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化疗作为恶性肿瘤临床治疗的主流手段,其疗效与应用却因目前抗癌药物的局限性而受到显著制约。在此背景下,诊疗型前药策略应运而生。这类前药兼具诊断与治疗双重功能,不仅能实现药物的精准可控释放,还可以实时反馈治疗效果,因而成为当前肿瘤化疗领域的研究热点。本文中,陈春英院士等人聚焦近期开发的有机小分子肿瘤诊疗前药,系统阐述其设计理念及各功能模块的作用机制。通过深入探讨,旨在帮助从事相关领域研究的学者把握该领域面临的挑战与潜在机遇,进而激发出更多的研究思路与灵感。

## 基于智能响应型前药递送系统的肿瘤诊疗一体化策略研究进展

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**摘要:** 化疗作为恶性肿瘤临床治疗领域的主流手段,在当代肿瘤综合治疗体系中具有不可替代的临床价值。但是当前化疗药物存在以下关键局限性:首先,其理化性质表现为普遍水溶性不佳,且缺乏肿瘤组织靶向性;其次,在药代动力学方面存在系统清除快、生物利用度低的问题,同时伴随显著的毒性反应;更重要的是,长期化疗会诱导肿瘤细胞产生多药耐药性,这已成为导致化疗方案失效的首要机制。针对以上局限性,前药递送系统作为一种新型的解决方案应运而生。该递送策略通可以明显增强药物溶解性、延长血液半衰期、实现肿瘤靶向递送。而且该策略支持多药共载,实现协同治疗。这种多维度优化使得化疗疗效获得系统性提升。尽管前药递送系统提升了传统药物的利用率,但该系统也面临着难以向肿瘤深处渗透,细胞摄取困难以及药物释放不完全等弊端。为此,当前研究热点聚焦于智能响应型前药递送系统的开发并经过功能调控来实现精准给药:(1)在体循环阶段维持结构完整性,确保药物封装稳定性,保护药物不被漏释;(2)通过感知肿瘤微环境特征参数(pH、活性氧水平、生物酶表达水平等因素)引发结构改变,从而区分肿瘤组织和正常组织,并释放药物,降低对正常组织的毒副作用;(3)动态调控递送系统的理化性质(表面电荷分布、粒径尺寸、分子拓扑结构等),从而同步优化肿瘤组织渗透性、细胞摄取效率及可控制释放的药代动力学。这种智能响应型递送系统有效突破了传统化疗药物的生物屏障限制。本文回顾了智能响应型前药递送系统在肿瘤诊疗一体化研究中的进展,并对该领域的未来发展趋势做了展望。

**关键词:** 前药递送系统;肿瘤诊疗一体化;智能响应型

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## Research progress on integrated strategy of the theranostics of tumor based on intelligent responsive prodrug delivery systems

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**Abstract:** Chemotherapy, as the mainstream therapeutic modality in clinical oncology, remains clinically irre-

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placeable in the contemporary multimodal cancer management frameworks. However, current chemotherapeutic agents have the following critical limitations: Firstly, their physicochemical characteristic includes unfavorable aqueous solubility and inadequate tumor-specific targeting capabilities; secondly, pharmacokinetic limitations are revealed in accelerated systemic clearance and diminished bioavailability with marked cytotoxic sequelae. Notably, long-term chemotherapy triggers the development of multidrug-resistant phenotypes in malignant tumor, which has become the primary mechanism of the failure of chemotherapy. To overcome the clinical limitations of chemotherapeutic agents, prodrug delivery systems have emerged as an innovative solution. This delivery strategy increases aqueous solubility, extends half-life of plasma, realizes tumor-targeted accumulation and enables synergistic treatment by co-delivery of multi-drug. This multidimensional optimization leads to a systematic improvement in chemotherapy efficacy. Although prodrug delivery systems have improved the utilization rate of traditional drugs, they have some drawbacks such as the difficulty in deeper tumor penetration depth, the difficulty in cellular uptake, and the incomplete release of drug. Therefore, the current research focuses on the development of intelligent responsive prodrug delivery systems and the precise drug-delivery through functional regulation: (1) In the systemic circulation phase, maintaining structural integrity ensures drug encapsulation stability and prevents premature drug leakage; (2) Drug delivery systems can distinguish tumor tissues from normal tissues by sensing characteristic parameters of the tumor microenvironment, such as pH, reactive oxygen species and enzyme expression levels, thereby triggering structural transformations to release drugs and reduce toxicity toward normal tissues; (3) Dynamic regulation of the physicochemical properties of prodrug delivery systems (surface charge distribution, particle size, and molecular topology) enables simultaneous optimization of tumor tissue penetration, cellular uptake efficiency, and controlled-release pharmacokinetics. This intelligent responsive prodrug delivery systems effectively overcomes the biological barrier limitations of traditional chemotherapy drugs. This review systematically summarizes the advancements of intelligent responsive prodrug delivery systems in the theranostics of tumor and provides insights into the future development trends of this field.

**Keywords:** prodrug delivery systems; the theranostics of tumor; intelligent responsiveness

## 0 引言

恶性肿瘤仍然是全球最严重的健康问题之一,由于其具有侵袭性和转移性,严重威胁着人类的健康和生命<sup>[1-2]</sup>。目前,癌症治疗的方法主要有化疗、放疗和手术治疗等<sup>[3]</sup>。其中化疗是治疗癌症患者的常规方法<sup>[4]</sup>。然而,目前的化疗方案在临床使用中也会遇到许多困难<sup>[5-8]</sup>。例如化疗药物缺乏对癌细胞的适当选择性;恶性肿瘤部位抗癌药物浓度不足;机体内容易产生耐药性并受到剂量限制毒性的困扰,最重要的是现在缺乏实时监控治疗效果的技术等。为了克服以上的种种困难,研究人员致力于开发有可能克服这些局限性的基于前药的策略。所谓前药,也称前体药物,是指药物经过化学结构修饰后得到的在体外无活性或活性较小,而能在体内经生物分子转化而释放出发挥药效的化合物<sup>[9]</sup>。在前药种类中,诊疗型前药兼备了诊断与治疗的功能,不仅实现了药物的可控释放,而且还可以及时反馈治疗效果<sup>[10-13]</sup>。因此,这种前药成为现在的研究热点。

诊疗型前药有很多种类,包括纳米前药、有机小分子前药等<sup>[14-17]</sup>。有机小分子诊疗前药具有结构简单、便于修饰、生物相容性好等特点,是前药设计与研究的优选。本综述将重点介绍最近开发的有机小分子癌症诊疗前药。我们讨论了每种前药的设计理念以及各个部分的功能。我们希望读者能够了解该领域当前的挑战和机遇,并激励大家为这一关键研究领域贡献更多的力量。

## 1 诊疗型有机小分子前药的简介

目前临床上使用的抗癌药物的局限性大大限制了化疗在肿瘤治疗中的效果与应用。因此,诊疗型前药策略应运而生。诊疗型前药就是把影像诊断和药物治疗结合起来的一种多功能药物<sup>[18-20]</sup>。在众多种类的前药中,诊疗型有机小分子前药因其制备简单,较高的肿瘤靶向性,较小的毒性以及较低的耐药性而得到了广泛的关注<sup>[21-23]</sup>。诊疗型有机小分子前药是将抗癌药物、荧光分子、响应基团以及靶向基团等不同功能片段通过共价作用连接起来。前药诊断

功能的实现依赖于肿瘤微环境与正常组织之间的差异,如pH、还原型谷胱甘肽(glutathione, GSH)、活性氧(Reactive oxygen species, ROS)和活性酶等<sup>[24-28]</sup>。这种方式可以有效提高治疗效果以及减少副作用。而前药治疗功能的实现则是依赖于开发出来的抗癌药物。传统的化疗药物包括具有细胞毒性的抗生素(如阿霉素和丝裂霉素)<sup>[29]</sup>、烷化试剂(氮芥类、顺铂和环磷酰胺)、抗代谢类(氟尿嘧啶、吉西他滨和甲氨蝶呤)<sup>[30]</sup>、微管蛋白抑制剂(长春新碱和紫杉醇)<sup>[31]</sup>和拓扑异构酶I抑制剂(喜树碱和伊立替康)<sup>[32]</sup>等。目前这些化疗药物均存在水溶性差、对癌细胞选择性较差以及多药耐药性和剂量限制毒性等问题<sup>[33-35]</sup>。但是,新型的抗癌药物(如光动力学药物、光热疗药物、磁热疗药物等)也为肿瘤的治疗提供了新的思路<sup>[36-38]</sup>。荧光分子则是诊断成像的发光分子,其可以根据治疗效果而改变荧光强度,从而指导药物治疗方案。荧光分子覆盖了可见光、近红外一区 and 近红外二区等波段的成像范围,随着波长的延长,组织穿透能力和空间分辨率也随之提高<sup>[39-40]</sup>。靶向基团会特异性识别肿瘤细胞表面过度表达的受体,以区分正常细胞与癌细胞<sup>[41-42]</sup>。利用靶向基团对药物分子进行结构修饰可提高药物分子的靶向性,降低对正常组织的毒性。总之,该前药策略可以改善靶向给药系统的物理和化学性质,从而构建智能化靶向给药系统,实现以低毒或无毒策略靶向肿瘤组织或细胞,按照设想方案释放药物,进行癌症治疗。

## 2 有机小分子前药的激活分类

诊疗型有机小分子前药需要在特定的刺激下,才能够发挥诊断与治疗双重功能。肿瘤微环境响应性的诊疗前药一般在肿瘤区域被选择性激活而同时释放抗肿瘤药物分子及成像分子。因此这种肿瘤微环境响应性诊疗前药在抗肿瘤药物的个体化给药中发挥了巨大的作用。下面是常见的几种肿瘤微环境介导的响应型有机小分子前药的具体介绍。

### 2.1 生物酶介导响应性前药

氧化还原酶是一类调节生命系统中氧化还原稳态的重要生物酶,其活性的异常表达与各种疾病息息相关<sup>[43]</sup>。例如,酪氨酸酶与黑色素瘤和帕金森病有关。此外,细胞色素P450酶是一种血红素蛋白酶,在生命体中的代谢降解过程中起着重要作用,它的异常表达是肝损伤性疾病的生理指标。此外,单

胺氧化酶也是帕金森病和阿尔茨海默病的生物标志物。在癌细胞内,多种蛋白酶过度表达,活性升高,常被作为肿瘤标志物<sup>[44]</sup>。比如NADH醌氧化还原酶(NADH quinone oxidoreductase, NQO)、硝基还原酶(Nitroreductase, NTR)和偶氮还原酶(Azoreductase, AzoR)在肿瘤中的缺氧条件下会过度表达;碱性磷酸酶、氨基肽酶N、 $\gamma$ -谷氨酰转移酶等活性酶也会在癌细胞中过度表达。

