内嵌佐剂在肿瘤糖疫苗及糖肽疫苗中的应用

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摘要: 糖疫苗及糖肽疫苗在肿瘤的预防与治疗领域展现出重要潜力, 但其面临免疫原性较低等问题。佐剂被广泛用于增强对特定抗原的免疫响应, 但外加佐剂仍存在诸多局限性, 如免疫增强能力有限, 分子扩散引发一定程度的安全性问题等。内嵌佐剂策略是将佐剂与疫苗抗原通过特定方式结合, 使佐剂在疫苗体系中能更充分发挥效用的一种方法。本文归纳了不同激动剂分子 (Toll样受体激动剂、iNKT细胞激动剂、干扰素基因刺激因子激动剂、核苷酸结合寡聚结构域样受体激动剂, 以及C型凝集素受体激动剂等) 作为内嵌佐剂在提升疫苗免疫原性方面的应用和研究进展, 旨在为开发更高效、安全的糖疫苗提供参考。

关键词: 抗肿瘤疫苗; 佐剂−抗原偶联物; 糖抗原; 糖肽抗原; 免疫治疗

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Applications of built-in adjuvants in anti-tumor carbohydrate and glycopeptide vaccines

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Abstract: Carbohydrate and glycopeptide vaccines have shown great promise in tumor prevention and treatment. However, their relatively low immunogenicity remains a significant challenge. To address this, adjuvants are commonly employed to enhance immune responses against specific antigens. Despite their widespread use, conventional adjuvants still face several limitations, including limited immune stimulation and potential safety concerns associated with molecular diffusion. A promising strategy to overcome these challenges is the incorporation of built-in adjuvants, which promotes adjuvants to augment their immunostimulatory effect. This review highlights the application of various immune agonists as built-in adjuvants to enhance vaccine immunogenicity, including Toll-like receptor (TLR) agonists, invariant natural killer T (iNKT) cell agonists, stimulator of interferon genes (STING) agonists, nucleotide-binding oligomerization domain-like receptors (NLR) agonists, and C-type lectin receptor (CLR) agonists. By summarizing recent advancements in this field, we provide valuable insights into the development of more effective and safer carbohydrate vaccines.

Key words: anti-tumor vaccine; adjuvant-antigen conjugate; carbohydrate antigen; glycopeptide antigen; immunotherapy

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随着免疫学和生物技术的发展, 疫苗作为预防和治疗癌症的重要手段, 其研发受到广泛关注。疫苗接种的目的是诱导针对特定抗原的强大免疫反应, 从而防止生物体受到感染。理论上, 利用肿瘤细胞自身或者肿瘤细胞特有的抗原作为靶标即可制备肿瘤疫苗[1]。糖类作为生物体内重要的分子标志物, 其结构与功能的异常表达与多种重大疾病的病理进程具有复杂而深刻的关联性。早期研究发现, 糖类分子可通过模式识别受体 (pattern recognition receptors, PRRs) 触发宿主的先天性免疫应答[2,3]。在抗细菌感染领域[4-10], 具有免疫原性的糖类分子已被成功应用于开发针对多种多糖包膜细菌病原体的疫苗, 其中具有代表性的包括肺炎链球菌 (*Streptococcus pneumoniae*)[8,9]、b 型流感嗜血杆菌 (*Haemophilus influenzae* type b)[10]等。近年来, 随着糖化学和糖化学生物学的发展, 基于糖类抗原的亚单位疫苗, 包括糖疫苗和糖肽疫苗被相继开发出来。

1 肿瘤相关糖抗原

在肿瘤的发生发展过程中, 肿瘤细胞表面糖链的异常修饰形成了具有免疫原性的糖抗原, 也被称为肿瘤相关糖抗原 (tumor associated carbohydrate antigen, TACA), 不仅参与肿瘤细胞的黏附、侵袭和转移, 还能通过逃避免疫监视机制, 为肿瘤的免疫逃逸提供助力。因此, TACA 作为抗肿瘤疫苗的有效靶点已被广泛研究[11-14]。目前 TACA 主要包括 4 类重要成员: 神经节苷脂[15] (如 GD2、GD3 和 GM3)、球状丝氨酸聚糖[16] (如 Globo H)、Lewis 抗原[17,18] (如 Lewis Y、sialyl-Lewis A 和 sialyl-Lewis X), 以及截短的 O-聚糖[19] (如 Tn、TF、STn 和 STF) (图 1)。

尽管这些糖抗原可刺激机体产生相应的抗体, 但其免疫原性较弱, 难以有效打破机体的免疫耐受, 且它们大多属于非 T 细胞依赖性抗原, 在免疫过程中缺乏 T 细胞的参与, 这导致多糖疫苗产生的抗体以亲和力较低的 IgM 为主, 且难以形成较为长效的免疫记忆[2]。为实现 IgM 到 IgG 的抗体类型转换, 产生高滴度的 IgG 抗体, 辅助 T 细胞对 B 细胞的帮助十分关键。将 TACA 和含有辅助 T 细胞表位的载体蛋白共价连接, 能够激活 CD4+ T 细胞, 实现对 B 细胞的辅助活化, 促进 B 细胞成熟分化为浆细胞, 从而产生高亲和力的抗体。此外, 使用佐剂也是疫苗增效的重要手段和突破口。病原体相关分子模式 (pathogen-associated molecular patterns, PAMPs) 作为一类重要的免疫激动剂已被广泛用于疫苗佐剂的研究中。目前所鉴定的模式识别受体主要包括 Toll 样受体 (Toll-like receptors, TLRs)、核苷酸结合寡聚结构域 (nucleotide-binding oligomeriza-

