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基于荧光方法的肿瘤标志物检测研究进展

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摘要 癌症的早期发现可以显著降低死亡率，所以癌症的早期检测与诊断已成为生物医学领域最关注的研究方向之一。肿瘤生物标志物在癌症风险评估、筛查诊断、预测治疗、预后监测等方面具有重要作用和巨大的应用潜力。目前，生物标志物的检测方法主要有放射免疫分析法、酶联免疫分析法、质谱法、光学方法、纳米技术、电化学法、微流控技术等。荧光方法具有高灵敏度、高分辨率、操作简便等特点，在无创检测和快速检测上具有重要的应用前景。本文介绍了荧光方法在肿瘤生物标志物检测方面的最新进展，从循环肿瘤细胞、循环肿瘤 DNA、外泌体及肿瘤标志物蛋白(癌胚抗原、甲胎蛋白、前列腺特异性抗原)等方面进行了综述，总结了最新的荧光检测技术，对基于液体活检技术的肿瘤早期检测研究具有指导意义。

关键词 医用光学；荧光；肿瘤生物标志物；纳米材料；液体活检

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1 引 言

癌症是复杂的系统性疾病，具有维持细胞增殖信号、躲避生长抑制因子和抵抗细胞凋亡、无限复制增殖、诱导血管生成、激起侵袭和转移扩散等特征[1]。长期以来，癌症一直是世界各国人口死亡的主要原因。根据国际癌症研究机构的统计，2020 年全球范围内有 1930 万新增癌症病例和近 1000 万癌症死亡病例[2]。癌症的早期发现和准确检测对于临床诊断、有效的毒性监测、降低癌症死亡率以及最终成功治疗癌症非常重要[3-4]。

肿瘤生物标志物，例如，核酸、蛋白质、酶、代谢物、细胞表面受体以及整个肿瘤细胞等，存在于肿瘤组织或血清中。它们可用于癌症风险评估、早期癌症检测和诊断、疗效评估、预后及复发监测[3-4]。基于肿瘤生物标志物和肿瘤微环境参数特异性识别的有效检测方法包括放射免疫分析、聚合酶链式反应、酶联免疫吸附、电泳、质谱分析、表面等离子体共振、表面增强拉曼光谱、电化学测定、流式细胞术等。虽然检测方法在不断发展和完善，但大部分方法仍然不能满足临床诊断应用要求的准确性、敏感性和特异性，并且部分检测方法操作复杂，需要在设备完善的临床和专业实验室中进行[3-5]。荧光方法以其高灵敏度、高稳定性、良好的生物相容性、高信噪比、快速简单和无创监测等优势受到国内外的广泛关注，具有广阔的应用前景[4-6]。近年来，新型荧光探针材料不断被开发出来，如上转换纳米粒子、量子点、碳点等荧光纳米材料，研究人员基于这些材料发展了多种检测技术，而且这些技术已被成功应用于肿瘤疾病的检测中[7-8]。本文针对荧光方法在肿瘤生物标志物检测方面的应用以及该方法在近几年的发展进行了综述，并介绍了该方法在液体活检生物标志物、其他生物标志物以及同时检测多个生物标志物方面的应用。

2 肿瘤生物标志物检测

生物标志物是指存在于生物体或其产物内，能够反映生物体的结构或功能在生理、生化、免疫和遗传等方面变化的物质。肿瘤生物标志物作为生物标志物的一个亚类，是指在肿瘤发生和增殖过程中，由肿瘤细胞自身产生，或由机体对肿瘤细胞反应而异常产生和(或)升高，反映肿瘤存在和生长的一类物质[3-4,9-10]。DNA 突变、转录变化或转录后修饰的改变，可以用于生物体正常或异常生物状态的区分、风险评估、疾病筛查、鉴别诊断、预后判断、疗效监测等。如图 1 所示，肿瘤生物标志物[4,10-14]包括：1)酶、蛋白质、核酸(DNA 和 RNA)、多肽、碳水化合物、激素和代谢产物等生物