Fan等人合成了一种氨基肽酶N(Aminopeptidase N, APN)响应的前药(NBFMeI)<sup>[45]</sup>。其中,尼罗蓝衍生物作为前药的荧光团,用来进行荧光诊断。抗癌药物美法仑被用来进行化学治疗。药物美法仑和尼罗蓝荧光团之间由于光诱导电子转移(Photoinduced electron transfer, PET)效应而表现出可忽略的细胞毒性和非常弱的荧光。当前药与APN发生反应之后, PET过程被阻断,尼罗蓝荧光团恢复近红外荧光发射,与此同时美法仑药物被释放并激活其活性,达到诊断和治疗的相统一(图1a)。前药对APN具有灵敏的响应性,随着APN浓度的逐渐增加,尼罗蓝的荧光强度逐渐增强(图1b)。前药NBFMeI对APN阳性癌症细胞也表现出了显著的细胞毒性,这是细胞摄取前药和细胞内前药被活化增强的结果(图1c)。更重要的是,小鼠经过尾静脉注射前药NBFMeI后,其肿瘤处有明显增强的荧光,说明前药在肿瘤内可以被特异性激活(图1d)。同时,小鼠肿瘤的体积也在逐渐变小,这说明该前药具有优异的抗癌作用(图1e)。小鼠的体重在整个治疗过程中保持稳定,说明前药具有优异的生物相容性,且无明显的毒副作用(图1f)。因此,这种治疗前药在个性化诊疗中具有广阔的应用前景。

Wang等人开发了一种基于硝基还原酶(NTR)激活的前药IMC-FDU-TZBC-NO<sub>2</sub>,用于在荧光成像技术指导下进行诊断与治疗<sup>[46]</sup>。该前药的设计如图2a所示,作者选择5-氟-2-脱氧尿苷(5-fluoro-2-deoxyuridine, FDU)作为抗癌药物,因为其具有较高的抗肿瘤活性、亲水性,以及在癌症治疗中的广泛应用。吲哚美辛(Indomethacin, IMC)被用作靶向基团,来提高前药的靶向效果、细胞摄取效率和药物积累。4-硝基苄基被用作NTR的响应基团,可以根据NTR的活性来控制药物的释放。联苯-羧酸酯衍生物用作荧光染料前体,该衍生物在响应基团和抗癌药物离去后可以形成具有荧光发射的染料。前药IMC-FDU-TZBC-NO<sub>2</sub>在体外对NTR表现出了优异的响应特性(图2b)。然后作者研究了前药在细

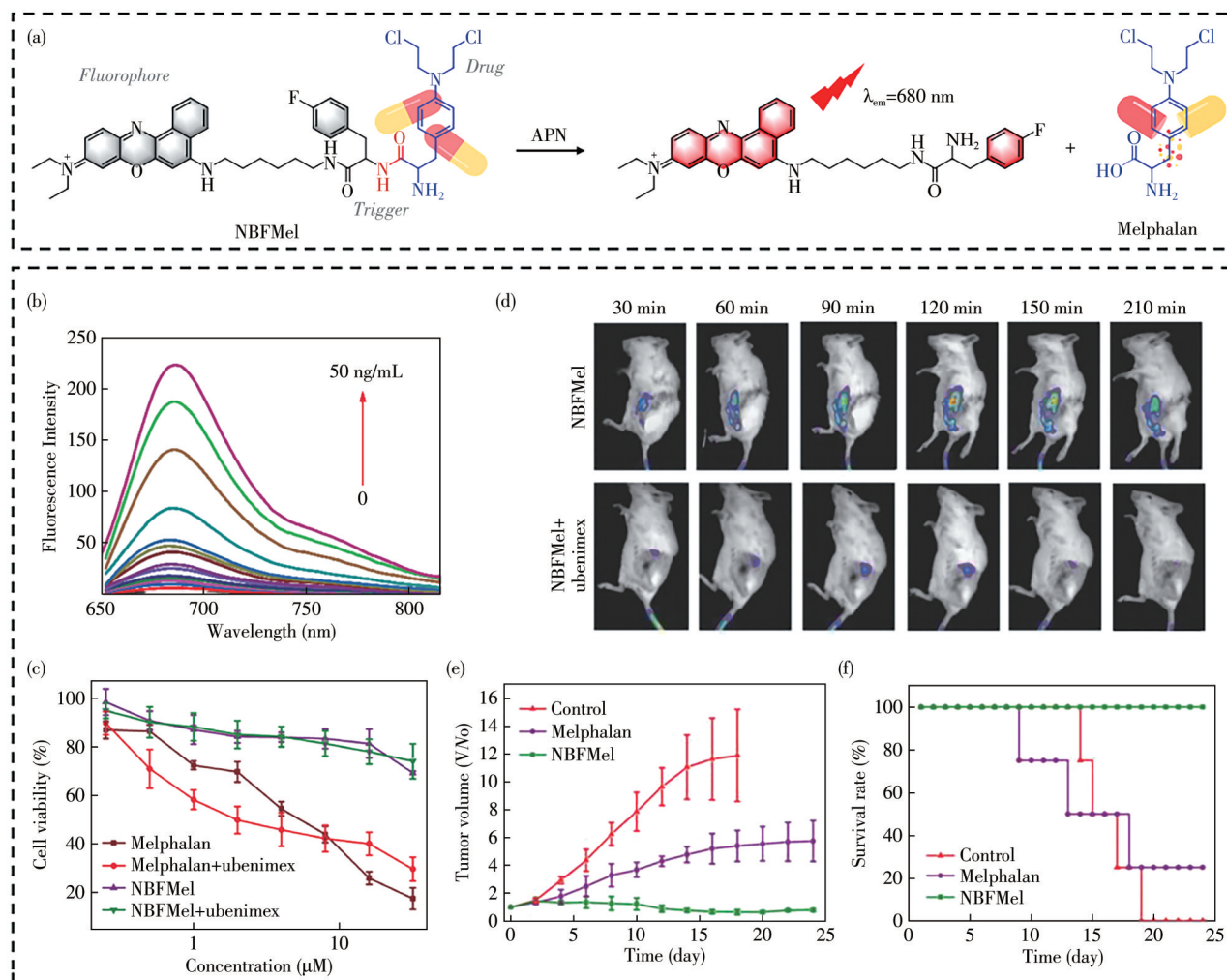


图1 APN响应的前药(NBFMeI)在肿瘤诊疗一体化中的应用<sup>[45]</sup>

(a)前药NBFMeI与APN的传感机制;(b)前药NBFMeI与不同浓度APN的荧光响应光谱图;(c)MDA-MB-231细胞在经过不同处理后的细胞存活率;(d)各组荷瘤小鼠的荧光成像图(NBFMeI组:瘤内注射PBS,然后尾静脉注射NBFMeI的PBS溶液到小鼠体内。NBFMeI+ubenimex组:瘤内注射ubenimex的PBS溶液,然后尾静脉注射NBFMeI的PBS溶液到小鼠体内);(e)静脉注射NBFMeI组、Melphalan组和PBS组的荷瘤小鼠的肿瘤体积变化图;(f)不同小组中小鼠的存活率图

Figure 1 Application of APN responsive prodrug (NBFMeI) in the theranostics of tumor<sup>[45]</sup>

(a) The sensing mechanism of prodrug NBFMeI and APN; (b) Emission spectra of NBFMeI after treatment with APN; (c) Cell viability of MDA-MB-231 cells after different treatments; (d) In vivo fluorescence imaging of tumor-bearing mice after different treatments (NBFMeI group: the tumor was pretreated with PBS by intratumoral injection. Then, NBFMeI was intravenously administered into the mice via the tail vein. NBFMeI+ubenimex group: the tumor was pretreated with ubenimex by intratumoral injection. Then, NBFMeI was intravenously administered into the mice via the tail vein); (e) Changes in tumor volume after intravenous injection of the NBFMeI group, melphalan group, and PBS group; (f) Survival rate of the mice from different groups

胞中的靶向性和响应性,结果显示前药不仅对肿瘤细胞显示出高度的特异靶向性,而且也可以响应细胞内的NTR(图2c)。此外,IMC基团的引入也提高了前药IMC-FDU-TZBC-NO<sub>2</sub>的细胞摄取效率。最后,为了进一步研究前药的治疗作用,作者把前药IMC-FDU-TZBC-NO<sub>2</sub>注射到荷瘤小鼠中,结果显示前药对小鼠肿瘤生长具有显著抑制作用,对各个器官也没有毒副作用(图2d和图2e)。

## 2.2 活性氧(ROS)介导响应性前药

癌症在发展进程中的病理过程(如转移、凋亡、增殖和血管生成等)都离不开活性氧(ROS)的参与<sup>[47]</sup>。癌细胞中ROS(如过氧化氢、羟基自由基等)水平要高于正常细胞,这可能与线粒体基因组完整性的丧失密切相关<sup>[48-49]</sup>。此外,肿瘤细胞内ROS水平的提高诱导了转移相关生长因子的表达,导致癌细胞的侵袭和迁移,难以完全治愈。最重要的是,肿

