•专题报道•

# 刺激响应型PROTAC用于肿瘤精准治疗

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**摘要**: 蛋白降解靶向嵌合体 (proteolysis-targeting chimeras, PROTAC) 凭借其“事件驱动”特性, 能够靶向传统小分子抑制剂无法成药的靶点, 从而大大拓宽了药物开发空间。此外, PROTAC通过完全降解靶蛋白而非仅仅抑制其功能, 有效克服了小分子抑制剂的耐药性问题。然而, PROTAC的持续催化活性可能导致脱靶毒性。为解决这一问题, 刺激响应型PROTAC应运而生。PROTAC前药能够通过光、酶或活性氧等内源性或外源性刺激, 在肿瘤微环境中被特异性激活、释放PROTAC, 从而减少PROTAC的脱靶毒性并保持对靶蛋白的降解效率。本文系统总结了刺激响应型PROTAC领域的最新进展, 讨论了该领域的不足与挑战, 并展望了未来的发展方向, 以推动癌症精准治疗的进步。

**关键词**: 刺激响应; 蛋白降解靶向嵌合体; 蛋白降解; 癌症; 精准治疗

**中图分类号**: R941 **文献标识码**: A **文章编号**: 0513-4870(2025)11-3263-20

# Stimuli-activatable PROTAC for precise cancer therapy

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**Abstract**: Proteolysis-targeting chimeras (PROTACs) leverage their "event-driven" mechanism to target traditionally undruggable proteins inaccessible to conventional small molecule inhibitors, thereby significantly expanding the therapeutic landscape of drug discovery. Furthermore, PROTACs overcome the resistance associated with small molecule inhibitors by achieving complete degradation of target proteins rather than transient functional inhibition. However, their sustained catalytic activity may induce off-target effects and systemic toxicity. To mitigate these limitations, stimuli-responsive PROTAC prodrug systems have emerged as a promising strategy to spatiotemporally control PROTAC activation within tumor microenvironments. PROTAC prodrug systems demonstrate tumor microenvironment-responsive activation through endogenous/exogenous stimuli (e. g., light, enzymes, reactive oxygen species), enabling spatiotemporal control of PROTAC liberation. This precision engineering strategy significantly reduces off-target toxicity while maintaining potent on-target protein degradation efficacy. This review provides a systematic overview of cutting-edge advances in stimuli-activatable PROTAC platforms, critically examines current limitations and challenges in the field, and proposes strategic directions for future development to accelerate the evolution of precision cancer therapeutics.

**Key words**: stimuli-activatable prodrug; PROTAC; protein degradation; cancer; precision therapy

恶性肿瘤 (癌症) 作为全球人类因病致死的十大死因之一, 其持续攀升的发病率和死亡率对公共卫生体系构成严峻挑战[1,2]。蛋白质的异常调控是癌症发生发展的核心驱动力[3,4]。肿瘤细胞通过受体酪氨酸激酶 [如表皮生长因子受体 (epidermal growth factor receptor, EGFR)] 的过度活化实现自主增殖[5,6], 同时伴随p53等肿瘤抑制因子的表观遗传沉默或功能缺失, 导致细胞周期检查点的失调并加剧基因组的不稳定

收稿日期: 2025-05-12; 修回日期: 2025-07-24.
基金项目: 国家自然科学基金资助项目 (22474042).
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DOI: 10.16438/j.0513-4870.2025-0540

性[7,8]。Warburg效应驱动的代谢重构、程序性死亡配体1 (programmed cell death ligand 1, PD-L1)/细胞毒性T淋巴细胞抗原4 (cytotoxic T-lymphocyte antigen 4, CTLA-4)等免疫检查点分子的异常表达, 以及上皮−间质转化相关蛋白, 如神经钙黏蛋白 (neural cadherin, N-cadherin)、基质金属蛋白酶 (matrix metalloproteinases, MMPs)的调控失衡, 共同构成了肿瘤微环境重塑和转移扩散的分子基础[9-11]。这些病理特征相关的蛋白质动态变化也为临床诊断提供了特异性生物标志物, 更催生了以聚 (ADP-核糖) 聚合酶 [poly(ADP-ribose) polymerase, PARP] 抑制剂[12,13]、PD-1单抗[14,15]等为代表的靶向治疗模式, 推动了肿瘤治疗向精准医学模式的转变。

传统小分子抑制剂基于“占位驱动”(occupancy-driven)的作用模式, 通过竞争性结合致病蛋白的活性位点或催化结构域实现功能抑制[16], 但该策略在应对具有宽泛结合表面、平滑拓扑特征或浅层结合口袋的靶蛋白时存在显著局限[17-19]。尤为突出的是, 约80%的人类蛋白质组被归类为“难成药”(undruggable)靶标, 包括转录因子家族、信号调控蛋白和支架蛋白等, 因缺乏明确的小分子结合位点而长期制约药物开发[4,20]。即便对可成药靶点, 小分子抑制剂的动态可逆结合特性与快速系统清除率导致靶组织有效浓度难以维持, 往往需要高剂量给药才能达到临床疗效所需的靶点占有率[21,22]。而这种高剂量策略不仅增加脱靶效应风险, 还可能引发剂量限制性毒性。与此同时, 靶蛋白适应性突变导致的获得性耐药问题也进一步限制了小分子抑制剂的临床应用[23], 这促使当前药物开发模式从传统的功能抑制转向更彻底的致病蛋白消除策略。

相较于传统“占位驱动”的小分子抑制剂, 蛋白降解靶向嵌合体 (proteolysis targeting chimera, PROTAC)通过创新的“事件驱动”(event-driven)模式实现了治疗突破[17,24,25]。其核心机制依赖于由靶蛋白配体、E3泛素连接酶配体及连接子构成的异双功能分子结构。当PROTAC同时结合目标蛋白 (protein of interest, POI)与E3连接酶形成三元复合物后, 触发POI的泛素化标记并引导其通过泛素−蛋白酶体系统 (ubiquitin-proteasome system, UPS)降解。PROTAC在完成单个降解事件后可循环参与多轮反应, 这种催化特性使其在低剂量下即可实现POI的持续清除, 显著降低脱靶毒性和剂量依赖性不良反应[26]。尤为关键的是, 该技术不仅能克服传统抑制剂面临的耐药性问题, 例如靶点过表达、功能获得性突变 (如激酶结构域突变)及支架蛋白适应性重塑, 更通过全蛋白消除策略将“可成药”靶标范围扩展至传统难治性靶点 (如转录因子和非酶功能蛋白)[27]。PROTAC突破了传统抑制剂对靶点结合位点的依赖, 即使POI发生点突变或缺乏催化活性域, 只要保留最低限度的结合能力即可触发降解, 这为攻克肿瘤耐药和拓展治疗模式提供了全新的思路。

尽管PROTAC技术通过“事件驱动”的蛋白降解机制突破了传统抑制剂的治疗瓶颈, 但其临床转化仍面临双重挑战, 即系统性毒性风险与药代动力学 (pharmacokinetics, PK)缺陷[28]。研究显示, 完全降解溴结构域和额外末端域 (bromodomain and extra-terminal domain, BET)家族蛋白虽可抑制肿瘤进展, 但会导致皮肤屏障破坏、脊柱形态异常等脱靶毒性, 这在ARV771治疗的小鼠模型中已得到验证[29,30]。此外, PROTAC分子通常具有高分子质量、大极性表面积及多氢键供体等特性, 使其偏离类药五原则 (Rule of Five), 导致膜渗透性低、溶解度差和系统清除过快, 难以在靶组织有效富集[31-33]。为突破这些局限, 刺激响应型PROTAC应运而生, 通过设计仅在病理微环境或外源刺激下释放活性PROTAC, 实现时空可控的POI清除 (图1)。例如, 光、X射线、乏氧、活性氧 (reactive oxygen species, ROS)以及肿瘤微环境酶响应型PROTAC (表1)。此外, 基于纳米递送系统构建的刺激响应型PROTAC, 能够通过主动靶向机制或增强通透性和滞留 (enhanced permeability and retention, EPR)效应增强药物在肿瘤组织中的积累, 并改善PK特性。本文综述了刺激响应型PROTAC的最新研究进展, 探讨了其在肿瘤选择性蛋白降解和精准医疗中的应用潜力, 希望推动基于PROTAC的癌症精准治疗发展。

## 1 光控型PROTAC

近年来, 光学调控技术在精确控制生物过程方面展现出显著优势, 其应用范围不断扩大。这一发展趋势主要得益于光学调控技术具备的三大核心特性[34-36]: ① 非侵入性, 可最大限度减少对生物系统的干扰; ② 时空可调性, 能够实现亚细胞级别的精准定位和时空调控; ③ 快速响应性, 通常在毫秒至秒级时间尺度内即可实现功能切换。因此, 通过光控技术使PROTAC在特定位置或特定时间发挥蛋白降解效果, 可有效提高其靶向能力并降低潜在毒性。目前, 通过光学调控PROTAC的策略主要分为两大类[22,37,38]: ① 基于笼状化合物的光控系统, 通过光解作用去除掩蔽降解活性的笼状基团后, PROTAC的蛋白降解能力被还原; ② 基于光开关基团的光药理学策略, 利用光致异构化和热弛豫实现分子构象的转变, 进而实现功能的可逆调控。

基于光控技术独特的非侵入性和时空精准性优

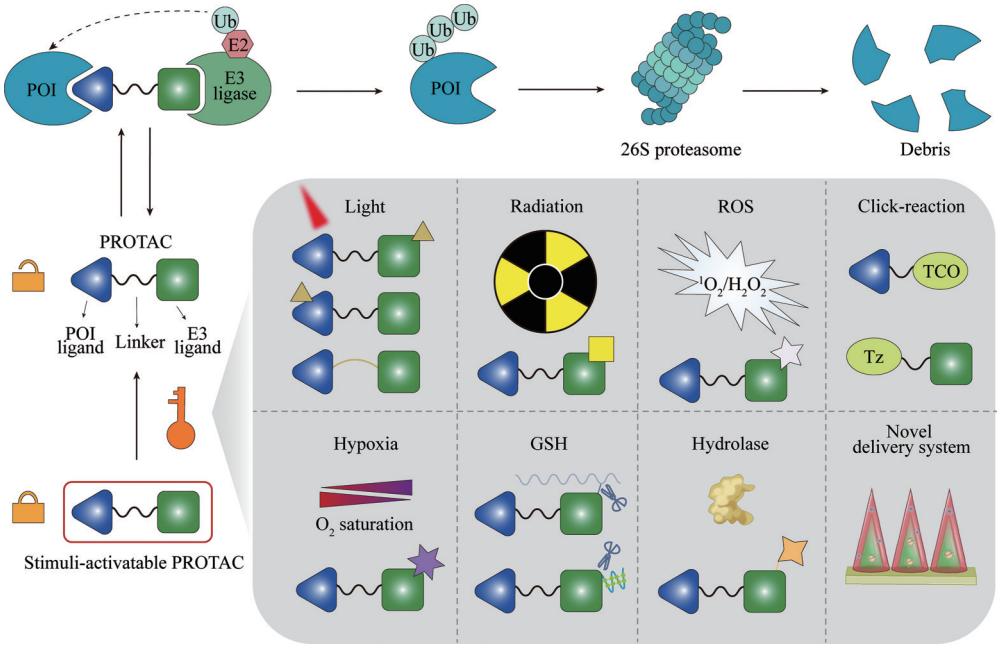



**Figure 1** Upon receiving a specific stimulus, stimuli-activatable PROTAC is reduced to activated PROTAC, which subsequently mediate protein degradation *via* UPS pathway. The heterobifunctional PROTAC binds to both the POI and E3 ligase, forming a stable ternary complex. Ubiquitin is then transferred from the E2 enzyme to the POI through the E3 ligase, resulting in the ubiquitination of the POI. The ubiquitin-tagged POI is recognized by the proteasome and ultimately degraded into small fragments. The PROTAC can then enter a new cycle, binding to other POIs in a catalytic manner. POI: Protein of interest; Ub: Ubiquitin; PROTAC: Proteolysis targeting chimera; ROS: Reactive oxygen species; TCO: Trans-cyclooctene; Tz: Tetrazine; GSH: Glutathione