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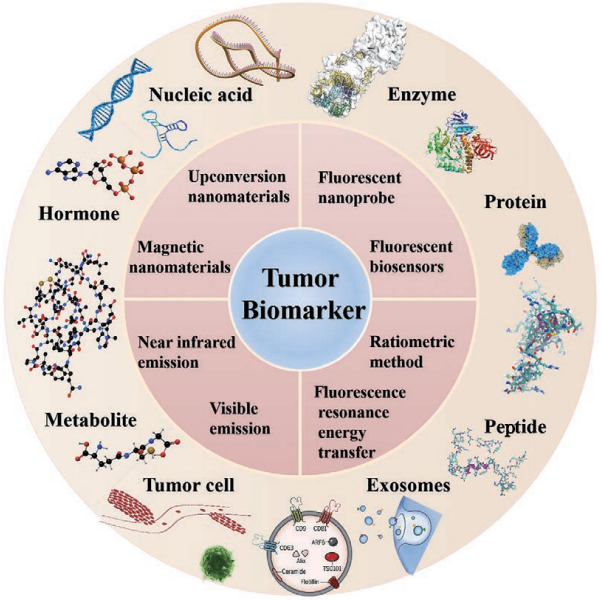

图1 肿瘤标志物的主要类型[4,10-14]

Fig. 1 Main types of tumor biomarkers[4,10-14]

分子;2)整个细胞或其他生物结构,如循环肿瘤细胞和外泌体;3)基因组学、蛋白质组学和代谢组学等[4,10-11]。

近年来,以体液样本(主要是血液)中与肿瘤相关的生物标志物为分析目标的液体活检逐渐兴起。肿瘤生物标志物分布广泛,可存在于血液(全血、血清或血浆)、尿液、唾液、脑脊液等液体中。表1列举了目前针对肿瘤标志物进行检测的方法及其原理、特点[15-35]。总体来说,随着材料科学、生物科学、化学等多种学科的发展,放射免疫分析、聚合酶链式反应(PCR)、酶联免疫吸附(ELISA)、质谱等传统肿瘤标志物检测技术克服固有局限,在安全性、可操作性、便携性等方面有所突破。与传统的活组织检查相比,液体活检侵入性低、成本低、耗时短且无风险,在癌症筛查、诊断和预后中具有巨大的应用潜力[23,35-36]。荧光、表面拉曼散射(SERS)、表面等离子体共振(SPR)、电化学等检测技术使得肿瘤标志物检测不断趋于小型化、集成化。此外,各检测技术在液体活检中的综合应用,在提高肿瘤标志物检测效率和准确度的同时,也推动了癌症早期筛查诊断和精准医疗的发展[15,17,27,21,32,36]。

表1 肿瘤标志物检测技术对比

Table 1 Comparison of detection technique for tumor marker

Technology	Basic principle	Advantage	Disadvantage	Ref.
Radioimmunoassay	Radiolabelled antigen and radioactive measurement	Accurate and sensitive; reliable and applicable; easy operation; absolute quantification	Health hazards; radioactive contamination; limited stability	[15-16]
ELISA	Enzyme-labeled antigens or antibodies, enzyme-mediated visible color change or fluorescence to quantitative and qualitative measurements	Simplicity and scalability; ease operation; automated high-throughput; visual inspection; detection of a molecule at a low concentration	Time-consuming; high cost; moderate sensitivity; relatively complex strategy; specific equipment	[17-18]
Chemiluminescence immunoassay	Chemiluminescent substance labeled antigen or antibody, luminescence signal detection	High sensitivity and specificity; wide linear range; rapid and simple analysis; no scattered light interference	Weak signals and short luminescence time; limited precision for small molecules; matrix interference in complex samples	[19-20]
PCR	Enzymatic synthesis and amplification of specific DNA fragment; product analyze	High sensitivity; small amount of sample; multiplex; precise and accurate target quantification	Expensive with poor integration and multiplexing capability	[21-22]
Next-generation sequencing	Sequence millions of DNA molecule in one single run	Fast and efficient; high throughput; suitable for individual therapy of cancer	Expensive and mass processing of data; multiple steps of sample preparation; prone to mistakes	[23-24]

（续表）

Technology	Basic principle	Advantage	Disadvantage	Ref.
Mass spectrometry	Ionizing chemical species, separating and analyzing based on their mass-to-charge ratio	Rapid and sensitivity; high-throughput; structure identification; able to find new tumor biomarkers	Expensive equipment and complicated operation; invasive; low abundant proteins detection; prone to interference	[25-26]
SPR	Refractive index changes occurring from the capture of a molecule on the plasmonic surface	Label-free and real-time; high sensitivity and accuracy; suitable for a broad range of biofluids	Interference from complex samples; affects specificity and detection limits; most of them are in the proof-of-concept level	[27-29]
SERS	Difference in Raman scattering spectra of different molecule	Multiplexing capacity; high sensitivity and specificity; non-destructive and non-invasive	Expensive and expert-dependent equipment; batch to batch reproducibility of SERS substrates	[30-31]
Electrochemical sensors	Converts an interaction signal between a biometric element and a recognition target into a detectable electrical signal	High sensitivity; rapid and low cost; simple and suitable for microfabrication; mass production and integration	Lack of specificity for the captured cancer cells; lack the ability to detect intracellular protein markers	[32-33]
Flow cytometry	Measurement of cell size and cell granularity, expression of cell surface and intracellular molecules	Sorting capability; high-throughput measurement; rapidly counts	Indirect and *in vitro* measurement; time-consuming preparation	[34]
Fluorescence	Change of fluorescence spectrum and fluorescence intensity	High sensitivity and stability; simplicity and rapidity; biocompatibility; accurate data collection	Spectral overlap; background fluorescence; photobleaching; non-specific binding labeling	[35,31]

