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利用纳米技术提高癌症免疫治疗效果的研究进展

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摘要 免疫治疗是一种通过增强免疫应答反应, 或解除免疫抑制作用来治疗癌症的方法. 利用纳米技术能够防止抗原、免疫刺激分子等降解, 提高免疫刺激分子等药物在肿瘤部位的富集, 改善其生物分布和释放动力学, 同时使其靶向作用于肿瘤微环境内的基质细胞、肿瘤细胞和免疫细胞, 进行局部免疫调节, 从而更有效地治疗癌症和预防全身免疫毒性. 本文主要对免疫治疗的现状以及利用纳米技术提高免疫治疗效果的研究进行了总结, 并对纳米技术在免疫治疗中应用的挑战进行了分析与展望.

关键词 免疫治疗, 纳米技术, 癌症, 肿瘤微环境, 临床转化

癌症是一类严重威胁人类生命健康的重大疾病. 尽管人们对癌症潜在病因的探索已经取得了极大的进展, 但是如何让患者获得对癌症持久抵抗的能力, 仍然是肿瘤临床治疗中存在的一个主要问题. 癌症是细胞自主性疾病, 具有复杂性和异质性, 肿瘤微环境中的细胞外基质和基质细胞, 以及浸润其中的免疫细胞, 都会抑制或促进疾病的发生、发展和侵袭[1]. 因此, 在癌症治疗中, 对机体免疫系统和肿瘤微环境的调节显得尤为重要.

免疫治疗是继手术切除、化疗和放疗后, 一种通过激发免疫功能或解除免疫抑制来抵抗癌症的新兴癌症治疗策略. 对一些不能进行手术切除或转移性肿瘤进行免疫治疗, 既能够有效抑制肿瘤发展, 又可以使机体产生免疫记忆, 有效抑制耐药性恶性肿瘤的增殖肿瘤并防止复发[2]. 2013年, Roche/Genetech公司肿瘤免疫学研究团队的Chen和Mellman[3]提出了“癌症免疫循环”的概念, 以简单的图绘形式说明了免疫系统识别和杀死癌细胞的正反馈路径, 包括癌细胞死亡释放肿瘤相关抗原(tumor associated antigen, TAA)、肿瘤抗原呈递、启动和激活免疫细胞, 以及T细胞通过体液循环浸润肿瘤, 最终识别并杀死癌细胞这几个部分.

目前临床中所应用的免疫治疗主要有单克隆抗体治疗和过继细胞疗法(adoptive cell therapy, ACT). 单克隆抗体可以通过阻断或刺激T细胞受体的免疫检查点以调节信号通路, 抑制或激活T细胞功能, 激活患者的免疫系统[4]. 目前, 美国食品药品管理局(FDA)批准的免疫检查点抑制剂主要有细胞毒性T淋巴细胞抗原4(cytotoxic T-lymphocyte antigen 4, CTLA-4)阻断剂的单克隆抗体药物Ipilimumab, 转移性黑色素瘤和肾小细胞癌等癌症[5], 以及阻断程序性死亡受体1(programmed death-1, PD-1)及PD-1配体(PD-1 ligand, PD-L1)的单抗药物, Pembrolizumab和Nivolumab也在治疗晚期黑色素瘤[6]和非小细胞癌患者[7]的身上得到应用. ACT是通过在体外改造免疫细胞增强其免疫效力, 再将其输回患者体内, 从而产生抗肿瘤免疫的治疗方法[8]. 肿瘤浸润淋巴细胞(tumor infiltrating lymphocytes, TILs)疗法是利用患者手术切除的肿瘤中提

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取的TILs, 在体外进行选择和扩增, 在极度难治的黑色素瘤的临床试验中极大地提高了肿瘤控制率[9~11]. 嵌合抗原受体-T细胞(chimeric antigen receptor T-cell immunotherapy, CAR-T)疗法是一种将衍生自抗体的靶向细胞外抗原复合物转移到T细胞[12]或其他免疫细胞中[13]的极具潜力的ACT疗法. 目前主流的第二代CAR能够同时激活第一信号(抗原-主要组织相容性抗原(major histocompatibility complex, MHC)复合物)和第二信号(共刺激分子的结合)[14], 虽然对实体瘤的治疗效果并不理想, 但在治疗淋巴瘤[14]和B细胞白血病[15]的临床试验中均有不错的疗效. 然而, 这两种类型的疗法均具有明显的免疫相关副作用[16]. 临床前试验表明, 免疫检查点阻断会在瘤内病毒治疗和冷冻消融治疗过程中引发全身毒性反应[17]和严重的免疫相关炎症反应[18]. 而为了满足ACT疗法所需的细胞数量和质量, ACT疗法的治疗周期相对较长且成本高昂, 且易引发免疫毒性[19~21].

随着现代科学技术的进步, 纳米技术的应用极大地推动了癌症临床诊断和治疗的发展. 医学研究者们利用各种纳米结构的载体, 不仅提高疏水性药物的溶解度和生物利用度, 能够改善药物代谢动力学性质, 避免药物在体内循环中降解, 实现控制释放, 还可以将分子探针、细胞抑制剂、细胞毒性药物等一种或多种化合物递送到病灶部位[22]. 同时, 纳米颗粒可以借助不同的靶向分子或修饰连接各种配体(抗体或适配体)[23]或通过肿瘤血管结构异常所导致的增强的通透和滞留(enhanced permeation and retention, EPR)效应[24], 使得包载的药物在肿瘤局部富集, 进而开展检查点抑制剂、细胞疗法、溶瘤病毒和癌症疫苗的研发和临床转化.

