中国激光

近红外光免疫治疗策略靶向肿瘤微环境的研究进展

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摘要 近红外光免疫治疗(NIR-PIT)是一种结合抗体和光吸收剂IRDye700DX的新型肿瘤疗法，它既能够激活局部免疫效应，又能够增强肿瘤靶向性，已在不同肿瘤类型的治疗中显示出巨大的应用潜力。大量研究已经证实肿瘤微环境是导致肿瘤不断发展的重要原因，因此NIR-PIT中的光免疫偶联物靶点也已经扩展至肿瘤微环境中非肿瘤细胞的表面蛋白中。利用NIR-PIT局部消除肿瘤微环境中某些具有特定标志物的免疫抑制细胞、血管或肿瘤成纤维细胞，将解除免疫抑制，最大效率发挥机体的正常免疫功能，取得最佳的疗效。主要综述了NIR-PIT的治疗策略和靶向肿瘤微环境的最新研究进展。

关键词 医用光学；恶性肿瘤；近红外光免疫疗法；抗体-光吸收剂偶联物；免疫治疗；近红外光

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1 引 言

半个世纪以来，传统肿瘤疗法的三大支柱是手术治疗、化疗和放疗。然而，在这些治疗过程中不可避免地会对包括免疫细胞在内的正常细胞造成严重损害，不利于患者康复[1]。近些年，随着研究者们对肿瘤免疫研究的不断深入，免疫疗法可以通过调节不同免疫细胞群之间的平衡控制免疫反应，具有广阔的临床应用前景，包括使用T细胞激活型细胞因子、免疫检查点抑制剂、消耗免疫抑制细胞(例如负调节性T细胞，Tregs)和髓源性抑制细胞(MDSCs)等[2-4]。然而，这些疗法经常使免疫系统被过度激活或过度抑制，导致相关临床试验有严重的副反应，例如脱靶效应导致的免疫相关不良事件(irAEs)[5-6]。研究者们已经不断尝试了一些局部免疫调节方法并取得诸多进展，包括器官特异性基因敲除、利用纳米颗粒实现基因转移以及改良工程生物材料等等，但这些技术存在伦理等问题，导致其在临床应用中依旧存在很大障碍[1,7-8]。因此寻求既能够激活局部免疫效应，又能够增强肿瘤靶向性的治疗策略是肿瘤研究领域的热点问题。

近红外光免疫治疗(NIR-PIT)是近年来出现的一种新型肿瘤治疗方法，可以有效、特异性地根除和远处转移原发肿瘤，并抑制癌症复发[9]。NIR-PIT技术的核心是靶向肿瘤细胞表面标志物的单克隆抗体(McAb)与光激活化学物质IRDye700DX(IR700)所形成的抗体-光吸收偶联物(APC)，因此NIR-PIT本质上是一种光靶向治疗与免疫治疗相结合的技术。在690 nm近红外光(NIR)激发下，APC与肿瘤细胞高度选择性地结合，并且发生强烈的抗肿瘤免疫反应[10](在几分钟内细胞迅速肿胀、起泡、膜破裂，最终死亡)。与传统光动力疗法(PDT)相比，NIR-PIT的优势在于它不依赖于活性氧(ROS)的产生，对周围细胞的副作用很少，具有更好的疗效[11]，并且在NIR-PIT中使用不同的靶向片段可进一步提高NIR-PIT药物的肿瘤免疫原性、靶向能力、稳定性和灵活性[9,12-14]。NIR-PIT已在不同肿瘤类型的治疗中显示出巨大的应用潜力[15]。目前一项针对局部复发性头颈部鳞状细胞癌(HNSCC)的国际III期临床试验正在进行中(LUZERA301，NCT03769506)[11,16]。本文将从NIR-PIT发挥抗肿瘤效应的机制原理、靶向肿瘤微环境的新进展、NIR-PIT与免疫检查点阻断剂(ICB)联合治疗、相关临床研究进展、治疗效果监测与评价手段，以及目前所面临的局限性和挑战等方面进行综述，为其临床研究与应用提供参考。

2 NIR-PIT发挥抗肿瘤效应的机制和特点

2.1 细胞毒性作用

NIR-PIT的特异性主要来源于APC中的McAb的高度靶向性，而细胞杀伤效应包括多种途径，其中最重要的途径为细胞毒性作用。研究者发现，在近红外光下，APC中的IR700发生光化学反应，化学性质由亲

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水性迅速转变为疏水性并在水溶液中聚集，使与之结合的细胞膜抗原发生变性，物理性诱导细胞膜损伤，从而导致细胞膜破裂、跨膜水流增加和细胞死亡（如图 1 所示）。这一新型光诱导细胞死亡方式既不依赖氧气，也不同于传统光疗中提出的氧化应激，是 NIR-PIT 的独特优势。