3 生物标志物的荧光检测

3.1 荧光检测

荧光是物质受到激发后从单重激发态经辐射跃迁返回基态时所发射的光[37-38]。荧光检测不仅可以规避处理放射性物质时的危险，还具有高灵敏度和低成本的特点，能够同时检测多个信号以及跟踪单个分子，阐述许多疾病的生理过程，在生物医学领域得到了广泛应用[37-40]。荧光检测技术是传感领域的主要检测方法，具有快速、简单、方便、高灵敏度等特点。它通常需要用荧光染料标记目标分子，通过检测荧光特性对标记物进行定性或定量分析[39,41]。基于有机染料的荧光检测方法具有简单多样、实时无损的特点，已有许多有机染料、荧光蛋白和发光金属络合物被用于荧光检测，促进了光学传感、光学成像等领域的进步。然而，有机染料的标记率低、荧光寿命短、耐光漂白性能差，很难获得优良的检测性能[41-42]。近年来，纳米技术快速发展，荧光纳米材料的质量和数量显著增加，可以通过控制纳米颗粒的尺寸和形状直接控制纳米粒子的荧光特性，使其具有更高的灵敏度和更优良的信噪比，并允许多重分析和多模式诊断[38-40]。

目前的荧光检测大多是基于荧光纳米材料的光电特性，与其他技术相结合进行综合测量的。广泛应用的荧光纳米材料包括量子点（QDs）[43]、上转换纳米粒子（UCNPs）[44]、金属纳米团簇[45]、聚合物点（Pdots）[46]、荧光硅纳米材料[47-48]以及荧光碳纳米材料[49-51]。量子点是最早应用于生物科学领域的纳米材料之一，与传统的有机染料相比具有更高的量子产率、更好的光稳定性和更长的发光寿命，但其毒性和宽吸收带严重限制了它在生物医学方面的使用[43,52]。上转换纳米粒子、聚合物点、金属纳米团簇等材料具有优异的光电特性和物理特性，且毒副作用小[53-58]。荧光硅纳米材料具有光稳定性好、表面易修饰、可生物降解等特性，且储量丰富，在生物检测和生物成像等领域被广泛应用[47,59]。碳点（CDs）、石墨烯量子点

(GQD)、碳纳米管(CNT)、氧化石墨烯(GO)等荧光碳纳米材料具有合成来源环保、可生物降解、光学性质优良等特点[60-67]。这些独特的性质使得荧光检测方法成为最有效的检测方法之一,被广泛应用于物质的定性与定量测量、分子结构与特性分析、疾病检测和诊断等领域。针对肿瘤标志物的检测,目前研究人们已经探索了各种基于以上荧光纳米材料的检测方法,并取得了重要进展,如表2所示。