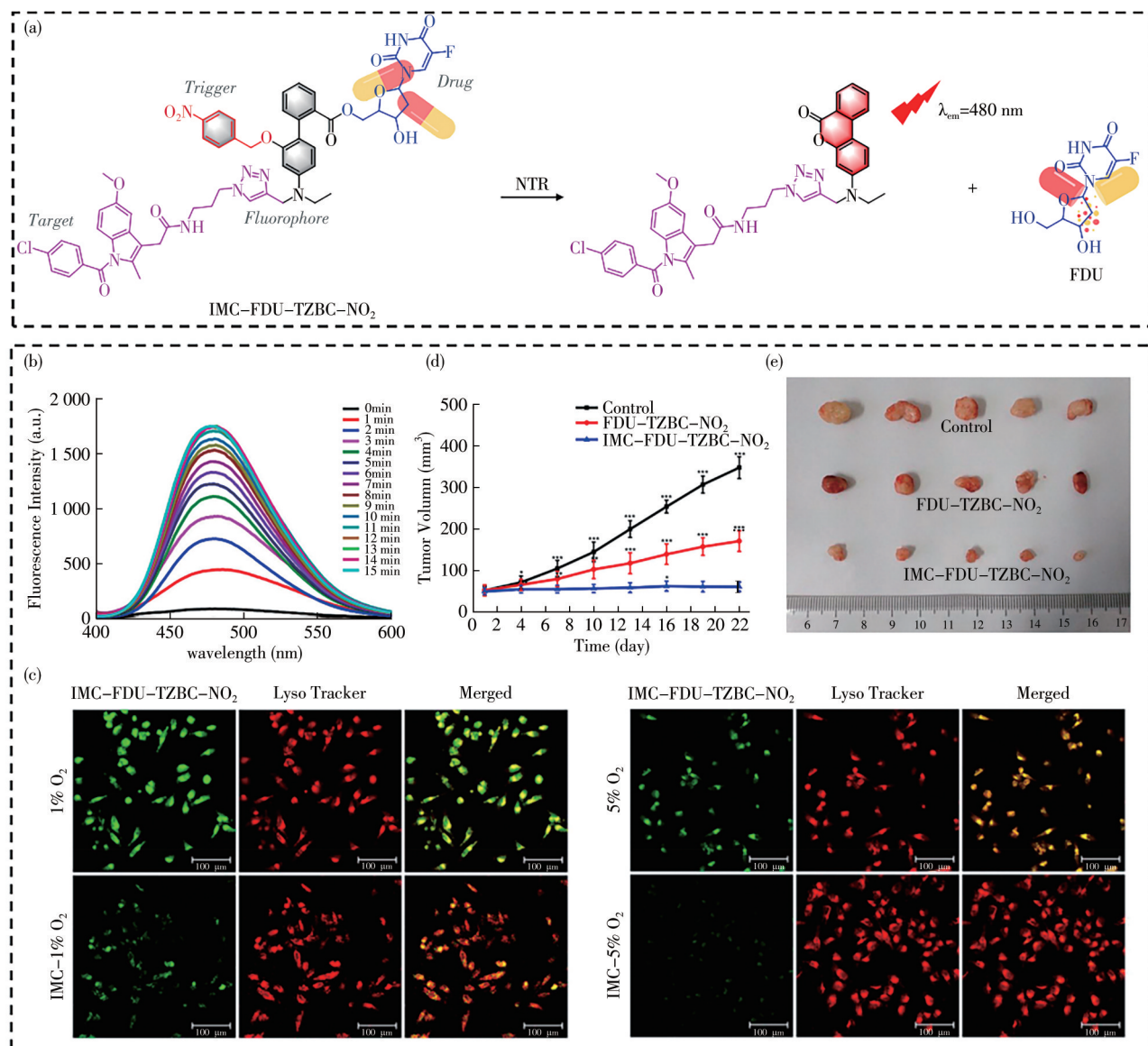


图2 NTR响应的前药(IMC-FDU-TZBC-NO<sub>2</sub>)在肿瘤诊疗一体化中的应用<sup>[46]</sup>

(a)前药IMC-FDU-TZBC-NO<sub>2</sub>与NTR的传感机制;(b)前药IMC-FDU-TZBC-NO<sub>2</sub>与NTR在不同的孵育时间下的荧光光谱图;(c)引入靶向基团IMC和不引入靶向基团IMC的情况下,HepG-2细胞与前药IMC-FDU-TZBC-NO<sub>2</sub>共孵育12 h的荧光成像图;(d)在22天治疗期内,各组小鼠的相对肿瘤体积变化图,数据以平均值±标准误差表示,\*、\*\*和\*\*\*分别表示 $P$ 值 $<0.05$ 、 $<0.01$ 或 $<0.001$ ;(e)注射IMC-FDU-TZBC-NO<sub>2</sub>组、FDU-TZBC-NO<sub>2</sub>组和PBS组的荷瘤小鼠在解剖后的肿瘤大小的实物图

Figure 2 Application of NTR responsive prodrug (IMC-FDU-TZBC-NO<sub>2</sub>) in the theranostics of tumor<sup>[46]</sup>

(a) The sensing mechanism of prodrug IMC-FDU-TZBC-NO<sub>2</sub> and NTR; (b) Time-dependent fluorescence spectra of IMC-FDU-TZBC-NO<sub>2</sub> incubation with NTR; (c) Images of the HepG-2 cells coincubated with IMC-FDU-TZBC-NO<sub>2</sub> in the absence and presence of IMC under hypoxic conditions for 12 h; (d) Relative tumor volume changes of each groups in 22 day, Data expressed as average  $\pm$  SE, \*, \*\*, and \*\*\* means  $P < 0.05$ ,  $< 0.01$ , or  $< 0.001$ , respectively; (e) Representative histological photomicrograph of xenograft mice injected with IMC-FDU-TZBC-NO<sub>2</sub>, FDU-TZBC-NO<sub>2</sub>, and PBS

瘤细胞中的ROS水平(50~100 μmol/L)显著高于正常细胞(~20 nmol/L),这种差异高达100倍以上的特点赋予了ROS响应型前药具有高选择性的优势<sup>[50-51]</sup>。

Kim等人开发了一种H<sub>2</sub>O<sub>2</sub>激活的前药7(Pro-

drug 7)<sup>[52]</sup>。其结构设计如图3a所示,前药中含有H<sub>2</sub>O<sub>2</sub>响应的可裂解硼酸酯基团,香豆素荧光团以及强效抗癌药物SN-38。SN-38是一种抗癌药物喜树碱(Camptothecin, CPT)的衍生物,用于通过抑制拓扑异构酶I治疗各种类型的癌症。该前药与H<sub>2</sub>O<sub>2</sub>反应

时,芳基硼酸酯基团被氧化为苯酚基团,进而引发前药7的分解,释放出游离的香豆素荧光团,并且开启荧光,实现诊断功能。同时,SN-38药物分子也被激活,可以发挥其抗癌效果。前药7在体外可以高灵敏地响应 $\text{H}_2\text{O}_2$ ,随着 $\text{H}_2\text{O}_2$ 浓度的增大,荧光强度也逐渐增强(图3b)。然后作者研究了前药7在细胞中的作用,从

图3c可以看到加入前药7后细胞存活率明显降低,这说明前药7可以选择性抑制肿瘤细胞生长,表明抗癌药物SN-38在细胞内被释放出来并发挥了治疗作用。最后作者将前药7通过气管内给药的方式注射到小鼠体内,最后前药7在转移性肺肿瘤中选择性积聚,同时在转移肿瘤处释放出活性抗癌药物SN-38(图3d)。

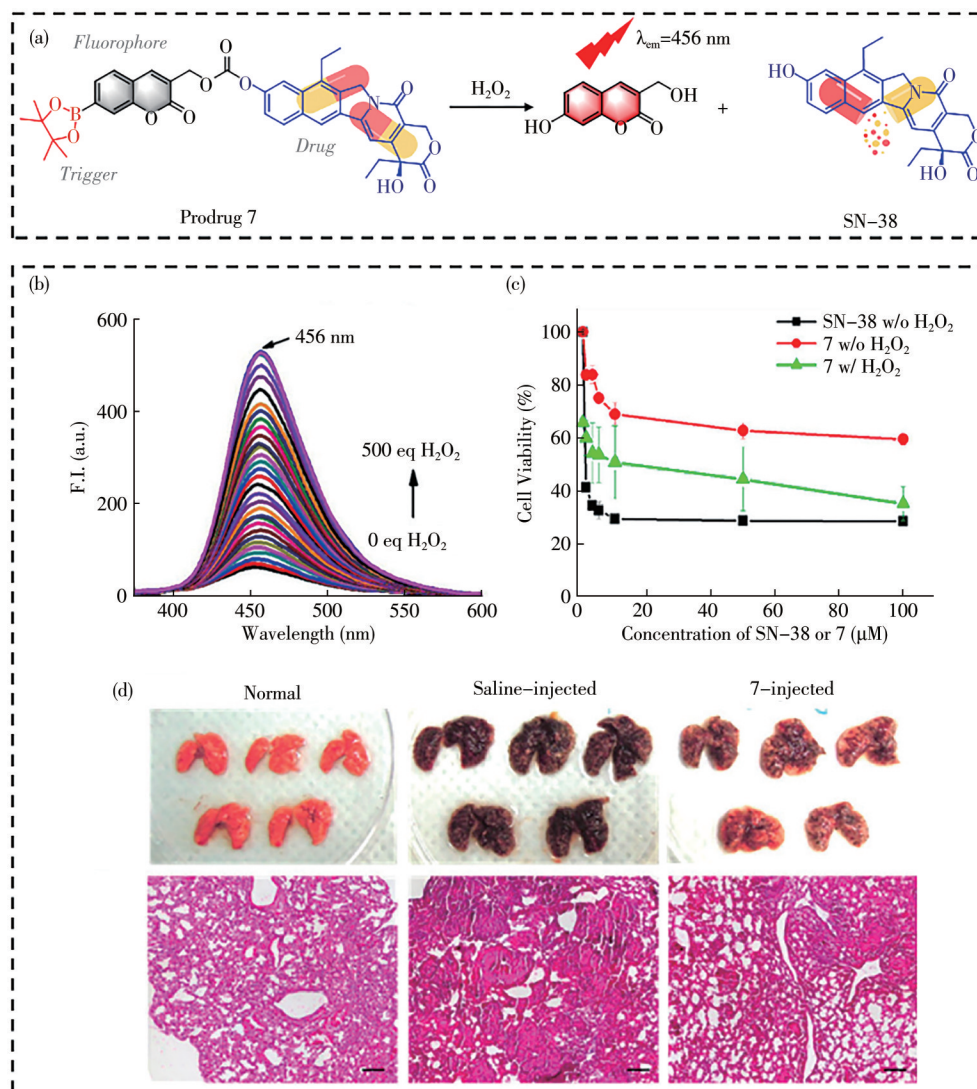


图3  $\text{H}_2\text{O}_2$ 响应的前药(Prodrug 7)在肿瘤诊疗一体化中的应用<sup>[52]</sup>

(a)前药7与 $\text{H}_2\text{O}_2$ 的传感机制;(b)前药7与不同浓度 $\text{H}_2\text{O}_2$ 响应的荧光光谱图;(c)药物SN-38和前药7在B16F10细胞系中的细胞存活率图;(d)正常小鼠、气管内注射生理盐水的肺癌小鼠和气管内注射前药7的肺癌小鼠被解剖后,分离出的肺实物图像以及肺组织的H&E染色切片

Figure 3 Application of  $\text{H}_2\text{O}_2$  responsive prodrug 7 in the theranostics of tumor<sup>[52]</sup>