因此, 本文将总结近年来基于纳米技术对免疫治疗增效研究的进展, 并分析免疫治疗联合纳米技术发展中存在的挑战与展望.

1 基于纳米载体的癌症疫苗

癌症疫苗是利用TAA、免疫细胞或免疫分子调节免疫系统、激活特异性免疫应答的一种治疗方式, 在单次给药后能够模拟初次和增强注射的免疫应答效果[25], 是免疫治疗中一个越来越重要的研究领域.

根据物理学和生物学特性, 二级淋巴器官, 例如脾脏的窗孔结构中有许多树突状细胞(dendritic cell, DC), 纳米颗粒的尺寸和病原体十分接近, 能够很容易被抗原呈递细胞(antigen presenting cell, APC)摄取[26], 并且二级淋巴器官不具有进入具有实体肿瘤特征的物理屏障, 例如间质压升高和异常密集的细胞外基质而引起的弥散障碍[27], 纳米疫苗可以靶向“癌症免疫循环”中相对应的激动或抑制因子, 共同递送抗原和免疫激活剂到所需的特异性免疫细胞中[2], 并产生肿瘤特异性T细胞, 通过TAA在APC的交叉呈递和捕获来激活T细胞[28], 或直接激活TAA特异性T细胞[29], 从而引发获得性免疫应答. 其中, 纳米颗粒可以保护有效包封抗原和联合的佐剂, 使其免受生物环境的影响[30], 可以主动地促进DC的抗原呈递功能, 增强对抗原的免疫应答[31], 还可以减少肿瘤对免疫的耐受或屏蔽, 让肿瘤细胞无法躲避免疫监视而是让APC或其他吞噬细胞对肿瘤重新识别, 从而诱导T细胞攻击肿瘤细胞[32]. 这一方式在过去几十年的癌症免疫治疗研究中一直处于前沿, 从注射抗原疫苗到利用纳米载体同时注射佐剂和抗原的转变, 是目前免疫治疗的一大发展.

1.1 靶向DC的纳米疫苗

DC是获得性免疫应答的关键引发剂, 因此也是抗肿瘤纳米药物的主要作用点. 如果共同递送游离的抗原和佐剂, 会导致抗原被递送至DC而佐剂被递送至其他细胞, 不能共同作用[33], 而在没有佐剂的情况下, 递送抗原会诱导免疫耐受, 从而抑制抗肿瘤反应. 同时包裹抗原和佐剂的纳米颗粒可以实现两种化合物对DC的共同递送, 能够有效地提高对抗原特异性$CD8^+$细胞毒性T细胞(cytotoxic T lymphocyte, CTL)的激活. 在DC中的粒子持续释放抗原, 可以增强DC对抗原的呈递, 进一步激活CTL[34]. 另一方面, DC靶向的细胞亚群对于定义免疫反应的诱发和调节也至关重要. 在摄取了Toll样受体(toll-like receptor, TLR)激动剂时, 浆细胞样DC可以从免疫耐受状态转化为激活的先天性免疫状态[35]. 为了实现获得性免疫反应, 利用偶联CD40抗体的聚乳酸-羟基乙酸共聚物(PLGA)纳米颗粒靶向DC表面的C型凝集素受体, 产生免疫应答, Cruz等人[36]发现靶向DC特异性细胞间黏连分子3结合非整合素(Dendritic cell specific intracellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin, DC-SIGN), DC内吞受体(DEC-205), DC/自然杀伤凝集素受体1(DC NK lectin group receptor-1, DNGR-1)和兰格素(Langerin), 有利于增强$CD8^+$ T细

胞免疫应答；而靶向DC免疫受体2(DC immunoreceptor 2, DCIR2)，有利于增强$CD4^+$ T细胞和B细胞体液免疫应答；纳米疫苗也可以通过靶向多个DC亚群使其效力最大化，从而同时诱导细胞和体液免疫应答[37]. 另外，TAA是免疫系统识别和攻击的靶点，化学合成的肿瘤相关抗原多肽较自体分离纯度更高，然而体内稳定性低[38,39]. 张志平教授研究团队[40]构建了以红细胞膜为载体的黑色素瘤相关抗原多肽递送系统，利用红细胞膜的免疫特性，实现对多肽抗原在体内的保护作用，促进DC成熟和T细胞活化，进而用于肿瘤的预防和治疗.

1.2 靶向T细胞的纳米疫苗

除了向DC传递关于特异性和活性的信息，针对肿瘤特异性T细胞作用的纳米疫苗会产生对癌症更有效的免疫治疗. 一些研究者在考虑设计可以直接交叉引发抗原特异性的$CD8^+$ T细胞的人造抗原呈递细胞(aAPC). 合成的aAPC是结合了T细胞活化所需蛋白质的颗粒，如结合MHC-表位或CD3抗体(第一信号)和CD28抗体(第二信号). 研究者通过改变纳米颗粒的形状和几何结构，发现aAPC活性与长宽比相关，从机制层面上讲，$CD8^+$ T细胞优先迁移到aAPC的长轴上，并且这种延长的接触长度促进了T细胞的增殖，能够更好地预防肿瘤的发生[41].