表2 基于荧光纳米材料的生物标志物检测

Table 2 Biomarkers detection based on fluorescent nanomaterial

Target	Material	Method	Linear range	Limits of detection	Time analysis	Sample	Ref.
CA19-9	QDs	Fluorescence quenching	2.76×10^{-2}–5.23×10^{2} U·mL^{-1}	1.58×10^{-3} U·mL^{-1}	21 min	Human serum	[68]
GPC3 or DKK1 or AFP	QDs	Fluorescent nanoprobes	0.625–2.5 ng·mL^{-1}			Solution	[69]
CA125	UCNPs	Luminescence resonance energy transfer	0.01–100 U·mL^{-1}	9.0×10^{-3} U·mL^{-1}		Human serum	[70]
GPC-1 Leptin OPN VEGF	UCNPs	UCNPs-assisted single-molecule sandwich immunoassay	90–0.37 ng·mL^{-1} 100–0.412 ng·mL^{-1} 33.333–0.137 ng·mL^{-1} 10–0.041 ng·mL^{-1}	0.0123 ng·mL^{-1} 0.2711 ng·mL^{-1} 0.1238 ng·mL^{-1} 0.0158 ng·mL^{-1}		Human serum	[71]
Cyt c	CDs	Inner filter effect	0.5–25 μmol·L^{-1}	0.25 μmol·L^{-1}		Solution	[72]
HE4 Ovarian cancer cells	CDs	Metal-enhanced fluorescence effect	0.01–200 nmol·L^{-1} 1.72×10^{5}–2.3×10^{6} cell·mL^{-1}	2.3 pmol·L^{-1} 196 cell·mL^{-1}		Solution	[73]
HE4	GQD	Ratiometric FRET	4.3 pmol·L^{-1}–300 nmol·L^{-1}	4.8 pmol·L^{-1}		Solution	[74]
AFP	GQD	Electrochemical immunosensor	0.001–200 ng·mL^{-1}	0.25 pg·mL^{-1}		Solution	[75]
CEA or AFP CA199 or CA125 or CA153	GO	Integrated microfluidic immunofluorescence micro assays chip	5 pg–0.5 mg 0.5–5000 U·mL^{-1}	1 pg·mL^{-1} 0.01 U·mL^{-1}	40 min	Human serum	[76]
MMP-7	CNT	Electrochemical sensor and differential pulse voltammetry	0.01–1000 ng·mL^{-1} 0–1000 ng·mL^{-1}	6 pg·mL^{-1}	30 min	Solution Human serum; synthetic urine	[77]
AKT2 gene	CNT	All-CNT thin-film transistor biosensors incorporated with tetrahedral DNA nanostructures	1 pmol·L^{-1}–1 μmol·L^{-1}	2 fmol·L^{-1}		Solution	[78]
PSA	Pdots	FRET-based immunochromatographic strip	2–10 ng·mL^{-1}	0.32 ng·mL^{-1}	10 min	Whole blood	[79]
Exosomes	Pdots	Localized surface plasmon resonance	1.0×10^{3}–1.0×10^{6} particle·mL^{-1}	400 particle·mL^{-1}		Solution	[80]
CEA CYFRA 21-1	Pdots	Fluorometric immunochromatographic test strips	0–15 ng·mL^{-1} 0–10 ng·mL^{-1}	0.12 ng·mL^{-1} 0.07 ng·mL^{-1}	15 min	Solution	[81]

（续表）

Target	Material	Method	Linear range	Limits of detection	Time analysis	Sample	Ref.
GSH	Metal nanoclusters	Fluorescence quenching interactions	0–1.75 μmol·L^{-1}	0.1 μmol·L^{-1}		Solution	[82]
MicroRNA-21	Metal nanoclusters	Paper colorimetric assay by nanocluster catalytic activity	1.0–700 pmol·L^{-1}	0.6 pmol·L^{-1}		Solution	[83]
MicroRNA-141 MicroRNA-155	Metal nanoclusters	Entropy-driven amplification system and multiplexed analysis	0–50 nmol·L^{-1} 0–50 nmol·L^{-1}	6.1 pmol·L^{-1} 8.7 pmol·L^{-1}		Human serum	[84]
ACP	Fluorescent silicon Nanomaterials	Inner filtering effect	1.0–50 mU·L^{-1}	0.3 mU·L^{-1}	20 min	Human serum	[85]
PIK3CA E542K	Fluorescent silicon nanomaterials	SiNW array field effect transistor biosensor	0.1 fmol·L^{-1}–100 pmol·L^{-1} 1 pmol·L^{-1}–1 nmol·L^{-1}	10 amol·L^{-1} 10 fmol·L^{-1}		Solution Human serum	[86]

Notes: CA19-9 represents carbohydrate antigen 19-9; GPC3 represents glypican-3; DKK1 represents dickkopf-1; AFP represents α-fetoprotein; CA125 represents carbohydrate antigen 125; GPC-1 represents glypican-1; OPN represents osteopontin; VGEF represents vascular endothelial growth factor; Cyt c represents cytochrome c; HE4 represents human epididymis protein 4; CEA represents carcinoembryonic antigen; CA199 represents carbohydrate antigen 199; CA153 represents carbohydrate antigen 153; MMP-7 represents matrix metalloproteinase-7; PSA represents prostate-specific antigen; CYFRA 21-1 represents cytokeratin 19 fragment; GSH represents glutathione; ACP represents acid phosphatase; FRET represents fluorescence resonance energy transfer; SiNW represents silicon nanowires