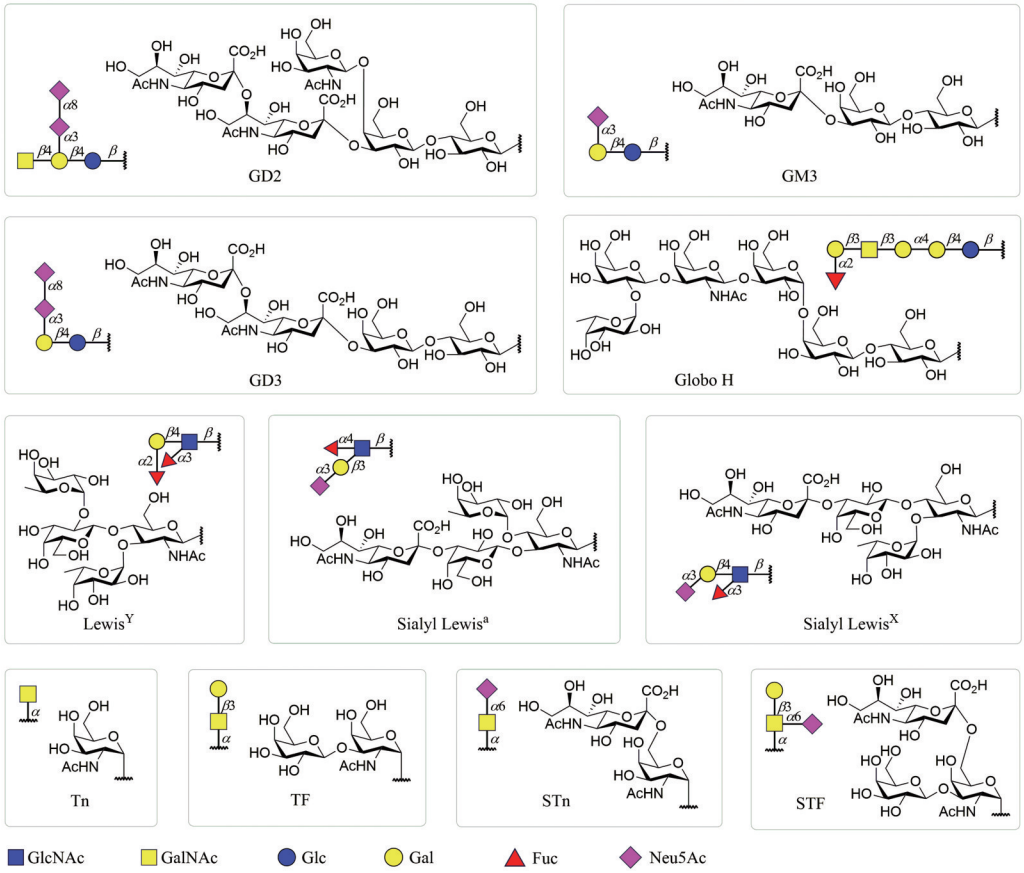


Figure 1 Representative tumor-associated carbohydrate antigens

tion domain, NOD) 样受体 (NOD-like receptors, NLRs)、干扰素基因刺激因子 (stimulator of interferon genes, STING)、C 型凝集素受体 (C-type lectin-like receptors, CLRs) 等。PRRs 识别病原体相关分子模式后, 迅速触发下游信号转导级联反应, 诱导抗原呈递细胞 (antigen-presenting cell, APC) 产生并分泌细胞因子, 同时显著上调细胞表面共刺激分子的表达, 进而调控优化 T 细胞活化、增殖, 最终激活适应性免疫系统[20]。

2 内嵌佐剂策略的优势

传统糖抗原疫苗以外加佐剂的形式为主, 虽能在一定程度上增强免疫效果, 但存在诸多局限性, 如分子扩散引发安全性问题以及可能影响疫苗稳定性等。内嵌佐剂策略的出现为疫苗的开发提供了新的方向 (图 2): 佐剂与糖抗原或糖肽抗原紧密结合, 确保抗原和佐剂同时到达同一 APC, 促进它们对抗原的摄取、加工和呈递, 从而增强机体对抗原的免疫应答; 此外, 内嵌佐剂可以在一定程度上改变抗原的物理性质和空间结构, 保护抗原免受外界环境因素 (酶解、氧化等) 的影响, 增加抗原在体内的稳定性, 从而持续刺激免疫系统, 增强记忆免疫的形成, 包括记忆 B 细胞和记忆 T 细胞。对于一些分子佐剂来说, 通过合理化的设计可以使佐剂的作用更集中于抗原所在部位, 更精准地调控免疫反应的强度和范围, 减少佐剂在其他组织和器官中的非特异性分布和作用, 避免过度的免疫反应导致的炎症等不良反应, 提高疫苗的安全性[21,22]。本综述将根据不同的激动剂种类, 结合近 5 年内最新的研究成果和本课题组的部分工作, 介绍相关基于内嵌佐剂策略的糖疫苗的研究进展, 以期为新型糖疫苗的开发提供思路。

3 内嵌佐剂在肿瘤疫苗中的应用

3.1 TLR 激动剂作为内嵌佐剂

TLRs 是一类重要的模式识别受体, 既可以参与先天免疫反应, 又可以在被 PAMP 激活后调节适应性免疫系统, 是连接先天性免疫和适应性免疫的桥梁。目前, 在人类体内共发现 10 种 TLRs。根据所处的位置不同, TLRs 被大概分为两大类[23], 其中 TLR1、TLR2、TLR4、TLR5、TLR6、TLR10 主要为位于细胞膜表面, TLR3、TLR7、TLR8、TLR9 主要位于细胞内体膜上。TLR1、2 和 6 的配体主要为一些特定的脂质, TLR3 则能被双链 RNA 激活。TLR4 是目前研究最为广泛的受体, 已发现其可以被多种配体激活, 包括脂多糖 (lipopolysaccharide)、热休克蛋白 (heat shock proteins) 等。TLR7、8 主要识别单链 RNA (ssRNA)。不同 TLR 在与适当的配体结合并激活后, 其构象发生变化, 从而导致下游分子的收敛, 触发相关的免疫信号通路, 并诱导细胞因子以及其他共刺激因子的上调[23,24]。

3.1.1 脂类 TLR 激动剂 TLR1/2 激动剂 Pam_3Cys 及其衍生物是一类最常用的内嵌佐剂, 主要由 3 个脂肪酸链与一个半胱氨酸残基相连。该结构与 TLR2 结合后, 通过 MyD88 依赖的信号通路, 诱导 NF-κB 等转录因子的活化, 进而促进炎症细胞因子如肿瘤坏死因子-α (tumor necrosis factor-α, TNF-α)、白细胞介素-6 (interleukin-6, IL-6) 等的表达和释放, 从而启动免疫应答[24-26]。早在 1994 年, Toyokuni 等[27]将肿瘤相关糖抗原 Tn 与 Pam_3Cys 的偶联物免疫小鼠后观察到了高水平的 IgM 抗体。随后, Scott 等[28]制备了基于簇状 Tn 抗

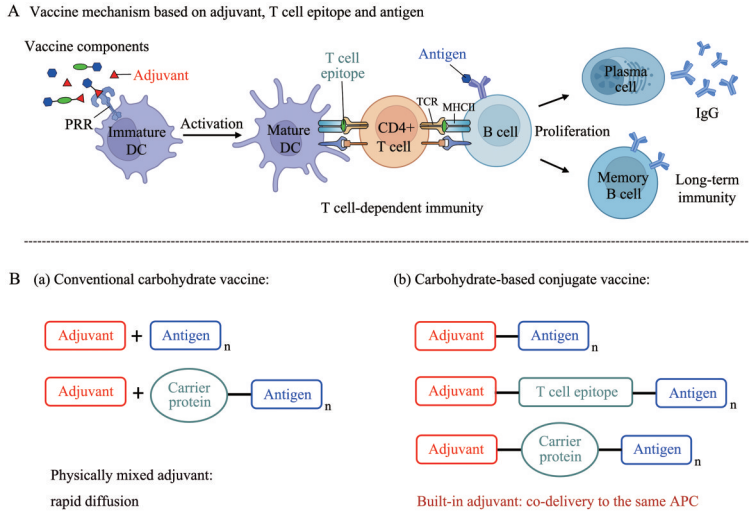


Figure 2 Vaccine mechanism and carbohydrate vaccine design strategies. A: Vaccine mechanism based on adjuvant-activation and T cell-dependent immunity; B: Carbohydrate vaccine design strategies. PRR: Pattern recognition receptor; DC: Dendritic cell; TCR: T cell receptor; MHC: Major histocompatibility complex; APC: Antigen-presenting cell