(a) The sensing mechanism of prodrug 7 and  $\text{H}_2\text{O}_2$ ; (b) Fluorescence changes of prodrug 7 after treatment with increasing concentrations of  $\text{H}_2\text{O}_2$ ; (c) Antiproliferative activity of SN-38 and prodrug 7 in B16F10 cell lines; (d) Representative lungs isolated from normal mice and mice with lung tumors administered saline or prodrug 7 intratracheally and Histological sections of lung tissues with H&E staining

Tan等人首次开发了线粒体靶向前药PNPS,用于近红外荧光成像指导下的化学/光动力协同精确

治疗肿瘤<sup>[53]</sup>。作者选择半花菁染料NPS作为近红外荧光指示剂,同时NPS还具有光动力效果。作者选

择5'-脱氧-5-氟尿苷(5'-DFUR)作为抗癌药物,然后通过硼酸酯基团连接了这两部分并淬灭了NPS的光毒性和固有的近红外荧光以及药物(5'-DFUR)的细胞毒性。当前药与 $H_2O_2$ 发生反应后,硼酸酯基团被破坏,导致NPS的光动力疗法被激活,同时提供NIR荧光信号。抗癌药物5'-DFUR活性被激活并用于化疗(图4a)。作者研究了前药与 $H_2O_2$ 的响应效果。结果如图4b所示,随着 $H_2O_2$ 浓度逐渐增大,近红外一区荧光逐渐增强,这说明前药具有很高的灵敏性。作者也验证了NPS具有优异的光动力效果。然后利用癌症细胞中高 $H_2O_2$ 浓度的优势,前药PNPS对癌症细胞的细胞毒性明显高于正常细胞,且副作用较低,这归功于化疗和光动力治疗的互补结合(图4c和图4d)。此外,基于其线粒体靶向能力,与游离的5'-DFUR相比,PNPS显示出更高的治疗效率。此外,PNPS在荷瘤小鼠体内也表现出优异的诊断与治疗效果。

### 2.3 活性硫(Reactive sulfur species, RSS)介导响应性前药

生物硫醇在生物系统中起着许多关键作用。生物硫醇包括半胱氨酸(Cysteine, Cys)、同型半胱氨酸(Homocysteine, Hcy)和谷胱甘肽(GSH)等,这些含硫化合物在生物系统中的代谢和运输与一系列重要的酶和蛋白质有关<sup>[54]</sup>。这些硫醇的浓度与相应的酶和蛋白质异常有关<sup>[55]</sup>。例如,胱硫醚 $\beta$ -合酶和胱硫醚 $\gamma$ -裂解酶等相关酶表达不足可能导致同型半胱氨酸的异常积累,这是同型胱氨酸尿症和心血管异常等遗传疾病的特征。GSH是细胞内最丰富的非蛋白巯基化合物。游离GSH与其氧化型GSH二硫化物的比率(>100:1)是相应酶活性和细胞氧化还原状态的指标<sup>[56]</sup>。研究表明,肿瘤组织具有高度还原性,肿瘤细胞内部的GSH含量远高于正常细胞的含量。所以GSH浓度的这种剧烈差异使得开发出可以在肿瘤组织中选择性激活的GSH介导前药成为可能<sup>[57]</sup>。

Tang等人开发了一种GSH激活的肿瘤前药4(Prodrug 4)<sup>[58]</sup>。前药4通过二硫键将抗癌药物喜树碱(CPT)和近红外花菁荧光团连接起来,既屏蔽掉了花菁染料的荧光信号,还抑制了喜树碱的活性。其中,二硫键可以被GSH特异性识别并断裂。利用肿瘤细胞中高浓度的GSH,前药4中的二硫键可以被GSH特异性裂解,然后开启近红外荧光信号并释放活性抗癌药物(图5a)。作者首先测试了前药4与GSH的响应性,结果如图5b所示,前药4与不同浓

度GSH反应后,释放出了逐渐增强的荧光信号。这说明前药可以灵敏地响应GSH。同时,前药4在细胞内具有优异的响应效果(图5c)。然后作者研究了前药4在细胞中的诊疗效果。与单独的抗癌药物CPT相比,前药4对肿瘤细胞表现出更高的细胞毒性,这说明前药4具有更好的选择性开启诊疗效果(图5d)。最后在荷瘤小鼠体内也验证了前药4可以在肿瘤中积累并特异性激活(图5e)。因此,前药4是一种有前景的肿瘤激活药物递送平台。

Kim等人合成了一种GSH激活的肿瘤前药1(Prodrug 1)<sup>[59]</sup>。该前药由四个不同基团组成。二硫键是GSH的响应基团,用于开启前药的激活。叶酸基团被用作肿瘤靶向配体,因为其受体在大多数肿瘤细胞表面过度表达。Cy7基团被用作NIR荧光信号分子,其中酰胺键可以在二硫键被GSH断裂后迅速分解为胺基。最后一个基团是吉西他滨化合物。吉西他滨是一种临床嘧啶类抗癌药物,其主要作用是在DNA复制中作为嘧啶碱基的有力竞争者,或通过与核糖核苷酸还原酶的相互作用作为凋亡诱导剂。当前药1的二硫键与GSH反应后,氨基甲酸酯基团迅速分解为胺,从而释放荧光染料和抗癌药物(图6a)。前药1在体外可以灵敏地响应GSH,产生明显增加的荧光强度(图6b)。相比于不表达叶酸受体的阴性A549细胞而言,表达叶酸受体的阳性KB细胞表现出优异的细胞毒性,这也证明了前药1具有优异的肿瘤靶向能力(图6c)。最后作者考察了前药1在细胞中的治疗能力,结果显示前药1在细胞中具有良好的抑制作用(图6d和图6e)。

### 2.4 缺氧(Hypoxia)介导响应性前药

缺氧是指由于氧气消耗和供应不平衡,组织呈现低氧状态的情况。包括中风、炎症和癌症在内的各种疾病都与缺氧有关<sup>[60-61]</sup>。缺氧是实体瘤的共同特征。随着肿瘤的恶性增殖,肿瘤内部对氧气的需求远远超出血液供应,导致氧气的输送受到很大的抑制<sup>[62]</sup>,使肿瘤微环境呈现缺氧的状态<sup>[63]</sup>。缺氧会导致还原应激的增加,导致细胞内还原酶的过度表达,如偶氮还原酶、硝基还原酶和细胞色素P450酶等<sup>[64-65]</sup>。大量的研究表明,缺氧性肿瘤患者的总体生存率经常降低。尽管已经开发了各种类型的缺氧激活前药,以尽量减少化疗药物的毒性,提高肿瘤治疗的选择性。然而,缺氧也被发现会增加肿瘤细胞对化疗药物毒性的耐受性,诱导耐药基因的高表达,并降低肿瘤细胞中的药物浓度。因此,缺氧阻碍了化疗药物向实体瘤核心的运输,导致对此类肿瘤的

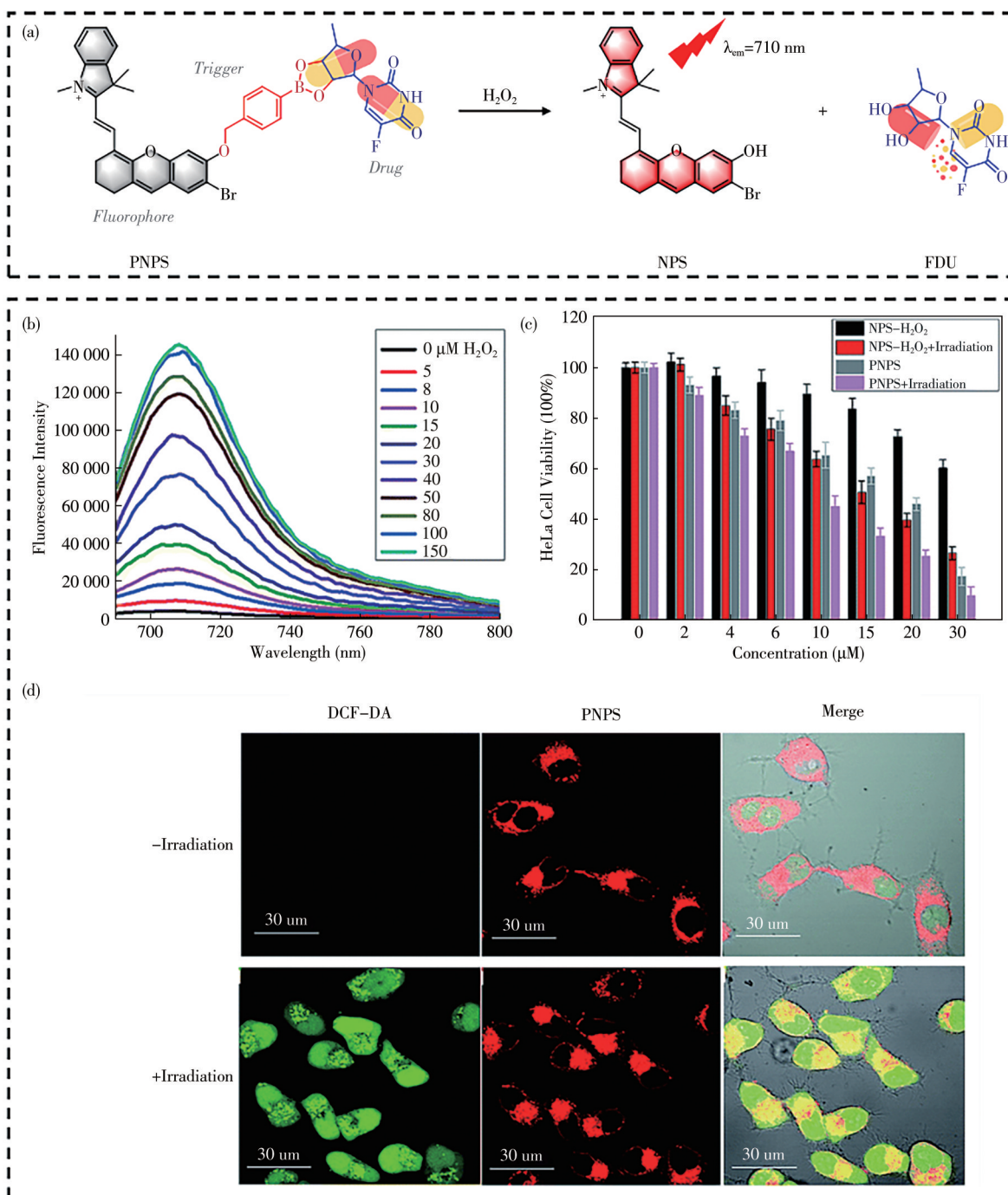


图4  $H_2O_2$ 响应的 prodrug (PNPS) 在肿瘤诊疗一体化中的应用<sup>[53]</sup>

(a)前药 PNPS 与  $H_2O_2$  的传感机制;(b)前药 PNPS 与不同浓度  $H_2O_2$  响应的荧光发射光谱;(c)在光照或无光照下,不同浓度的 NPS- $H_2O_2$  或 PNPS 与 HeLa 细胞共孵育后的细胞存活率图;(d)HeLa 细胞与 PNPS 孵育后在有光照和无光照条件下,产生  $^1O_2$  的荧光成像图。用 DCF-DA 作为  $^1O_2$  指示剂,绿色通道来自 DCF-DA;红色通道来自 PNPS