3.2 循环肿瘤细胞检测

循环肿瘤细胞（CTC）是存在于外周血中的各类肿瘤细胞的统称。该细胞来自原发肿瘤或转移肿瘤，可破坏基底膜进入附近的血管和淋巴管，随后通过血液转移到其他器官。CTC是癌症患者出现术后复发和远处转移的重要原因，也是导致其死亡的重要因素[87-89]。作为液体活检的重要生物标志物，CTC在癌症诊断、监测转移、治疗评估和预后等方面具有不可替代的作用[90-91]。但CTC的分离和检测一直受到准确性和特异性的限制，主要原因在于CTC十分罕见，在数十亿个血细胞中只有一到几个CTC，且会受到血液中大量血细胞的干扰[92-93]。

过去几十年间，随着科技的不断进步，免疫磁分离、电化学、光学、微流控等技术在CTC检测中被广泛应用[92-95]。CTC技术通常包括捕获并富集、检测识别以及释放三个主要步骤，其中释放的CTC主要用于下游分析，如基因组学、蛋白质组学和CTC培养[87-91]。

CTC捕获并富集通常是基于CTC的大小、密度、电荷等物理性质以及免疫亲和性实现的。CTC在预处理过程中易出现破损，针对这一问题，孙佳姝研究团队[88]利用惯性升力和界面弹性升力之间的竞争，开发了界面黏弹性微流体系统，如图2(a)所示。该系统可直接从全血中通过尺寸选择来分离肿瘤细胞，实现了95.1%的分离效率和77.5%的回收率，不需要细胞标记和其他处理。Li课题组[92]开发了一种新型材料——磁性纳米粒子-量子点-适体共聚物（MQAP），它能够在20 min内实现血样中稀有CTC的简便分离和计数，捕获效率和捕获纯度均能达到80%以上。硅纳米线（SiNWs）场效应管凭借其对肿瘤标志物检测的敏感度高、检测范围宽、实时性好等优势，在早期极低质量浓度的肿瘤标志物检测方面得到了广泛应用[48]。本课题组[87]设计了一种硅纳米线与多功能磁性纳米复合材料（Fe_3O_4@C6/Ce6@硅烷）相结合的倒置芯片，如图2(b)所示，它不仅可以实现CTC的高捕获纯度（90%）和高捕获效率（培养基中90.3%，血液中82%），还可以在对CTC进行实时监测的同时通过原位光动力疗法直接灭活捕获的CTC，阻止肿瘤扩散。如图2(c)所示，彭孝军课题组[96]将抗上皮细胞黏附分子（EpCAM）抗体和小分子近红外荧光剂MLP组装合成到Fe_3O_4磁珠（MB-MLP-EpCAM）表面，既提高了CTC的捕获效率（大于85%），又保证了细胞活力（大于90%），而且规避了单一靶标带来的假阳性信号

的干扰，降低了成本，实现了 CTC 的特异性识别和动态监测。如图 2(d)所示，Shen 等[97]在微流控芯片上构建了抗体工程红细胞(RBC-Ab)亲和界面，用于 CTC 的高效捕获和释放；该设计利用红细胞膜的横向流动性使抗体聚集，从而高效地捕获 CTC(捕获效率为 96.5%)；该设计通过红细胞裂解缓冲液破坏红细胞界面，在温和释放 CTC 的同时避免了 DNA 污染(存活率为 96.1%)。