原和Pam_3Cys偶联的疫苗, 结果也同样只观察到了有限的抗体免疫响应, 这可能是由于免疫过程中缺乏T细胞的参与, 从而限制了B细胞的充分活化及抗体的高效产生 (图3A)。MUC1是肿瘤糖肽疫苗研究的有效靶点[29,30]。Kunz课题组将不同Th细胞表位 (TT[31]、$OVA_{323-339}$[32]) 分别与肿瘤相关糖肽MUC1共价连接合成了两组分糖肽疫苗, 免疫结果显示这种疫苗产生了抗原特异性免疫响应。2005年, Buskas等[33]在Pam_3Cys-Tn偶联物中引入了辅助性T细胞表位YAF从而合成了一种三组分的全合成糖疫苗, 该三组分的疫苗相较于二组分确实实现了更高比例的IgM向IgG类别的转化, 但只展示出了中等滴度的IgG抗体, 造成该现象的原因被归结为所使用的T辅助细胞表位不匹配 (图3B)。为改进这一设计, Ingale等[34]进一步构建了由Pam_3CSK_4作为内嵌佐剂, 包含MUC1糖肽和来源于脊髓灰质炎病毒的Th细胞表位的三组分候选疫苗, 该疫苗成功诱导产生了高滴度的IgG抗体, 并有效抑制了肿瘤的生长。此外, 该研究也证实了这种自佐剂疫苗无需添加额外的佐剂 (图3C)。同一时期, Payne课题组[35,36]及李艳梅课题组[37,38]也分别以Pam_3CS为内嵌佐剂成功制备了抗肿瘤疫苗 (图3D、E)。随后, TLR2激动剂被不同课题组广泛应用在多组分抗肿瘤疫苗的设计开发中[39-48]。Zhou等[43]将Pam_3CSK_4、辅助T细胞表位与MUC1糖肽三聚体进行共价连接, 设计了一种自佐剂多价糖肽疫苗, 研究结果表明接受三聚体疫苗治疗的野生型和转基因小鼠均表现出更好的肿瘤抑制效果。为探究偶联方式与位点对佐剂效用的影响, Shi等[42]使用MAGE-A1肽和TLR2激动剂通过不同的共价键设计合成了12种具有自组装特性的偶联物 (N-Ac PamCS-M-1～12)。所有偶联物都能有效地被树突状细胞摄取并诱导IL-6、IL-12和TNF-α等因子的分泌。其中, 与非共价的混合组相比, 偶联物N-Ac PamCS-M-6在促进树突状细胞成熟、CD8+ T细胞活化以及杀死肿瘤细胞方面具有更显著的能力。此外, 本课题组[44]基于*N*-端转氨反应成功将Pam_3CSK_4作为内嵌佐剂负载于RBD糖蛋白制备了Pam_3CSK_4-RBD抗病毒疫苗, 并用于对抗新型冠状病毒SARS-CoV-2感染。此后, 本课题组[45]通过转氨反应将Pam_3CSK_4结合到负载了MUC1糖肽抗原的载体蛋白BSA的N端, 构建了一种含内嵌脂质佐剂的新型抗肿瘤疫苗。免疫的结果表明Pam_3CSK_4-BSA-MUC1偶联物疫苗显著增强了APC对MUC1糖肽抗原的摄取, 并引发了强大的体液和细胞免疫, 显著抑制了肿瘤的生长 (图3F)。从这些研究中可以清楚地看出, TLR1/2激动剂作为内嵌佐剂是增强抗原免疫原性的有前景的策略。

TLR4激动剂MPLA是另一种被常用作内嵌佐剂的脂类激动剂, 其可与细胞表面的TLR4受体结合, 启动细胞内的信号传导通路, 激活巨噬细胞、树突状细胞等抗原呈递细胞。这些激活的抗原呈递细胞能够摄取、加工和呈递糖疫苗或糖肽疫苗中的抗原, 将其呈递给T细胞, 从而启动特异性免疫应答[49]。郭忠武课题组围绕“MPLA偶联肿瘤相关糖抗原二组分全合成疫苗”进行了一系列研究: 对分别与肿瘤相关糖抗原STn[50]、GM3[51]、Globo H[52]、GM2[53]等偶联的全合成疫苗进行免疫测试, 在不使用外部佐剂的情况下这些疫苗均展现出能引起更强的针对糖抗原的免疫反应, 产生具有更高滴度、高亲和力的IgG抗体和具有记忆力的T细胞调节免疫反应的能力。最近, Gao等[54]使用5种不同MPLA类似物作为内嵌佐剂设计和合成了基于TF的癌症候选疫苗分子。所有合成的偶联物在小鼠体内引起了强大的TF特异性免疫反应, 所产生的抗血清可以特异性识别、结合和诱导TF阳性癌细胞的裂解 (图3G)。此外, 基于抗原肽与TLR4配体的偶联疫苗方面, Reintjens等[55]探索了不同的连接系统和连接模式对疫苗活性的影响, 结果表明, TLR4配体CRX-527偶联到模型肽抗原的N端提供了一种有效的疫苗模式, 该模式在激活树突状细胞, 促进抗原呈递和启动特异性CD8+ T细胞介导的靶细胞杀伤方面是有效的。以上研究都充分表明TLR4配体作为内嵌佐剂在增强癌症疫苗效用方面具有巨大的前景。