Figure 4 Application of  $H_2O_2$  responsive prodrug (PNPS) in the theranostics of tumor<sup>[53]</sup>

(a) The sensing mechanism of prodrug PNPS and  $H_2O_2$ ; (b) Fluorescence emission spectra of PNPS with different concentrations of  $H_2O_2$ ; (c) Viability of HeLa cells upon treatment with different concentrations of NPS- $H_2O_2$  or PNPS showed under white light irradiation or in dark; (d) Fluorescence imaging showing the  $^1O_2$  generation of PNPS in HeLa cells after different treatments using DCF-DA as indicator. The green channel is from DCF-DA; the red channel is from PNPS

治疗效果降低<sup>[66-68]</sup>。因此,当务之急是设计一种能够克服肿瘤缺氧的肿瘤靶向和可激活的前药,这将

降低与化疗相关的全身毒性,并提高药物输送到实体瘤缺氧核心的有效性。

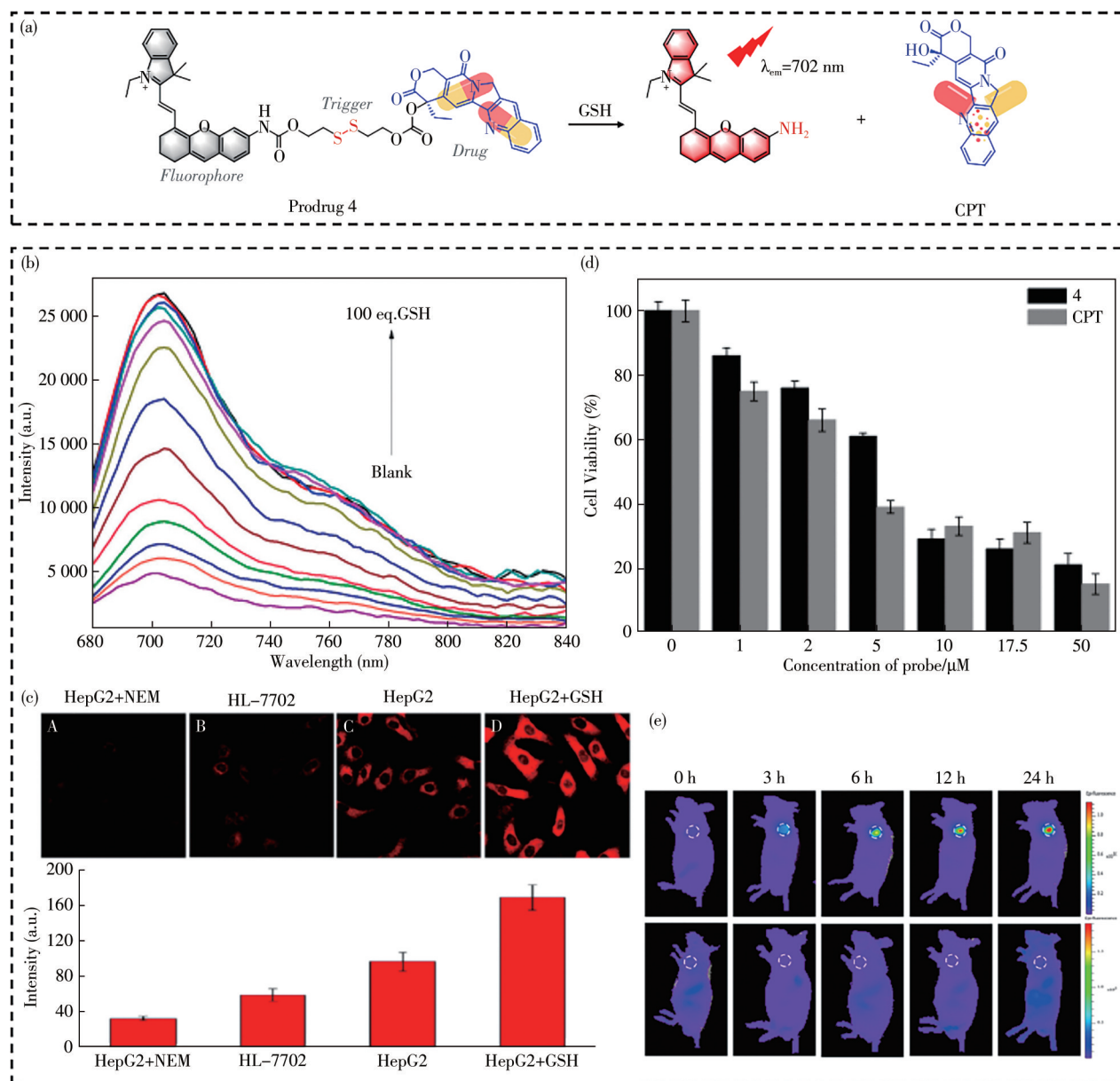


图5 GSH响应的前药(Prodrug 4)在肿瘤诊疗一体化中的应用<sup>[58]</sup>

(a)前药4与GSH的传感机制;(b)前药4与不同浓度的GSH响应的荧光光谱图;(c)不同处理过的细胞(NEM预处理的HepG2细胞、HL-7702细胞与前药4孵育、HepG2细胞与前药4孵育、用GSH预处理HepG2细胞后再与前药4孵育)的共聚焦荧光成像图和荧光强度的定量分析图;(d)不同浓度的前药4和单独的CPT与HepG2细胞共孵育后的细胞存活率图;(e)荷瘤小鼠在静脉注射前药4(上)和生理盐水(下)后不同时间点的荧光成像图

Figure 5 Application of GSH responsive prodrug 4 in the theranostics of tumor<sup>[58]</sup>

(a) The sensing mechanism of prodrug 4 and GSH;(b) Fluorescence changes of prodrug 4 after treatment with increasing concentrations of GSH;(c) Cellular fluorescence images and quantitative analysis of fluorescence intensity of prodrug 4-treated HepG2 and HL-7702 cells(HepG2 cells pretreated by NEM, HL-7702 cells, HepG2 cells were incubated with prodrug 4, HepG2 cells were pretreated with GSH followed incubated with prodrug 4);(d) Cell viability of HepG2 cells with different concentrations of prodrug 4 and free CPT;(e) In vivo imaging of tumor-bearing mice at various times after intravenous injection of prodrug 4 (top) and (bottom)

Liu等人合成了一种具有肿瘤靶向和缺氧激活的自荧光前药(DHQ-AZO-BIO)<sup>[69]</sup>。DHQ-AZO-BIO由四个不同的结构连接而成:(i)偶氮双键作为肿瘤

内缺氧环境的激活位点;(ii)近红外荧光指示剂(DHQ-Rd)用于实时监测化疗药物的释放;(iii)生物素基团作为一种水溶性维生素,用于靶向肿瘤细

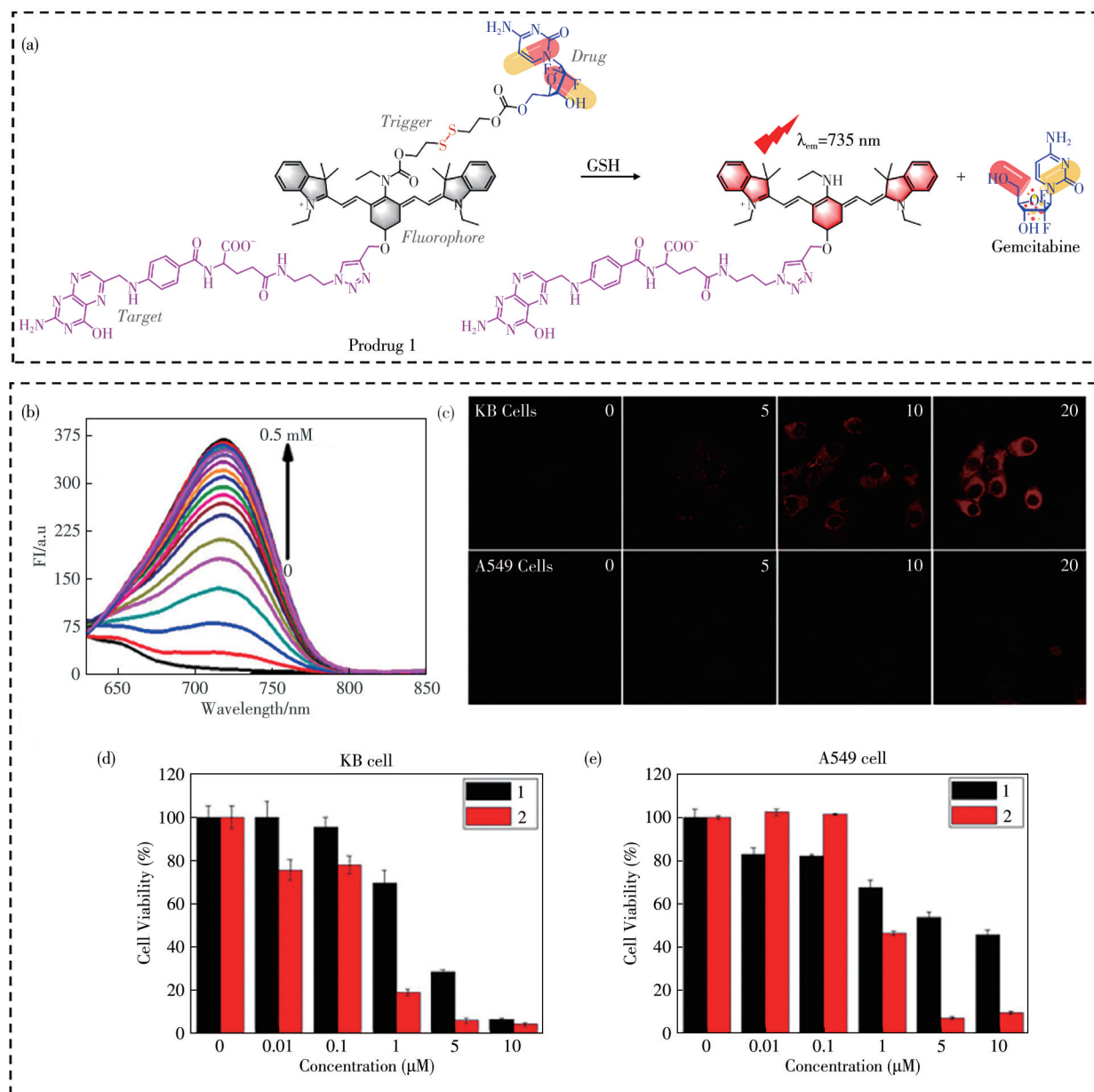


图6 GSH响应的前药(Prodrug 1)在肿瘤诊疗一体化中的应用<sup>[59]</sup>

(a)前药1与GSH的传感机制;(b)前药1与不同浓度GSH响应的荧光光谱图;(c)前药1与KB细胞和A549细胞在不同时间孵育后的共聚焦显微镜图像;(d)KB细胞和(E)A459细胞与前药1和2共孵育后的细胞存活率图