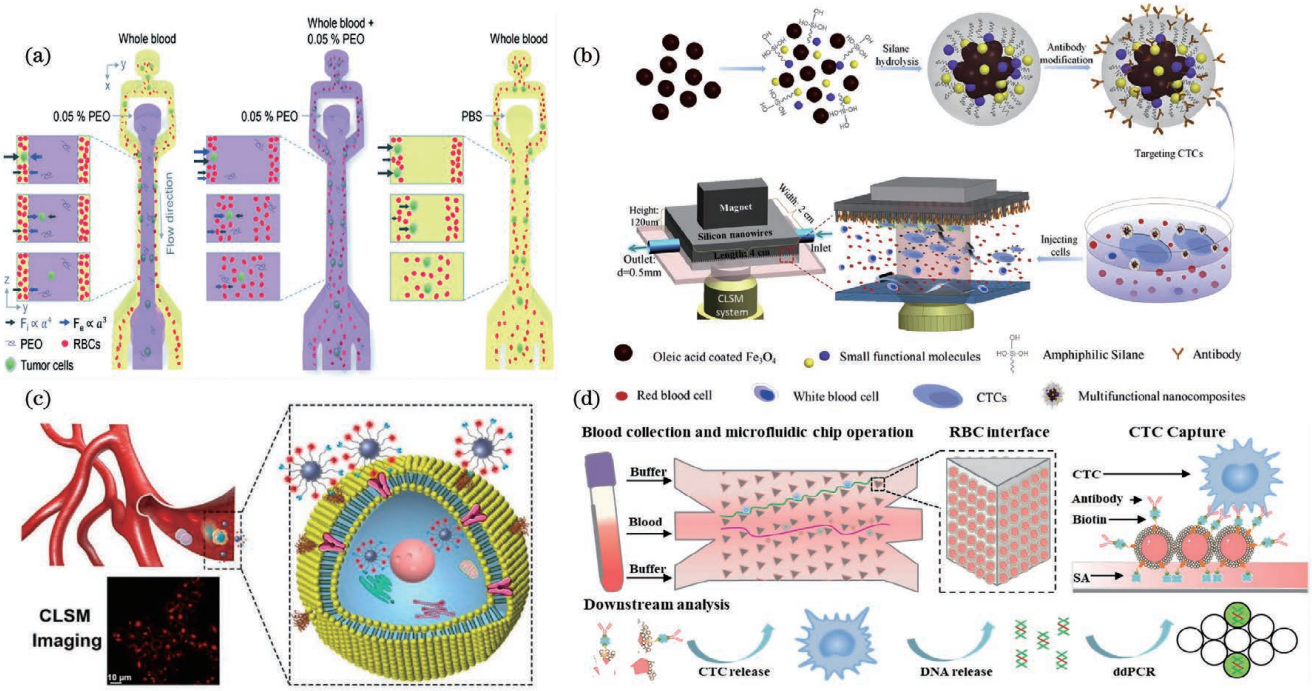

图 2 CTC 的捕获和富集方案。(a)基于 CTC 物理性质的界面黏弹微流控系统[88]；(b)基于多功能磁性材料的 CTC 微流控分选平台[87]；(c)基于双靶向磁性荧光纳米珠的 CTC 体内识别[96]；(d)用于 CTC 高效捕获和释放的抗体工程红细胞界面[97]

Fig. 2 Strategy of circulating tumor cell (CTC) capture and enrichment. (a) Interfacial viscoelastic microfluidics system based on CTC physical properties[88]; (b) microfluidic sorting platform for CTC based on multifunctional magnetic composites[87]; (c) *in vivo* identification of CTC by dual-targeting magnetic-fluorescent nanobeads[96]; (d) antibody-engineered red blood cell interface for high-performance capture and release of CTC[97]

许多可见光发射的荧光纳米材料已被应用于 CTC 的后续检测。如图 3(a)所示，Chen 等[98]基于 CdTe 量子点选择性识别 Ag^+ 和 C-Ag^+-C 的特点，以黏蛋白 1 为 CTC 标志物，以适体为识别探针，提出了一种灵敏、简单且低成本的 CTC 检测策略。该策略对黏蛋白 1 和 A549 细胞的检出限分别为 0.15 fg/mL 和 3 cell/mL，并且肉眼可分辨出质量浓度为 1 fg/mL 的黏蛋白 1 和 100 cell/mL 的 A549 细胞。Yu 等[99]将石墨氮化碳量子点、金纳米团簇结合体与抗 EpCAM 抗体连接，获得了 CTC 特异性比率荧光免疫探针，该探针可以有效捕获和准确测定 CTC。

CTC 荧光检测的灵敏度往往会受到光的散射、吸收以及自发荧光的限制。近年来，具有较高组织穿透深度且生物特性较好的近红外发光材料被逐渐应用于 CTC 检测[100]。$CuInSe_2$(CISe)是一种具有发展潜力的近红外纳米探针，但 CISe 量子点容易形成铜/铟间隙等缺陷，使得化学计量偏差较大，降低了其在近红外二区(NIR-Ⅱ)的光致发光量子产率，从而使得其在敏感发光生物传感中的应用受到严重阻碍。陈学元研究团队[89]通过控制 Se 和 In 的化学计量比，合成了近红外发射峰在 920～1224 nm 范围内的可调 $CuInSe_2$@ZnS 量子点，如图 3(b)所示。$CuInSe_2$@ZnS 量子点不仅具有 21.8%的 NIR-Ⅱ光致发光量子产率，而且可以通过与 EpCAM 抗体的生物结合，实现全血中 MCF-7 细胞的高灵敏度检测，检出限低至 96 孔板的 12 cell/孔。Pons 等[101]用涂有多齿咪唑-两性离子嵌段共聚物的近红外发射 ZnCuInSe/ZnS 量子点标记红细胞和淋巴瘤细胞，通过时间门控成像技术，对活体大鼠血管中标记的肿瘤细胞(循环速度低于 1 mm/s)进行了有效检测和计数。此外，作为近红外荧光纳米材料，Ag_2S 纳米颗粒以其独特的性质被用于生物传感和生物成像中[102]。鲜跃仲课题组[103]将多价适体功能化的近红外 Ag_2S 纳米点与杂化细胞膜包裹的磁性纳米颗粒相结合，开发了一种可以抗背景干扰且可以高效分离和超灵敏检测 CTC 的纳米平台，CTC 的捕获效率和捕获纯度分别高达 97.63%和 96.96%，该平台对血液样本中 CTC 的检测范围为6～10 cell/mL。