APC与抗原交叉呈递到淋巴结的有效输送是纳米疫苗激活T细胞的关键因素. 因此，Luo等人[42]设计了一种由酸响应聚合物(PC7A)组成的纳米颗粒与卵清蛋白(ovalbumin, OVA)模式抗原组成的简单复合物疫苗，不仅能够让抗原在APC中的胞质输送、抗原的呈递和淋巴结输送更加高效，还可以通过激活Ⅰ型干扰素刺激因子(stimulator of interferon gene, STING)产生Ⅰ型干扰素(Interferon, IFN)和IFN-γ，从而激活OVA特异性的CTL(图1). 研究结果表明，PC7A纳米疫苗对黑色素瘤、结肠癌和人乳突状瘤病毒E6/E7瘤的模型小鼠都有显著的肿瘤抑制效果，平均延长了60天的生存期.

此外，精准治疗是抗肿瘤免疫治疗的一个重要发展方向. 基于新兴的肿瘤外显子测序技术，密歇根大学Moon课题组[43]设计了携带肿瘤细胞特有变异抗原的纳米盘疫苗sHDL，能够刺激机体产生特异性识别这些肿瘤变异抗原的CTL，从而抑制肿瘤生长(图2). 实验结果显示，和水溶性疫苗相比，在给予黑素瘤B16F10和结肠癌MC-38的荷瘤小鼠sHDL后，小鼠血液中平均有47倍的CTL被激活并表达肿瘤抗原特异性的受体，并且多表位的疫苗能够产生更广泛的T细胞免疫应答，同时，sHDL疫苗和免疫检查点阻断剂的联用也可以显著放大抗癌效果，防止肿瘤的复发.

传统的癌症疫苗在向免疫系统的APC输送和T细胞激活中仍然面临许多技术性挑战，例如靶向性差、血浆清除率高等. 而基于纳米颗粒的癌症疫苗的研发，使得疫苗在体内系统性给药后，能够优先以DC或T细胞作为目标，从而实现持久、有效的全身免疫.

2 基于纳米技术的新型免疫治疗

2.1 新型ACT疗法

除了纳米颗粒之外，也可以植入聚合物支架来治疗癌症，且支架也提供了类似传统ACT的实用性和功能性的优点. 传统ACT需要首先分离出免疫细胞(DC或T细胞)进行离体操作，然后重新输回给患者，而植入或注射纳米聚合物或水凝胶支架用来产生模式化、定制化的局部微环境，可以在原位产生共定位炎症因子、肿瘤抗原和免疫信号[44].

例如，通过将结合粒细胞-巨噬细胞集落刺激因子(granulocyte-macrophage colony-stimulating factor,

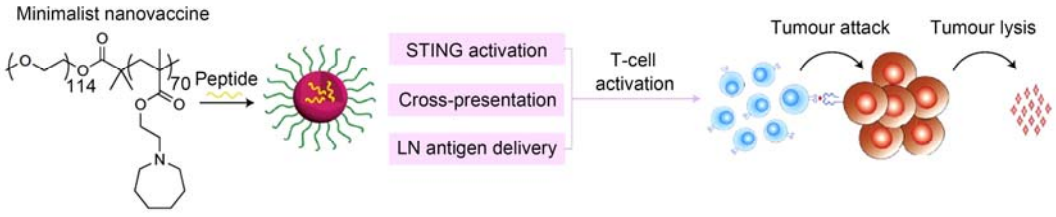

图 1 (网络版彩色)PC7A纳米疫苗作用示意图[42]

Figure 1 (Color online) Schematic graph of the minimalist design of the PC7A nanovaccine[42]

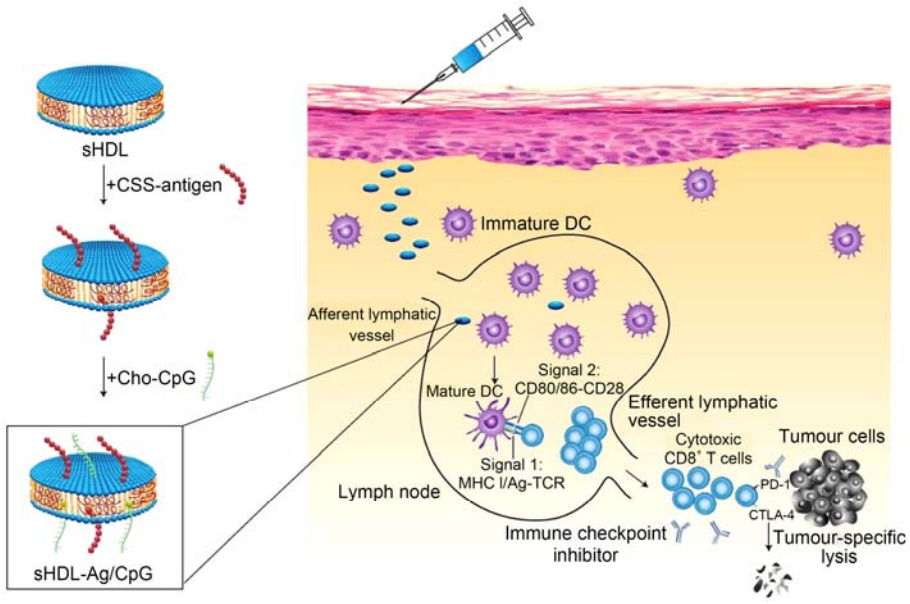

图 2 (网络版彩色)癌症疫苗sHDL纳米盘设计和作用示意图[43]