Figure 6 Application of GSH responsive prodrug 1 in the theranostics of tumor<sup>[59]</sup>

(a) The sensing mechanism of prodrug 1 and GSH; (b) Fluorescence spectral changes of prodrug 1 in the presence of different concentrations of GSH; (c) Confocal microscopy images of KB and A549 cells treated with prodrug 1 at various times; (d) Cell viability assay of 1 and 2 in KB cell lines; (e) Cell viability assay of 1 and 2 in A459 cell lines

胞表面,并增强肿瘤细胞对它们的摄取;(iv)氮芥作为抗癌药物,用于肿瘤细胞的治疗。DHQ-AZO-BIO具有强烈的分子内非共价相互作用,因此降低了单线态激发态和三重态激发态之间的能隙,提高了自旋轨道耦合常数,从而使其成为一种新型的无重原子光敏剂。该前药的生物素基团对肿瘤细胞具有高亲和力,它可以与细胞表面受体结合,选择性地靶向

肿瘤细胞。然后产生光动力效果而发挥治疗作用。随着前药逐渐进入实体瘤内,化疗药物氮芥通过肿瘤缺氧微环境中过度表达的偶氮还原酶还原偶氮键而释放。化疗前药的释放也可以通过近红外荧光团DHQ-Rd的释放来追踪(图7a)。作者研究了前药在肝微粒体中的还原能力,结果显示随着时间的延长,其荧光强度逐渐增加,这个结果验证了其偶氮键

被还原为胺基的能力(图7b)。然后作者研究了前药在细胞中的诊疗效果。前药在无光照的条件下,缺氧环境下的细胞存活率低于常氧环境;而加入光照后,缺氧环境下的细胞存活率变得更低(图7c)。同时作者还研究了前药在荷瘤小鼠中的诊疗作用。接受光照和前药注射的小鼠的肿瘤消融效果最好

(图7d—图7f)。这说明缺氧环境激活化学/光动力的协同治疗最大限度地提高了对实体瘤的治疗效果。在光动力治疗期间,实体瘤中的缺氧水平逐渐升高,过度表达的偶氮还原酶进一步裂解前药的偶氮键来提高前药的按需释放效率。这种联合治疗方式克服了肿瘤缺氧的局限性,提高肿瘤的治疗指数。

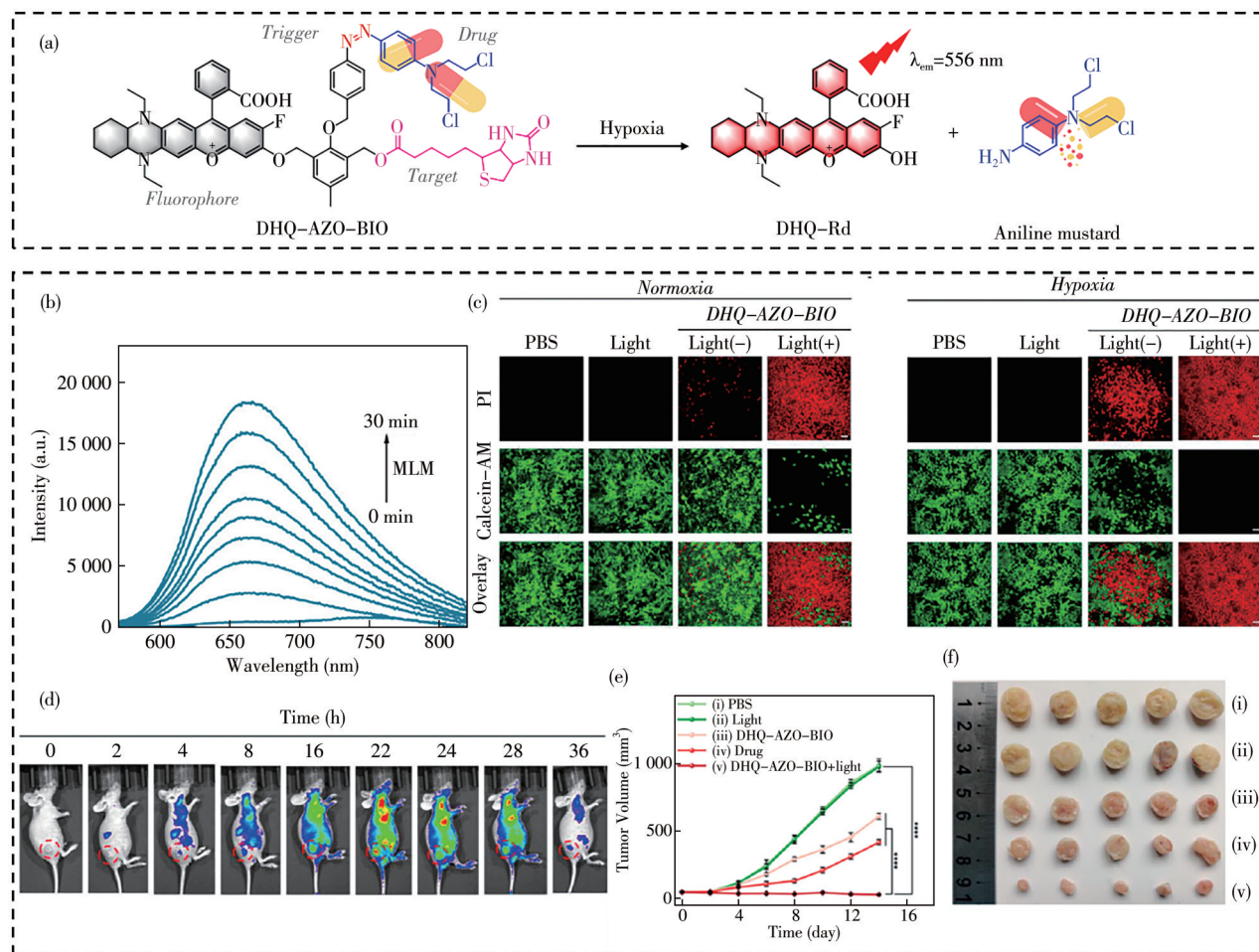


图7 缺氧环境响应的前药(DHQ-AZO-BIO)在肿瘤诊疗一体化中的应用<sup>[69]</sup>

(a)前药DHQ-AZO-BIO在缺氧环境下的传感机制;(b)前药DHQ-AZO-BIO与肝微粒体随时间响应的荧光光谱图;(c)在常氧和缺氧条件下,DHQ-AZO-BIO与癌细胞共孵育后,激光照射前后的荧光成像图;(d)前药DHQ-AZO-BIO在荷瘤小鼠体内随时间变化的荧光成像图;(e)小鼠在14天治疗期内肿瘤体积的变化图;(f)小鼠在14天治疗期后,解剖出来的肿瘤实物图

Figure 7 Application of hypoxia responsive prodrug (DHQ-AZO-BIO) in the theranostics of tumor<sup>[69]</sup>

(a) The sensing mechanism of prodrug (DHQ-AZO-BIO) under hypoxia environment; (b) Fluorescence spectra changes of DHQ-AZO-BIO with time after addition of mouse liver microsomes; (c) Fluorescence imaging of the DHQ-AZO-BIO-cultured cancerous cells under normoxia environment and hypoxia environment with light; (d) Time-dependent in vivo fluorescence imaging of DHQ-AZO-BIO in 4T1 tumor-bearing mice; (e) Tumor volume changes during 14 days of treatment; (f) Photographs of tumor morphology under treatments were recorded after a 14-day treatment cycle

Kim 等人合成了一种缺氧激活的线粒体靶向前药(Probe 1)<sup>[70]</sup>。该前药由四个不同的结构连接而成:(i)罗丹明衍生物被用来作为荧光信号报告分子,实时监测化疗效果;(ii)偶氮双键作为肿瘤中缺

氧环境的激活位点;(iii)三苯基膦盐衍生物作为一种正电荷化合物,用于靶向细胞内线粒体;(iv)氮芥作为抗癌药物,用于肿瘤细胞的治疗。在缺氧条件下,偶氮键被还原,氮芥药物和罗丹明衍生物荧光

团被释放出来,荧光的变化来监测药物的激活效果。该前药靶向线粒体后,发生烷基化损伤随后导致细胞死亡(图 8a)。作者研究了前药在体外被还原的效果。连二亚硫酸钠是一种强还原剂,可以裂解偶氮基团,形成两分子胺基衍生物。前药在加入连二亚硫酸钠后,随着时间的延长,其荧光强度逐渐增强,这个结果表明探针具有被还原的能力(图 8b)。细胞实验表明,与正常细胞相比,肿

瘤细胞在缺氧环境下具有明显的荧光信号,这说明前药可以通过荧光信号来诊断正常细胞和肿瘤细胞。另外,图 8c 的荧光信号对比说明了线粒体靶向基团促进了前药在肿瘤处的积聚。接受前药治疗后,各组小鼠被解剖并分离出肿瘤组织,结果显示前药具有优异的治疗效果(图 8d)。离体组织分析实验也证实了前药在肿瘤组织中的特异性激活(图 8e)。