3.1.2 非脂类TLR激动剂 TLR7/8能够识别来自细菌或病毒的ssRNA并被激活, 然后通过MyD88途径发出信号, 快速启动信号级联, 导致NF-κB的激活以及细胞因子的产生。研究表明[56], 核苷类物质, 包括咪唑喹啉类似物、鸟苷类似物和嘌呤类似物等都是有效的免疫刺激剂。TLR7激动剂的一个主要代表是咪喹莫特, 其已被用于各种皮肤病的局部治疗[57,58]。然而, 在用作疫苗佐剂时, 这些小分子面临着从注射部位扩散从而导致效力下降以及引发全身不良反应等问题。Gao等[59]的早期研究揭示了基于8-羟基腺嘌呤的TLR7激动剂通过其9-苄基部分连接到寡肽上能有效介导DC活化。Gential等[60]描述了一类腺嘌呤类TLR7配体连接到肽抗原序列中不同位置的二组分疫苗。这些偶联物的生物学评估显示偶联能诱导树突状细胞成熟, 表明了TLR信号的传导。值得注意的是, 配体偶联的位置会影响免疫原性, 两个N端偶联物诱导了更高水平的T细胞增殖, 这可能是由于C端位置的TLR7配体干扰了蛋白水解过程。2020年, Wang等[61]应用一种小分子TLR7激动剂T7和MG7-Ag表位抗原肽构建了多肽疫苗, 结果表明内嵌T7的疫苗能更有效地诱导免疫应

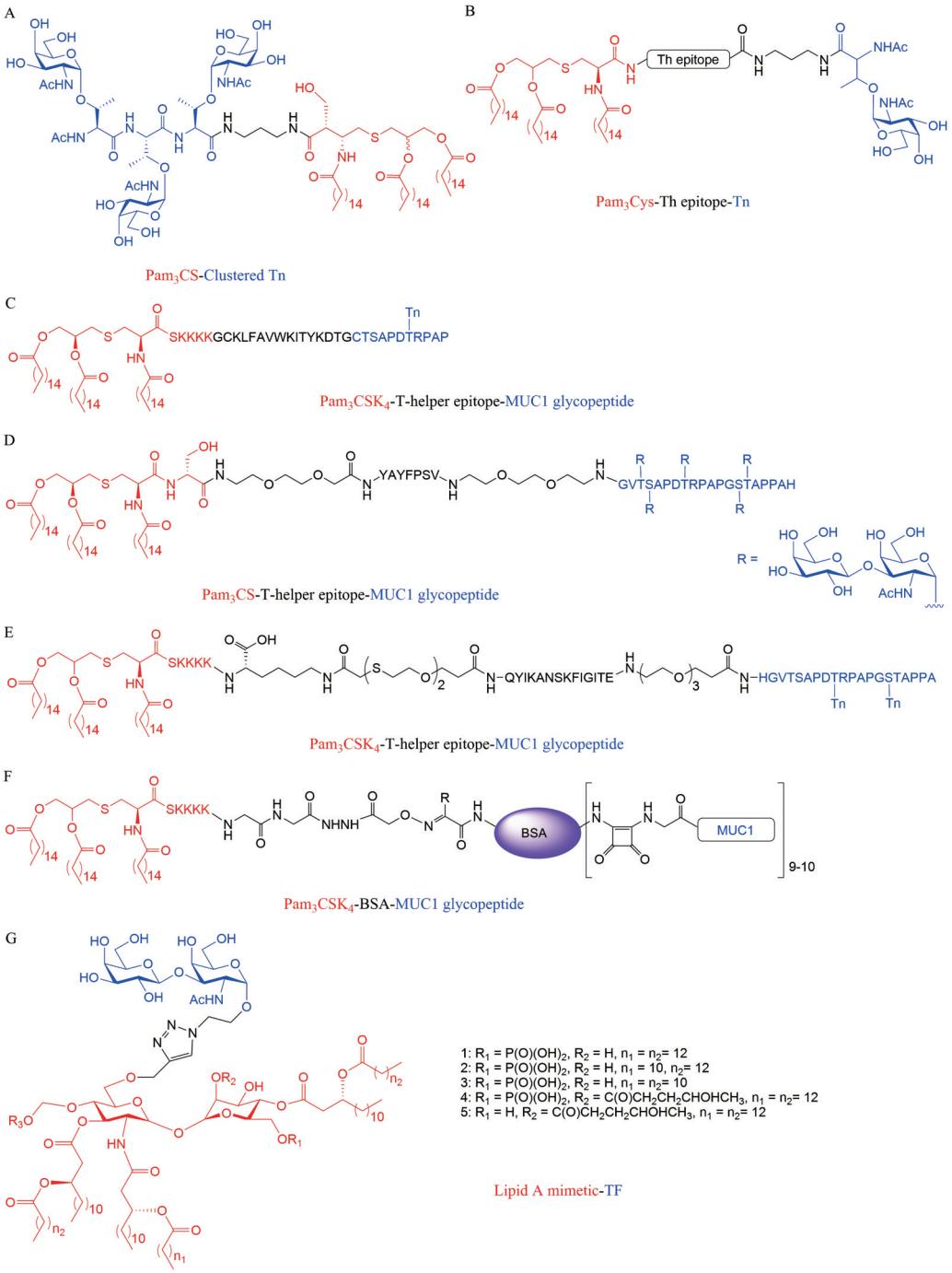


Figure 3 Anti-tumor vaccines based on the built-in lipid Toll-like receptor (TLR) agonists and tumor associated carbohydrate antigens ($TACA_S$). A: Two-component vaccine of Pam_3CS and trivalent Tn; B: Three-component vaccine of Pam_3Cys, YAF peptide, and Tn; C: Three-component vaccine of Pam_3CSK_4, Th epitope, and MUC1 glycopeptide; D: Three-component vaccine of Pam_3CS, Th epitope, and MUC1 glycopeptide; E: Three-component vaccine of Pam_3CSK_4, Th epitope, and MUC1 glycopeptide; F: Three-component vaccine of Pam_3CS, BSA, and MUC1 glycopeptide; G: Two-component vaccine of lipid A mimetics and TF antigen

答。有趣的是, 相较于直链的疫苗结构T7-ML, 具有特别分支结构的疫苗T7-MB在引发细胞因子分泌、抗体产生、ADCC和CTL等方面更加有效, 且与5-氟尿嘧啶协同使用时显著抑制了肿瘤的生长, 并延长小鼠的生存时间。随后, Liu等[62]以T7为内嵌佐剂, 通过将多个T7与MUC1糖肽抗原共价偶联, 以一种T7佐剂簇的形式制备了一种二组分全合成抗肿瘤疫苗T7-MUC1, 该疫苗表现出了良好的抗肿瘤活性 (图4A)。在内嵌TLR7激动剂方面, 本课题组[63]通过偶联TLR7激动剂TLR7a、牛血清白蛋白BSA和MUC1糖肽构建了三合

一疫苗。三合一疫苗在IgG抗体滴度上相较于BSA-MUC1对照组展现了两个数量级的优势, 且与非共价疫苗相比, 三合一疫苗诱导了明显的Th1偏向免疫反应 (图4B)。随后, 通过改变TLR7a与MUC1糖肽的偶联方式, 本课题组[64]将TLR7a与MUC1偶联后再整体连接在载体蛋白BSA上, 构建出了新型的MUC1疫苗。经过Alum佐剂的共同施用, 疫苗展现出了强效的抗肿瘤活性 (图4C)。同样的, 内嵌TLR7a在SARS-CoV-2疫苗中依旧能引发高滴度的S1抗原特异性IgG抗体以及中和抗体, 表现出良好的病毒中和能力[65,66]。

