗原和迁移性抗原呈递细胞 (antigen-presenting cells, APC) 转运至引流淋巴结, 推动适应性免疫启动。肠道相关淋巴组织 (包括派尔集合淋巴结、阑尾和孤立淋巴滤泡) 则是口服疫苗抗原刺激初始 T 细胞和 B 细胞的主要场所。其中 DC 是 GALT 中最常见且重要的 APC, 它们位于滤泡相关上皮 (follicular-associated epithelium, FAE) 下方的上皮下穹窿区域, 可直接从 M 细胞摄取抗原, 启动后续免疫级联反应[11]。

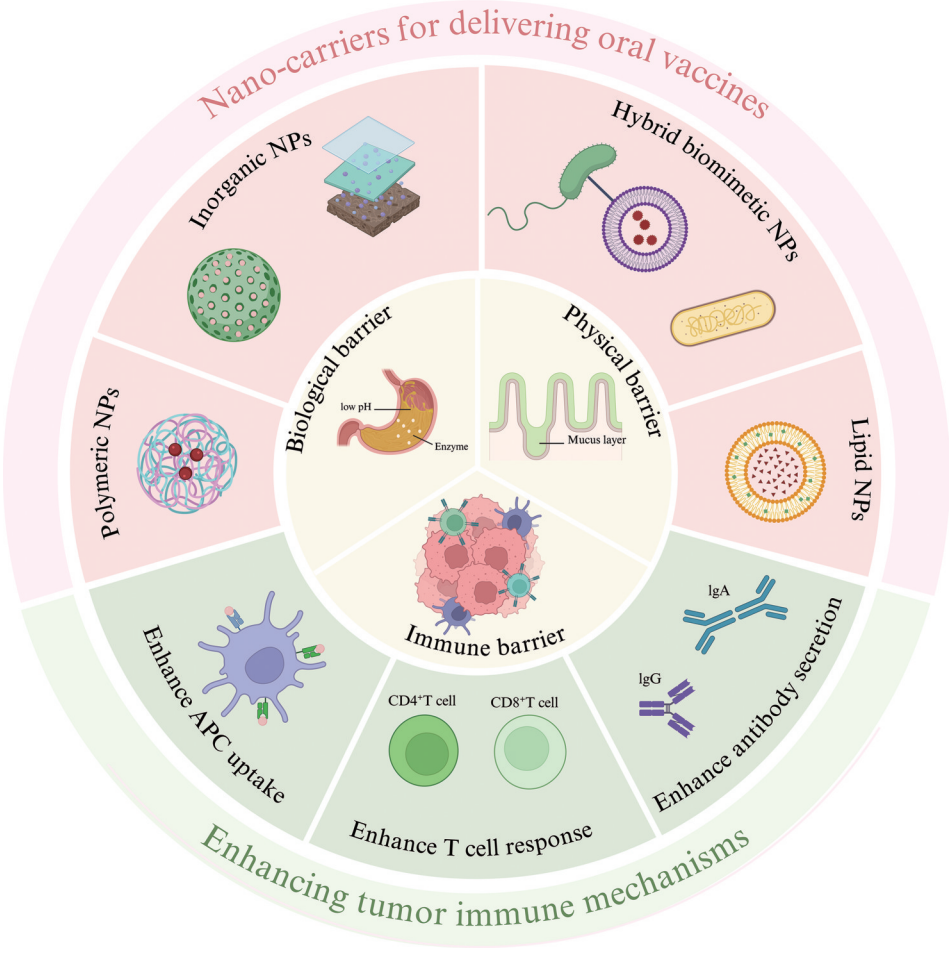


Figure 1 Oral tumor vaccine delivery systems and immunological targets for tumor therapy (created with BioRender.com). NPs: Nanoparticles; APC: Antigen-presenting cells

口服肿瘤疫苗进入胃肠道后, 其抗原成分在小肠内被M细胞摄取并转运至APC。随后, DC摄取并加工这些抗原, 将抗原呈递给初始T细胞并激活其分化为辅助性T细胞, 活化的辅助性T细胞通过细胞因子和共刺激信号辅助B细胞活化, 促使B细胞发生类别转换, 在肠道固有层分化为浆细胞, 分泌IgA, IgA可通过与肿瘤细胞的特异性结合, 介导抗体依赖性细胞介导的吞噬作用, 促进中性粒细胞吞噬清除肿瘤细胞[12]。此外, IgA在黏膜组织中经过加工修饰可形成sIgA, sIgA通过黏膜上皮释放至肠腔, 形成局部免疫屏障[11]。另有部分活化的B细胞会随循环迁移至骨髓或外周淋巴器官, 进一步分化为分泌IgG的浆细胞, IgG抗体通过血液循环到达全身, 通过抗体依赖性细胞介导的细胞毒性靶向杀伤远处肿瘤细胞[13]。同时, $CD8^+$ T细胞在细胞因子的作用下分化出细胞毒性T细胞 (cytotoxic T cells, CTLs), CTLs会精确地在肿瘤组织中积累并启动肿瘤细胞的特异性杀伤[14](图2)。

1.2 口服肿瘤疫苗的优势 口服肿瘤疫苗相较传统注射肿瘤疫苗具有显著优势。首先, 其为非侵入性接种方式, 无需专业医护人员操作即可自行服用, 规避了注射带来的疼痛与创伤, 降低了因针头重复使用引发交叉感染的风险[15], 显著提升了患者依从性, 并减少了大规模免疫接种的人力与时间成本。并且口服疫苗能模拟病原体自然感染路径, 直接激活肠道等黏膜免疫系统, 诱导分泌型IgA在黏膜表面形成第一道免疫屏障, 同时促使记忆T细胞在黏膜组织长期驻留[16], 持续发挥局部微环境的免疫监视作用, 利于早期清除入侵病原体或肿瘤复发、隐匿转移灶。此外, 口服肿瘤疫苗储存和运输要求较低, 无需像注射疫苗一样严格依赖低温条件, 所需经济成本更低[2]。