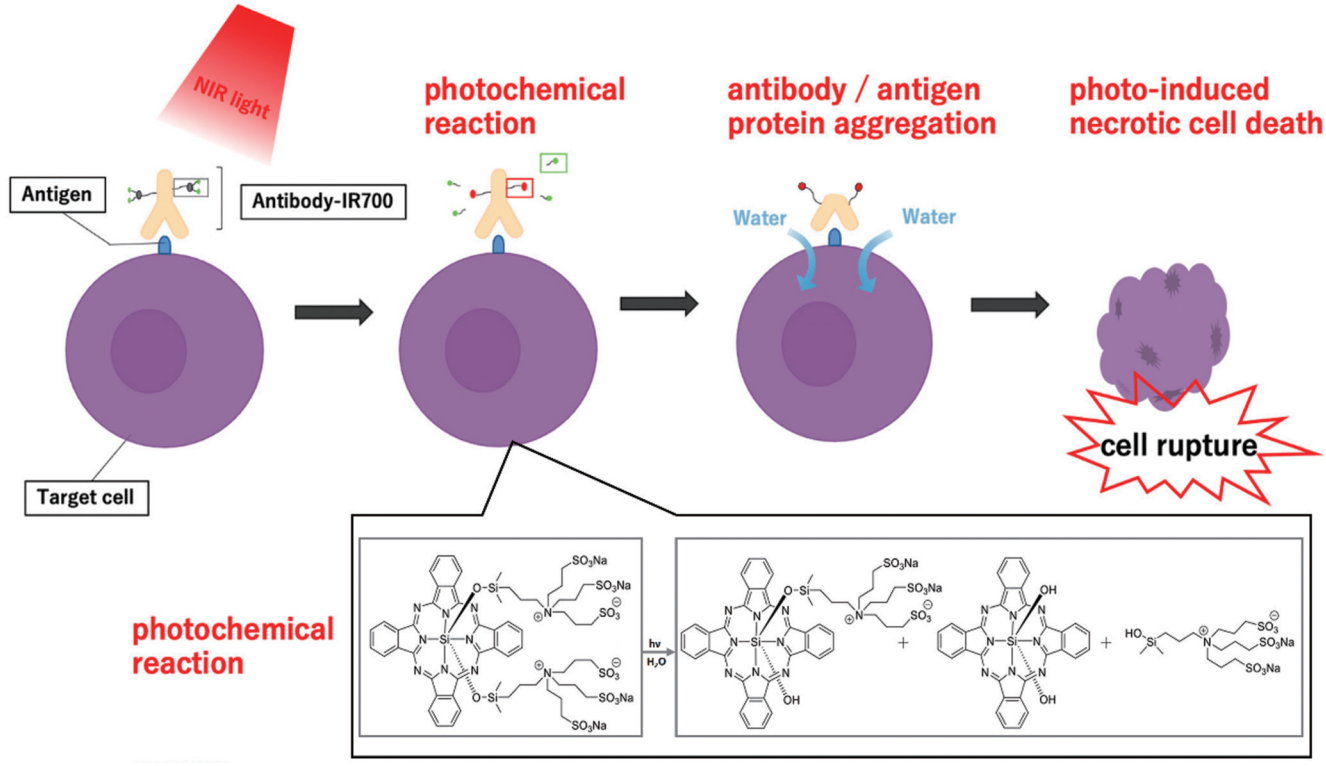

图 1　NIR-PIT 诱导细胞死亡示意图[17]

Fig. 1　Schematic diagram of cell death induced by NIR-PIT[17]

2.2　免疫原性死亡

与其他传统疗法不同，NIR-PIT 不仅不会损害宿主的免疫功能，甚至会激活多克隆肿瘤特异性免疫反应，这也是 NIR-PIT 发挥抗肿瘤效应的另一重要途径。在 NIR-PIT 过程中，被 NIR-PIT 效应直接损伤的肿瘤细胞快速释放肿瘤相关抗原（TAAs）和损伤相关分子模式（DAMPs），诱导免疫原性细胞死亡（ICD）并促进初始树突状细胞成熟，从而吞噬即将死亡的肿瘤细胞，激活宿主的抗肿瘤免疫反应。幼稚 T 淋巴细胞被诱导成为特异性 $CD8^+$ T 淋巴细胞，适应性全身免疫反应被激活以攻击其他癌细胞，这些变化进一步放大了 NIR-PIT 的治疗效果，具体过程如图 2 所示[18-21]。研究表明这种效果不局限于采用 NIR-PIT 治疗的肿瘤，还使位于身体其他部位的相似来源肿瘤停止了生长，这意味着 NIR-PIT 具有远期治疗效果，可以有效控制肿瘤的转移和复发；这也在一定程度上说明，即使靶抗原存在表达不均匀、递送不均匀或剂量不足等问题，可能导致 APC 与肿瘤细胞结合不足，但 NIR-PIT 诱导的多克隆免疫反应可消除治疗后暂时存活的肿瘤细胞，因此与传统基于抗体的治疗手段相比，NIR-PIT 具有巨大的优势和临床应用潜力。

2.3　超高通透性和滞留（SUPR）效应与结合位点屏障

NIR-PIT 的另一个独特之处在于它对血液药物输送的直接影响[22]。事实上，大多数肿瘤相关血管具有结构异常、基底膜不成熟和高通透性的特点，并且本身就存在一定程度的渗透性和滞留性增强（EPR）效应。但令人惊喜的是，NIR-PIT 通过诱导血管周围肿瘤细胞立即坏死，在血管和剩余肿瘤细胞之间形成一个允许血管进一步扩张的空间，导致该部分血管内的血容量增加和血流速度降低，进而使药物快速渗透到肿瘤细胞内部，更有利于发挥杀伤效应[23]，这种效应被称为超高通透性和滞留（SUPR）效应。

理论上，与 APC 结合的分子靶标在肿瘤组织中的表达水平越高，APC 在肿瘤部位的积累越多，NIR-PIT 所发挥的抗肿瘤作用就越强。然而，使用由高亲和力抗体组成的 APC 结合高表达水平的分子靶标并不总是有益的。有研究发现，高亲和力结合配体/抗体会在血管附近积累，可能会阻碍治疗、深入肿瘤实质，这种现象被称为“结合位点屏障”（如图 3 所示）[24]。因此，使用具有低亲和力的抗体或靶向低表达水平抗原的抗体，可能有利于实现 APC 在肿瘤实质中更均匀的分布[25]。