**Table 1** Key features and advantages of stimuli-responsive strategies in PROTAC. NTR: Nitroreductase

| Strategy | Key feature | Advantage |
|---|---|---|
| Photo | UV light activation, reversible switches | Non-invasive spatiotemporal, rapid on/off kinetics |
| X-ray | Activated by clinical X-ray irradiation, ROS generation | Deep tissue penetration, synergy with radiotherapy, non-invasive spatiotemporal, self-amplifying in high-ROS tumor microenvironment |
| Hypoxia | Activated by NTR overexpression in hypoxia | Tumor selective activation, minimizes on-target/off tumor toxicity |
| ROS | Activated by ROS, combined with oxidative stress-inducing therapy | Tumor selective activation, synergy therapy |
| Enzyme | Enzyme substrate specificity, modular design, tumor microenvironment adaptation, prodrug stability | Precision targeting, enhanced tissue penetration |
| Multi-stimulation | Logic-gated activation, modular stimuli-responsive unit, region-confined targeting | Enhanced tumor selectivity, improved safety profile, adaptability to tumor heterogeneity, synergistic activation mechanisms |

势，Liu 等[39]开发了光诱导型 PROTAC 分子 (opto-PROTAC) (图 2A)。该研究通过在泊马度胺关键活性位点引入光敏笼蔽基团——硝基藜芦氧羰基 (nitroveratryloxycarbonyl, NVOC)，成功阻断了其与 E3 泛素连接酶 cereblon (CRBN) 的相互作用。具体而言，该研究团队选择在泊马度胺戊二酰亚胺环的氮原子进行化学修饰。该位点原本通过与 CRBN His380 主链羰基形成关键氢键介导分子识别。未激活的 opto-泊马度胺几乎丧失与 CRBN 的结合能力，但在紫外光 (ultraviolet light, UV) 照射后发生高效光裂解反应，其结合活性可恢复至接近天然配体水平，且光激活效率呈现显著的时间依赖性，表明光控开关具备优异的“off-on”调控特性。研究者进一步选取靶向溴结构域蛋白 (bromodomain, BRD) 2/3 的 PROTAC 分子 dBET1 进行光敏化改造，构建 opto-dBET1。实验结果表明，未激活状态下 opto-dBET1 因 CRBN 结合域被封闭而无法介导 BRD2/3 的泛素化修饰。经 UV 照射后，其诱导靶蛋白泛素化的能力恢复 (图 2B)。既往研究显示，dBET1 在完全降解 BRD 家族蛋白时会产生显著细胞毒性，这可能与其干扰增强子调控及转录组稳态有关。通过光控激活策略，opto-dBET1 可实现亚细胞区域特异性降解，从而在维持疗效的同时降低系统性毒性，为临床转化提供了新的技术路径。

作为 PROTAC 设计中另一类重要的 E3 泛素连接

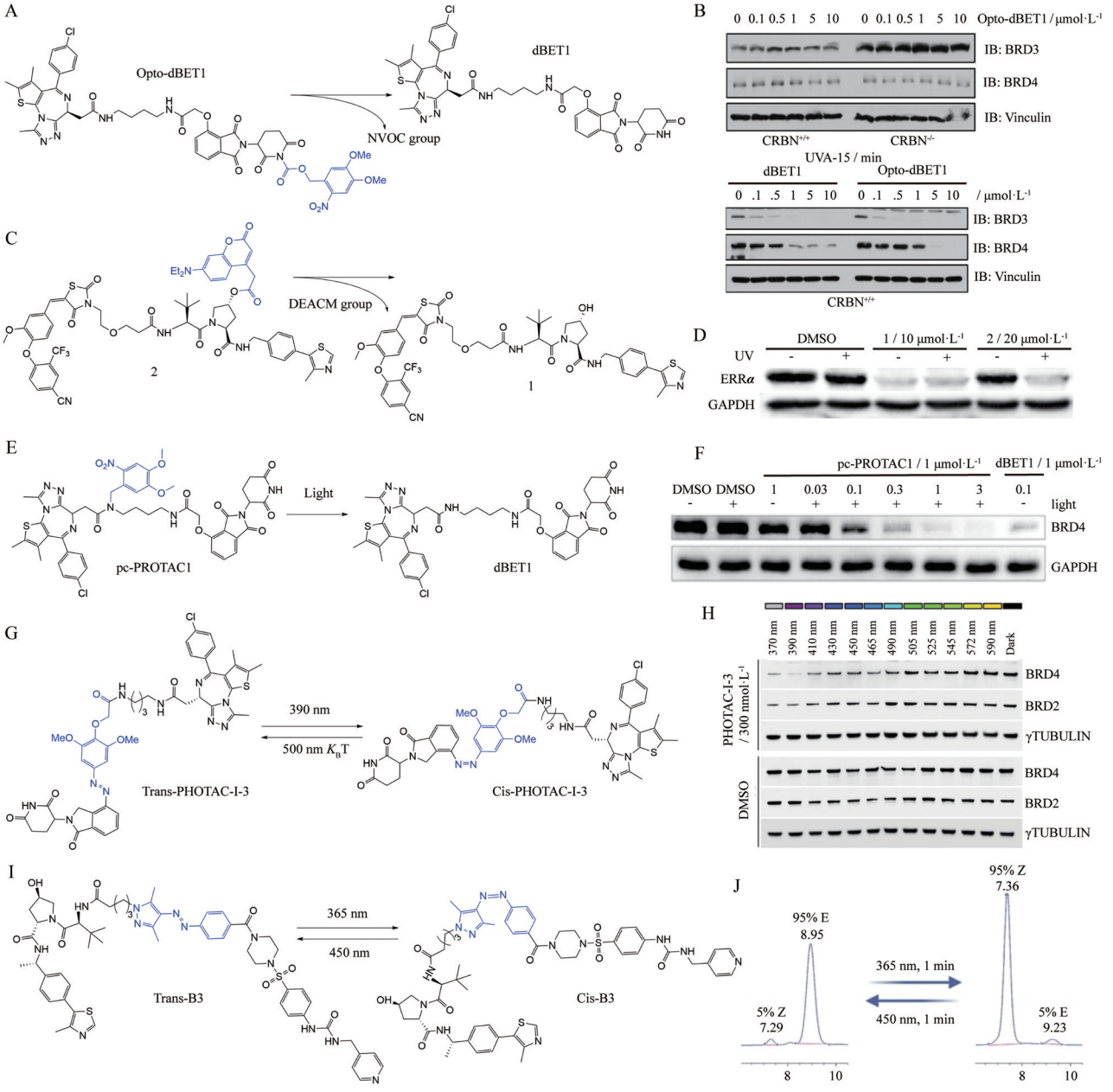



**Figure 2** Photo-activated PROTACs for spatiotemporal control POI degradation under light. A: Opto-dBET1 can be uncaged by UV irradiation (365 nm); B: UV irradiation activates opto-dBET1 to promote the degradation of BRDs. Adapted from Ref. 39 with permission. Copyright © 2020 American Association for the Advancement of Science; C: PROTAC 2 can be uncaged by UV irradiation; D: UV irradiation activates PROTAC 2 to promote the degradation of ERR*α*. Adapted from Ref. 29 with permission. Copyright © 2020 American Chemical Society; E: pc-PROTAC1 can be uncaged by UV irradiation; F: UV irradiation activates pc-PROTAC1 to promote the degradation of BRD4. Adapted from Ref. 42 with permission. Copyright © 2019 American Chemical Society; G: Switching of PHOTAC-I-3 between the trans isomer (left) and the cis isomer (right); H: Wavelength dependence of BRD2/4 degradation promoted by PHOTAC-I-3. Adapted from Ref. 43 with permission. Copyright © 2020 American Association for the Advancement of Science; I: Switching of B3 between the trans isomer (left) and the cis isomer (right); J: HPLC chromatograms of B3 under the stated irradiation conditions. Adapted from Ref. 47 with permission. Copyright © 2024 Wiley-VCH GmbH. NVOC: Nitroveratryloxycarbonyl; DEACM: 7-Diethylaminocoumarin-4-ylmethyl; DMSO: Dimethyl sulfoxide

酶配体, VHL (von Hippel-Lindau) 蛋白配体的结合特性已被深入研究[40]。其关键识别基团——羟脯氨酸深埋于 VHL 的结合口袋中, 通过与 Ser111 和 His115 残基形成两个关键氢键来维持稳定的相互作用网络[29]。基于这一结构特征, 研究人员提出了一种创新的光控策略: 在羟脯氨酸的羟基上引入大体积光敏遮蔽基团, 使其在

去遮蔽前无法与VHL有效结合, 从而实现光控激活。为验证这一假设, Naro等[29]选取了靶向雌激素相关受体 $\alpha$ (estrogen-related receptor $\alpha$, ERR$\alpha$) 的PROTAC 1作为研究对象。ERR$\alpha$作为孤儿核受体家族成员, 在细胞代谢调控和肿瘤发生发展中发挥重要作用[41]。该研究团队通过碳酸酯键将光敏基团二乙氨基香豆素 (7-diethylaminocoumarin-4-ylmethyl, DEACM) 连接到羟脯氨酸的羟基上, 成功构建了光控型PROTAC 2 (图2C)。DEACM基团具有以下优势: ① 可被 ≤ 405 nm波长的光高效激活; ② 去遮蔽后释放的酸性基团 p$K_a$ < 5, 有利于维持分子稳定性。研究人员通过色谱分析证实了ERR$\alpha$ PROTAC 2在紫外光照射3 min内即可高效转化为天然PROTAC 1, 同时释放出香豆素片段。这一结果不仅验证了该策略的快速去遮蔽特性, 还具有优异的时间分辨率。在功能验证实验中, 光激活的PROTAC 2可显著降低MCF-7细胞中ERR$\alpha$蛋白水平, 而未光照组则没有观察到明显降解效应 (图2D)。但是, PROTAC 2达到同等降解效率所需的浓度高于PROTAC 1, 这可能是光转化效率未达100%, 也可能是光敏基团修饰影响细胞摄取效率和部分分子在细胞内发生非特异性降解。