TLR9同样是机体天然免疫的重要识别受体, 主要识别含有非甲基化胞嘧啶–磷酸–鸟嘌呤 (CpG) 基序的双链DNA。为了研究和应用的需要, 研究者合成了模拟天然CpG DNA的CpG寡脱氧核苷酸 (CpG oligodeoxynucleotide, CpG ODN)。在一些病毒疫苗、肿瘤疫苗的研发中[67-70], 将CpG ODN作为疫苗佐剂与抗原联合使用, 可以诱导机体产生更强的细胞免疫和体液免疫反应。尽管如此, 仅有少量研究使用了CpG ODN作为内置佐剂。Abdel-Aal等[71]合成了含有MUC1糖肽、Th表位和CpG ODN的三组分肿瘤疫苗 (图4D)。然而, 内嵌CpG ODN的三组分疫苗并没有取得预期的结果。最近, TLR9激动剂作为癌症疫苗佐剂的研究主要集中在新的递送策略以及多佐剂联用方面[72-75]。Chen等[76]发表的一项研究中, 通过将OCT4特异性肽与载体蛋白KLH和TLR9激动剂化学偶联, 设计了一种半合成疫苗来靶向干细胞样癌细胞, 抑瘤效果评估显示携带F9畸胎癌的小鼠在接种该疫苗后导致了肿瘤生长变慢, 生存期延长。肿瘤分析显示CD8+ T细胞浸润增加, 并

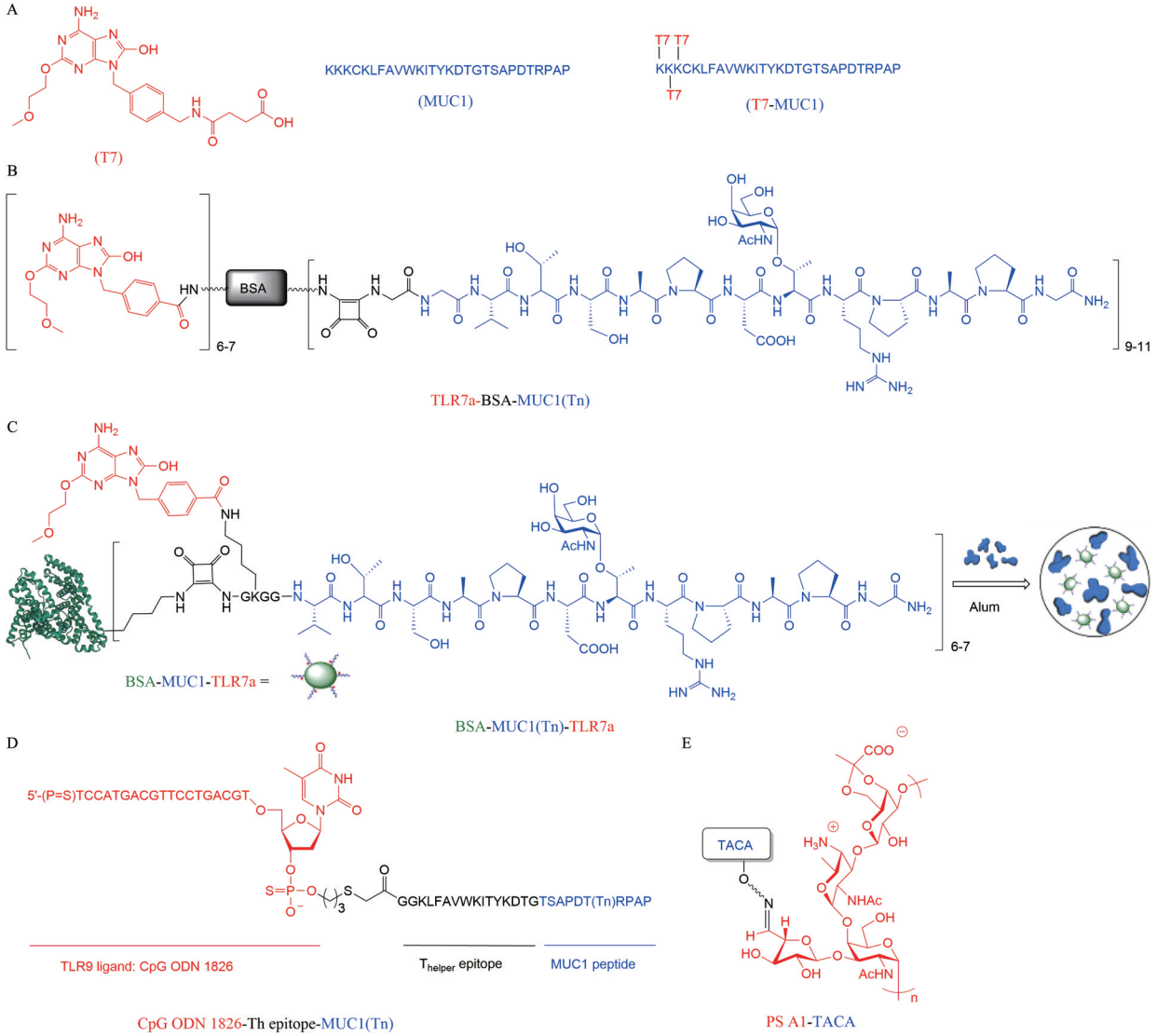


Figure 4 Anti-tumor vaccines based on the built-in non-lipid TLR agonists and $TACA_S$. A: Two-component vaccine of T7 and MUC1 glycopeptide; B, C: Three-in-one protein conjugate vaccine of TLR7a, BSA protein, and MUC1 peptide; D: Three-component vaccine of CpG ODN 1826, T_{helper} epitope, and MUC1 peptide; E: Two-component vaccine of PS A1 and TACA

分泌了大量促炎细胞因子, 如IL-2、IL-6和IL-12。

荚膜多糖PS A1是一种两性离子多糖, 其能够在缺乏蛋白质/肽或脂质的情况下引发MHC II类分子依赖的T细胞免疫应答, 同时增强目标糖抗原的免疫原性。研究表明[77,78], PS A1能够与TLR2结合, 触发先天免疫反应, 诱导IL-12、IFN-γ等细胞因子的释放。Andreana团队将其作为一种特殊的内嵌佐剂分别与肿瘤相关糖抗原Tn[79,80]、TF[81,82]、STn[83]、Globo-H[84]、GM3[85]等抗原偶联制备了新型的内嵌佐剂糖疫苗 (图4E)。这类疫苗均显示出了免疫活性, 在没有T细胞表位的参与下同样引发了T细胞介导的免疫反应, 并选择性地识别并区分相关肿瘤细胞。