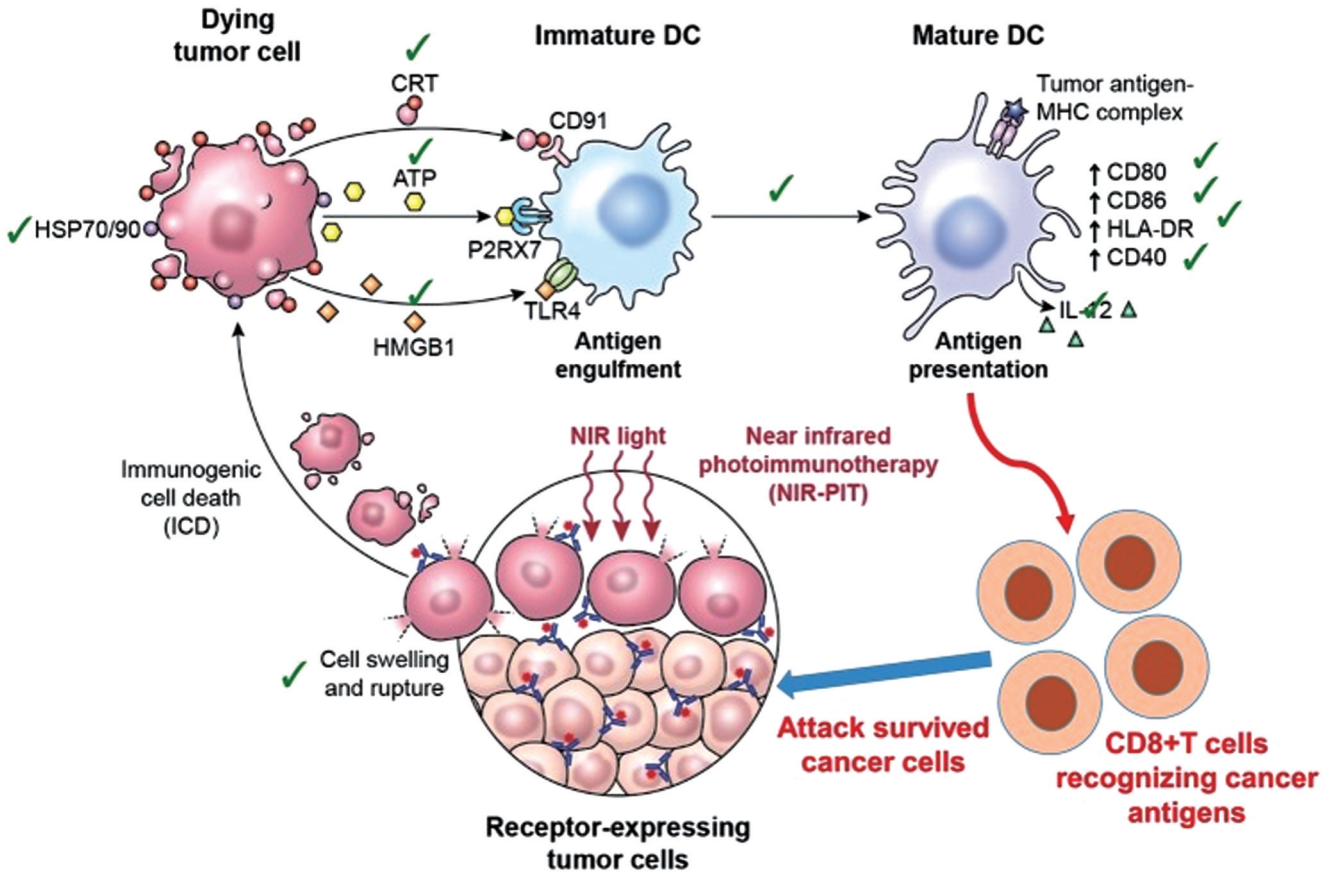

图 2　NIR-PIT 诱导的免疫原性细胞死亡的生物学机制[11]

Fig. 2　Biological mechanism of immunogenic cell death induced by NIR-PIT[11]

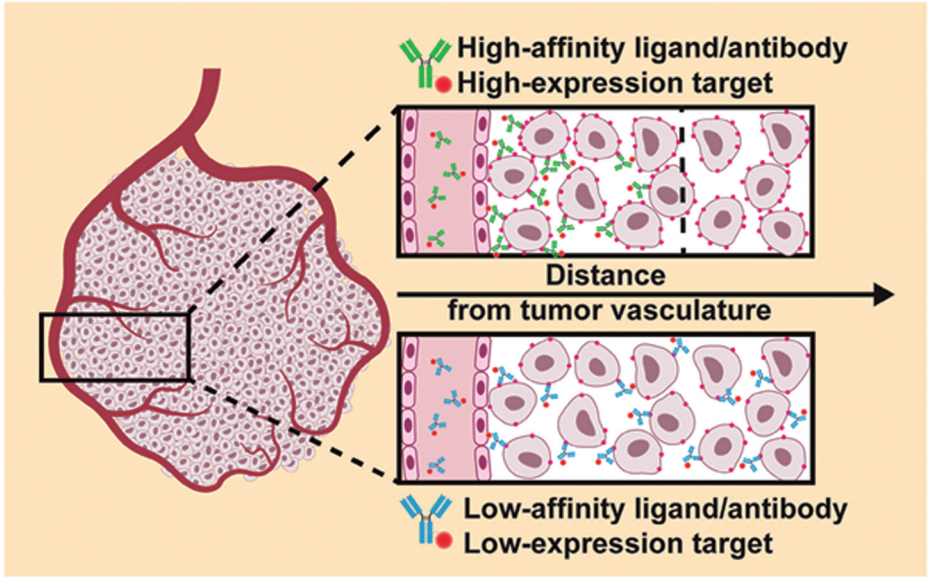

图 3　肿瘤中 APC 的结合位点屏障[22]