在PROTAC的光控策略研究中, 除了对E3连接酶配体进行修饰外, 对POI配体进行可逆性光敏修饰同样是一种有效的设计思路。基于这一理念, Xue等[42]巧妙地利用CRBN–沙利度胺复合物 (PDB ID: 4CI1) 和BRD4-JQ1复合物 (PDB ID: 3MXF) 的晶体结构信息, 在JQ1配体的酰胺氮原子上引入大体积的4,5-二甲氧基-2-硝基苄基光敏基团, 成功开发了光控型pc-PROTAC1 (图2E)。这一设计策略通过空间位阻有效阻断了pc-PROTAC1与POI的结合能力, 从而实现了降解活性的光控调节。通过紫外–可见光谱分析, pc-PROTAC1在365 nm处显示出特征性吸收峰, 表明其具有良好的光响应特性。色谱分析进一步证实, 在365 nm紫外光 (3 mW·cm$^{-2}$) 照射下, pc-PROTAC1可快速转化为活性dBET1, 其光解半衰期 ($t_{1/2}$) 为60 s。值得注意的是, pc-PROTAC在黑暗条件下表现出优异的稳定性。在无光条件的PBS缓冲液中孵育24 h后, 仍保持88%的完整结构。为评估pc-PROTAC的光控降解效果, 研究团队选用Ramos细胞系进行功能验证, 实验结果显示, 在无光照条件下, 即使将pc-PROTAC1浓度提高至3 μmol·L$^{-1}$, 仍未能显著降低BRD4蛋白水平。然而, 经365 nm紫外光照射3 min后, pc-PROTAC1展现出显著的剂量依赖性降解活性, 0.3 μmol·L$^{-1}$ pc-PROTAC1即可实现与0.1 μmol·L$^{-1}$ dBET1相当的降解效果 (图2F)。这一结果不仅证实了光控策略的有效性, 还表明pc-PROTAC1具有与原型分子相当的降解活性。

在光控PROTAC技术领域, 利用光响应基团动态调节PROTAC分子构象正成为新兴研究方向。基于此, Reynders等[43]将偶氮苯这一经典光开关分子整合至PROTAC的Linker部分, 成功开发出可逆光控型PHOtochemically TArgeting Chimeras (PHOTAC)。该设计具有三重核心优势: ① 偶氮苯基团作为目前已知最小的光开关单元, 其引入不会显著增加分子复杂度; ② 顺式 (*cis*) 与反式 (*trans*) 异构体间的几何差异能有效调控三元复合物的形成效率; ③ 具有优异的光稳定性和可预测的光热响应特性。研究团队通过系统性筛选偶氮苯连接链的长度和取代基, 结合分子动力学模拟与构效关系分析, 最终确定PHOTAC-I-3为最优候选分子。该分子在390 nm蓝紫光照射下可高效转化为顺式构型, 而 > 450 nm光照或热弛豫则促使其恢复反式构型 (图2G)。该策略激活所需的低能量光照已被证实不会引起细胞毒性, 且工作浓度范围显著低于传统PROTACs, 有效降低了脱靶风险。Western blot分析显示, 在相同浓度下, PHOTAC-I-3能以光波长依赖方式降解BRD4蛋白 (图2H)。

烟酰胺腺嘌呤二核苷酸 (nicotinamide adenine dinucleotide, NAD$^+$) 作为细胞内不可或缺的辅酶, 在DNA生物合成和氧化还原代谢中发挥核心作用[44]。研究表明, NAD$^+$对维持肿瘤细胞增殖至关重要, 其通过清除ROS来维持肿瘤微环境的稳态[45,46]。基于这一发现, Cheng等[47]开发了光控型PS-PROTAC系统, 旨在通过精准调控烟酰胺磷酸核糖转移酶 (nicotinamide phosphoribosyltransferase, NAMPT) 的降解来实现NAD$^+$水平的时空调控 (图2I)。为实现NAMPT的高效降解和双稳态调控, 研究团队在PS-PROTACs设计中采用了二甲基吡唑偶氮苯作为光开关模块, 这是因为其具有优异的光异构化效率, 且其热弛豫半衰期显著优于传统偶氮苯。通过优化linker长度, 最终确定三碳链PS-PROTAC B3为最优候选分子。功能验证实验显示, B3在人卵巢癌A2780细胞中展现出剂量依赖性NAMPT降解活性和细胞毒性。光控特性研究表明在450 nm光照或无光照条件下, B3以E构型存在并维持NAMPT降解活性; 而在365 nm光照下, B3可逆转化为Z构型, 导致降解活性丧失 (图2J)。这一特性使得B3能够实现NAMPT-NAD$^+$轴的双向精准调控。

总的来说, 光控技术能够实现PROTAC蛋白降解活性的无创精准时空调控, 显著降低潜在的系统毒性。然而, 目前研究中所使用的激活光主要集中在紫外–可见光波段, 而紫外光可能引发氧化应激和DNA损

伤, 并且穿透皮肤的能力差, 这极大地限制了光控PROTAC在深层肿瘤治疗中的应用。因此, 利用更长波长的光源, 尤其是近红外光, 实现PROTAC蛋白降解活性的精准调控具有重要的研究意义; 其次, 通过化学发光的方式原位产生紫外光[48], 也可以作为一种实现深层肿瘤光控PROTAC治疗的可行策略。另外, 光控PROTAC可以与光动力疗法或其他光控激活的前药联合应用, 通过局部光照引发肿瘤的精准联合治疗, 从而达到增效减毒的治疗效果。最后, 光控PROTAC的研究大多集中于细胞水平, 仍需在动物模型中进一步验证其安全性和可行性。

## 2 X射线激活型PROTAC

X射线具有卓越的精确度和深层组织穿透能力, 被广泛应用于放射治疗[49]。因此, 临床相关剂量的X射线可用于控制射线响应基团在放疗中的释放。Yang等[50]开发了一种放疗诱导的PROTAC (radiotherapy triggered PROTAC prodrug, RT-PROTAC) 策略, 通过X射线时空控制PROTAC前药的激活与蛋白降解。该团队将 (4-叠氮-2,3,5,6-四氟苯基)甲醇基团修饰至PROTAC分子中VHL配体的羟基上, 形成RT-PROTAC化合物 (图3A)。RT-PROTAC的蛋白降解活性被遮蔽。但在X射线照射下, 笼状遮蔽基团可转化为4-(羟甲基)-2,3,5,6-四氟苯胺, 从PROTAC分子中离去, 导致PROTAC的还原。随后, PROTAC与内源性VHL E3泛素连接酶及BRD蛋白结合, 通过UPS泛素化途径降解目标蛋白。Western blot结果显示, 在没有辐射的情况下, RT-PROTAC几乎不影响目标蛋白的表达。而在X射线辐射作用下, 目标蛋白显著降解。值得注意的是, X射线不仅能够定点激活PROTAC的蛋白降解作用, 还能通过放疗作用协同抑制小鼠模型中MCF-7乳腺肿瘤的生长。

## 3 乏氧激活型PROTAC

乏氧是大多数实体肿瘤的典型特征, 并与肿瘤的多种生理活动密切相关[51-54]。通常, 乏氧状态伴随着硝基还原酶 (nitroreductase, NTR) 水平的升高[55-57]。而NTR在实体肿瘤的乏氧区域过表达, 在正常组织或细

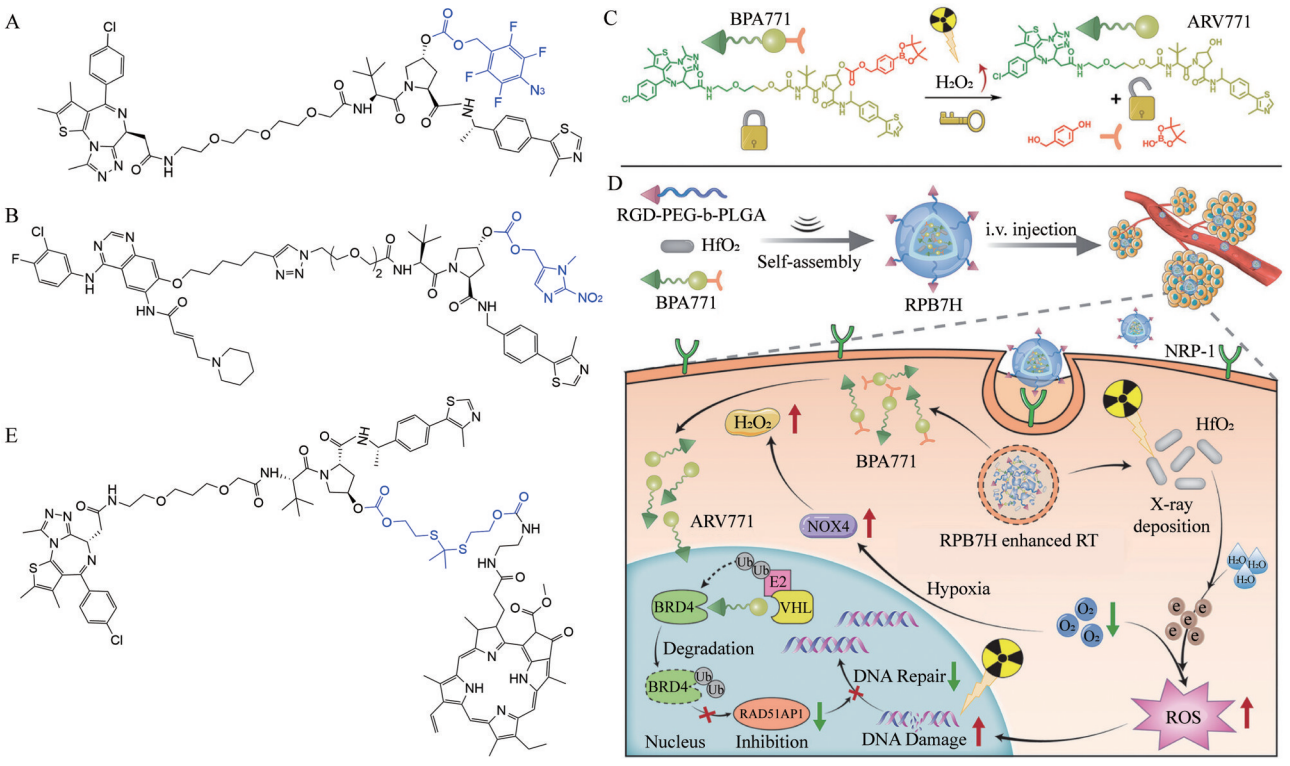



**Figure 3** Stimuli-activatable PROTACs for spatiotemporal control POI degradation. A: Chemical structure of the X-ray-activatable PROTAC. Adapted from Ref. 50 with permission. Copyright © 2023 American Chemical Society; B: Chemical structure of the hypoxia-responsive PROTAC. Adapted from Ref. 60 with permission. Copyright © 2022 American Chemical Society; C: The reaction mechanism of $H_2O_2$-activatable PROTAC; D: The anti-tumor mechanism of $H_2O_2$-activatable PROTAC-integrated nanosensitizer for potentiating radiation therapy of cancer. Adapted from Ref. 65 with permission. Copyright © 2024 Wiley-VCH GmbH; E: Chemical structure of ROS-responsive PROTAC. Adapted from Ref. 66 with permission. Copyright © 2023 American Chemical Society. i. v. injection: Intravenous injection; NOX4: Nicotinamide adenine dinucleotide phosphate oxidase 4; VHL: Von Hippel-Lindau; BRD4: Bromodomain-containing protein 4; DNA: Deoxyribonucleic acid; RT: Radiation therapy; NRP-1: Neuropilin-1