虽然 CTC 的检测已经有了很大突破，但是 CTC 的分离和鉴定会损害其活性和功能，并会导致大量的 CTC 损失，因此，在血液样本中实现直接和超灵敏度的 CTC 检测仍然是一个艰巨的挑战。如图 4(a)所示，Wu 等[104]利用免疫纳米球的荧光特性及其在复

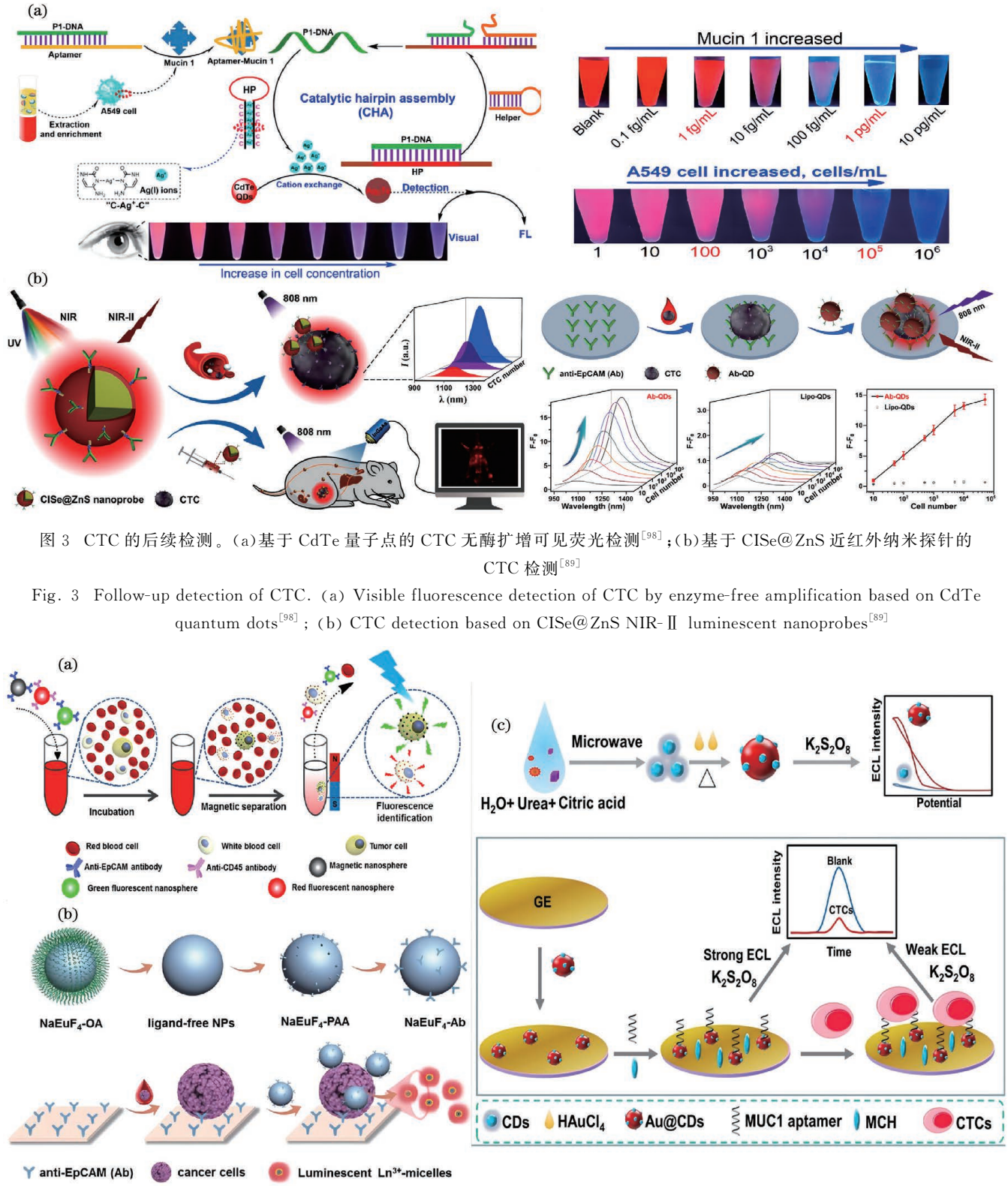

图3　CTC的后续检测。(a)基于CdTe量子点的CTC无酶扩增可见荧光检测[98]；(b)基于CISe@ZnS近红外纳米探针的CTC检测[89]

Fig. 3　Follow-up detection of CTC. (a) Visible fluorescence detection of CTC by enzyme-free amplification based on CdTe quantum dots[98]; (b) CTC detection based on CISe@ZnS NIR-Ⅱ luminescent nanoprobes[89]

图4　CTC的直接检测。(a)基于免疫纳米球的CTC一步检测策略[104]；(b)基于Ln^{3+}纳米探针的CTC直接检测策略[90]；(c)基于Au@CDs的CTC直接检测策略[105]

Fig. 4　Direct detection of CTC. (a) Immunonanospheres-based one-step strategy for efficient detection of CTC[104]; (b) direct detection strategy of CTC based on Ln^{3+} nanoprobes[90]; (c) direct detection of CTC based on Au@CDs[105]

杂基质中的非凡稳定性，开发了对细胞友好的一步CTC检测策略。该检测策略省时且可靠，仅需20 min孵育即可在约1 mL的全血中有效检测到5个肿瘤细胞，检测结果的相对标准偏差为8.7%，且检测到的93.8%±0.1%的肿瘤细胞保持着细胞活力和增殖能力。陈学元研究团队[90]开发了一种基于时间分辨发光的超灵敏Ln^{3+}纳米探针及CTC靶向平台，如图4(b)所示，并将该平台用于直接检测全血中的CTC。该平台