Fig. 3　Binding site barrier of APC in tumor[22]

3　靶向肿瘤微环境的 NIR-PIT 探索

研究表明，抑制性肿瘤微环境（TME）与肿瘤细胞的生长和发展密切相关[26]。在过去的几年中，NIR-PIT 中的 APC 靶点也已经扩展至 TME 中非肿瘤细胞的表面蛋白中[25,27]。

3.1　靶向免疫抑制细胞

肿瘤微环境中的免疫细胞功能是影响肿瘤治疗有效性的重要因素，其中 Tregs 发挥着关键的抑制作用。由于 CD25 受体在 Tregs 表面高度表达，肿瘤浸润性 $CD25^{+}$ Tregs 耗损被认为是增强抗癌免疫的重要步骤[28]。Sato 等[29]用抗 CD25 抗体-IR700 组成的 APC 选择性地消

耗浸润肿瘤微环境中的 $CD4^+CD25^+Foxp3^+$Tregs，诱导 $CD8^+$T 细胞和自然杀伤(NK)细胞重新激活，从而产生局部抗肿瘤免疫。在治疗中 Tregs 被选择性杀死，而肿瘤微环境中的非活化 $CD8^+$T 细胞和 NK 细胞不受影响。该研究结果在一定程度上证明：局部基于靶向 CD25 的 NIR-PIT 可在未系统性地清除免疫抑制细胞的基础上激活免疫效应细胞，与之前通过全身性应用抗 CD25 抗体或 IL-2 偶联物消耗全身 Tregs 的研究结果形成显著对比[30-31]。此外，Okada 等[32]发现，相较于基于抗 CD25-IgG 组成的 APC，使用基于抗 CD25-F(ab′)2 组成的 APC 可促进更强烈的活化 T 细胞反应，发挥更为显著的抑制肿瘤生长效应。细胞毒性 T 淋巴细胞相关蛋白 4(CTLA-4)是一种在 Tregs 和活化后的 T 细胞中组成性表达非常强效的共抑制分子。CTLA-4 抗体偶联的 NIR-PIT 制剂会选择性地清除局部 Tregs，从而增强 T 细胞介导的抗肿瘤免疫[33]。另外，细胞外腺苷的积累可能导致 TME 中 $CD8^+$T 细胞和 NK 细胞丧失细胞毒性，CD73 是腺苷的主要细胞外来源[34]。CD73 在肿瘤细胞、Tregs 或 MDSCs 上均有高水平表达，但对 T 淋巴细胞、NK 细胞或树突状细胞(DC)无影响。基于抗 CD73 抗体的 NIR-PIT 可同时清除肿瘤细胞和微环境中的免疫抑制细胞[35]。T 细胞活化的 V 结构域免疫球蛋白抑制分子(VISTA)是一种抑制性免疫检查点分子，广泛表达于 Tregs 和 MDSCs[36]。Wakiyama[37]等合成偶联物 anti-VISTA-IR700，在两种小鼠肿瘤模型 MC38-luc 和 LL2-luc 中评价其抗肿瘤作用，发现在这两种模型中基于靶向 VISTA 的 NIR-PIT 均可有效耗尽 VISTA 表达细胞，抑制肿瘤发展并延长小鼠存活时间。并且在区域淋巴结中观察到 $CD8^+$T 细胞和 DCs 活化，证明基于靶向 VISTA 的 NIR-PIT 通过减少 TME 中表达 VISTA 的免疫抑制细胞来增强治疗肿瘤的有效性[37]。此外，基于靶向趋化受体因子 2(CXCR2)的 NIR-PIT 可消除肿瘤微环境中的 MDSCs，从而激活 $CD8^+$T 细胞和自然杀伤细胞，增强局部抗肿瘤免疫反应[38]。综上所述，NIR-PIT 局部靶向微环境中的免疫调节细胞将有助于人体的免疫系统功能恢复正常，克服常规免疫药物的瓶颈，并且使全身副作用最小化(如图 4 所示)[17]。