1.3 口服肿瘤疫苗的递送屏障 口服肿瘤疫苗的递送面临多重胃肠道屏障, 严重制约其活性与吸收效率。首先是复杂的pH环境屏障, 胃肠道pH值在1.0～8.0间大幅波动, 胃内酸性极强 (pH 1.0～3.0)[17], 易导致蛋白质抗原变性、核酸类疫苗 (DNA、RNA) 脱嘌呤或降解[18], 而肠道不同区段pH差异也会影响疫苗稳定性与

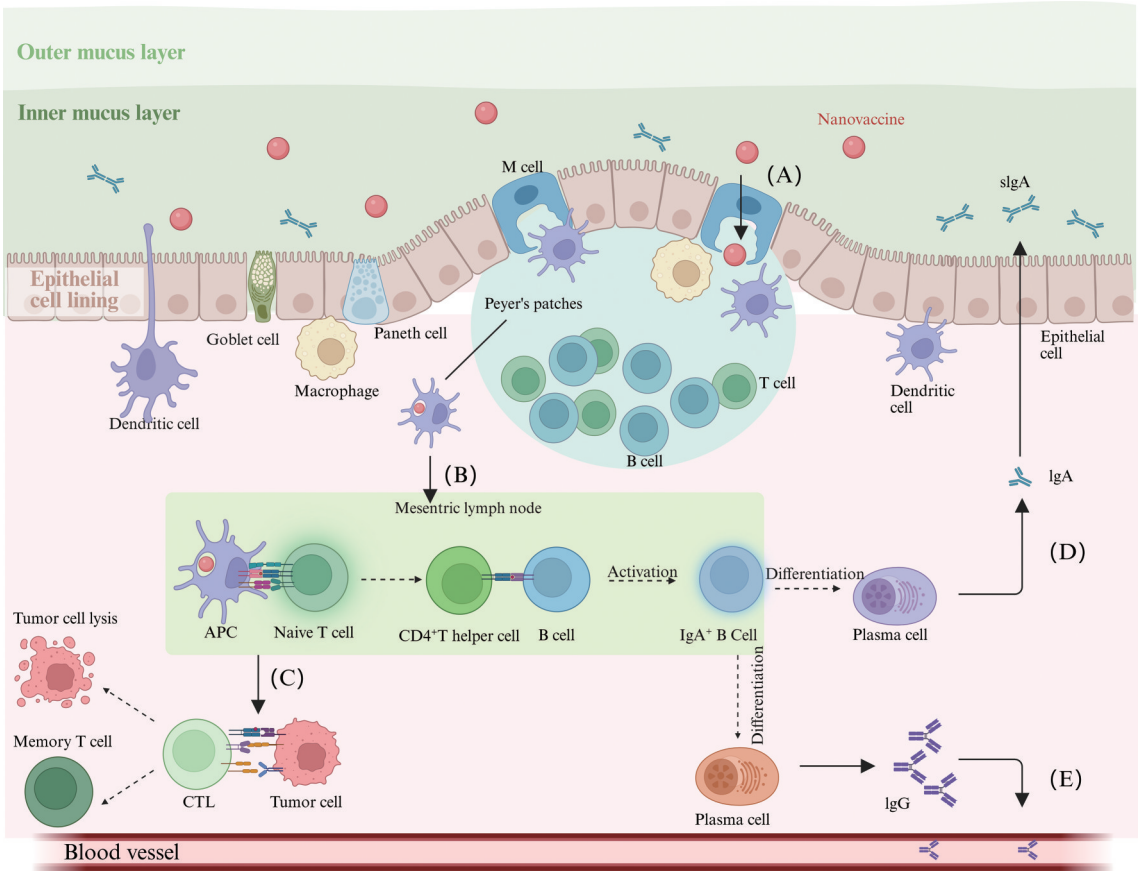


Figure 2 Intestinal immune system and nanovaccine anti-tumor mechanisms. A: Antigens are transported to DCs *via* the M cell pathway and taken up by DCs; B: Antigen-bearing DCs migrate to mesenteric lymph nodes (MLNs) to activate B cells and T cells; C: Naive T cells differentiate into cytotoxic T cells (CTLs) to kill tumors; D: Activated B cells differentiate into plasma cells that secrete IgA and release it into the intestinal lumen to form a local immune barrier; E: Activated B cells differentiate into plasma cells that secrete IgG and release it into the bloodstream (created with BioRender.com). DCs: Dendritic cells

溶解性。其次是酶解屏障, 胃内胃蛋白酶在酸性条件下活性增强, 可通过水解肽键将蛋白质降解成更小的肽片段[19]; 十二指肠中胰蛋白酶、羧肽酶和胰凝乳蛋白酶等协同作用, 能降解约20%的摄入蛋白质[20]; 此外, 核酸酶、脂肪酶等还会消化核酸、脂质等生物分子[21], 导致疫苗活性丧失。胃肠道的渗透应力、肌肉蠕动及管腔内液体流动产生的剪切应力, 也可能通过机械降解降低疫苗生物活力[17]。再者是黏液与吸收屏障, 胃肠道黏液层因黏蛋白的负电荷与疏水结构[22], 易通过静电或疏水作用捕获颗粒, 阻碍疫苗向上皮运输; 而肠上皮紧密连接限制细胞旁途径吸收, 跨细胞途径又面临细胞膜极性阻碍及胞内酶降解风险, 进一步降低疫苗吸收效率[17]。此外, 胃肠道微生物群还可通过影响药物代谢、导致药物失活及改变代谢酶表达等方式, 对治疗性蛋白质和肽类疫苗产生不利影响[14]。

2 纳米医学应对口服疫苗挑战的策略

上述胃肠道屏障不仅限制了抗原的肠道吸收与有效利用, 更阻碍了口服疫苗激活机体免疫应答。为解决这一瓶颈, 纳米医学凭借其可设计的载体特性, 为克服这些递送障碍及高效启动免疫应答提供了解决方案 (图3)。下文将系统阐述纳米技术应对这些挑战的具体策略。