Figure 2 (Color online) Schematic illustration of the design for personalized sHDL nanodisc cancer vaccines[43]

GM-CSF)和CpG寡核苷酸(TLR9激动剂)的多孔纳米PLGA支架植入到小鼠皮下，可以促进DC的招募和激活，消除局部和远端的肿瘤，并且能够在微环境中持续进行抗原呈递和佐剂信号传导，诱发持续的免疫反应[45]. Mooney课题组[46]也设计了一种可注射的自组装三维(3D)支架，高长宽比的介孔纳米硅棒(mesoporous nano-rods, MSRs)注射到小鼠皮下后可以自组装形成大孔结构，为DC提供了理想的微环境，使DC随后流向淋巴结并引发免疫应答(图3).

由于实体瘤具有免疫抑制性的肿瘤微环境，会阻碍ACT疗效，支架就可以用来在将CAR引入T细胞时提高ACT功能. 肿瘤切除后，将T细胞置入可降解纳米聚合物的支架中，可以使得肿瘤切除部位的T细胞在局部不断扩增和激活，从而提高抗肿瘤疗效[47]. 相较于全身或局部注射游离的T细胞，T细胞支架也适用于不能手术或不能被手术完全移除的肿瘤，可以减少残留的癌细胞和癌症复发，也为靶向递送细胞、小分子或生物制剂提供了概念性的佐证.

对于血液恶性肿瘤，无法通过ACT支架的方法来实现局部微环境的治疗. 而纳米技术可以不通过植入ACT支架，直接在体内将药物递送至血液循环中的T细胞并对其进行调节和改造，这不仅优化了

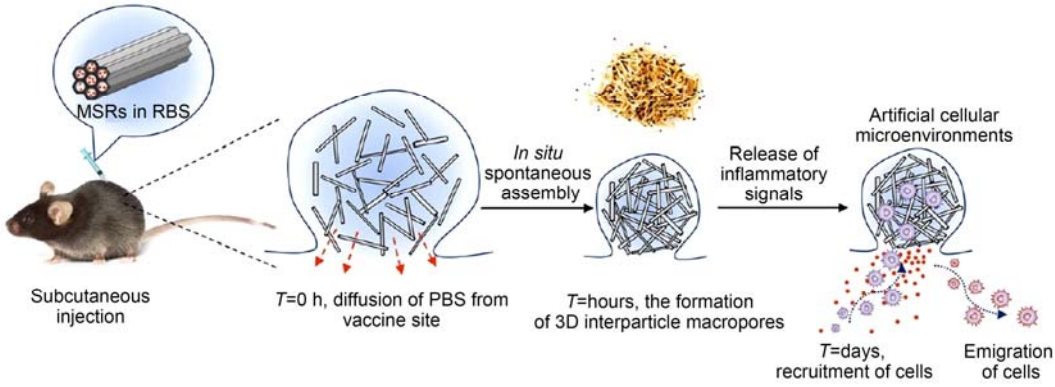

图 3 (网络版彩色)MSRs免疫支架作用过程示意图[46]

Figure 3 (Color online) Schematic graph of spontaneous assembly of MSRs[46]

ACT疗法，并且比上述ACT支架更易于临床转化. Stephan课题组[48,49]通过将包载了药物、单克隆抗体(monoantibody, mAb)、配体-抗体片段或佐剂的纳米颗粒连接到T细胞表面，向细胞提供持续的类自分泌刺激信号，避免了转移T细胞的活力和功能的快速下降，增强了ACT的抗淋巴瘤效果，并通过引发全身免疫调节消除远端肿瘤细胞. 这种方法操作简单、可推广，能使佐剂的全身毒副作用最小化，且不需要为每位患者进行细胞离体操作. 另一方面，由于产生大量肿瘤特异性T细胞所需的体外扩增方法复杂且十分耗时，Stephan课题组[50]又设计了一种可以改造循环T细胞的方法，使其具有识别肿瘤的能力(图4). 利用负载DNA的纳米颗粒有效地将白血病靶向的CAR基因引入小鼠T细胞核，对T细胞进行快速基因编辑，使白血病小鼠的生存期平均延长了58 d，同时也避免了并发症. 这些聚合物纳米粒子性质稳定，易于储存，成本低，较CAR-T疗法更适合广泛应用.

2.2 调节免疫细胞分化的免疫治疗

免疫治疗不仅可以通过激活免疫应答，还可以通过抑制或防止免疫抑制作用来实现. 例如，肿瘤相关巨噬细胞(tumor-associated macrophages, TAMs)有两种细胞分型，抗肿瘤的M1型和抑制免疫的M2型，因此，也有研究学者针对TAMs的细胞分型转化进行了研究. 氧化铁纳米粒子ferumoxytol是FDA批准的用于治疗慢性肾脏疾病导致的缺铁性贫血的药物，还可以用作肝脏、脾脏、淋巴等器官的磁共振成像(magnetic resonance imaging, MRI)造影剂. Link课题组[51]利用ferumoxytol递送铁来调节免疫细胞内的铁含量，并发现ferumoxytol能使TAMs从M2型向M1型转化，而M1型的巨噬细胞能够产生一系列炎症因子及活性氧族(reactive oxygen species, ROS)，进而激活促凋亡基因半胱氨酰天冬氨酸特异性蛋白酶3(caspase-3)的活性，诱使肿瘤细胞凋亡，从而有效抑制早期乳腺癌细胞的生长以及肺癌细胞向肝和肺的转移.