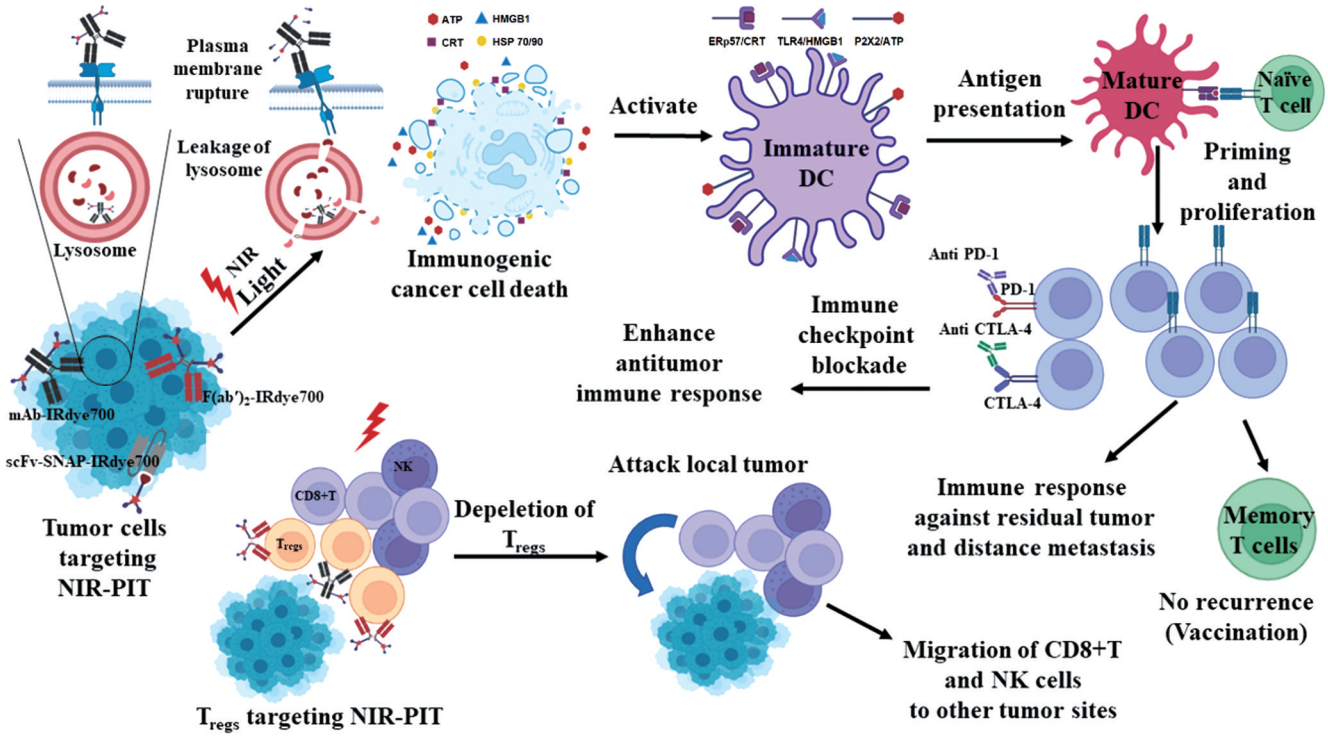

图 4　不同靶点 NIR-PIT 发挥作用的机制及相关免疫学效应[10]

Fig. 4　Mechanism and related immunological effects of different target NIR-PIT[10]

3.2　靶向肿瘤相关成纤维细胞

肿瘤相关成纤维细胞(CAFs)是 TME 的主要组成部分之一，在肿瘤侵袭、血管生成和免疫抑制中发挥重要作用[39-42]。成纤维细胞活化蛋白(FAP)在活化 CAFs 表面过度表达，因此 Jin 等[43]构建了偶联物 FAP-α-IR700，发现其在 NIR 光的诱发下可剂量依赖性杀伤过表达 FAP-α 人源乳腺癌肿瘤细胞。体内外研究均证实基于靶向 FAP-α 的 NIR-PIT 具有显著的抗肿瘤作用。此外，Katsube 等[44]和 Watanabe 等[45]在食管癌中的研究同样证实了基于 FAP 的 NIR-PIT 的有效性，并且有助于克服食管癌细胞对化疗的耐药性。Podoplanin(PDPN)，也称为 gp38，已被证实能够在多种肿瘤细胞和 CAFs 中广泛表达且与预后不良有关[46]。研究者们合成 anti-PDPN 抗体 IR700，发现偶联

物与PDPN表达阳性的肿瘤细胞和CAFs发生特异性结合。经NIR-PIT处理后的小鼠口腔癌肿物体积显著缩小，延长小鼠生存期，同时肿瘤微环境中细胞毒性T细胞数量增加，宿主抗肿瘤免疫能力增强。总之，这些结果均表明基于靶向CAFs的NIR-PIT有可能解决传统肿瘤治疗中产生的耐药问题，是一种具有广阔前景的肿瘤治疗策略。

3.3 靶向肿瘤血管

除了以Tregs或CAFs表面的抗原为靶点外，NIR-PIT还可以作用于微环境中的其他细胞（例如血管内皮细胞和周细胞）以达到肿瘤治疗的目的[47]。

肿瘤血管异常是导致免疫抑制性TME的重要原因之一。NIR-PIT靶向肿瘤血管将通过阻断肿瘤血管的营养和氧气供应介导肿瘤细胞死亡[48-52]。Nishimura等[48]将抗血管内皮生长因子受体2（VEGFR-2）的抗体DC101与IR700结合制备出DC101-IR700，用于治疗胃癌。在异种移植肿瘤模型中发现，该APC可使肿瘤微血管密度降低和破坏肿瘤新脉管系统，不仅效果优于偶联物trastuzumab-IR700（Tra-IR700），还可以通过控制NIR光照部位来限制正常组织毒性。此外，Shi等[49]首先制备二聚体$Z_{PDGFR\beta}$亲和体，随后将IR700与$Z_{PDGFR\beta}$偶联产生Z_{IR700}，在异种移植物小鼠模型中评估其与表达PDGFRβ受体的周细胞结合和肿瘤归巢能力，发现Z_{IR700}在NIR光的激活下能特异性杀死血管周细胞，破坏肿瘤血管，从而诱导小鼠肿瘤细胞死亡。并且，Z_{IR700}介导光免疫治疗（PIT）通过增加肿瘤对肿瘤坏死因子相关凋亡诱导配体（TRAIL）的摄取，增强其抗肿瘤作用，在一定程度上证实了Z_{IR700}介导NIR-PIT作为单一或联合疗法发挥抗肿瘤作用的潜在可能性。因此，针对肿瘤脉管系统的NIR-PIT可应用于大多数实体瘤，并有望实现临床转化。表1为最近开发的NIR-PIT中APC靶点扩展至TME中非肿瘤细胞的表面蛋白中的具体靶点。