2.1 抗胃肠道屏障策略 针对疫苗在胃肠道中易被降解的问题, 可采用耐酸生物材料封装与涂层技术来提升疫苗在胃肠道 (gastrointestinal tract, GIT) 中的稳定性, 例如可应用pH敏感型材料来封装疫苗, 如藻酸盐、丙烯酸、明胶等。这类聚合物含有酯键、羧基等pH敏感基团, 可在中性或弱碱性条件下发生结构解离, 将负载的疫苗递送至肠道, 避免其在胃部被酸降解[23]。Liu等[24]用海藻酸盐包被DNA疫苗, 其使得DNA疫苗可以在pH 1.5条件下免受降解, 而且还可以积累在Peyer斑 (Peyer patches, PPs) 中并使得CTL介导的免疫反应增强。Eudragit L100是一种阴离子聚合物, 广泛用于胃肠道递送系统[23], 经Eudragit L100包被的甘露糖基化壳聚糖纳米颗粒 (Eud L100-MCS NPs), 在胃肠道液中表现出优异的抗酸降解能力和酶抵抗力, 并且负载牛血清白蛋白的Eud L100-MCS NPs可引起强烈的黏膜IgA反应及全身性IgG抗体应答[25]。此外, 某些坚固材料, 如金属有机框架 (metal-organic frameworks, MOF) 也被应用于疫苗保护: 研究表明, Al-MOF可通过金属离子与肽骨架的相互作用在蛋白质表面形成矿化层, 从而保护卵清蛋白抗原 (ovalbumin, OVA) 免受胃消化液的降解[26]。

2.2 黏液渗透策略 黏液是一种黏弹性凝胶, 由黏蛋白、抗体、细胞碎片、无机盐和水组成, 其中黏蛋白作为核心大分子成分, 直接决定黏液的黏弹特性[27]。黏蛋白纤维含有许多带高负电荷的糖基化片段, 通过静电相互作用易捕获带正电荷的颗粒。同时, 黏蛋白纤维中存在丰富的疏水链段, 因此, 亲水性聚合物可避免被黏液包裹从而实现更好的黏液渗透[27]。聚乙二醇 (polyethylene glycol, PEG) 因具有良好的亲水性被广泛用作黏液惰性聚合物, 疫苗可通过涂覆亲水颗粒或修饰PEG衍生物, 以渗透黏液到达肠上皮处。细胞穿透肽 (cell-penetrating peptide, CPP) 与PEG衍生物 (PEG-Suc) 结合后, 既能实现快速穿透黏液, 又能促进抗原被有效吸收[28]。此外, 其他化合物也可促进黏液渗透, 如两亲性化合物Pluronic包被的颗粒可在黏液层中迅速扩散[29], 此外, 其亲水单元和疏水单元的相对长度也会影响黏液渗透性, 疏水基团与亲水基团的比例不应太高或太低, 否则会影响与黏蛋白的疏水相互作用[30]。

2.3 黏膜黏附策略 黏膜黏附系统能够延长药物在肠道中的停留时间, 从而改善黏膜部位对药物的摄取。其中, 具有高分子质量、高电荷和具有氢键/疏水相互作用官能团等特性的聚合物能够实现良好的黏膜黏附作用, 如纤维素衍生物、透明质酸、藻酸盐和壳聚糖

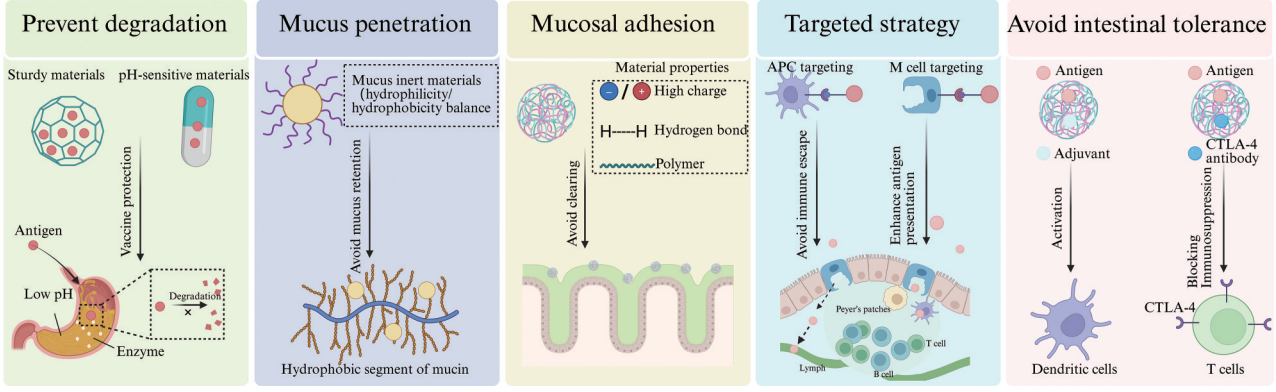


Figure 3 Oral delivery barriers for vaccines and material design strategy (created with BioRender.com)

(chitosan, CS) 等[1]。CS是口服免疫中最常用的黏附材料, 因为它具有高正电荷、破坏上皮紧密连接的能力[31]和佐剂效应等特性[32]。CS制备的口服DNA疫苗, 对疫苗有良好的保护作用, 并可以诱导Th1型免疫反应[33]。然而, CS在酸性环境中可被降解, 因此可通过修饰来增强胃肠道稳定性。例如, 海藻酸钠修饰的CS NPs可以防止抗原在pH 1.2的介质中突释, 并在pH 6.8和7.4的中性环境保持颗粒稳定性。此外, 该纳米粒还可被PPs内化, 继而使得抗原被呈递给APC[34]。硫醇基团则可与黏蛋白形成二硫键, 特异性地附着在黏膜表面。研究表明, 硫醇化颗粒相较于未硫醇化颗粒的黏附性增加了2倍, 并且可以导致强烈的sIgA及全身性IgG抗体反应[35]。