3.2 αGalCer作为内嵌佐剂

iNKT是一类兼具T细胞和NK细胞特征的固有免疫样T细胞。区别于主要识别肽段抗原的传统T细胞, iNKT细胞最显著的特征是其主要识别由CD1d分子呈递的糖脂类抗原[86]。αGalCer是一类结构独特的糖脂, 最初是从冲绳海域的海绵提取物中发现的, 是自然界中发现的第一类CD1d介导的小分子NKT细胞激动剂。αGalCer作为一种具有独特免疫调节作用的糖脂类化合物, 能够与抗原呈递细胞表面的CD1d蛋白结合形成二元复合物从而被呈递, 该复合物进一步被iNKT细胞的TCR识别, 形成一个具有免疫活性的“CD1d/αGalCer/TCR”三元复合物。NKT细胞被迅速激活, 快速释放大量细胞因子, 参与调节先天性免疫和获得性免疫[87,88]。因此, αGalCer作为NKT细胞激动剂在免疫治疗方面具有广泛的研究和应用价值。

在肿瘤免疫治疗中[89-91], αGalCer作为一种内嵌佐剂, 能够激活NKT细胞, 分泌IFN-γ等细胞因子, 增强T细胞和NK细胞对肿瘤细胞的杀伤活性, 有效抑制肿瘤的生长, 增强细胞免疫功能。Broecker等[92]将肿瘤相关的Tn抗原与αGalCer偶联构建了糖疫苗候选物, 这种结合使糖抗原更有效地呈递给免疫系统, 提高糖抗原的免疫原性 (图5A)。本课题组通过酰胺键在αGalCer的C6位置引入抗原成分, 包括STn[93]、GM3[94]、RBD[95]等构建了含有αGalCer的双组分疫苗 (图5B)。在STn疫苗方面[93], αGalCer-STn迅速诱导分泌细胞因子, 如IFN-γ、IL-4, 从而增强机体的免疫应答, 包括提高抗体滴度, 展示出了共价疫苗的优势。同样的结果在GM3疫苗里也得到了验证[94], 此外在GM3疫苗的设计方面, 还评估了抗原的各种脂质化策略的影响, 发现β-GalCer-GM3是GM3糖抗原修饰的最佳脂质形式。在糖肽疫苗的研究中, Chen等[96]利用点击化学形成三唑环, 将αGalCer与MUC1糖肽抗原共价偶联制备了一种新型的二组分抗肿瘤疫苗 (图5C)。该疫苗有效激活了CD11+树突细胞和F4/80+巨噬细胞, 小鼠免疫的结果显示了血清中含有高滴度的抗MUC1特异性IgG抗体, 并且抗血清表现出了对MCF-7细胞的有效识别。此外, Yang等[91]设计合成了含有TLR4配体MPLA和iNKT细胞激动剂KRN7000的三组分疫苗MPLA-Tn-KRN7000 (图5D)。免疫学评估发现MPLA-Tn-KRN7000会引发强大的Tn特异性和T细胞依赖性免疫。TLR4敲除小鼠的免疫学研究以及与CD1d蛋白结合亲和力的测试表明, 共价连接的MPLA-KRN7000免疫刺激剂诱导TLR4和iNKT细胞的协同激活, 从而提高Tn的免疫原性。这项工作表明MPLA-Tn-KRN7000具有成为候选疫苗的潜力, 并为全合成疫苗设计提供了新的方向。Liu等[97]以αGalCer作为免疫佐剂, 金纳米颗粒为多价载体, MUC1糖肽为抗原表位构建的疫苗产生了较高的抗体滴度, 并且抗原与佐剂的比例与免疫效果正相关, 这表明αGalCer能够有效增强糖肽疫苗的免疫原性, 通过激活NKT细胞, 诱导机体产生强烈的免疫应答, 对小鼠黑色素瘤的生长产生明显的抑制作用。

3.3 STING激动剂作为内嵌佐剂

STING是天然免疫信号通路中的关键调控分子。自2008年被发现以来, STING作为抗肿瘤免疫的核心接头蛋白逐渐被认为是免疫治疗的核心且有前途的靶点。正常状态下, STING位于内质网。细胞质中的环状二核苷酸 (cyclic dinucleotide, CDN), 如环鸟苷酸–腺苷酸 (cGAMP) 可作为STING通路的激活信号。STING与cGAMP等配体结合后, 从内质网转运至高尔基体, 并在此招募并激活TBK1, TBK1进而磷酸化转录因子IRF3, 使其发生二聚化并进入细胞核。在入核的IRF3与其他转录因子的协同作用下, 诱导产生I型干扰素等细胞因子, 同时促进APC的成熟, 激活适应性免疫[98,99]。

天然CDN包括CDG、CDA、3′,3′-cGAMP和2′,3′-cGAMP。其中, CDG、CDA和3′,3′-cGAMP由细菌合成, 2′,3′-cGAMP是由cGAS产生的内源性次级信使。然而, CDN的负电荷、亲水性和不稳定性阻碍了它们的进一步应用。为了克服这些缺点, 研究者逐渐将目光聚焦于化学设计作为内嵌佐剂来加强激动剂的应用。Wu等[100]通过CDG的2-OH位点将CDG与MUC1糖肽偶联开发了基于内嵌STING激动剂的抗肿瘤疫苗 (图5E)。该内嵌佐剂疫苗能有效激活RAW264.7和巨噬细胞。然而, 与CDG和MUC1糖肽直接物理混合的对照组相比, 该疫苗在激发高滴度抗体和诱导Th1和Th2细胞因子分泌方面没有表现出优势。值得注意的是, 肿瘤内注射CDG引起了良好的抗肿瘤作用。最新的研究