2.3 利用纳米系统递送酶的免疫治疗

除了递送小分子、寡核苷酸、抗原和细胞因子，也可以利用纳米颗粒在体内进行酶的递送. 中性粒细胞是术后感染期间的第一道防线，而中性粒细胞胞外陷阱(neutrophil extracellular traps, NETs)，一种细胞外DNA结构，在血管内形成时可以隐蔽循环肿瘤细胞(circulating tumor cells, CTCs)，从而协助肿瘤转移，进而促进癌症的发生、发展. 在Park等人[52]的研究中，通过包载DNase或中性粒细胞弹性蛋白酶的PLGA纳米颗粒，对NETs进行抑制和催化降解，相较于用游离的DNase消化NETs，能够更好地抑制肿瘤转移.

2.4 靶向肿瘤细胞和巨噬细胞的免疫治疗

纳米技术还可以同时实现对肿瘤细胞和免疫细胞的靶向治疗，不仅能特异性杀伤肿瘤，同时也能够降低免疫相关毒性. Kim课题组[53]设计了多价双特异

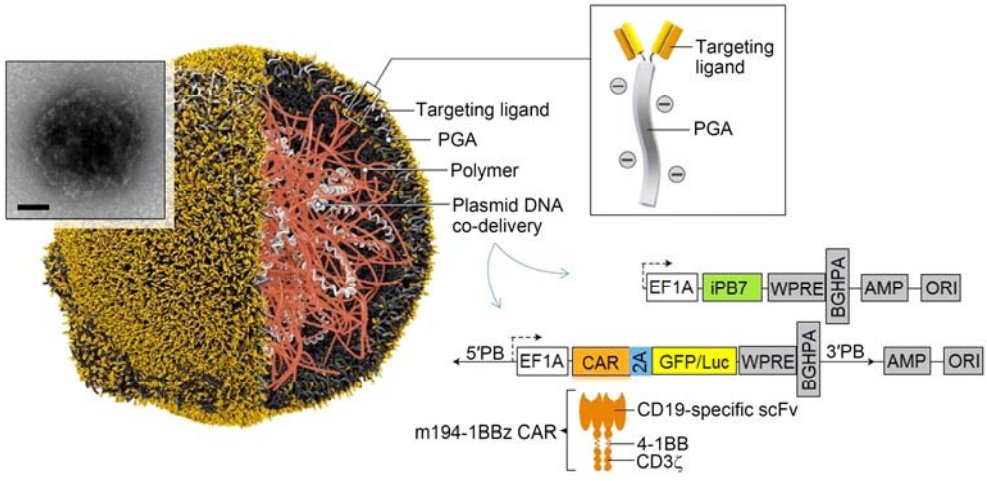

图 4 (网络版彩色)T细胞靶向DNA纳米粒子示意图[50]

Figure 4 (Color online) Schematic graph of the T-cell-targeted DNA nanocarrier for lymphocyte-programming[50]

性纳米生物共轭偶联物(mBiNE), 靶向人乳腺癌癌细胞SK-BR-3阳性表达的人表皮生长因子受体2(human epidermal growth factor receptor-2, HER-2)的同时, 表达钙网蛋白(calreticulin, CRT)介导的促吞噬作用信号(图5). 纳米粒子先识别肿瘤细胞表面的HER-2并与之结合, 随后协助巨噬细胞识别肿瘤细胞, 从而使巨噬细胞攻击肿瘤细胞, 刺激先天性免疫并引发获得性免疫反应, 产生抗HER-2表达的持久抗肿瘤免疫应答.

2.5 癌症的联合免疫治疗

目前癌症治疗主流的方法包括手术切除、化疗和放疗, 以及消融治疗、光疗等, 都对正常细胞有一定的杀伤作用, 且会致使残留的肿瘤细胞通过伤口处进入全身血液循环, 预后差、易复发. 而单独的免疫治疗对原发性实体瘤的杀伤作用具有局限性. 因此, 利用纳米颗粒进行癌症的联合治疗, 可以协同增效, 互相弥补. 装载免疫调节剂的纳米颗粒能够调节制剂的生物分布[54], 降低全身毒性, 改善那些极为有效的免疫刺激分子的治疗窗, 同时仍能促进全身的抗肿瘤免疫反应, 防止经手术、化疗等治疗后的癌症转移和复发[55].