胞中几乎没有表达, 且其水平与乏氧的程度密切相关[58,59]。Shi等[60]受硝基咪唑对NTR响应特性的启发, 通过将硝基咪唑单元与PROTAC结合, 开发了乏氧响应型PROTAC (图3B)。硝基咪唑单元可被NTR识别, 并将硝基还原为羟胺, 随后发生分子内重排并离去, 从而激活PROTAC。乏氧响应型PROTAC在正常组织中显示较低的抗肿瘤活性, 并且无法诱导蛋白降解, 但在乏氧肿瘤组织中通过NTR的过表达被激活, 选择性地诱导肿瘤组织中EGFR蛋白的降解。除此之外, Cheng等[61]通过将硝基咪唑基团作为乏氧激活离去基团 (hypoxia-activated leaving groups, HALG) 引入吉非替尼的4-NH位点, 开发了乏氧激活的PROTAC (ha-PROTAC), 展示了在肿瘤乏氧微环境中精确释放PROTAC的可行性。然而, 由于ha-PROTAC是通过在靶蛋白配体上引入笼体基团构建的, 这一策略无法直接应用于开发靶向其他蛋白的通用型乏氧激活PROTAC。

## 4 ROS激活型PROTAC

除乏氧外, ROS水平的升高也是肿瘤微环境中的一大特征[62]。肿瘤微环境中的ROS通常包括羟基自由基、超氧化物和过氧化氢 ($H_2O_2$)[63]。与正常细胞相比, 肿瘤细胞表现出异常较高的$H_2O_2$水平, 浓度可达100 μmol·$L^{-1}$ [64]。因此, 利用肿瘤微环境中高ROS水平开发ROS激活的前药或药物递送系统, 可实现癌症的精准治疗。Zhang等[65]设计了一种$H_2O_2$激活的BRD4降解剂前药 (BPA771, 图3C)。BPA771通过在VHL配体上的羟基与4-(羟甲基)苯硼酸反应, 从而实现对$H_2O_2$的响应。随后, BPA771与CRGDK肽连接的RGD-PEG-*b*-PLGA和物理放射增敏剂$HfO_2$纳米颗粒, 自组装形成RPB7H纳米颗粒 (图3D)。CRGDK配体能够识别过表达在血管表面和HNSCC肿瘤细胞膜上的神经纤毛蛋白-1 (neuropilin-1, NRP-1), 确保$HfO_2$纳米颗粒和BPA771前药的肿瘤特异性递送和深度肿瘤穿透。$HfO_2$纳米颗粒能够在肿瘤细胞内部沉积并通过X射线辐射增强放疗诱导的DNA损伤, 同时诱导$H_2O_2$的分泌。$H_2O_2$可将BPA771前药还原成PROTAC, 精准降解BRD4并下调下游的RAD51AP1, 从而增强肿瘤细胞对放射治疗的敏感性。

除了肿瘤微环境中的高ROS水平, 外源刺激生成的ROS也可用于开发激活型前药。Wang等[66]报道了一种纳米PROTAC (nanoformulated PROTAC, NAP) (图3E), 用于在肿瘤细胞中远程可控的蛋白降解。NAP通过分子自组装形成, 其中PROTAC与光敏剂 (photosensitizer, PS) 通过一个单线态氧 ($^1O_2$) 可裂解的连接键连接。由于共价交联, PROTAC的蛋白降解活性在初始阶段被沉默。在全身给药后, NAP通过EPR效应积聚在肿瘤组织中, 从而促进PROTAC在肿瘤中的富集。随后, 通过在肿瘤部位实施近红外光照射, 激活光敏剂生成$^1O_2$。这不仅可以通过裂解响应连接键释放PROTAC, 还可触发光动力疗法 (photodynamic therapy, PDT)。释放的PROTAC恢复其活性后, 能够招募E3泛素连接酶进行蛋白降解。该研究选择了BET蛋白BRD4作为PROTAC的靶标, 因为BRD4的抑制作用不仅能够逆转细胞对PDT的抗性, 还可提高治疗效果。这种策略为光激活PROTAC提供了新的思路, 通过光引发的$^1O_2$升高来调控PROTAC的蛋白降解活性, 可解决紫外/可见光激活带来的光毒性问题以及较差的组织穿透能力。

## 5 酶激活型PROTAC

肿瘤中有许多过表达的酶, 这些酶的过度表达为肿瘤治疗提供了独特的靶点[67]。通过设计酶激活型PROTAC前药, 可以实现肿瘤细胞的特异性降解, 从而提高抗肿瘤治疗的精准性和有效性。Zhang等[68]开发了一种新型半导体聚合物纳米PROTAC ($SPN_{pro}$), 用于癌症的光免疫代谢治疗。$SPN_{pro}$由半导体聚合物核心与PROTAC片段通过癌症生物标志物可裂解的肽连接而成, 具体组成为: ① 具有优异的生物相容性和可调的光学特性、作为光疗诊断部分的半导体聚合物纳米粒 (SPNs); ② 由吲哚胺2,3-双加氧酶 (indoleamine 2,3-dioxygenase, IDO) 靶向单元和与E3泛素连接酶VHL结合肽段组成的IDO靶向PROTAC肽 (IPP); ③ 在多种癌细胞中过度表达的癌症生物标志物组织蛋白酶B (cathepsin B, Cat B) 可裂解的肽段[69]。$SPN_{pro}$在全身给药后, 被动累积在小鼠肿瘤中。在近红外光照射下, 生成$^1O_2$以消除肿瘤细胞, 并释放肿瘤相关抗原 (tumor-associated antigen, TAA) 和诱导免疫原性细胞死亡 (immunogenic cell death, ICD)。TAA进一步诱导树突状细胞 (dendritic cells, DC) 成熟并促进T细胞激活, 从而增强抗肿瘤T细胞免疫反应。同时, 肿瘤中过度表达的Cat B裂解$SPN_{pro}$并局部释放IPP以持续降解IDO。IDO的降解缓解了色氨酸的过度消耗和犬尿氨酸的积累, 从而逆转免疫抑制。因此, $SPN_{pro}$介导的可激活光免疫代谢治疗通过协同作用有效抑制了4T1小鼠模型中肿瘤的生长。

鉴于半导体聚合物纳米PROTAC的模块化设计具有高度可编程性, 通过替换靶向单元即可实现对不同蛋白的特异性降解。鉴于此, Zhang等[70]还开发了一种靶向环氧合酶 (cyclooxygenase 1/2, COX-1/2) 的智能纳米系统$SPN_{COX}$, 其核心由半导体聚合物骨架、Cat B可裂解肽链及COX-1/2靶向PROTAC三部分组

成 (图4A)。静脉注射后, $SPN_{COX}$通过EPR效应富集于肿瘤部位, 肿瘤微环境中过表达的Cat B特异性切断肽链, 释放COX-1/2靶向PROTAC肽 (CPP) (图4B)。该活性肽通过吲哚美辛 (indomethacin, IND) 结构域靶向COX-1/2, 同时借助VHL配体片段 (GSGSALAPYIP) 招募E3泛素连接酶, 诱导COX-1/2降解 (图4C), 从而阻断其催化产物前列腺素$E_2$ (prostaglandin $E_2$, $PGE_2$) 的生成。$PGE_2$作为免疫抑制分子可促进调节性T细胞 (regulatory T cells, Tregs)、M2型巨噬细胞及髓源性抑制细胞 (myeloid-derived suppressor cells, MDSCs) 的扩增, 形成免疫抑制微环境[71-73]。COX-1/2的清除有效逆转了免疫抑制性细胞浸润, 同时$SPN_{COX}$在近红外光激发下产生的$^1O_2$可直接杀伤肿瘤细胞并诱导ICD, 释放TAAs以激活DC细胞并增强细胞毒性T淋巴细胞浸润 (图4D)。这种“代谢干预−光免疫激活”的双引擎策略, 在4T1三阴性乳腺癌模型中展现出显著的协同效应 (图4E), 更通过重塑肿瘤免疫微环境降低远端转移与复发风险。除此之外, 还开发了一种靶向Src同源2结构域含磷酸酶2 (src homology 2 domain-containing phosphatase 2, SHP2) 的免疫检查点纳米PROTAC (nano-PROTAC, NPRO)[74], 用于癌症光免疫治疗 (图5A)。NPRO通过EPR效应积聚在肿瘤部位。在660 nm激光照射下, NPRO生成$^1O_2$以消除肿瘤细胞, 通过释放TAAs和增强肿瘤细胞中caspase 3的表达来诱导ICD。过表达的caspase 3可激活PROTAC的释放以持续降解SHP2 (图5B)。SHP2的耗竭阻断了免疫抑制信号, 包括CD47/SIRP$\alpha$ (“别吃我”) 和PD-1/PD-L1信号, 从而激活M1型巨噬细胞介导的吞噬作用和效应T细胞介导的抗肿瘤免疫。此外, 该策略促使M2型巨噬细胞转化为M1型巨噬细胞, 并促进活化的效应T细胞浸润到肿瘤微环境。

将肿瘤微环境中高表达的酶激活策略与特定靶向载体结合, 不仅可以实现PROTAC的特异性激活, 还能增强其肿瘤靶向能力。适配体凭借其单链核酸固有的三维拓扑结构 (如茎环、G4四链体), 可通过多模式分子相互作用 (氢键、范德华力、碱基堆积及静电效应) 与靶蛋白实现高特异性结合[75,76]。相较于抗体或肽类载体, 适配体在体内展现出更优的组织渗透性、生物稳定性及低免疫原性特征[77]。以核仁素靶向适配体AS1411为例, 该分子通过富集鸟嘌呤碱基形成的特殊空间构象, 特异性识别肿瘤细胞表面过表达的核仁素蛋白, 为靶向递送提供了精准的“分子导航”[78,79]。He等[80]将适配体技术与酶响应型化学开关相结合, 构建了一种可编程的PROTAC控释系统 (图5C): 通过酯−二硫化物双响应连接子将AS1411共价偶联至VHL配体, 使PROTAC分子在循环过程中保持失活状态。当适配体引导复合物至肿瘤细胞表面并与核仁素结合后, 细胞内高浓度谷胱甘肽 (glutathione, GSH) 优先断裂二硫键, 暴露的自由巯基进一步触发邻近碳酸酐酯键的水解, 最终释放出活性PROTAC分子。这种级联激活机制巧妙地利用了肿瘤微环境特有的氧化还原失衡, 实现了PROTAC降解活性的时空限制性激活。在此过程中, 不仅适配体作为靶向载体增强肿瘤蓄积, 更通过分子开关设计避免了系统性毒性, 为克服传统PROTAC脱靶效应提供了新思路。

叶酸受体$\alpha$ (folate receptor $\alpha$, FOLR1) 作为肿瘤特异性递送的重要靶标, 在卵巢癌、肺癌及乳腺癌等实体瘤中呈现异常高表达, 而正常组织中的表达水平可忽略不计[81-83]。基于此差异化表达特征, Liu等[84]设计了一种叶酸−PROTAC偶联分子, 利用叶酸与FOLR1的高亲和力结合特性, 实现药物分子选择性内化至FOLR1过表达的肿瘤细胞 (图5D)。进入细胞后, 胞内水解酶催化叶酸基团的断裂, 释放出活性PROTAC分子, 后者通过招募VHL E3泛素连接酶对POI进行泛素标记, 最终经26S蛋白酶体途径完成降解。然而, 叶酸基团的引入使PROTAC分子质量突破1 000 Da, 可能限制其口服吸收及系统暴露量, 进而影响PK特性。分子尺寸的增大不仅会降低跨膜渗透效率, 导致肿瘤局部药物浓度不足。因此, 如何通过结构优化平衡靶向性与分子质量之间的矛盾, 成为后续研究的重点。此外, 需系统性评估叶酸−PROTAC在复杂体内环境中的稳定性及肿瘤特异性递送效率, 以验证其临床转化潜力。总之, 该研究既展现了受体介导靶向递送在PROTAC领域的前景, 也揭示了分子工程优化在推进治疗精准化中的必要性。