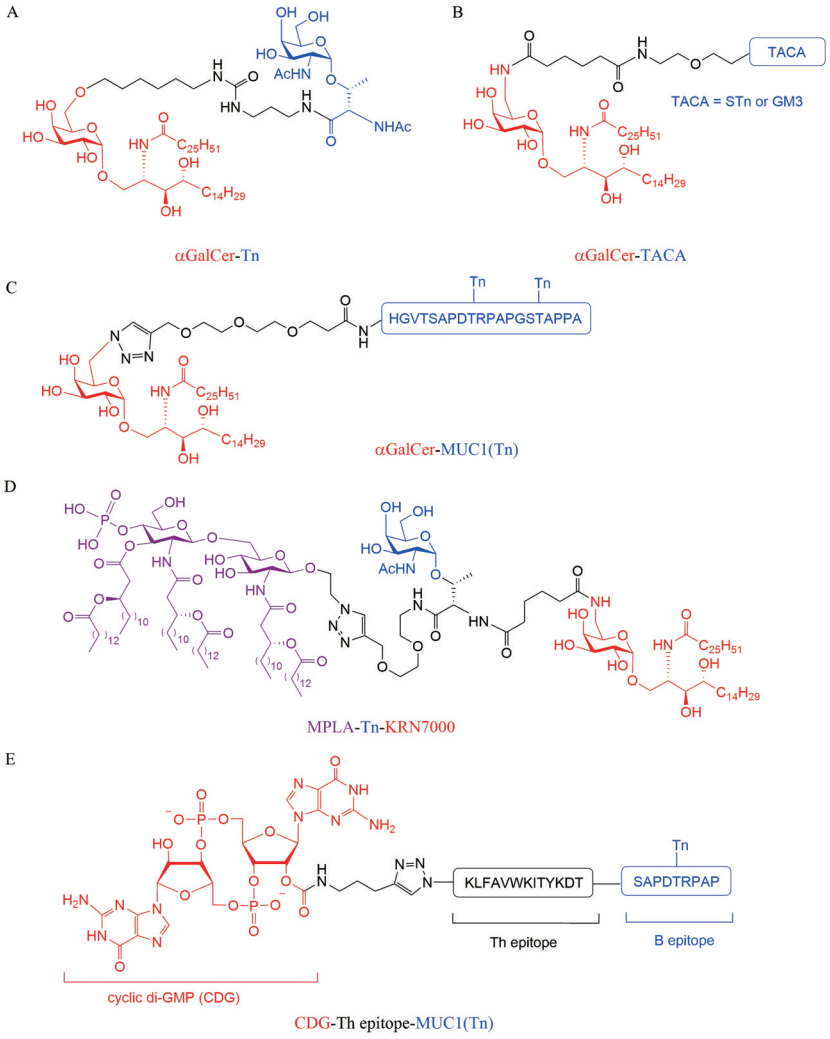


Figure 5 Anti-tumor vaccines based on the built-in αGalCer or STING agonists. A: Two-component vaccine of αGalCer and Tn; B: Two-component vaccine of αGalCer and TACAs; C: Two-component vaccine of αGalCer and MUC1 peptide; D: Vaccine of MPLA, KRN7000, and Tn; E: Three-component vaccine of CDG, Th epitope, and MUC1 peptide

将 Pam_3CSK_4 与 CDG^{SF} 偶联得到了新型的多靶点激动剂[101,102]。这种偶联通过协同激活 STING 和 TLR 途径, 引发了强效的体液和细胞免疫反应, 且对肿瘤生长产生了明显的抑制效果。2020 年, 研究者发现了一种非核苷酸 STING 激动剂 MSA-2[103-105], 其具有独特的结合模式和增强的生物利用度, 适合口服给药, 并在动物研究中表现出积极的抗肿瘤作用。

3.4 NLR 激动剂作为内嵌佐剂

NOD 样受体是一类重要的模式识别受体, 主要由 C 端亮氨酸重复序列结构域 (leucine rich repeat, LRR)、中间核苷酸结合寡聚化结构域和 N 端效应结构域构成。其中 LRR 通常由多个串联的亮氨酸重复序列组成, 具有较高的多样性, 能够识别多种 PAMPs 或 DAMPs, 如细菌的肽聚糖、病毒的核酸等, 而 N 端效应结构域则主要负责与下游信号分子相互作用, 招募并激活相关的信号转导蛋白, 从而启动下游的免疫和炎症信号通路[106]。NOD1 和 NOD2 是 NLR 最重要的代表, 它们主要识别构成细菌细胞壁的肽聚糖的特定部分。NOD1 主要识别细菌细胞壁肽聚糖的降解产物, 而 NOD2 的识别范围更为广泛, 有效激活 NOD2 所需的最小肽聚糖基序是胞壁酰二肽 (muramyl dipeptide, MDP)[107]。

2014 年, Willems 等[108]在 OVA 衍生肽抗原 $DEVA_3K$ 上评估了 MDP 作为内嵌佐剂的效用, 结果表明偶联确实能够激活 NOD2, 但并不能引发有效的免疫响应。此后, 该课题组对 MDP 进行脂质化修饰后再次用作疫苗内嵌佐剂[109]。最近, Zom 等[110]探究了嵌合 NOD2/TLR2 作为内嵌佐剂的潜力, 结果表明, MDP-SLP-Pam 具有强效的 NOD2 活化能力, 并以 TLR2 依赖性方式诱导 APC 成熟 (图 6A)。本课题组[111]最近制备了 NOD2-TLR4 多靶点免疫佐剂, 在基于 OVA 的模型抗