临床研究结果表明, 将免疫治疗与放疗联合可以提高抗癌效果. 放疗辐射诱发的"远端效应", 即放疗杀伤的肿瘤细胞会释放TAA和促炎性因子, 促进免疫细胞吞噬并呈递抗原, 诱导肿瘤特异性免疫反应, 在实现强大的局部抗肿瘤作用的同时, 也可以产生全身性抗肿瘤免疫应答, 促进远端转移灶的消退, 在病人中长期的免疫应答中十分重要[56]. Wang课题组[57]制备出了可以抓住抗原的纳米颗粒(AC-NP), 这种AC-NP可以吸附肿瘤来源的特异性抗原(tumor-derived protein antigens, TDPAs), 并且能够被吞噬细胞识别、捕获, 可以一定程度上增强放疗抗肿瘤免疫治疗效果以及放疗的远端效应(图6). 将放疗杀伤的B16F10肿瘤细胞裂解液与AC-NP共同孵育, AC-NP的颗粒表面电位和粒径都会发生变化, 证明吸附了相关蛋白. 经过质谱定量分析发现, AC-NP能够吸附较多的TAA和促炎因子, 主要为损伤相关分子模式蛋白(damage associated molecular patterns, DAMPs), 可以增强免疫反应. 体内研究中, 对接受PD-1抗体治疗的患B16F10黑色素瘤小鼠的原发灶进行放疗, 随后注射AC-NP, 发现疗效明显增强且荷瘤小鼠的生存期延长了约60%, 并且3个月后对这些小鼠再次接种B16F10细胞也不会长出肿瘤, 有效防止了肿瘤

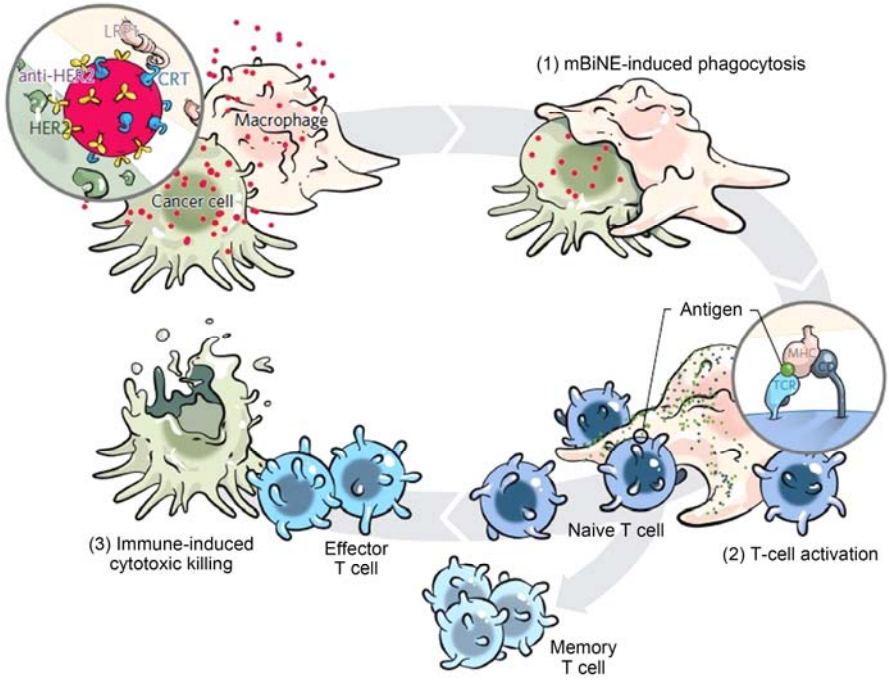

图 5 (网络版彩色)mBiNE的设计及其提出的作用机制示意图[53]

Figure 5 (Color online) Schematic graph illustrating the design of the mBiNE and the proposed action mechanisms[53]

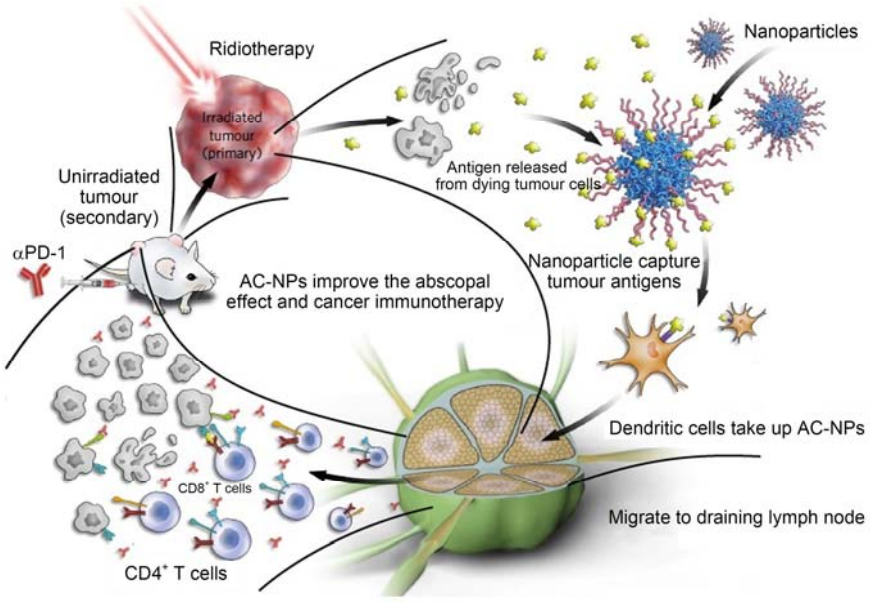

图 6 (网络版彩色)放疗的远端效应及AC-NP免疫联合疗法示意图[57]

Figure 6 (Color online) Schematic graph of applying AC-NPs to improve cancer immunotherapy[57]

复发[57].