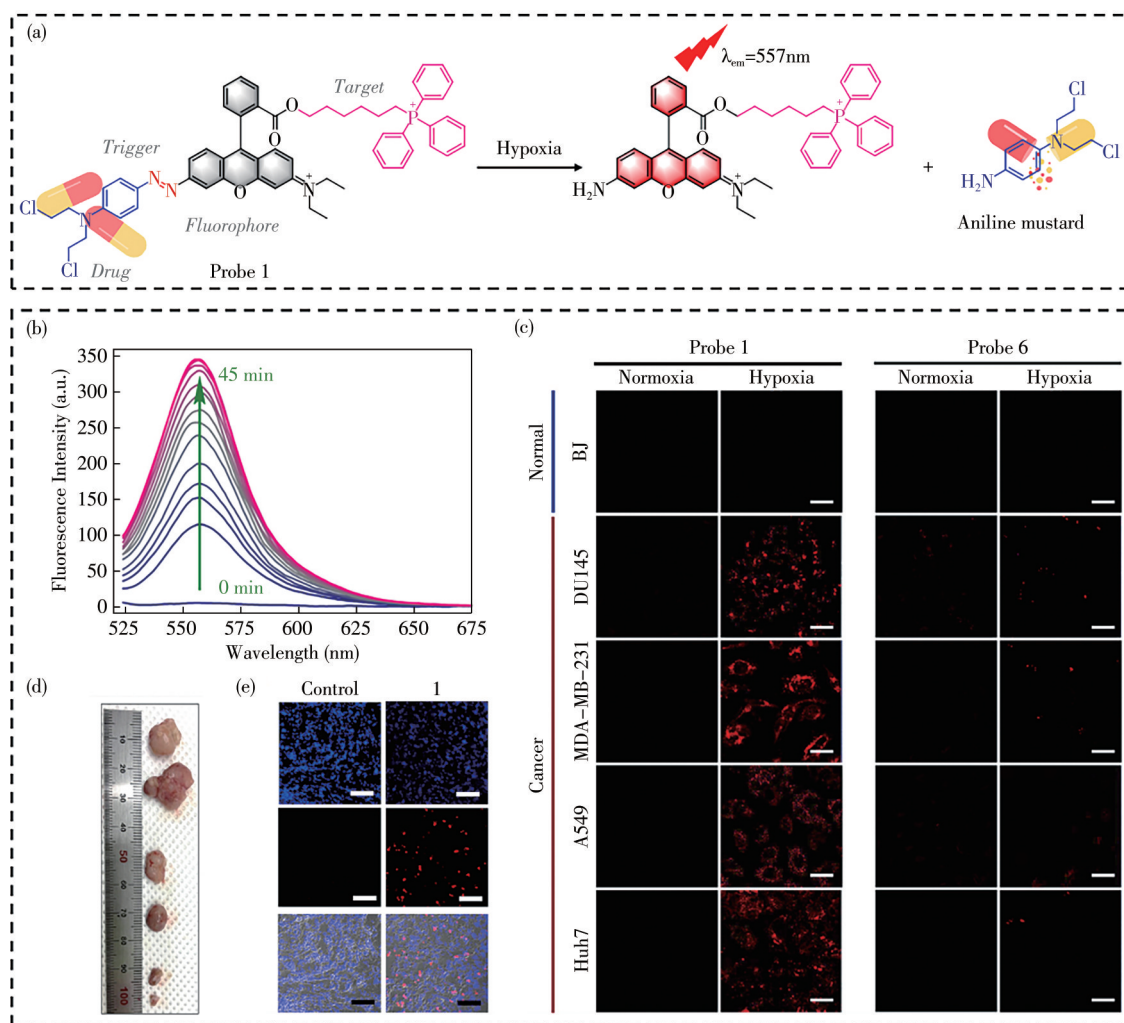


图 8 缺氧环境响应的前药 (Probe 1) 在肿瘤诊疗一体化中的应用<sup>[70]</sup>

(a)前药(Probe 1)在缺氧环境下的传感机制;(b)前药与连二亚硫酸钠随时间响应的荧光光谱图;(c)在常氧或者缺氧条件下,前药和探针 6 在不同细胞系中孵育 24 h 后的共聚焦荧光成像图,比例尺:10  $\mu\text{m}$ ;(d)各个小组在接受不同处理后的第 13 周解剖出的实体瘤图像;(e)对照组和前药组小鼠的肿瘤组织冷冻切片的荧光成像图,比例尺:100  $\mu\text{m}$

Figure 8 Application of hypoxia responsive prodrug (Probe 1) in the theranostics of tumor<sup>[70]</sup>

(a) The sensing mechanism of prodrug (Probe 1) under hypoxia environment;(b) Fluorescence spectra changes of prodrug with time after addition of sodium dithionite;(c) Fluorescence imaging of different cells treated with probe 1 or probe 6 under hypoxia conditions or normoxia conditions for 24 h, Scale bar: 10  $\mu\text{m}$ ;(d) Dissected solid tumors after termination at week 13;(e) Fluorescent images of cryosectioned tumor tissues dissected from the control group and probe 1 group, Scale bar: 100  $\mu\text{m}$

## 2.5 药物荧光团一体化前药

前面展示的前药都是由两种分子分别作为一种

荧光指示剂和一种抗癌药物而组成。但是有的抗癌药物本身就是一种荧光发光团,因为他们也具有较

大的共轭结构。所以如果将荧光指示剂和抗癌药物集中在一个分子结构上,这样会减弱前药的合成复杂度,同时还具有诊疗的功能。但是,药物本身的荧光性能也给诊疗一体化带来了许多挑战。比如药物的荧光性能是否能够满足荧光的成像反馈要求,又或者药物的代谢是否能够如实反馈荧光指导下的治疗效果。

Wu 等人报道了一种自焚的DT-黄递酶激活前药8(Prodrug 8),可以实现药物按需释放,同时监测药物释放/治疗效果和生物标志物水平<sup>[71]</sup>。该前药系统由两个喜树碱(CPT)基团、醌丙酸衍生物和一组自焚连接体组成。喜树碱基团既充当了荧光指示剂,又具有肿瘤治疗的功能。DT-黄递酶可以催化醌类结构的还原,并在一些恶性肿瘤中过度表达。当前药与DT-黄递酶发生反应时,醌丙酸结构以及自焚结构发生裂解,两个CPT分子被释放并恢复其自身的荧光,从而实现DT-黄递酶水平的荧光监测以及CPT释放的跟踪(图9a)。前药在体外可以灵敏地响应DT-黄递酶并释放CPT荧光(图9b)。最后,作者研究了前药8对肿瘤细胞的抑制作用,结果显示前药8表现出较高的细胞毒性(图9c—图9e)。这种前药有以下优点:首先,一个前药分子含有两个CPT部分,这大大提高了药物负载量,从而相应地提高了治疗效果;其次,前药中的CPT部分不仅用作活性药物,还用作荧光指示剂,用于监测释放的药物和生物标志物水平;第三,前药的醌丙酸部分不仅作为响应性按需释放药物的触发器,而且通过光诱导电子转移机理作为CPT荧光的强淬灭剂,确保成功实现荧光开启成像和跟踪。这一新策略可能为开发具有增强药物负载以及药物释放和跟踪可控性的前药提供有用的见解。

Kim 等人报道了一种缺氧响应型诊疗前药AzP1<sup>[72]</sup>。该前药选择SN-38作为抗癌药物和荧光报告分子。由于偶氮键的淬灭作用,SN-38没有药物活性和荧光发射。当前药在缺氧环境下被还原后,SN-38药物活性和荧光都被激活,成功实现诊疗作用(图10a)。作者首先研究了AzP1在体外的还原效果,结果显示随着肝微粒体的浓度增大,在560 nm处的荧光强度逐渐增强。这也意味着前药被逐渐还原(图10b)。同时前药AzP1在肿瘤细胞中也表现出了增强的细胞毒性。此外,作者将不同剂量的AzP1注射在荷瘤小鼠模型中,随着浓度的增大,小鼠肿瘤的抑制作用也逐渐加强(图10b)。最后各组小鼠在治疗期结束后被解剖出其肿瘤,从图中可以

看到相同的结果。

在这些前药激活机制中,缺氧激活机制可能会受到局部氧气浓度的差异而引起正常组织偶发假阳性激活,造成假阳性信号的干扰。pH激活机制会受到瘤内pH梯度不均匀的影响而导致药物释放不完全。RSS激活机制会受到肿瘤细胞内GSH浓度个体差异大的影响而导致前药的诊疗效果受限。未来的前药递送系统向临床转化需要重点验证标志物在瘤内的特异性表达强度及脱靶风险,优先选择多重响应型设计以应对微环境动态变化。

### 3 总结与展望

化疗是在临床上干预肿瘤最重要的手段之一。尽管化疗药物具有很强的抗肿瘤能力,但是同时也对正常组织产生了较强的毒副作用。因此前药策略可以有效改善药物的溶解性、毒性、药代动力学等性质,并且可以改善药物的靶向性,降低化疗药物的不良反应。在本综述中,我们主要回顾了可以被肿瘤微环境中的特定生物标志物(pH、缺氧、氧化还原环境、生物活性酶)特异性激活的前药。该前药通过分子设计将诊断与治疗功能整合于单一体系中,该体系通过影像学的指导可以进行诊断和疗效反馈,最终实现疾病靶向识别、原位激活与疗效反馈的协同闭环。尽管现在已经有大量前药被开发出来,但是目前还存在着一些问题,这也是该研究领域未来可以去攻克的一些问题:①有机小分子前药的生物相容性和负载量仍需改善。生物相容性差的前药难以发挥其作用,而且负载药物量低更是无法达到理想疗效,甚至诱发肿瘤产生耐药性等。②如果只用单一的刺激因素诱导药物释放,具有假信号存在的可能性,而且单一刺激也会导致药物释放率低,疗效差。在未来,多重刺激诱导药物释放前药的开发可以保证前药激活的有效性和真实性。③在设计前药的时候,我们不得不考虑肿瘤的异质性。肿瘤组织中存在着各种类型的细胞,而这些细胞具有相对特殊的性质、功能,肿瘤的异质性以及个体差异性均有可能影响前药的治疗效果。因此,我们在设计前药的时候需要“个性化”处理,这样才有望提高前药的作用效果。如果把前药递送系统真正应用在临床上还需要面临很多挑战,比如前药的规模化生产存在工艺复杂度高和质控标准缺失等问题、前药在人体内的代谢路径及潜在免疫原性尚需深入解析、缺乏统一标准评估靶向精度和释药动力学,这些挑战都将阻碍临床试验的推进。在未来,AI技术模型可以