## 6 多重响应激活型PROTAC

为实现精准时空控制蛋白质降解, An等[85]设计了一种多重响应型PROTAC分子 (C-$srP_{TCO}$), 其激活机制整合了肿瘤内源微环境信号 (硫化氢) 与外源性物理刺激 (X射线), 并通过生物正交策略实现靶向调控 (图6A)。该分子以 (叠氮基)-2,3,5,6-四氟苯甲基为动态保护基团, 其设计灵感源于芳基叠氮化物在硫化氢 ($H_2S$)、生物正交反应或辐射条件下可被特异性激活的特性。在低$H_2S$水平的HEK 293T或HepG2细胞中, 内源性$H_2S$仅能部分还原叠氮基为胺基, 导致C-$srP_{TCO}$的激活效率较低 (图6B)。然而, 在高$H_2S$肿瘤模型中, 如HCT116细胞或NaHS处理, 10 μmol·$L^{-1}$ C-$srP_{TCO}$在12 h内即可实现靶蛋白近乎完全降解, 这一差异通过荧光成像证实与$H_2S$在特定肿瘤 (如结直肠癌HCT116、乳腺癌MCF-7) 中的异常高表达密切相关。为突破$H_2S$依赖

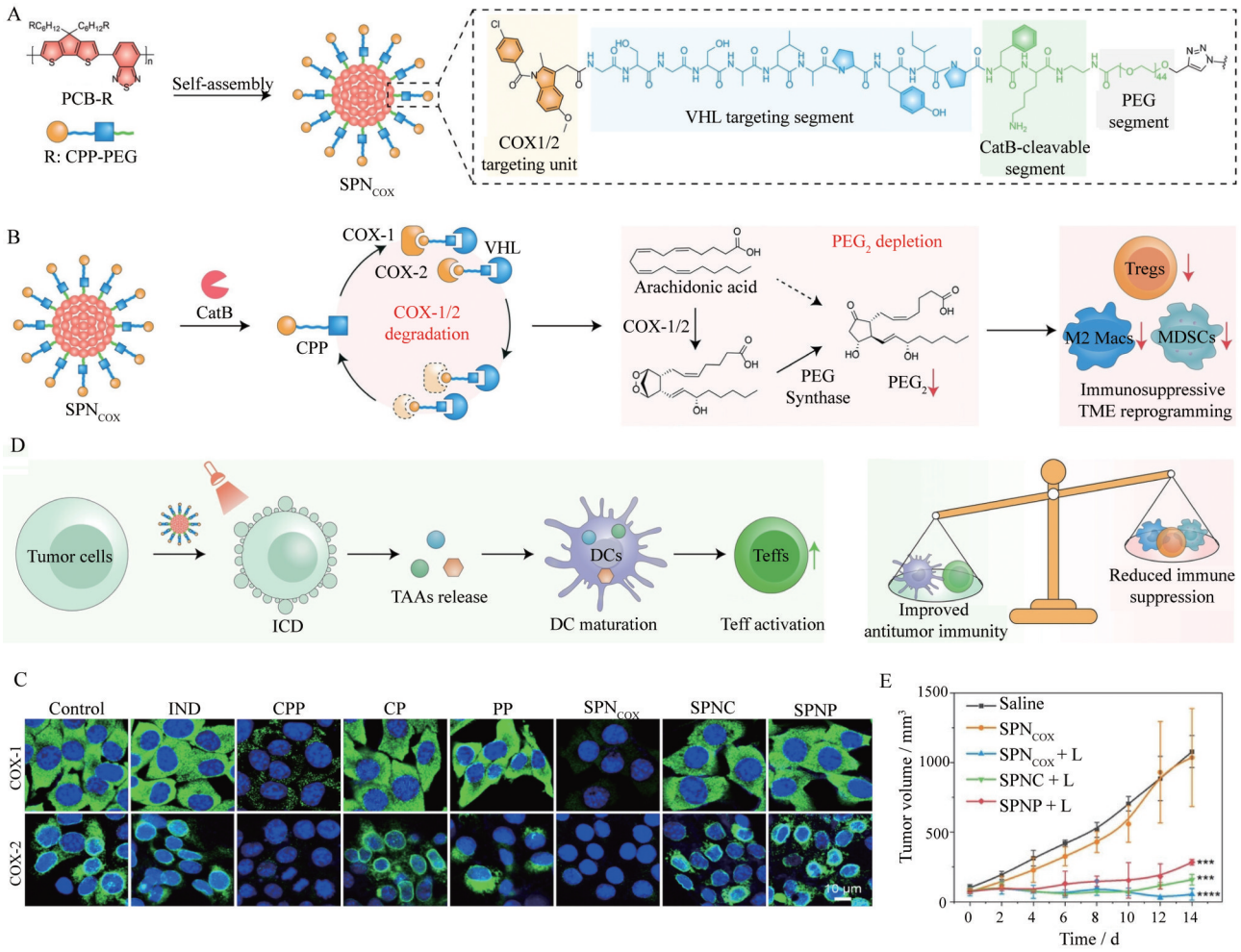



**Figure 4** TME-reprogramming CatB-responsive PROTAC peptide for photo-metabolic cancer immunotherapy. A: Structure of CatB-responsive SPN$_{COX}$; B: CatB-responsive SPN$_{COX}$ for persistent COX-1/2 degradation, PGE$_2$ depletion, and immunosuppressive TME reprogramming; C: Confocal fluorescence images of COX-1/2 expression of 4T1 cells after incubation with different nanoparticles for 12 h; D: The activation of antitumor immune responses *via* inducing ICD, promoting TAAs release, triggering DC maturation, and stimulating effector Teff activation by SPN$_{COX}$-mediated immunogenic phototherapy; E: Activated-SPN$_{COX}$ suppressed tumor growth in 4T1-tumor-bearing mice. Adapted from Ref. 70 with permission. Copyright © 2021 Wiley-VCH GmbH. TME: Tumor microenvironment; CatB: Cathepsin B; ICD: Immunogenic cell death; TAAs: Tumor-associated antigens; DC: Dendritic cell; Teff: Effector T cell; PEG: Polyethylene glycol; PEG$_2$: Prostaglandin E$_2$; COX: Cyclooxygenase; MDSCs: Myeloid-derived suppressor cells; Tregs: Regulatory T cells; M2 Macs: M2 macrophages

性的激活限制, 研究者进一步引入反式环辛烯 (*trans*-cyclooctene, TCO) 介导的生物正交释放系统。TCO与叠氮基反应生成不稳定的1,2,3-三氮唑中间体, 经自发水解后释放活性分子dBET1。实验表明, 直接添加TCO可显著增强C-srP$_{TCO}$的激活, 并呈现剂量/时间依赖性的BRD4-EGFP降解效果。为实现肿瘤靶向激活, 该团队将TCO标记于核仁素 (nucleolin) 靶向适配体AS1411 (AS1411$_{TCO}$), 利用适配体对肿瘤细胞的特异性识别, 将生物正交触发剂定向递送至病灶部位。尽管AS1411$_{TCO}$系统的降解动力学较慢, 但其通过空间定位特异性显著降低了脱靶风险。更引人注目的是, C-srP$_{TCO}$还可响应低剂量X射线, 通过辐射诱导的羟基自由基将叠氮基还原为苯胺, 在保证安全性的同时实现蛋白降解的物理调控。该多重响应系统展现出多维度的精准调控能力: 在$H_2S$富集的HCT116模型中实现肿瘤的特异性蛋白降解与增殖抑制; 通过适配体-TCO的生物正交重分布策略诱导靶向以降低系统毒性; 借助X射线实现时空可控的蛋白降解。这种“内源微环境响应 + 外源物理触发 + 靶向递送”的三位一体策略, 为开发下一代智能型PROTAC提供了创新性技术思路。

Do等[86]开发了一种基于酶促点击反应的智能型PROTAC系统 (enzyme-derived clicking PROTACs, ENCTACs), 通过NTR和GSH双特异性响应肿瘤乏氧

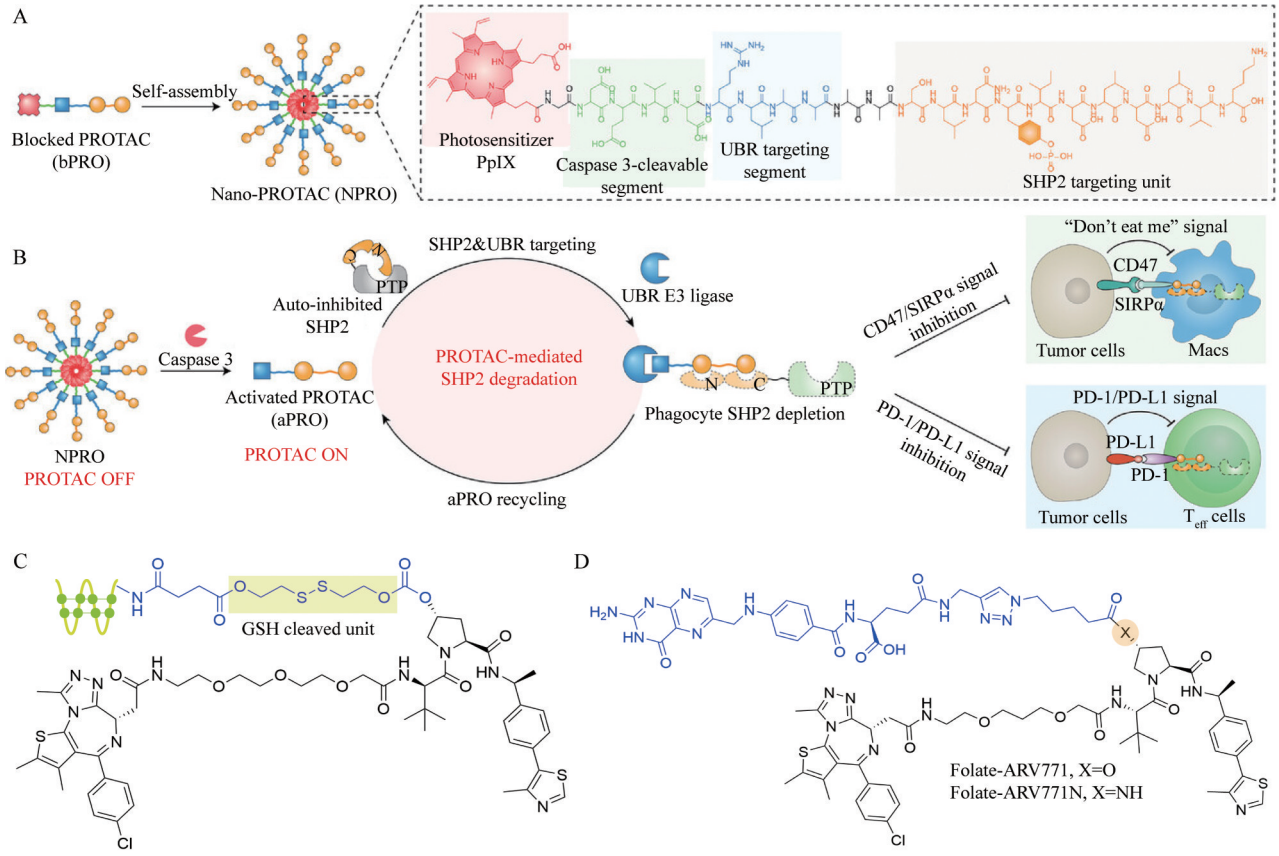



**Figure 5** Enzyme-responsive PROTAC for targeted protein degradation. A: Structure of caspase 3-responsive NPRO; B: Caspase 3-responsive NPRO for activatable cancer photo-immunotherapy. Adapted from Ref. 74 with permission. Copyright © 2022 Wiley-VCH GmbH; C: Chemical structure of GSH-responsive PROTAC. Adapted from Ref. 80 with permission. Copyright © 2021 Wiley-VCH GmbH; D: Chemical structure of Folate-ARV771. Adapted from Ref. 84 with permission. Copyright © 2021 American Chemical Society. SHP2: Src homology 2 domain-containing protein tyrosine phosphatase-2; PTP: Protein tyrosine phosphatase; UBR: Ubiquitin protein ligase E3 component N-recognin; PpIX: Protoporphyrin IX; CD47: Cluster of differentiation 47; SIRP*α*: Signal regulatory protein *α*; Teff cells: Effector T cells; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1