光动力疗法(photodynamic therapy, PDT)是利用光敏剂在特定波长的激光照射后, 引发光化学反应产生ROS, 治疗肿瘤的一种新方法. 而PDT产生的ROS可以破坏肿瘤细胞并使其暴露更多的TAA, 从而促进被DC的识别和捕获. Lin课题组[58]设计了包载光敏剂的极小纳米光敏药物(20~40 nm), 不仅可以在体内长循环, 同时可以让一些对免疫治疗反应差的癌症也能产生免疫应答. 纳米颗粒被肿瘤细胞吞噬后, 经过激光照射, 产生的ROS促使肿瘤细胞死亡, 产生的TAA被DC的识别和捕获. 这意味着即使在肿瘤组织周围T细胞数量不足的情况下, 免疫系统仍然可以发挥作用. 另外, 苏州大学Liu课题组[59]联合光热疗法(photothermal therapy, PTT)、免疫佐剂R837(TLR7受体的小分子激动剂)、免疫检验点抑制剂(CTLA-4抗体), 设计了纳米颗粒PLGA-ICG-R837, 对小鼠乳腺癌和结直肠癌进行了免疫治疗. 肿瘤在近红外光激发PLGA-ICG-R837所产生的PTT作用下, 裂解并释放TAA. 同时, 由R837和CTLA-4抗体协同增强机体免疫系统, 促进DC在肿瘤部位的抗原呈递功能, 继而显著提升$CD8^+$ CTL和$CD4^+$效应T细胞水平. 因此, 该联合免疫疗法不仅能够对原发性肿瘤进行杀伤, 还可产生免疫记忆效应, 防止肿瘤复发[59].

基于纳米技术的新型免疫疗法, 还包括模拟肿瘤相关中性粒细胞成分和功能的纳米颗粒(NEs)对术后脑胶质瘤的免疫治疗[60]、在酸性条件下可与DC内体膜融合的MGlu-Dex脂质体对抗原交叉呈递的调节[61]等, 尽管仍处于概念验证的初级阶段, 但是针对传统免疫疗法中存在的不足和局限, 提出了具有可行性的解决办法, 有巨大的发展潜力.

3 总结与展望

为了通过纳米技术有效提高肿瘤免疫疗法效果, 我们不仅需要了解免疫系统是如何感知和应对抗原和肿瘤威胁的, 也要加深对肿瘤细胞逃避免疫监视的机制, 以及抗肿瘤免疫反应中可能产生的副作用的理解[62]. 除了需要增强对肿瘤微环境中浸润的细胞和生化成分的认识之外, 还需要增加对肿瘤物理微环境及肿瘤引流淋巴结(tumor-draining lymph node, TDLN)的考虑[63]: 细胞外基质作为免疫抑制的物理介质, 会阻止免疫细胞渗透到肿瘤核心内[64], 而免疫工程可以通过改变肿瘤周围的细胞外基质来改变肿瘤的物理微环境[65], 促进免疫细胞的渗透. 然而, 如何让纳米技术增效的免疫疗法走向临床, 实现广泛应用, 还有许多的问题与挑战.

首先, 对于基于纳米技术的药物递送, 需要特别考虑纳米颗粒的尺寸和载体材料. 直径为10~40 nm的小纳米颗粒更容易在淋巴结中富集, 更可能被

APC摄取, 但是有效载药量低, 且在无佐剂的情况下, 无法促进抗原呈递并激活免疫反应[66]; 另外, 虽然靶向递送纳米疫苗可以利用相当低剂量的免疫刺激分子来实现同样程度的免疫应答, 但是这些纳米载体的材料本身可能就会引起宿主反应. 因此在载体材料的选择上, 需要研究更加安全的参数设置, 如评价血清中I型干扰素和白细胞介素-6(Interleukin, IL-6)的水平等[67], 并且根据其生物降解性、免疫介导和非免疫介导的毒性以及稳定性建立标准化生产[25].

另一方面, 基于纳米技术的癌症免疫治疗涵盖了药物细胞内运输、纳米载体构建, 及其与免疫系统作用、影像和医学转化所需的各个方面[68,69], 但是免疫耐受往往被忽视. 在免疫治疗的过程中, 克服肿瘤产生的T细胞抑制信号转化生长因子-β(transforming growth factor, TGF-β), 或由长期活化的T细胞表达的CTLA-4, 都可能导致免疫耐受问题[70]. 因此, 为了避免严重的不良反应, 直接靶向T细胞方法的开发和临床转化, 需要利用纳米技术高效包封免疫刺激分子等药物, 调整抗肿瘤的抗原特异性和非抗原特异性免疫激活剂的组合, 对这些T细胞特异性靶向和治疗, 诱导肿瘤内的炎症以招募免疫细胞, 从而消除免疫耐受.

其次, 免疫工程需要有更多新的检测工具和形式, 从而确定最有可能从T细胞刺激治疗中受益的患者, 同时避免不必要的免疫毒性和不良反应[71]. 例如, 用聚焦离子束(focused ion beam, FIB)成像分析在肿瘤微环境和血液循环中的多种不同细胞类型, 用放射性抗体纳米探针联合正电子发射计算机断层扫描(positron emission computed tomography, PET)影像诊断对肿瘤进行精确分型, 或是用Luminex液相芯片定量可溶性介质的刺激/抑制程度. 这些基于纳米技术的方法, 和流式细胞术、酶联免疫吸附测定(enzyme-linked immuno sorbent assay, ELISA)等传统检测方法相比, 可以建立、比较更多的变量和参数, 并且能够协调更加复杂的免疫系统, 从而更有效地提高免疫治疗效果[72].