2.4 靶向策略 M细胞位于肠道相关淋巴组织表面的滤泡相关上皮 (FAE) 中, 其顶端缺乏密集的微绒毛、细胞间连接较疏松, 其可有效地摄取抗原并转运到下方的淋巴滤泡[36], 诱导免疫反应, 因此是设计口服疫苗的理想靶标。M细胞可通过识别颗粒表面的特异性配体, 高效识别并摄取颗粒。M细胞表面表达独特的碳水化合物受体, 可与多种配体结合, 主要包括凝集素、肽配体 (如RGD等) 及葡聚糖等。凝集素可特异性结合M细胞表面蛋白质或脂质的碳水化合物残基[37]。研究表明, 与非靶向颗粒相比, 用凝集素修饰的颗粒进行口服免疫会导致sIgA显著升高, 并且还可增强免疫能力, 例如, Th1细胞因子IL-2和IFN-γ的显著增加[38]。凝集素家族成员众多, 如荆豆凝集素I (ulex europaeus agglutinin I, UEA I)、小麦胚芽凝集素 (wheat germ agglutinin, WGA)、番茄凝集素[39]等, 在选择配体时需注意凝集素的选择, 因部分凝集素有毒, 可能具有一定的免疫原性, 故可能引发针对靶向分子的免疫反应, 最终阻碍抗原的摄取[2]。肽配体RGD可识别在M细胞上过表达的*α5β1*整联蛋白, 但在GIT中容易被降解, 故可使用更为稳定的肽类似物, 有研究制备了一种精氨酸–甘氨酸–天冬氨酸拟肽 (RGD peptidomimetic, RGDp), 并将其接枝于聚乳酸–羟基乙酸共聚物纳米粒上, 结果证明, RGDp和RGD具有相同的M细胞亲和能力[40]。酵母细胞壁中含有的β-1,3-*D*-葡聚糖, 可被M细胞及其黏膜下巨噬细胞上表达的dectin-1受体识别, 使用该物质制备的纳米颗粒实现了对FAE表面上的M细胞和亚FAE口袋中的巨噬细胞的双重靶向, 并导致药物在Peyer斑中大量积累和运输[41]。

抗原被M细胞摄取后, 会被转运至其基底侧的细胞口袋中, APC正聚集于此, 准备捕获并处理抗原, 然而部分抗原颗粒可能通过规避APC的识别与摄取, 从而不引发免疫应答[42], 故可通过靶向APC细胞来减少免疫逃逸。巨噬细胞作为专业的APC, 用于将颗粒抗原呈递给未成熟的B细胞或T细胞, 通过利用巨噬细胞的抗原呈递功能, 药物载体可以用专门靶向巨噬细胞表面受体的配体进行修饰。巨噬细胞表达甘露糖和透明质酸等受体[43]。Xu等[44]开发了用甘露糖修饰的纳米颗粒, 口服该纳米颗粒后实现了良好的巨噬细胞靶向, 并诱发了强大的细胞免疫反应。DC是肠黏膜中最常见和最重要的APC, 对于驱动免疫反应至关重要。DC表面表达多种受体, 包括Toll样受体 (Toll like receptors, TLR)、C型凝集素受体、FcRn、整合素等, 这些受体与外来分子的识别和运输及免疫反应的激活密切相关[45]。例如, 通过甘露糖受体介导的内吞作用可增强DC细胞的抗原内化, 并将抗原呈递给$CD4^+$和$CD8^+$ T细胞, 实现了优异的免疫应答[46]。

2.5 避免肠道耐受策略 肠道黏膜持续暴露于来自食物蛋白质和微生物群的外源性抗原中, 会对某些抗原 (如食物蛋白和共生菌抗原等) 产生耐受反应[47], 表现为调节性免疫细胞的诱导及免疫抑制信号的激活, 无法引起抗肿瘤免疫应答。纳米技术可以从多维度解决肿瘤抗原耐受的问题: 一方面, 纳米载体可实现抗原与佐剂的共递送, 佐剂不仅能打破肿瘤抗原相关耐受, 还可引导免疫反应向Th1型发展。多种TLR配体作为佐剂与抗原共载于纳米载体时, 可通过激活先天免疫信号通路, 刺激相应黏膜DC, 进而诱导适应性免疫应答[48]。其中, CpG佐剂的存在明显促进了DC激活并增加了T细胞增殖[49]。另一方面, 可通过纳米载体调节肠道代谢微环境, 削弱短链脂肪酸 (short-chain fatty acids, SCFA)[50]对Treg的诱导作用[51]。此外, 细胞毒性T淋巴细胞相关蛋白4 (CTLA-4) 通路被认为在免疫应答早期 (尤其在淋巴结内) 抑制T细胞增殖, 因此, 可将肿瘤抗原与CTLA-4抗体偶联, 以打破免疫抑制。

3 纳米平台驱动免疫激活

尽管纳米平台可以通过黏液渗透策略及靶向策略等方式来克服口服疫苗在复杂胃肠道环境中所面临的递送挑战, 然而这仅仅是疫苗成功的第一步, 抗原的“抵达”并不等同于免疫反应的“激活”。疫苗效力的最终实现, 关键在于能否高效地激活宿主体内一系列复杂的免疫级联反应。因此, 本部分将聚焦纳米平台在免疫激活中的作用, 系统阐述其如何通过调控抗原呈递细胞功能、激活先天免疫等方式, 以实现口服疫苗的免疫保护效能 (图4)。

3.1 增强抗原呈递 DC能够激活初始T细胞, 启动适应性免疫反应, 其在连接先天免疫和获得性免疫反应方面发挥重要作用。DC在一系列信号分子的作用下发育和成熟, 并刺激T淋巴细胞和B淋巴细胞启动