微环境, 实现时空可控的蛋白质降解。该系统的核心设计在于利用乏氧相关酶NTR对硝基底物的选择性激活 (图6C), 在乏氧条件下, NTR催化ENCTACs的硝基基团还原, 触发保护基团移除, 暴露出半胱氨酸活性位点; 随后, GSH响应释放的硫醇基团与邻近预先偶联了2-氰基苯并噻唑的BRD4靶向配体JQ1-CBT进行点击反应, 原位生成具有完整功能的PROTAC分子。这种"酶激活−点击组装"的策略突破了传统PROTAC需预先化学合成的限制, 首次实现了降解剂在病灶部位的按需原位合成。通过将CRBN招募配体与BRD4抑制剂JQ1的解耦设计, 显著降低了分子质量, 改善了药代动力学特性; 其次, 利用肿瘤乏氧微环境特异性高表达的NTR作为分子开关, 确保降解活性仅在靶组织中被激活, 有效减少脱靶效应 (图6D)。正交点击交联反应实现了弹头与E3连接酶配体的可控自组装, 在维持高效降解能力的同时, 避免了传统PROTAC因结构刚性导致的细胞渗透性差等问题。这种"条件式合成−原位激活"的策略, 为开发微环境响应型PROTAC开辟了新的技术路径。

纳米药物递送系统 (nanosized drug delivery systems, NDDS) 凭借其延长血液循环时间、EPR效应以及可控的药物释放特性, 已成为突破传统肿瘤治疗瓶颈的重要技术平台[87-89]。Gao等[90]将NDDS与PROTAC技术融合, 构建了一种聚合物PROTAC (polymeric PROTAC, POLY-PROTAC) 纳米递送系统 (图7A)。POLY-PROTAC可对肿瘤微环境中高表达的MMP-2特异性响应并脱去PEG冠层以此增强POLY-PROTAC对肿瘤的渗透深度 (图7B), 随后在酸性条件下解离并通过高浓度GSH断裂二硫键释放活性PROTAC分子。实验证明, BRD4靶向的POLY-PROTAC在MDA-MB-231三阴性乳腺癌模型中可显著降解BRD4蛋白并抑制肿瘤生长。为进一步提升肿

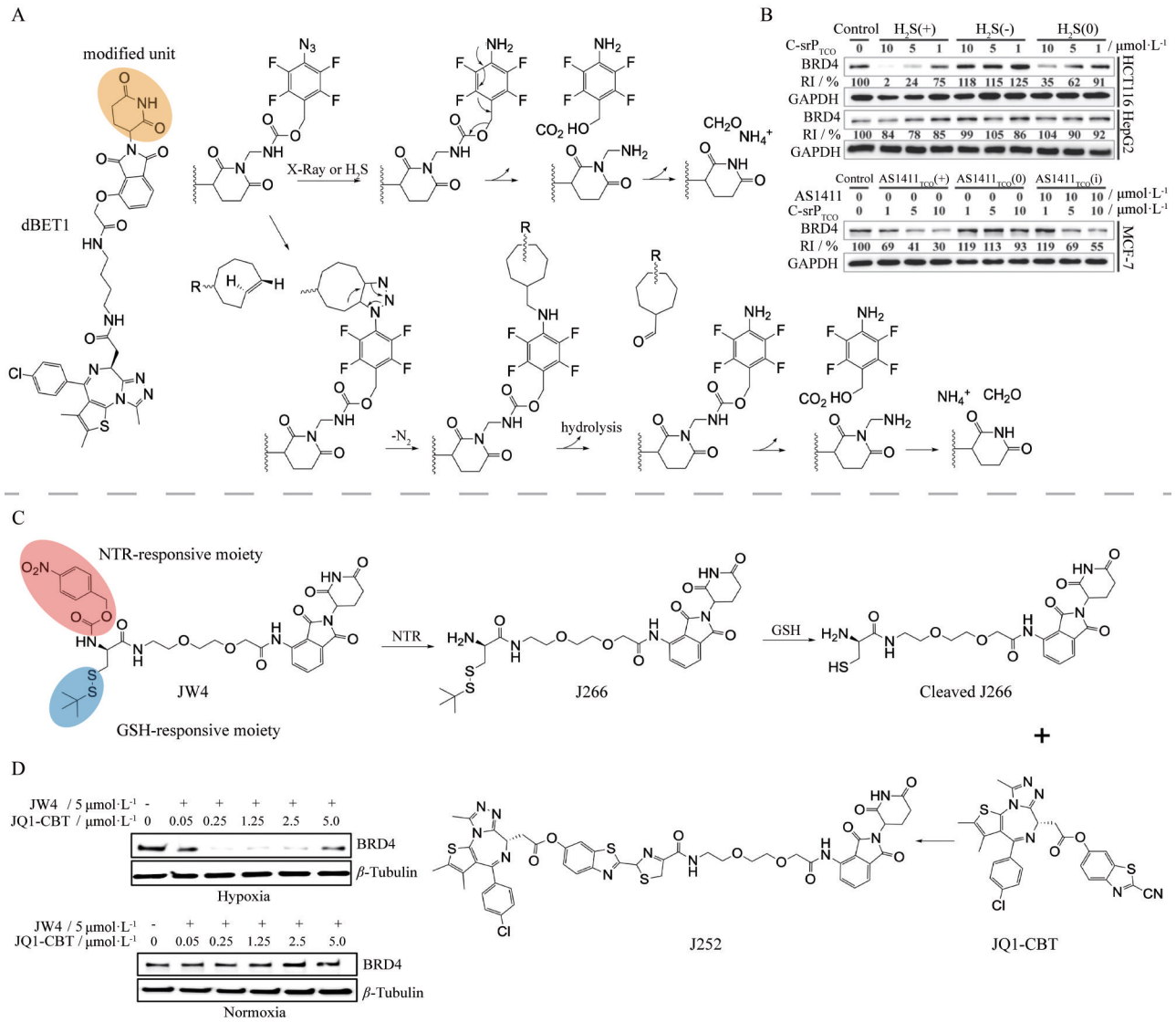



**Figure 6** Multiple stimuli-activatable PROTAC for precise protein degradation. A: Responsive mechanism of C-srP$_{TCO}$; B: C-srP$_{TCO}$ degrades BRD4 protein in different cells after activating with different stimuli. Adapted from Ref. 85 with permission. Copyright © 2023 Wiley-VCH GmbH; C: GSH and NTR dual-responsive CRBN ligand (JW4) activates and clicks with CBT-linked BRD4-targeting ligand (JQ1-CBT) to form BRD4 degrader (J252); D: BRD4 protein levels after treatments with JW4 and JQ1-CBT in concentration-dependent examinations of HeLa cancer cells under hypoxia and normoxia condition. Adapted from Ref. 86 with permission. Copyright © 2022 American Association for the Advancement of Science

瘤特异性积累效率, 该研究团队引入生物正交点击化学策略 (图 7C): 首先通过酸响应型纳米载体递送二苯并环辛烯 (dibenzocyclooctyne, DBCO) 至肿瘤部位, 其在酸性微环境中暴露活性基团; 随后注射叠氮修饰的 POLY-PROTAC ($N_3$@POLY-PROTAC), 通过与 DBCO-$N_3$ 发生肿瘤原位点击反应实现特异性锚定, 使 PROTAC 瘤内浓度较游离药物提升 3.9 倍 (图 7D)。此外, 该体系还在聚合物骨架上接枝了光敏剂焦脱镁叶绿酸 a (pyropheophorbide a, PPa)。在 650 nm 激光照射下, PPa 产生 ROS 引发 PDT, 与 BRD4 蛋白降解形成协同抗肿瘤效应。体内实验表明, 这种“蛋白降解−PDT”联合治疗模式可显著促进肿瘤细胞凋亡, 抑制 MDA-MB-231 荷瘤小鼠的肿瘤生长 (图 7E)。该策略通过多层级响应设计 (酶响应脱壳/酸响应预靶向/还原响应释放) 与协同治疗整合, 成功构建了首个 PROTAC-NDDS 协同治疗平台。该策略实现了 PROTAC 的时空可控释放, 规避了传统 PROTAC 分子质量过大导致的渗透障碍。生物正交预靶向策略突破了 EPR 效应的空间局限性, 显著提升了肿瘤选择性。PDT 与蛋白降解的协同作用通过表观遗传调控与直接细胞杀伤双重