表1 TME中非肿瘤细胞治疗靶点

Table 1 Therapeutic targets on non-tumor cells in TME

Target molecule	APC	Cancer	Tumor model	Ref.
CD25	Anti-CD25-IgG-IR700,	Colon cancer	*In vitro* and *in vivo*	[32]
	Anti-CD25-F(ab′)2-IR700	Colon cancer, lung carcinoma, and prostate cancer	*In vitro* and *in vivo*	[29]
CD73	αCD73-IR700	Pancreatic ductal adenocarcinoma subcutaneous and breast cancer (orthotopic)	*In vitro* and *in vivo*	[35]
FAP	Anti-FAP-IR700	Esophageal squamous cell carcinoma	*In vitro* and *in vivo*	[44]
	Anti-FAP-α-IR700	Esophageal cancer	*In vitro* and *in vivo*	[43]
Integrin αvβ3	cRGD-PEG-HSA-IR700	Ovarian tumor spheroid	*In vitro* (3D culture)	[50]
	IR700-PEG-PGlu-cRGDx	Glioblastoma	*In vitro* (3D culture)	[51]
	RGD-8PEG-IR700	Melanoma spheroid	*In vitro* (3D culture)	[52]
PDGFRβ	Dimeric Z-PDGFRβ-IR700	Colorectal cancer	*In vitro* and *in vivo*	[49]
Podoplanin	Anti PDPN-IR700	Oral cancer	*In vitro* and *in vivo*	[53]
VEGFR-2	DC101-IR700	Gastric cancer	*In vitro* and *in vivo*	[48]
VISTA	Anti-VISTA-IR700	Colon cancer and lung carcinoma	*In vitro* and *in vivo*	[37]

4 NIR-PIT与免疫检查点阻断剂的联合策略

虽然NIR-PIT具有显著的抗肿瘤疗效，但适应性免疫抵抗使其持久性较差，这往往会导致抗肿瘤效果不佳或肿瘤复发[54-55]。在这些情况下，联合治疗将有助于改善NIR-PIT的治疗效果。ICB是目前临床研究最成熟、应用最广泛的肿瘤免疫治疗手段[38]。已知的一种提高NIR-PIT抗肿瘤效果的常用策略是将NIR-PIT与免疫检查点抑制剂治疗药物联合使用，通过SUPR效应或协同抗肿瘤机制诱导抗肿瘤免疫应答，增强抗肿瘤作用[20,56]。

基于靶向程序性死亡受体-1（PD-1）/程序性死亡受体-配体1（PD-L1）的ICB在多种癌症模型中均能明显增强NIR-PIT诱导抗肿瘤免疫的能力。CD276是一种在肿瘤细胞和肿瘤血管中均过表达的跨膜糖蛋白。有一项研究将基于靶向CD276的NIR-PIT与PD-L1 ICB联用，结果发现基于靶向CD276的NIR-PIT促进DC激活和成熟并显著提高肿瘤细胞PD-L1的表达水平。当基于CD276的NIR-PIT与抗PD-L1联合治疗时，能显著抑制小鼠乳腺癌细胞4T1肿瘤生长并通过募集浸润性$CD8^+$T细胞阻止肿瘤细胞向肺部转移[56]。CD44是一种特性良好的癌症干细胞标志物，Nagaya[21]等将靶向CD44的PIT与免疫检查点抑制剂（PD-1或CTLA4）联合应用。与单一治疗相比，联合治疗可逆转自身适应性免疫抵抗，降低结肠癌和肺癌的肿瘤发展并显著提高患者生存率[54,57-59]。CD73在肿瘤细胞以及免疫相关细胞（如Tregs、MDSCs和TAMs）中过表达，而在效应$CD8^+$T细胞、NK细胞和DC中不存在[60]。Xue等[35]将基于靶向CD73的NIR-

PIT 与抗 PD-1 抗体联合治疗可克服肿瘤对 PD-1 治疗的获得性耐药问题，并且在无须考虑肿瘤细胞中 CD73 表达水平的情况下消除小鼠晚期肿瘤异种移植物。

针对不同肿瘤免疫细胞群的异质性，根据特异性免疫检查点分子在各自肿瘤中的表达水平来选择最合适的免疫检查点抑制剂并与 NIR-PIT 联合使用，这种联合治疗将获得更为理想的临床效果。NIR-PIT 联合 ICB 为靶向肿瘤免疫治疗提供了可行的思路，未来有望发现更多的免疫靶点，实现对晚期肿瘤患者的精准临床管理。