此外, 如何制造这些纳米器件可能是临床转化所面临的最大障碍, 可控的生产可以最大限度地减少纳米粒子的多分散性, 优化制剂的制备和生产工艺至关重要. 例如, 3D打印再生医学领域的一次革新, 在癌症免疫工程方面也有重大意义, 会使得特定细胞因子、免疫细胞和细胞基质的定位更加精准[73], 可以被应用到疫苗和支架的生产或创建可植入的人造的、具有相应特定免疫细胞的限定区域的三级淋巴结构[74].

免疫疗法不仅具有治愈癌症的潜能, 也是解析基础生物学非常有价值的工具. 利用纳米技术可以大幅提高肿瘤疫苗效力, 提高免疫疗法的疗效, 实现联合治疗, 同时克服免疫疗法本身缺陷, 减轻副作用. 随着纳米技术的不断发展, 免疫学理论的不断完善, 纳米技术联合免疫治疗在肿瘤的诊断、治疗以及预防等方面将会有更重大的突破. 因此, 联合纳米技术的癌症免疫工程是一个方兴未艾的领域.

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Summary for “利用纳米技术提高癌症免疫治疗效果的研究进展”

Advances in enhancing cancer immunotherapy by nanotechnology

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To date, the preliminary research for understanding the underlying cancer etiology has made great progress. However, due to the heterogeneity of cancer and the complexity of tumor microenvironment, as well as the evasion of tumor cells from the immune surveillance, only a few people are rehabilitated with the eradicative cancer therapy so far. In the recent few years, the immunotherapy to stimulate the immune response or inhibit the immunosuppression against cancer has achieved unprecedented efficacy in refractory patients. There are two main streams of the immunoregulation for cancer treatment, immune checkpoint monoclonal antibodies (mAbs) and adoptive cell therapy (ACT). Nowadays, three kinds of checkpoints-blockade inhibitors, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1)/PD-L1 mAbs, have been approved by the United States Food and Drug Administration (FDA) to treat several types of cancer in clinic, including melanoma, non-small cell lung cancer, renal cell carcinoma and leukemia. Although the current cancer immunotherapy can successfully lead to durable outcomes, the therapeutic effect is still limited and patients are even suffering from the adverse reactions. Thus, it is urgent to develop a localized and efficient immunoregulation strategy against cancer.

Encouragingly, nanotechnology is a promising tool to optimize the tumor co-localization, bio-distribution and pharmacokinetics for the molecular probes, cytotoxic pharmaceuticals, immunostimulators, various ligands (e.g., antibodies or aptamers) and other biological agents. The conventional cancer treatment with nanoparticle administration is to increase the cancer cellular uptake by enhanced permeation and retention (EPR) effect, which is a kind of passive accumulation due to the leaky vasculature but is proven somewhat elusive. In contrast, leukocytes of immune system *in vivo* can actively trace through chemokine gradients to the tumor cells, and then recognize and kill them by binding to the the tumor specific antigens. Besides, secondary lymphoid organs do not exhibit physical barriers as tumor microenvironment. Owing to the similar size to pathogens, nanoparticles are also able to accumulate in these fenestrated structures and readily uptaken by antigen-presenting cells (APCs), such as dendritic cells (DCs) and other natural phagocytes. The most typical application of nanotechnology on immunotherapy is cancer vaccines, and the nanoparticles are serving as antigen reservoirs to mimic both prime and boost injections after a single administration. The nanovaccines are able to induce robust DCs or $CD8^+$ T cells response and confer cross-priming efficacy observed in preclinical animal models, which is an important breakthrough in the development of the soluble vector-free cancer vaccines. In addition, nanotechnology-mediated immunotherapy can enhance the treatment efficacy in combination with other approaches, such as surgery, radio-/chemo-therapy or ablation therapy. Meanwhile, there are numerous novel nanotechnology-based strategies for the regulation of both immune system and tumor microenvironment. For example, polymer scaffold can be implanted *in vivo* to establish a condition for the T-cell engineering; nanocarriers are intended to increase the intercellular avidity by targeting the circulating T cells, macrophages and cancer cells; drug-loaded nanoparticles will deliver DNase to destroy the neutrophil extracellular trappings (NETs). Looking ahead, the field of enhancing immunotherapy by nanotechnology will be developed to permit the analysis of multiple cell subtypes or immune cell activation state. What is more important, the researchers should take the responsibility to place an emphasis on the study of profound theory for immunology, innovative biomaterials for nanoparticles and clinical translation for engineered immunotherapeutic products. Hence, the nanotechnology-enhanced immunotherapy will enable the evaluation of treatment suitability and consequently improve the personalized immunotherapy. In conclusion, the concentrated immune response realized by nanotechnology can not only lower the drug dose and improve the efficacy, but also prevent the systemic toxicity in patients. It will definitely become the mainstay in immunotherapy in clinic. Therefore, this review is going to summarize the current situation of immunotherapy, and to analyze the opportunities, challenges and development of nanotechnology-enhanced immunotherapy in the future.

immunotherapy, nanotechnology, cancer, tumor microenvironment, clinical transformation

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