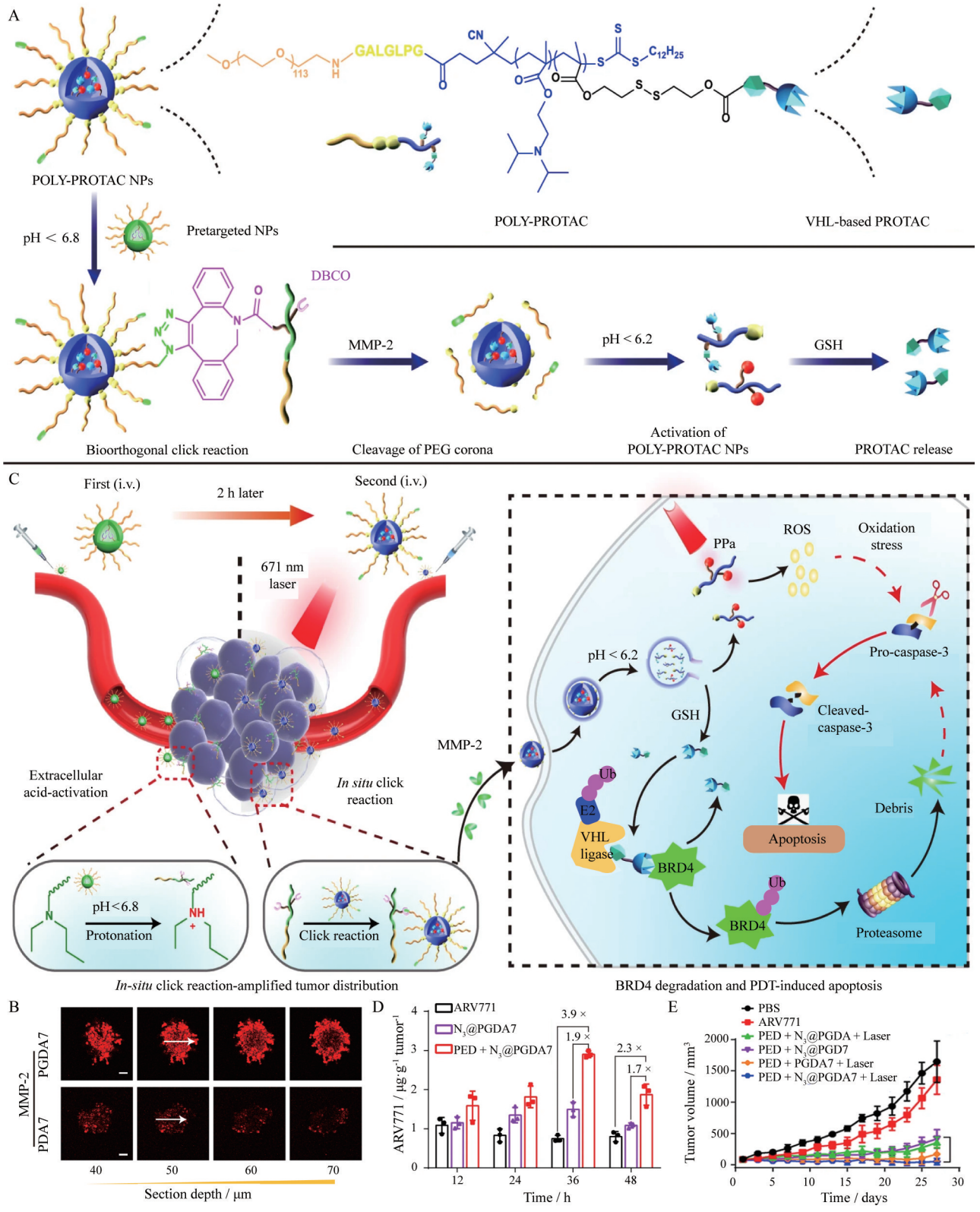



**Figure 7** POLY PROTAC for targeted protein degradation and precise cancer therapy. A: Composition of multiple stimuli-activatable POLY PROTAC and restoration process of PROTAC; B: MMP-2-responsive PGDA7 nanoparticles (POLY-PROTAC) displayed increased penetration into MDA-MB-231 MCSs *in vitro* compared with their MMP-2-nonresponsive PDA7 counterpart; C: The anti-tumor mechanism of multiple stimuli-activatable POLY-PROTAC; D: HPLC-determined intratumoural ARV771 distribution; E: Multiple stimuli-activatable POLY PROTAC suppressed tumor growth in MDA-MB-231 tumor-bearing mice. Adapted from Ref. 90 with permission. Copyright © 2022 Nature Publishing Group. POLY PROTAC: Polymeric PROTAC; DBCO: Dibenzocyclooctyne; MMP-2: Matrix metalloproteinase-2; PDT: Photodynamic therapy; PPa: Pyropheophorbide-a

路径增强疗效。这种智能化、可编程、多重响应的NDDS-PROTAC技术框架, 为实体瘤的精准治疗提供了开创性解决方案。

肿瘤异质性引发的治疗抵抗源于肿瘤微环境中细胞亚群的空间分布与功能差异, 其中常氧区的快速增殖细胞与乏氧区的癌症干细胞 (cancer stem cells, CSCs) 共存尤为关键[91-95]。CSCs凭借其定位于乏氧区域、维持低ROS水平以及高表达干性相关转录因子的特性, 成为肿瘤复发与转移的核心驱动因素[96-98]。然而, 传统治疗手段难以同步清除常氧区的肿瘤细胞与乏氧区的CSCs, 导致治疗盲区的存在。针对这一挑战, Gao等[99]开发了一种限域PROTAC纳米平台 (PGDAT@N) (图8A), 通过耦合肿瘤微环境异质性特征与智能响应型药物设计, 实现了空间选择性的精准治疗。该平台的核心创新在于构建了双环境响应型PROTAC前药系统。在常氧区域, ROS敏感的缩硫酮连接键 (thioketal, TK) 将BRD4靶向PROTAC (ARV771) 锁定为无活性状态, 当纳米载体在肿瘤酸性环境 (pH 6.5) 中激活光敏剂PPa后, 671 nm激光诱导的ROS风暴不仅直接杀伤增殖期肿瘤细胞, 同时特异性裂解TK连接键释放活性ARV771, 通过降解BRD4蛋白破坏癌基因转录; 而在乏氧区域, CSCs内高表达的NTR可将PROTAC前药ARV771-Nb的硝基苯基团还原为氨基, 触发药物活化并持续降解BRD4, 从而抑制CSCs的干性维持与耐药表型 (图8B、C)。为实现精准递送, 该研究团队采用RAFT聚合技术将ARV771-TK与酸响应单体DPA共聚, 并与含PPa的聚合物共组装形成多功能纳米颗粒 (PGDAT)。PGDAT通过EPR效应在肿瘤部位富集后, 肿瘤微环境中过表达的MMP-2酶脱去PEG壳层以增强组织渗透性。在MDA-MB-231乳腺癌和HN30头颈癌模型中, PGDAT@N展现出显著协同效应且无显著系统毒性。这种“光动力杀伤–表观遗传调控–干细胞清除”的多维治疗模式, 成功突破了肿瘤异质性导致的治疗局限。该研究的科学价值不仅在于开发了一种新型纳米PROTAC平台, 更在于建立了基于肿瘤生物学特性的精准治疗策略。通过整合内源性生物标志物 (ROS/NTR) 与外源性物理刺激 (激光), 实现了药物活性的空间编程化控制。未来, 通过替换响应模块 (如GSH敏感键、X射线激活基团) 或靶头 (如ER/AR配体), 该技术框架可扩展至其他难治性肿瘤类型, 为实体瘤的根治性治疗提供全新策略。

血脑屏障 (blood-brain barrier, BBB) 的限制[100]、肿瘤乏氧环境[101], 以及PROTAC在正常组织中引起的非预期蛋白质降解[102], 使得胶质母细胞瘤 (glioblastoma, GBM) 的治疗面临巨大挑战。与全身给药相比, 基于微针 (microneedle, MN) 的药物递送策略在局部递送治疗药物方面具有显著优势。MN介导的药物递送可以绕过多种生理屏障, 如胃肠道代谢[103]、肝脏首过效应[104]和血脑屏障[105], 显著提高药物的生物利用度。同时, MN还可将药物精准递送到病变部位, 减少药物在全身循环中的浓度, 从而最小化全身不良反应[106]。Jiang等[107]利用MN的局部递送特性, 开发了一种自供氧微针 (self-oxygenating PROTAC microneedle, SOP MN) 以辅助药物绕过血脑屏障并实现BRD4蛋白的时空可控降解。SOP MN分为内外两层 (图9A、C)。外层负载了由酸激活聚合光敏剂和光激活PROTAC前药自组装而成的酸敏感PPT7纳米粒, 内层负载了牛血清白蛋白包载的二氧化锰 (BSA-$MnO_2$, BM) 纳米粒。在手术植入GBM肿瘤后, SOP MN的外层迅速溶解, 使PPT7纳米粒区域特异性地递送到肿瘤部位, 并在溶酶体中酸解离 (图9B)。随后通过近红外光照射, 光敏剂的光动力活性被激活, 生成$^1O_2$并还原PROTAC前药, 在肿瘤病灶处实现时空可控的BRD4降解和PDT。与此同时, BM纳米粒在肿瘤内部持续释放 (图9D), 并将$H_2O_2$分解成$O_2$, 有效缓解肿瘤乏氧环境并增强PDT诱导的$^1O_2$生成, 促进PROTAC的还原 (图9E), 实现增强型GBM联合治疗。SOP MN在皮下和原位GBM肿瘤小鼠模型中显著抑制了肿瘤生长。利用微针局部递送特性成功克服了PROTAC难以穿透血脑屏障的难题, 为PROTAC在脑部疾病治疗中的应用提供了新的思路。未来研究可聚焦于整合PROTAC与新型智能递送系统, 如刺激响应型水凝胶、仿生纳米机器人, 通过化学偶联或物理包封策略实现病灶部位特异性蓄积, 突破PROTAC分子因亲脂性强导致的生物利用度低、系统毒性高等瓶颈。这对推动PROTAC从血液肿瘤拓展到实体瘤治疗具有转化医学意义, 特别是在胶质母细胞瘤等具有致密基质屏障的恶性肿瘤治疗中展现出独特的临床转化路径。

## 7 展望与挑战

PROTAC技术通过其独特的“事件驱动”作用机制, 实现了POI的持续性降解清除, 在克服传统小分子抑制剂耐药性难题方面展现出显著优势。与依赖“占位驱动”模式的传统抑制剂不同, 该技术不仅突破了POI结合位点的空间限制, 更能通过催化循环作用实现多次POI降解, 从而有效降低治疗剂量相关的脱靶毒性和不良反应风险。目前已有多个PROTAC分子进入临床试验阶段。靶向雌激素受体阳性乳腺癌的ARV-471已推进至III期临床, 有望成为全球首个上市的PROTAC药物[108]。此外, 用于治疗去势抵抗性前列腺癌的ARV-110 (靶向雄激素受体) 及其同类药物

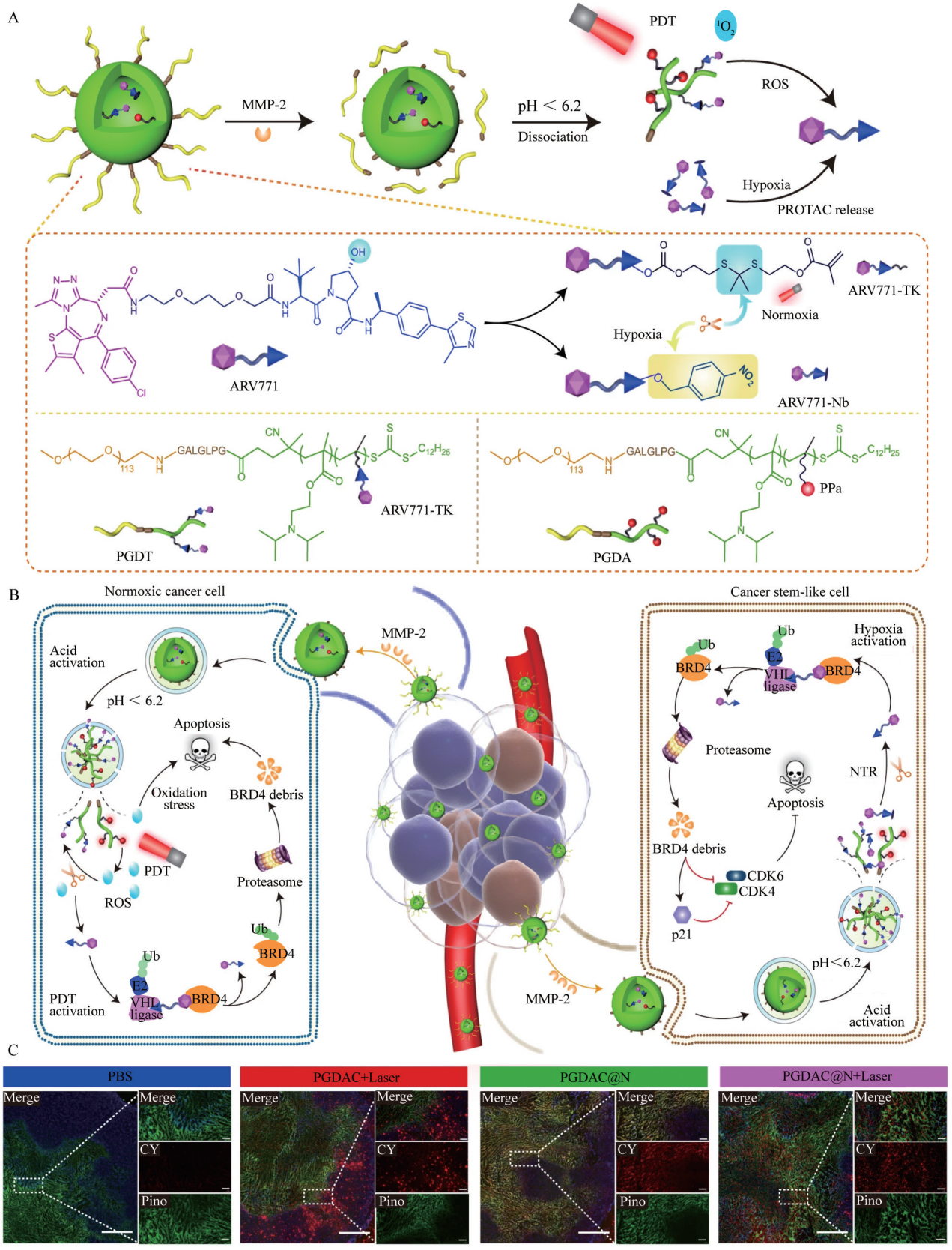



**Figure 8** Region-confined PROTAC nanoplatform for spatiotemporally tunable protein degradation and combinatory cancer therapy. A: Composition of region-confined PROTAC nanoplatform; B: The anti-tumor mechanism of region-confined PROTAC nanoplatform; C: Distribution of ROS/NTR-activated CY on tumor sections to simulate the distribution of ARV771. Adapted from Ref. 99 with permission. Copyright © 2024 Nature Publishing Group. CDK4: Cyclin-dependent kinase 4; CDK6: Cyclin-dependent kinase 6; CY: Hemicyanine