5 NIR-PIT 相关临床试验进展

NIR-PIT 的潜在疗效和安全性使其成为一种很有前途的癌症治疗方法，目前已在世界各地开展了临床试验。在美国，一项基于西妥昔单抗和 IR700（西妥昔单抗-IR700，RM-1929）的 NIR-PIT I/IIa 期临床试验已经结束。结果表明，NIR-PIT 的传统临床治疗效果不理想，但其在局部复发 HNSCC 患者中表现出有效的抗肿瘤反应[61]。在日本开展的一项基于 NIR-PIT（RM-1929）的 I 期、开放标签、单中心研究也得出与美国 I/IIa 期相似的研究结果[62]。鉴于这些具有临床意义的结果，目前正在进行一项包括 275 例复发或第二原发性 HNSCC 患者的全球 III 期试验（NCT03769506）。2020 年 9 月，西妥昔单抗-IR700 已被日本政府批准用于治疗不可切除的局部晚期或复发性 HNSCC[63]。2020 年 12 月，一项开放标签的 I/II 期肿瘤免疫治疗试验启动，即对复发或转移性 HNSCC 或皮肤鳞状细胞癌（NCT04305795）患者使用 RM-1929 介导的 NIR-PIT 联合靶向 PD-1 ICB。此外，基于动物模型试验结果，美国目前正在进行一项 Ib/II 期临床试验，以研究基于一种新的重组乳头瘤病毒样颗粒与 IR700 形成的缀合物（AU-011）介导的 NIR-PIT 在原发性小脉络膜黑色素瘤（NCT03052127）患者体内的安全性、免疫原性和有效性。此外，近红外光照射后 IR700 荧光降低，这种荧光变化目前正在临床试验（NCT05182866）中作为治疗性生物标志物。

6 NIR-PIT 效果监测与评价手段

实时监测 NIR-PIT 在靶组织中的积累情况、治疗反应和适当的近红外光照射对于精确治疗非常重要[27,64]。NIR-PIT 的治疗效果可以采用以下成像方法来监测。一种用于治疗期间成像的方法是 IR700 荧光成像，可用于确认缀合物与肿瘤的结合情况。监测部位在 690 nm 近红外光激活后，由于酞菁核的二聚体和低聚物的形成以及光化学配体释放反应后共轭蛋白的沉淀，IR700 荧光会消失。这种光漂白导致的荧光损失，表明该部位有足够的光传递，即已达到最大的光暴露。因此，IR700 荧光可以在光暴露期间用作适当的光剂量测定指标[65]。此外，SUPR 效应可作为 NIR-PIT 的药效学生物标志物。已有报道表明定量 SUPR 效应的方法使用了吲哚菁绿（ICG）-荧光和磁共振成像（MRI）造影剂[16]。有研究发现 SUPR 效应可增强直径约为 5 μm 的微颗粒在肿瘤内的持久性，即“微尺寸 SUPR 效应”。微泡和对比度增强超声造影（CEUS）可作为一种基于图像的标志物来评估 SUPR 效应，还能监测和确认 NIR-PIT 的有效性[66]。NIR-PIT 治疗完成后，18F-氟代脱氧葡萄糖-正电子发射断层扫描（18F-FDG-PET）是评估 NIR-PIT 急性治疗效果的一种极好的方法。采用 18F-FDG-PET 观察到的肿瘤大小变化比物理观察到的变化发生得更早[67]。对于浅表病变或使用内窥镜检查，荧光寿命成像和生物发光成像（BLI）也可用于评估急性 PIT 的治疗效果[68-69]。除此之外，采用光学相干断层扫描（OCT）进行生物医学成像时，可显示 NIR-PIT 期间肿瘤血管的剧烈血流动力学变化，治疗后的肿瘤与未治疗的肿瘤有显著差异[70]。此外，分别使用双通道荧光纤维成像系统和带微棱镜与不带微棱镜的双光子显微镜监测 NIR-PIT 在治疗期间和治疗后从肿瘤表面到肿瘤深部的微观分布及其治疗效果[41]。NIR-PIT 的杀瘤作用和血流动力学变化可通过 ^{13}C MRI、血氧水平依赖（BOLD）MRI 和光声成像监测[64,71]。

7 NIR-PIT 面临的挑战与未来展望

NIR-PIT 可通过靶向肿瘤细胞或免疫调节细胞高度特异性消除肿瘤细胞，且具有极弱的脱靶效应。当与免疫抑制剂联合使用时，NIR-PIT 可发生更强烈的抗肿瘤免疫反应，减少肿瘤细胞转移和局部复发。NIR-PIT 尽管具有广阔的应用前景，也面临着巨大挑战。首先，研究发现使用单一的光免疫治疗制剂并不能完全治愈肿瘤，并且不同类型的肿瘤会表达不同特点的肿瘤相关蛋白。为了生成多功能 NIR-PIT 制剂，Shirasu 等[72]将生物素化抗体（BioAbs）与 AvIR（IRdye700-conjugated NeutrAvidin）结合，开发出新型 NIR-PIT 制剂以实现无限靶向特异性，这种制剂能够克服重复制备 IRdye700-McAb 偶联物的局限性。其次，近红外光无法均匀照射到较大的肿瘤肿块和位于组织深处的肿瘤。在动物模型中肿瘤复发的大部分原因也是 IRdye700-McAb 在肿瘤内分布不均匀。为了克服这一局限性并解决“结合位点屏障”问题，重复近红外光照射或加入亲和力较低的第二种单抗，从而促进抗体偶联物渗透到肿瘤细胞深部。[73-74]。除此之外，NIR-PIT 依赖于特异性配体，仅限于靶向高表达的细胞表面抗原，但这些抗原在持续发展的癌症中并不总是过表达，因此进一步研究靶标分子的种类与表达，也是需要关注的重点[15]。此外，NIR-PIT 中 APC 剂量的安全范围有待进一步探讨，其毒性和安全性必须在临床应用之前进行充分的研究。