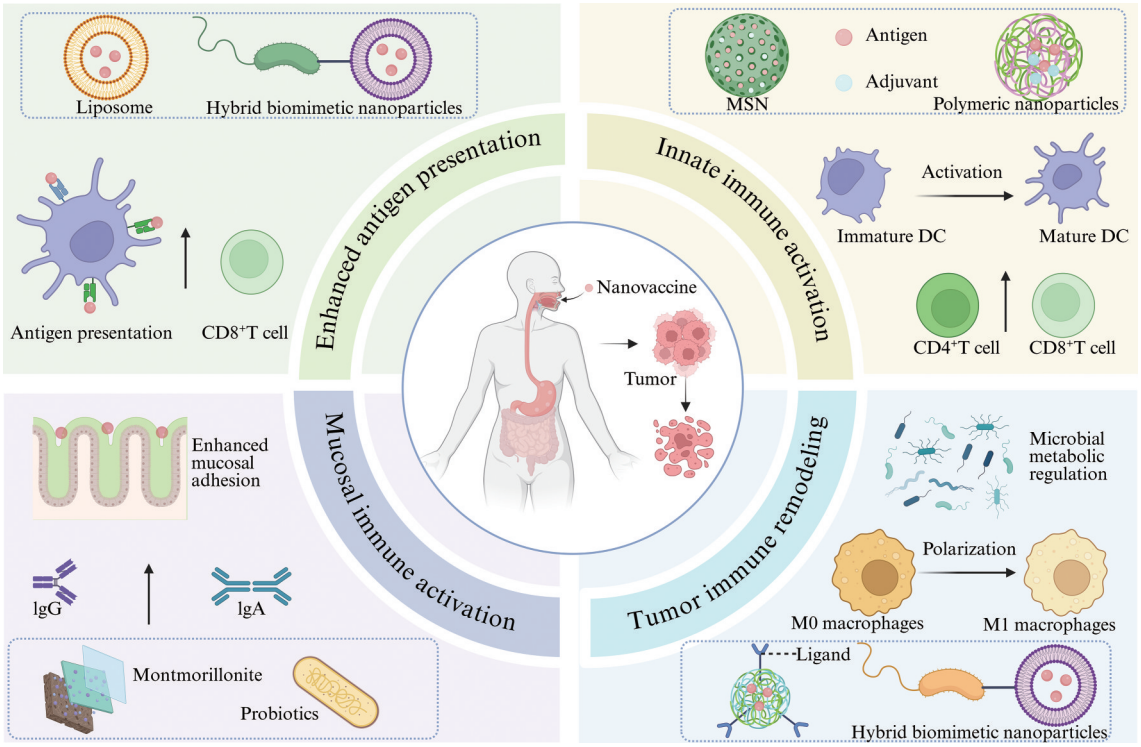


Figure 4 Schematic diagram of the oral vaccine nanodelivery systems and their enhancement of antitumor immunity (created with BioRender.com)

免疫反应[52]。阳离子脂质体可将抗原高效递送至PPs, 促进APC对其摄取, 进而激活局部及远端淋巴器官的免疫应答[53]。Naciute等[54]开发了一种阳离子脂质体用于口服递送结肠癌免疫显性肽, 以刺激局部及全身免疫反应实现肿瘤治疗。结果显示, 该脂质肽疫苗可以促进DC细胞标志物CD11c表达, 表明了MLN中先天免疫反应的激活, 还刺激淋巴结中淋巴细胞 ($CD4^+$ T细胞、$CD8^+$ T细胞及B细胞) 的积累, 通过诱导抗肿瘤免疫反应发挥了结直肠癌治疗的作用。此外, 纳米乳液也可以促进APC呈递抗原并被动靶向淋巴系统[55]。Long等[39]用纳米乳包载抗原, 并用番茄凝集素进行修饰, 该纳米乳不仅可以保护抗原缓慢释放, 有效促进抗原摄取和呈递, 还可增强CTL活性, 提高抗肿瘤免疫作用。

细菌含有许多被称为微生物病原相关分子模式的细胞壁结构, 这些结构可被APC识别[56], 以刺激免疫反应。Naciute等[57]将含有疫苗成分的阳离子脂质体与大肠杆菌偶联, 形成脂质体−细菌生物杂交体 (Bac L-Vac)。Bac L-Vac不仅毒性较低, 还能有效激活小鼠骨髓来源的树突状细胞; 同时可增加肿瘤组织中 $CD8^+$ T细胞的浸润, 从而显著抑制小鼠模型中原位肿瘤的生长。

3.2 先天免疫激活 先天免疫激活是口服肿瘤疫苗诱导有效免疫应答的关键环节, 其有效激活可显著提升抗肿瘤疗效。据报道, 酵母来源的β-葡聚糖 (β-glucan, βGlus) 可以被DCs和巨噬细胞等特异性识别[58], 从而激活T细胞以增强抗肿瘤功效[59], 还可作为佐剂增强免疫应答[60]。Mao等[61]选用了具有高比表面积、大开孔体积及易于表面改性特点的介孔二氧化硅纳米颗粒 (mesoporous silica nanoparticles, MSN) 作为核心, 包载卵清蛋白 (OVA) 和乐伐替尼 (lenvatinib, LEN), 并用葡聚糖进行涂层 (即Len/OVA/yMSN)。Len/OVA/yMSN经口服后, 不仅可以将抗原有效递送至淋巴组织内的APC, 还可通过激活TLR2/4信号通路, 增强疫苗诱导的免疫应答, 逆转肿瘤微环境 (tumor microenvironment, TME) 中巨噬细胞介导的免疫抑制状态, 安全地激活先天免疫反应。

免疫系统的激活是设计抗原/药物递送系统以开发新疗法和研究疾病发病机制所必需的, 仅靠抗原不足以激活免疫系统。有时, 需要负载佐剂来有效激活免疫系统[62]。纳米颗粒可以封装佐剂, 确保抗原和佐剂的共同递送。Luo等[63]构建了一种用于负载βGlus与mRNA复合物的脂质纳米粒 (lipid nanoparticle, LNP) —— βGlus/mRNA@LNPs, LNP充当递送载体, 封闭和保护βGlus/mRNA复合物, 促进其稳定递送至DC及肿瘤细胞。βGlus作为佐剂和保护剂, 与缺乏