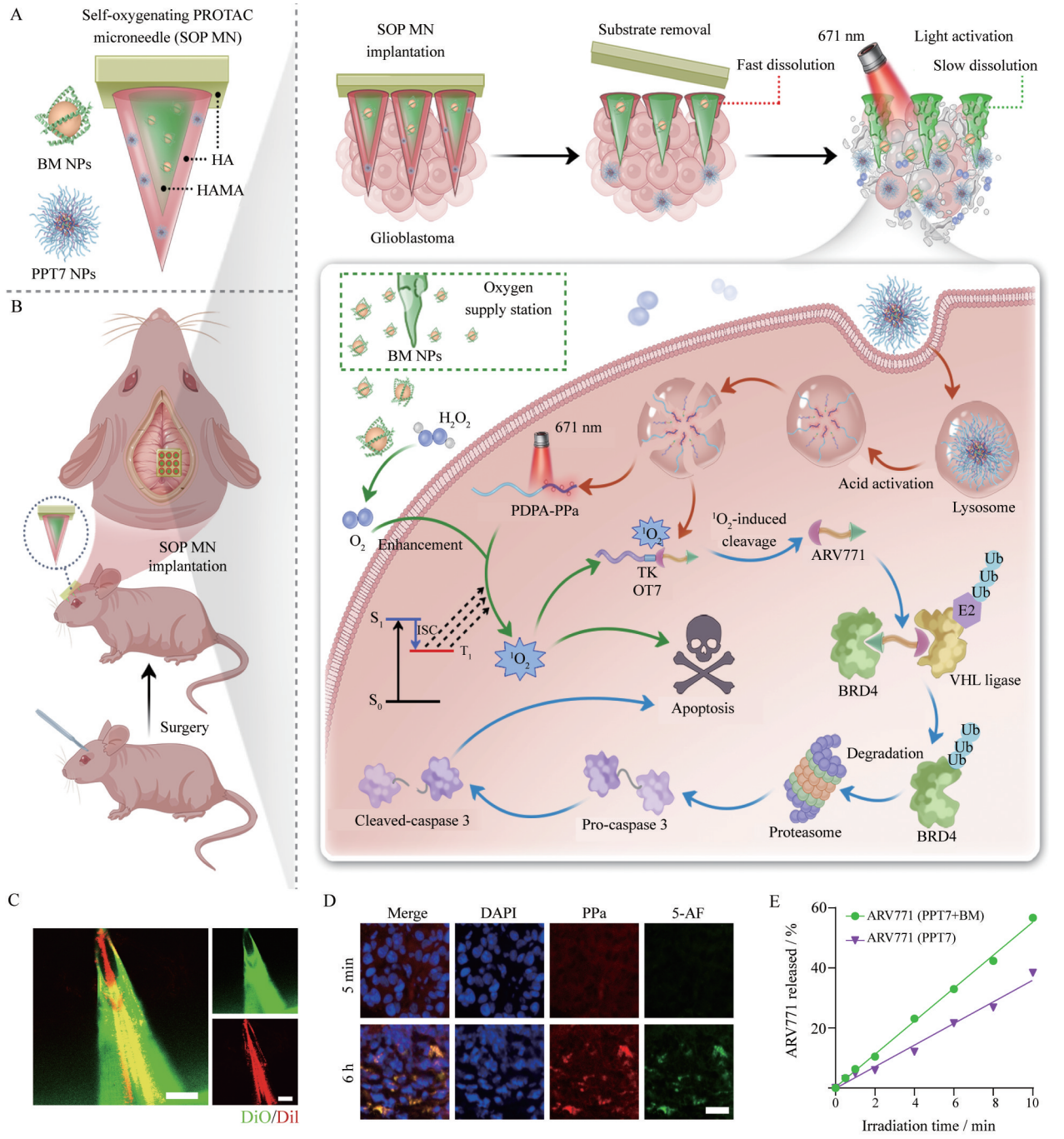



**Figure 9** SOP MN platform for *in situ* protein degradation and potentiated therapy of GBM. A: A schematic of SOP MN; B: SOP MN is surgically implanted into the tumor site and antitumor application of PPT7 and BM nanoparticles *via* SOP MN; C: Representative confocal images of double-layered SOP MN (scale bar = 100 μm); D: Tumor imaging revealed differential release of PPT7 NPs (PPa staining) and BM NPs (5-AF staining) at 5 min and 6 h after the MN application (scale bar = 20 μm); E: Exposure to light following the co-incubation of PPT7 and BM nanoparticles resulted in a significantly higher release of ARV771. Adapted from Ref. 107 with permission. Copyright © 2025 Wiley-VCH GmbH. SOP MN: Self-oxygenating PROTAC microneedle; DiO: 3, 3′-Dioctadecyloxacarbocyanine perchlorate; DiI: 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DAPI: 4′,6-Diamidino-2-phenylindole; 5-AF: 5-Aminofluorescein; ISC: Intersystem crossing; HA: Hyaluronic acid; HAMA: Hyaluronic acid methacrylate

ARV-766、CC-94676、AC-0176和HP518也显示出良好的临床前景[109,110]。针对血液系统恶性肿瘤, 靶向布鲁顿酪氨酸激酶 (Bruton's tyrosine kinase, BTK) 的NX-2127和NX-5948、靶向白细胞介素-1受体相关激酶4 (interleukin-1 receptor-associated kinase 4, IRAK4) 的KT-474/KT-413, 以及靶向信号转导和转录激活因子3

(signal transducer and activator of transcription 3, STAT3)的KT-333等分子正在开展I/II期试验[111-115]。针对特殊靶点的PROTAC也取得突破, 如靶向BRD9的FHD-609用于滑膜肉瘤治疗, 以及靶向B细胞淋巴瘤-extra large蛋白 (B-cell lymphoma-extra large protein, BCL-XL) 的DT2216用于T细胞淋巴瘤治疗等[116,117]。这些临床案例充分展现了PROTAC技术在肿瘤治疗领域的广阔应用前景。

然而, 这类双功能降解剂在临床转化过程中仍面临多重挑战, 主要受限于其固有的分子结构特征。典型PROTAC分子通常具有高分子质量、大极性表面积以及多重氢键供体等理化性质, 这些性质显著偏离类药五原则, 导致PROTAC存在膜渗透效率低下、水溶性欠佳以及系统清除速率过快等关键缺陷, 最终造成药物在靶组织中的有效暴露浓度难以达到治疗窗要求。为突破这些局限, 众多学者开发了刺激响应型PROTAC前药策略, 通过整合内源性或外源性激活机制实现肿瘤选择性蛋白降解。光控型PROTAC采用光敏基团或光致异构元件, 可在特定波长照射下触发笼状基团离去或分子构象变化从而激活降解功能。然而, 临床转化受限于紫外−可见光的组织穿透深度不足及潜在光毒性风险。肿瘤微环境响应系统通过设计对乏氧或过表达酶敏感的化学连接臂, 可在病灶部位特异性释放活性PROTAC, 但响应基团的引入往往导致分子质量突破1 000 Da, 显著影响化合物的吸收、分布、代谢和排泄特性。通过整合多模态刺激响应机制构建的刺激响应型PROTAC, 能够实现肿瘤微环境特异性激活与精准药物释放。这类策略不仅显著改善PROTAC分子的PK特征和肿瘤组织蓄积效率, 还可通过级联响应降低非靶组织中的活性药物暴露以降低脱靶效应。然而, 多重响应模块的协同整合导致分子结构复杂, 需维持较高给药剂量才能达到半数有效降解浓度, 这种现象暗示仍需系统评估长期风险。同时, 基于纳米递送系统设计的刺激响应PROTAC可通过EPR效应和多重刺激相应手段实现PROTAC分子在肿瘤内的蓄积和时空蛋白降解, 但纳米制剂的异质性批次控制、载体材料的潜在免疫原性以及是否符合GMP标准的规模化生产工艺, 仍是阻碍其临床转化的核心问题。

未来, 刺激响应型PROTAC的临床开发可从多个方面进行优化, 以进一步提升其在肿瘤治疗中的潜力。首先, 可以结合现有的其他外源性治疗手段, 如高强度聚焦超声 (high intensity focused ultrasound, HIFU), 进一步开发刺激响应型PROTAC。HIFU技术通过集中超声波能量, 能够在肿瘤区域产生高温, 导致肿瘤组织的坏死或凝固[118]。这一热效应不仅对肿瘤细胞造成直接损伤, 还能通过局部温度的变化调节药物释放。超声波的机械作用和空化效应也可促进PROTAC的膜渗透性, 增强递送效率[119]。此外, 超声波的实时可控性使得治疗过程能够精确监测和调整, 进一步提高了治疗的准确性和个体化[120]。结合HIFU等超声技术与刺激响应型PROTAC, 可进一步推动肿瘤治疗向非侵入性、靶向性和个性化的方向发展。在靶向递送系统方面, 深入挖掘肿瘤特异性生物标志物, 将为刺激响应型PROTAC的精准治疗提供重要支持。通过利用这些标志物, 可以实现更加精准的药物递送, 减少药物在正常组织的分布, 从而提高药物的选择性和疗效。这种靶向性递送不仅能够降低不良反应, 还能够提高治疗的特异性, 使治疗更加个性化和有效。

未来刺激响应型PROTAC的开发将依赖于多学科技术的整合。通过结合化学修饰、生物靶向策略以及智能材料, 可以进一步优化PROTAC的结构和功能。这些技术的融合不仅能够提高药物的稳定性和靶向性, 还能够赋予PROTAC智能化特性, 如在特定环境下的自我调节、响应激活等能力, 为肿瘤精准治疗提供新的突破。通过这些创新的技术手段, 刺激响应型PROTAC有望成为未来肿瘤治疗领域的重要工具, 推动肿瘤治疗向更加个性化、精准化的方向发展。

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**利益冲突**: 本文所有作者声明不存在利益冲突关系。

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