总的来说，NIR-PIT已在各种类型的肿瘤中进行了广泛的体外和体内模型研究，并显示出良好的治疗效果。NIR-PIT具有广泛且灵活的应用范围和多种增强其抗肿瘤能力的途径，还具有成为高效肿瘤治疗方法的巨大潜力。

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Recent Advancements in Near-Infrared Light Immunotherapy Targeted on Tumor Microenvironment

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Abstract

Significance For over half a century, the three main pillars of conventional cancer therapy are surgery, chemotherapy, and radiotherapy. However, these treatment methods have inherent limitations, as they inevitably cause severe damage to normal cells, particularly immune cells. The discovery and development of immunotherapy show promising clinical applications. Nonetheless, immunotherapy is a double-edged sword, often leading to the occurrence of immune-related adverse events (irAEs) because of off-target effects. Therefore, the current focus in cancer research is to explore treatment strategies that can activate local immune responses while enhancing tumor specificity.

Near-infrared photoimmunotherapy (NIR-PIT) is a novel tumor therapy, and it depends on a single antibody-photo absorber conjugate (APC), which combines a monoclonal antibody (McAb) targeted on tumor features with IRDye700DX (IR700). Except for its specific antitumor mechanisms, a unique aspect of NIR-PIT is its direct impact on blood drug delivery. The super-enhanced permeability and retention (SUPR) effects facilitate the rapid leakage of drugs into the tumor, favoring the induction of cytotoxic effects. However, the presence of the "binding site barrier" indicates that using antibodies with low affinity or targeting antibodies with low antigen expression may promote a more even distribution of APCs within the tumor parenchyma. In recent years, researchers have investigated the use of different targeting segments in NIR-PIT, enhancing the tumor immunogenicity, targeting ability, stability, and flexibility of NIR-PIT drugs. This approach has shown considerable potential for application in various types of tumors, with some related clinical trials yielding satisfactory results.

Studies have shown a close association between the suppressive tumor microenvironment (TME) and the growth and progression of cancer. In recent years, the targets of APCs in NIR-PIT have expanded to surface proteins of non-tumor cells in TME. The combination therapy of NIR-PIT with immune checkpoint blockade (ICB) has also shown promising experimental results. The development and continuous improvement of optical devices also facilitate the monitoring and evaluation of the therapeutic effects of NIR-PIT. Therefore, it is necessary to summarize previous relevant research to provide a rational reference for the clinical research and application of NIR-PIT.

Progress The primary mechanism through which NIR-PIT exerts its cytotoxic effects is via a photochemical reaction from IR700. Under near-infrared light, IR700 in APCs undergoes a photocatalytic transformation, changing its chemical properties from hydrophilic to hydrophobic, and aggregating in an aqueous solution. This process leads to the denaturation of the cell membrane antigens bound to it, physical damage to the cell membrane and cell rupture, increased transmembrane water flow, and cell death (Fig. 1). Simultaneously, the rapid release of tumor-associated antigens (TAAs) and damage-associated molecular patterns (DAMPs) during NIR-PIT induces immunogenic cell death (ICD), and subsequently, activates the antitumor immune response of the host, enhancing the activation of systemic immune responses to attack other cancer cells and further amplifying the therapeutic effects of NIR-PIT (Fig. 2). Current NIR-PIT treatment strategies targeting key components in TME, including immune inhibitory cells (Tregs and MDSCs), cancer-associated fibroblasts (CAFs), and blood vessels, are listed in Table 1. The design principles of APCs and relevant experimental results are also presented. Subsequently, the combined therapeutic strategies and efficacy of immune checkpoint inhibitors with NIR-PIT targeting different cell surface proteins are elucidated. Given the heterogeneity of immune cell populations in different tumors, the choice of ICBs can be based on the expression levels of specific immune checkpoint molecules in their respective tumors.

In addition, the progress of clinical trials related to NIR-PIT is summarized, demonstrating that cetuximab-IR700 (RM-1929) can elicit effective antitumor responses in patients with locally recurrent HNSCC where conventional clinical treatments are less

effective. Furthermore, the SUPR effect can be quantified using indocyanine green (ICG)-fluorescence and magnetic resonance imaging (MRI) contrast agents to monitor and identify the viability of NIR-PIT. 18F-fluorode-oxyglucose positron emission tomography (18F-FDG-PET), fluorescence lifetime imaging, and bioluminescence imaging can evaluate acute NIR-PIT treatment in preclinical studies. Moreover, the micro distribution of the NIR-PIT agent and its therapeutic effects is monitored using a two-channel fluorescence fiber-imaging system and two-photon microscopy with and without a microprism. The tumoricidal effects and hemodynamic changes induced by NIR-PIT can be monitored by ^{13}C MRI, blood oxygenation level dependent (BOLD) MRI, and photoacoustic imaging.

The current investigation of NIR-PIT is relatively limited. In summary, the limitations of replicating IRdye700-McAb conjugates in NIR-PIT, the penetration and uniformity of near-infrared light irradiation, differences in the types and expression of target molecules in different types of tumors, and the safe range of APC dosage in NIR-PIT still require detailed investigations.

Conclusions and Prospects Extensive *in vitro* and *in vivo* models study on NIR-PIT have been conducted for various types of tumors, with promising therapeutic outcomes. With its broad and flexible application scope, and various approaches to enhance its efficacy, NIR-PIT has significant potential as a valuable method for cancer treatment.

Key words medical optics; malignant tumor; near-infrared photo immunotherapy; antibody-photo absorber conjugate; immunotherapy; near-infrared