βGlus (mOVA@LNPs) 的口服对照组相比, 将βGlus掺入LNP中可以有效地激活未成熟的DC并启动它们向MLN的迁移。Ma等[64]则通过调节MSN的孔径和疏水性来实现抗原卵清蛋白 (OVA) 和佐剂R848的共同负载, 并用酵母胶囊包被, 该纳米粒通过酵母胶囊及佐剂共同实现Dectin-1、TLR2、TLR7、TLR8等多途径完全激活DC, 有效增加$CD8^+$和$CD4^+$细胞的比例, 实现了80%早期肿瘤的治愈及有效抑制了肺转移。Kim等[65]构建了一种用硫酸软骨素-g-甘露糖与硫酸软骨素-g-糖胆酸包被的阳离子脂质体, 以共负载OVA和佐剂聚肌苷–聚胞苷酸 (即OPGMN)。OPGMN经口服后, 可直接进入淋巴结, 诱导淋巴结中的DC成熟增加, 并诱导Th1型和Th2型辅助T细胞免疫应答, 从而增加血液中的白细胞介素2 (IL-2) 和干扰素-γ (IFN-γ) 水平。并通过增加血液中中央记忆T细胞、细胞毒性T细胞和自然杀伤细胞的数量及减少调节性T细胞 (Tregs) 的数量来实现免疫激活, 从而完全抑制B16F10黑色素瘤。

3.3 黏膜局部免疫应答激活 传统通过皮下或肌肉注射给药的肿瘤疫苗往往无法有效刺激针对黏膜的强效免疫反应, 而口服肿瘤疫苗可利用肠道广阔的黏膜表面实现免疫激活, 并且纳米技术可以帮助黏膜疫苗诱发强大的免疫反应。蒙脱石是一种由含水硅酸铝构成的二维层状矿物, 广泛用于治疗胃肠道疾病。研究表明, 基于蒙脱石的纳米载体具有作为疫苗抗原递送载体的潜力[66]。由于其天然结构特性, 蒙脱石能耐受胃肠道的恶劣环境, 还可通过与黏蛋白的相互作用黏附于小肠黏膜。此外, 蒙脱石晶体层内含有丰富的可交换阳离子, 这使其能够作为高效的抗原储存库, 通过离子交换反应将蛋白质分子嵌入层间[67]。Yang等[68]开发了一种基于锰离子掺杂蒙脱石纳米片 (Mn-MMT@OVA) 的口服肿瘤疫苗。锰离子和肿瘤抗原通过离子交换掺入纳米片层, 口服后, 疫苗能够稳定抵达小肠, 通过黏附于黏膜, 并将其集中在较小的表面积上, 以实现OVA抗原与锰离子的持续释放, 锰离子可激活DC内的cGAS-STING通路, 导致DC成熟增强并迁移到淋巴结, 将肿瘤抗原呈递给T淋巴细胞, 从而有效地激活全身和黏膜免疫反应, 最终实现对结直肠癌的特异性清除。抗原特异性IgA的显著升高强调了疫苗能够引发强大的黏膜免疫响应, IgG水平的同时升高进一步凸显了系统性的免疫激活, 这表明疫苗不仅能够启动局部保护, 还可进一步增强全身性的免疫防御能力。

双歧杆菌可以刺激宿主的黏膜免疫系统和全身免疫反应, 可在预防感染、调节脂质代谢和减轻过敏症状方面发挥作用[69]。Nakagawa等[70]开发了一种由双歧杆菌组成的口服蛋白疫苗平台, 其不仅可以黏附在肠上皮细胞上以诱导小鼠PPs中DC的激活, 还增强了$CD4^+$和$CD8^+$ T细胞强烈的瘤内浸润, 显著提高了抗肿瘤活性。

3.4 肿瘤免疫重塑 肿瘤微环境 (TME) 常因肿瘤细胞、免疫抑制细胞及免疫抑制相关细胞因子的共同作用, 形成免疫抑制状态, 纳米颗粒凭借易于修饰性及多功能性, 可重塑免疫抑制性TME和提高抗癌免疫治疗的效率[71]。肿瘤相关巨噬细胞 (tumor-associated macrophages, TAM) 通常会优先表达为M2表型巨噬细胞, 而M2巨噬细胞的高表达通常会对抗肿瘤免疫疗法产生抵抗力, 利用纳米药物诱导TAM向具有促炎、抗肿瘤活性的M1型巨噬细胞极化, 可有效逆转TME中的免疫抑制状态[72]。Xu等[44]设计了甘露糖和糖胆酸修饰的三甲基壳聚糖纳米颗粒, 可用于SIRPα siRNA (siSIRPα) 和MUC1 pDNA (pMUC1) 的共递送。siSIRPα是一种巨噬细胞检查点抑制剂, 其不仅增强了pMUC1疫苗诱导的全身细胞免疫, 而且可以促进TAM的M1表型极化, 逆转了免疫抑制TME, 从而抑制了三阴性乳腺癌的生长和转移。

程序性细胞死亡受体1 (PD-1) 或细胞毒性T淋巴细胞相关蛋白4 (CTLA-4) 抗体等多种免疫检查点抑制剂, 在临床上治疗癌症中已显示出令人满意的结果, 但仍存在反应率低、患者耐药、非特异性毒性等诸多问题[73]。双歧杆菌癌症疫苗可通过调节肿瘤微生物组, 诱导$CD8^+$ T细胞产生分泌干扰素γ (IFN-γ), 以增强免疫检查点抑制剂抗PD-1抗体和抗CTLA-4抗体的抗肿瘤活性[74]。Zu等[75]则开发了一种负载声敏剂Ce6和免疫佐剂R837的核壳纳米药物, 通过声动力学疗法治疗结直肠癌。结果表明, 该纳米药物不仅可以诱导肿瘤细胞的免疫原性细胞死亡 (immunogenic cell death, ICD)、巨噬细胞M1型极化、促炎细胞因子TNF-α的分泌, 还可联合免疫检查点阻断剂PD-L1抗体 (αPD-L1) 发挥强大的系统性抗肿瘤免疫。

益生菌可以改变肠道微生物群并调节免疫反应, 在结直肠癌中有着良好的应用前景[76]。Xu等[77]构建了免疫佐剂 (CpG) 和碳点 (carbon dot, CD) 共负载的植物脂质纳米颗粒, 并与乳酸菌 (Lactobacillus reuteri, LR) 偶联 (LR-S-CD/CpG@LNPs), 该纳米药物实现了转移性结直肠癌的光免疫治疗和微生物代谢物调节。LR-S-CD/CpG@LNPs经口服后可在结直肠区域富集, 经近红外照射后, 肿瘤细胞发生免疫原性细胞死亡 (ICD), 并伴随释放肿瘤相关抗原 (tumor-associated antigen, TAA), TAAs和CpG形成原位疫苗, 促进DCs