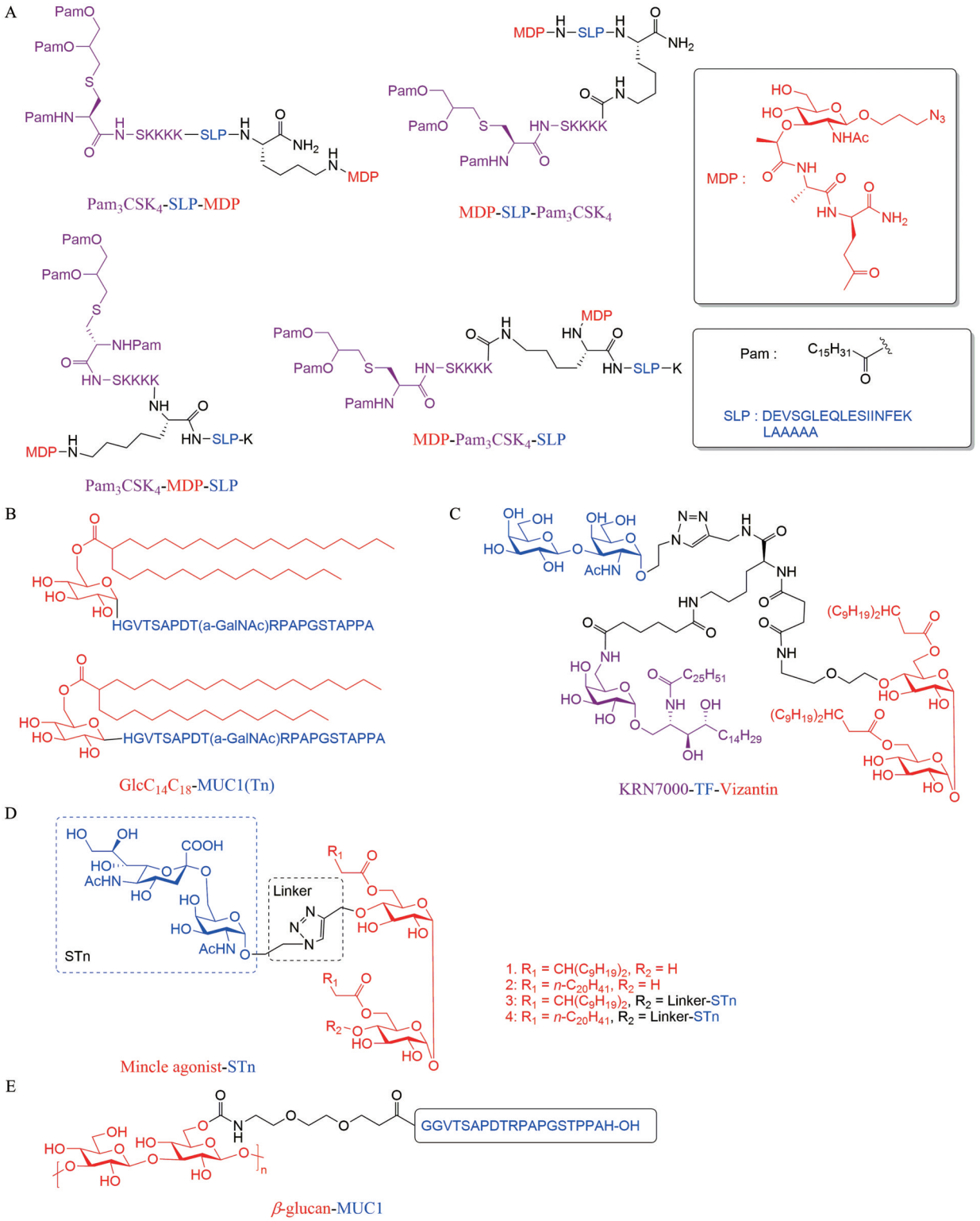


Figure 6 Anti-tumor vaccines based on the built-in NLR or CLR agonists. A: Vaccine of MDP, Pam_3CSK_4, and SLP peptide; B: Vaccine of $GlcC_{14}C_{18}$ and MUC1 peptide; C: Vaccine of Vizantin, KRN7000, and TF antigen; D: Two-component vaccine of Mincle agonist and STn; E: Vaccine of *β*-glucan, and MUC1 peptide

原疫苗中显示出了强效的佐剂刺激活性。

3.5 C型凝集素激动剂作为内嵌佐剂

巨噬细胞诱导的C型凝集素 (macrophage-inducible C-type lectin, Mincle) 是一种模式识别受体, 其配体主要包括细胞非自然死亡时释放的某些蛋白质以及非蛋白质的海藻糖-6,6′-二霉菌酸酯 (trehalose-6,6-

dimycolate, TDM)、海藻糖-6,6′-二山嵛酸酯 (trehalose-6,6-dibehenate, TDB)、葡萄糖神经酰胺等脂质分子。脂质TDM与Mincle的胞外C型凝集素结构域具有较高的亲和力, 结合后引起Mincle的构象变化, 使其与Fc受体γ链结合形成复合物, 从而启动下游的信号传导, 激活巨噬细胞、树突状细胞等免疫细胞, 诱导产生炎症细胞因子, 启动免疫反应。非蛋白的Mincle配体作为开发疫苗佐剂的分子靶点显示出了巨大的潜力[112]。

$GlcC_{14}C_{18}$是一种新型Mincle配体。2023年, Wang等[113]将具有不同α, β构型的$GlcC_{14}C_{18}$与MUC1糖肽偶联并评估其免疫作用 (图6B)。结果表明, 疫苗的两种构型都具有良好的免疫效果, 但在一定程度上, β-$GlcC_{14}C_{18}$-MUC1优于α-$GlcC_{14}C_{18}$-MUC1。最近, Yang等[114]提出了TF抗原与KRN7000和Vizantin共价偶联的新策略 (图6C)。由此产生的三组分疫苗KRN7000-TF-Vizantin能同时触发iNKT细胞和Mincle信号通路, 引发更高滴度的TF特异性IgG以及诱导Th1/Th2混合免疫应答, 体内荷瘤实验表明, KRN7000-TF-Vizantin增加了荷瘤小鼠的存活率和生存时间。此外, Luo等[115]以STn为模型抗原, 设计并合成了4种以Mincle激动剂为内嵌佐剂的偶联物肿瘤疫苗 (图6D)。所有的偶联物在小鼠中均单独引起了强大的体液和T细胞依赖免疫反应。相应的抗体能够识别、结合STn阳性癌细胞并表现出补体依赖性细胞毒性, 导致肿瘤细胞裂解。此外, 所有结合物均能有效抑制肿瘤生长, 延长小鼠生存时间。值得注意的是, 与传统的糖蛋白结合疫苗相比, 这些全合成的结合疫苗并不会引起“表位抑制”。TDB是一种靶向Mincle的有效糖脂佐剂。Hanna等[116]构建了通过PEG偶联$ESAT_6$肽抗原与TDB的二组分疫苗, 其引发了有效的免疫应答。巨噬细胞半乳糖型凝集素MGL是一种C型凝集素受体, 可结合Tn抗原, 是抗癌疫苗的有吸引力的靶标。Gabba等[117]通过多肽固相合成方法制备了一种基于四苯乙烯支架的高亲和力糖簇靶向树突细胞的抗肿瘤糖肽疫苗。体内测试表明, 与单独的TACA相比, 带有GalNAc糖簇的疫苗可诱导更高水平的抗MUC1抗体滴度。此外, 获得的抗体可有效结合MUC1阳性乳腺癌细胞上的肿瘤相关糖结构。GalNAc糖簇与MUC1糖肽抗原的偶联对抗体产生协同影响。

除此以外, β-葡聚糖同样是一种具有广泛应用价值的免疫激活剂。研究表明, 其主要与C型凝集素受体dectin-1相互作用。Wang等[118]通过将MUC1肽抗原与β-葡聚糖偶联构建了一种基于β-葡聚糖作为的佐剂及抗原载体的糖肽疫苗。该疫苗促进了抗原的呈递, 有效地激活了先天免疫和适应性免疫 (图6E)。

4 展望与挑战

疫苗是人类对抗病原体的重要武器。佐剂是增强疫苗免疫原性、延长免疫反应和调节免疫的关键成分。内嵌佐剂在糖疫苗及糖肽疫苗的应用研究为提高疫苗免疫原性、打破免疫耐受、克服传统疫苗的局限性提供了新的思路和方法。未来需要进一步探索和开发具有更高安全性、更强免疫激活能力的新型内嵌佐剂, 或将不同类型的具有协同增效作用的内嵌佐剂联合使用, 进一步增强疫苗的免疫效果。尽管目前还面临一些挑战, 但随着研究的不断深入和技术的不断进步, 相信在未来能够开发出更多安全、高效的肿瘤疫苗。

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