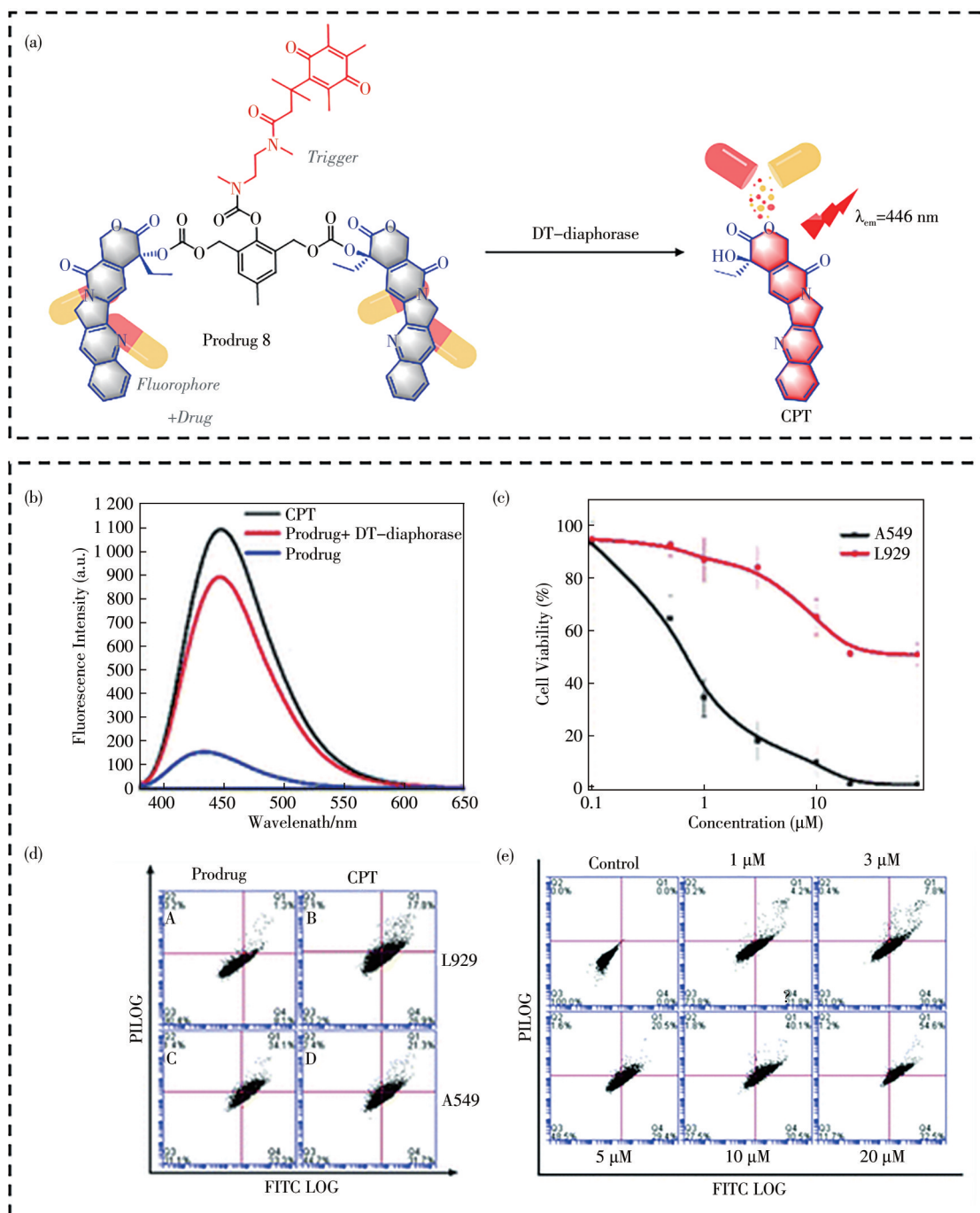


图9 DT-黄递酶响应的前药(Prodrug 8)在肿瘤诊疗一体化中的应用<sup>[71]</sup>

(a)前药8与DT-黄递酶的传感机制;(b)单一的前药8、单一的CPT和前药8与DT-黄递酶反应后的荧光光谱图;(c)不同浓度的前药8与A549和L929细胞孵育48 h后的细胞存活率图;(d)流式细胞术分析:通过膜联蛋白V-FITC/PI染色分析A549细胞和L929细胞的百分比分布。L929细胞用前药(a)和CPT(b)孵育24 h;A549细胞用前剂(c)和CPT(d)孵育24 h;(e)不同浓度的前药与A549细胞孵育24 h的百分比分布图

Figure 9 Application of DT-diaphorase responsive prodrug 8 in the theranostics of tumor<sup>[71]</sup>

(a) The sensing mechanism of prodrug 8 and DT-diaphorase;(b) Fluorescence spectra of the prodrug, CPT and the prodrug with DT-diaphorase;(c) Cell viabilities of A549 and L929 cell lines upon treatment with the varied concentrations of prodrug after 48 h incubation;(d) Flow cytometry analysis: representative percent distribution of A549 and L929 cells analyzed by using annexin V-FITC/PI staining. L929 cells were treated with the prodrug (A) and CPT (B) for 24 h and A549 cells were treated with the prodrug (C) and CPT (D) for 24 h;(e) Representative percentage distribution of A549 cells treated with the varied concentrations of prodrug for 24 h

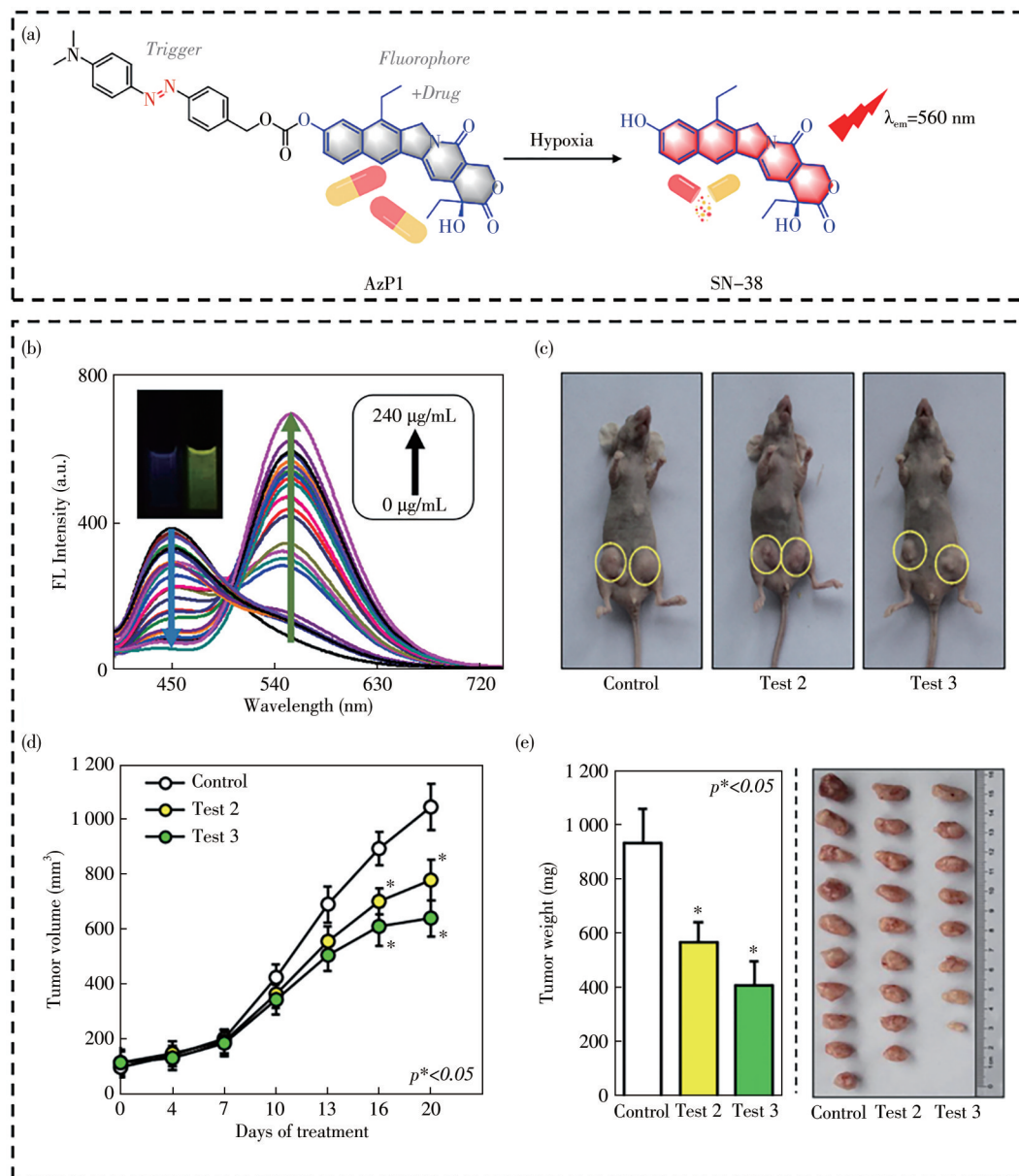


图10 缺氧环境响应的前药(AzP1)在肿瘤诊疗一体化中的应用<sup>[72]</sup>

(a)前药 AzP1 在缺氧环境下的传感机制;(b)前药 AzP1 与不同剂量肝微粒体响应的荧光光谱图;(c)用 PBS(对照)、10  $\mu\text{mol/L}$  AzP1(试验2)或 20  $\mu\text{mol/L}$  AzP1(试验3)处理的荷瘤小鼠图;(d)三个小组的小鼠在治疗期内的肿瘤体积变化图;(e)三个小组的小鼠肿瘤重量及解剖后肿瘤实物图

**Figure 10 Application of hypoxia responsive prodrug (AzP1) in the theranostics of tumor<sup>[72]</sup>**

(a) The sensing mechanism of prodrug (AzP1) under hypoxia environment; (b) Fluorescence emission spectrum of AzP1 in the presence of varying amount of rat liver microsomes in buffer system; (c) The image of tumor bearing xenograft mouse which was treated with PBS (control), 10  $\mu\text{mol/L}$  (Test 2) or 20  $\mu\text{mol/L}$  (Test 3) of AzP1; (d) Tumor volume change of all three test groups during the period of treatment; (e) Tumors weight and image of tumors of mice which was treated with PBS (control), 10  $\mu\text{mol/L}$  (Test 2) or 20  $\mu\text{mol/L}$  (Test 3) of AzP1

预测前药代谢行为,缩短研发周期;微流控技术可以优化纳米载体合成工艺,提升生产重现性;通过建立涵盖靶向效率、生物分布及长期毒性的临床前评价模型,为转化提供可靠数据支撑。我们相信,通过很多科技工作者的努力研究,在不久的将来,诊疗型有

机小分子前药将会真正地服务于临床医学,为肿瘤的诊疗研究领域带来福音。

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