的募集和成熟, 同时, LR催化一种增强抗肿瘤免疫反应代谢物I3A的产生, 进一步增强了抗肿瘤免疫效果, 有效抑制了原位结直肠肿瘤和转移性肿瘤的生长。

最后, 总结了各种纳米递药系统的特性及促进疫苗发挥免疫作用的机制, 见表1[24-26,39,44,53,54,57,61,63,65,68,70,75,77-80], 旨在为口服纳米疫苗的开发提供新途径新方法。

Table 1 Summary of the oral vaccine delivery system: Types, characteristics and mechanisms of immunity. PPs: Peyer patches; CTLs: Cytotoxic T cells; BSA: Bovine serum albumin; PLA: Polylactic acid; PLGA: Poly (lactic-co-glycolic acid); LN: Lymph nodes; OVA: Ovalbumin; PEG: Polyethylene glycol; ICD: Immunogenic cell death; GIT: Gastrointestinal tract; TME: Tumor microenvironment; ROS: Reactive oxygen species; DPPC: Dipalmitoyl-phosphatidylcholine; DPPE: 1,2-Bis (diphenylphosphino) ethane; DSPC: 1,2-Distearoyl-sn-glycero-3-phosphocholine; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine; DMG-PEG: 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol; SM-102: Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate; DOTAP: 1,2-Dioleoyl-3-trimethylammonium-propane; DOPC: 1, 2-Dioleoyl-*sn*-glycero-3-phosphocholine; APCs: Antigen-presenting cells; cGAS-STING: Cyclic GMP-AMP synthase-stimulator of interferon genes; CpG: Cytosine-phosphate-guanine; IL-10: Interleukin-10

Type	Composition	Load antigen/ adjuvant	Characteristic	Disease	Mechanisms of immunity	Ref.
Polymer nanoparticles	Alginate-coated chitosan nanoparticles	DNA plasmid	Protect DNA against degradation in gastric fluid	Breast cancer	Increase DNA uptake and expression by macrophages and DCs in the intestinal PPs, enhances the autoimmune response of CTLs	[24]
	Mannosylated chitosan nanoparticles coated with Eudragit® L100	BSA	Protection against enzymes and gastric acid degradation, reduce release into gastrointestinal fluids	-----	Enhanced antigen accumulation on PPs, elicit strong systemic IgG antibody and mucosal IgA responses	[25]
	Mannose and glycocholic acid-modified trimethyl chitosan nanoparticles	SIRPα siRNA and MUC1 pDNA	Gastrointestinal stability, effectively penetrate across the intestinal epithelia, reach the basolateral side with structural integrity	Triple-negative breast cancer	Antigen enrichment in LN, promote M1-type polarization, promote systemic immunity	[44]
	PLA/PLGA nanoparticles	Rotavirus	Slow-release antigen	------	Elicited long-lasting IgA and IgG antibody titer	[78]
Lipid nanoparticles	Soybean oil, Pluronic 188, Span-20, tomato lectin, phosphatidyl ethanolamines	Melanoma antigen, heat shock protein, staphylococcal enterotoxins A	Stable under pepsin and trypsin conditions	MAGE-1 expression in tumors	Targeted migration to mesenteric lymph nodes and PPs, preventing postoperative tumor metastasis and recurrence	[39]
	DPPC, DPPE, DC-cholesterol, α-GalCer	OVA	Gastric juice stability	------	Induction of systemic immune stimulation, APC uptake enhancement	[53]
	DSPC, DC-cholesterol, DSPE-PEG2000	Long peptide antigen and Pam2Cys-SKKKK adjuvant	Remains stable for long periods at 4 ℃	Colorectal cancer	The activation of innate immune responses in mLN	[54]
	DSPC, DMG-PEG, cholesterol, SM-102	βGlus/mRNA complex	pH response characteristics	E.G7-OVA tumor model	Uptaken by DCs in the gastrointestinal tract, inducing adaptive immune responses	[63]
	DOTAP, DOPC, chondroitin sulfate-*g*-mannose, chondroitin sulfate-*g*-glycocholic acid	OVA and polyinosine-polycytidylic acid	Higher drug encapsulation efficiency	Melanoma and colorectal cancer	Induce DC maturation and promote Th1 and Th2 immune responses	[65]
	Pluronic F127, plant-extracted lipids, silk fibroin	Ce6 and R837	Gastric and intestinal fluid stability, mucus permeability	Colorectal cancer	Induce ICD, promoting the polarization of M1 macrophages, regulate the intestinal microbiota	[75]
Inorganic nanoparticles	Aluminum metal-organic framework, yeast capsules	OVA	Long-term storage stability, gastric juice stability, controlled release	-----	Targeting intestinal M cells, inducing effective and persistent immunity	[26]

Continued

Type	Composition	Load antigen/ adjuvant	Characteristic	Disease	Mechanisms of immunity	Ref.
	Mesoporous silica nanoparticles, *β*-glucan	Len and OVA	Reduce drug leakage and ensure structural stability in the GIT	OVA-Hepa1-6 tumor model	Targeted delivery to tumors and APCs, activate the TLR2/4 signaling pathway, enhance immune responses, and reverse macrophage-related immune suppression in the TME	[61]
	Montmorillonite nanosheets	OVA	Gastrointestinal stability, mucosal adhesion	Colorectal cancer	Activate the cGAS-STING pathway within DCs to promote DC maturation and activate systemic and mucosal immune responses	[68]
Hybrid biomimetic nanoparticles	Cationic liposome conjugated *Escherichia coli*	Immunodominant peptides of colon adenocarcinoma	Low toxicity and good uniformity	Colorectal cancer	Activate DCs and enhance innate immune responses	[57]
	Bifidobacterium longum	Murine WT1 fusion gene	-----	Acute myeloid leukemia	Activate intestinal DCs and promote $CD4^+$ and $CD8^+$ T cell infiltration at tumor sites	[70]
	Plant lipid nanoparticles coupled with *Limosilactobacillus reuteri*	Immunoadjuvant CpG and carbon dot	Gastric and intestinal fluid stability, ROS responsiveness	Colorectal cancer	Stimulate dendritic cell maturation, promote tumor infiltration of cytotoxic T lymphocytes, and regulate the gut microbiota and its metabolites	[77]
	Crosslinked *β*-cyclodextrin-PEI600 (CP) and polyethyleneimine network adsorption of Salmonella	pDNA	Efficient phagosome escape and enhanced acid resistance	Melanoma	Induce systemic T cell responses and promote anti-tumor immunity	[79]
	Escherichia coli Nissle 1917	Tumor-associated antigen Glypican-1	-----	Pancreatic cancer	Increased levels of IgG and regulatory cytokine IL-10, stimulating mucosal and systemic immunity	[80]

4 临床转化现状

口服纳米疫苗凭借其无创给药、高效激活黏膜免疫的优势, 正成为疫苗研发的前沿热点, 但目前主要处于临床前研究阶段, 临床转化仍面临技术、监管与产业化的多重瓶颈。近期研究者们开发了多项口服递送系统来实现技术突破: 目前临床上批准的所有mRNA疫苗均以液体形式提供[81]。Huang等[82]设计了一种胶囊(RNACap), 采用pH敏感涂层与肠道蠕动驱动释放机制相结合, 成功在大鼠和猪模型中实现液态mRNA纳米颗粒的口服递送, 避免了冻干mRNA制剂所需的昂贵且耗时的冻干过程。此外, 由于抗原的生物利用度差, 单独使用时免疫原性较低[83], 可使用纳米佐剂与抗原共递送, Guo等[84]开发了一种由*β*-葡聚糖修饰的功能性介孔二氧化硅构成的纳米佐剂, 其不仅可促进DC中的代谢重编程, 增强与DC激活、交叉呈递、成熟和迁移相关的基因的表达, 还可将抗原OVA靶向递送到肠道M细胞和DC细胞, 为疫苗开发提供了新的方向。

安全性是临床前研究、临床试验及国家药监机构审批的核心考量, 在纳米技术领域尤为突出。由于纳米颗粒具有较小的尺寸及与生物系统的复杂相互作用, 其安全性面临着特殊的挑战[85]。纳米颗粒的小尺寸使其可能通过跨膜转运、细胞内吞等途径进入生物组织, 且其生物学行为会因成分、形态、表面电荷等理化特性的差异而有所不同, 表现出不同的体内分布、代谢速率和毒性风险, 具体而言, 脂质类载体的阳离子特性可能破坏肠黏膜完整性[86], 聚合物载体的合成残留易诱发局部炎症, 无机纳米颗粒则存在肝肾长期蓄积隐患[87], 同时表面修饰剂与佐剂的协同作用可能增强免疫原性, 引发过敏反应或过度免疫激活。此外, 纳米颗粒若与蛋白质、核酸等生物分子发生非特异性相互作用, 可能引发免疫激活紊乱、细胞功能损伤甚至遗传毒性等潜在健康风险。更需关注的是, 从实验室研究

到工业化放大生产过程中, 工艺参数波动 (如流速比、pH 偏差等) 易导致粒径不均、抗原包封率下降, 原材料批间差异 (如脂质纯度、聚合物分子质量分布) 及储存稳定性问题 (如肠溶包衣破损、颗粒聚集) 会进一步放大上述风险, 显著增加质量控制难度。因此, 必须建立合适的标准化方法, 以便在临床转化前对其进行安全性评估及质量控制的动态监测。

由于纳米医学缺乏通用的报告系统及表征方法, 其临床转化还面临着重复性较低的问题。此外, 纳米颗粒生产工艺、抗原负载效率等关键环节还缺乏统一的标准化方案[88]。应对这些挑战需要构建多维度的协同解决方案, 例如强化多学科交叉合作, 共同制定覆盖纳米颗粒从设计到应用全程的技术规范, 并建立基于过程分析技术的实时监测体系, 减少批次间差异, 保证纳米颗粒的安全性、稳定性和有效性。

5 总结与展望

口服疫苗凭借同步激活肠道黏膜与全身免疫的优势, 在抗肿瘤领域具有显著优势。纳米技术可通过抗胃肠道屏障、黏液渗透、黏膜黏附等策略破解其递送难题, 并通过增强抗原呈递、激活先天免疫等方式驱动免疫激活。然而, 绝大多数成果仍局限于动物模型。由于人体与动物在肠道解剖结构、菌群组成及免疫系统等方面存在显著差异, 推动其临床转化亟需在以下关键环节取得突破: 首先, 应优先建立更贴近人体肠道环境的体外 (如肠道类器官与免疫细胞共培养模型) 与体内评价体系, 以精准预测纳米疫苗在体内的效能; 其次, 须构建标准化的纳米载体安全性评价规范, 系统评估其长期生物相容性、体内代谢过程及对复杂肠道菌群的潜在影响; 此外, 应建立转化性评价体系, 如生理药代动力学模型, 以提升临床预测性。

针对不同肿瘤类型的免疫治疗需求, 未来口服纳米疫苗的设计应更具“适配性”。对于肠道相关肿瘤 (如结直肠癌), 疫苗设计应侧重于强化黏膜免疫与局部耐受打破。而对于非肠道肿瘤 (如乳腺癌、黑色素瘤等), 应侧重于激发全身性系统免疫, 可通过优化纳米载体的淋巴归巢能力, 促进高水平的 IgG 抗体和循环记忆 T 细胞的产生, 以实现对远端肿瘤的控制。

未来随着材料科学、纳米技术与免疫学的深度交叉融合, 口服纳米疫苗有望取得重大突破。同时, 可借助多组学技术, 实现个性化口服纳米疫苗的开发, 根据患者个体肿瘤特征与免疫状态, 定制精准的免疫治疗方案, 为肿瘤患者带来更为便捷、高效且安全的治疗选择, 推动肿瘤免疫治疗领域的快速发展。

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