通过溶解增强的时间分辨荧光(TRPL)进行信号放大,规避了传统荧光检测过程中的低灵敏度和高自发荧光干扰,对全血表现出优异的实际检测能力,检出限低至1 cell/孔,并且能够以93.9%的检测率诊断出癌症患者。如图4(c)所示,Liu课题组[105]通过碳点和$AuCl_4$水溶液直接加热沸腾合成了新型的Au@CDs。该材料不需要复杂的分离和标记处理就能实现对MCF-7细胞的检测,检测范围为100～10000 cell/mL,检出限为34 cell/mL,在常规的超灵敏度常规临床分析中具有广阔的应用前景。

3.3 ctDNA检测

循环肿瘤DNA(ctDNA)是血液系统中携带肿瘤基因组的DNA片段。这些肿瘤DNA往往含有肿瘤基因组所特有的基因突变,因此,作为一种即时、无创的液体活检标志物,ctDNA在癌症诊断、分子特征分析、治疗检测、疗效评估及术后监测等阶段都可能发挥作用[106-107]。ctDNA可以在血液及其他体液(例如,脑脊液、尿液、唾液、粪便、胸膜液和腹水)中被识别。血液ctDNA可以捕获肿瘤异质性,并反映从多个转移部位脱落的DNA。但与肿瘤组织相比,血液样品中ctDNA的丰度相对较低,区分血液中的ctDNA、正常DNA及野生型DNA面临巨大挑战[108]。

许多方法被用于癌症患者血液中ctDNA分子的检测,目前主要使用的ctDNA检测方法包括下一代测序(NGS)、液滴数字聚合酶链式反应(ddPCR)。尽管这些方法非常精确,但是需要依托昂贵的专用设备和严格的测定条件,并且检测过程复杂、操作时间长,不利于在资源有限的环境中进行检测[109-113]。针对快速、简便、准确的检测需求,Zhao课题组[114]结合微流体液滴分离、荧光多重聚合酶链化学反应和快速3D大体积液滴计数技术,开发了一个全面综合的液滴数字检测平台。如图5(a)所示,该平台以单细胞或单分子的灵敏度从mL体积(数千万个液滴)中选择性地检测生物标志物,可用于野生型基因组背景下致癌KRAS G12D突变等位基因的检测,检测灵敏度为0.00125%～0.005%,假阳性率为0%,检测能力远高于现有的商业液体活检平台。此外,该平台还可用于分析血液样品中被分离出的ctDNA及血液样本中

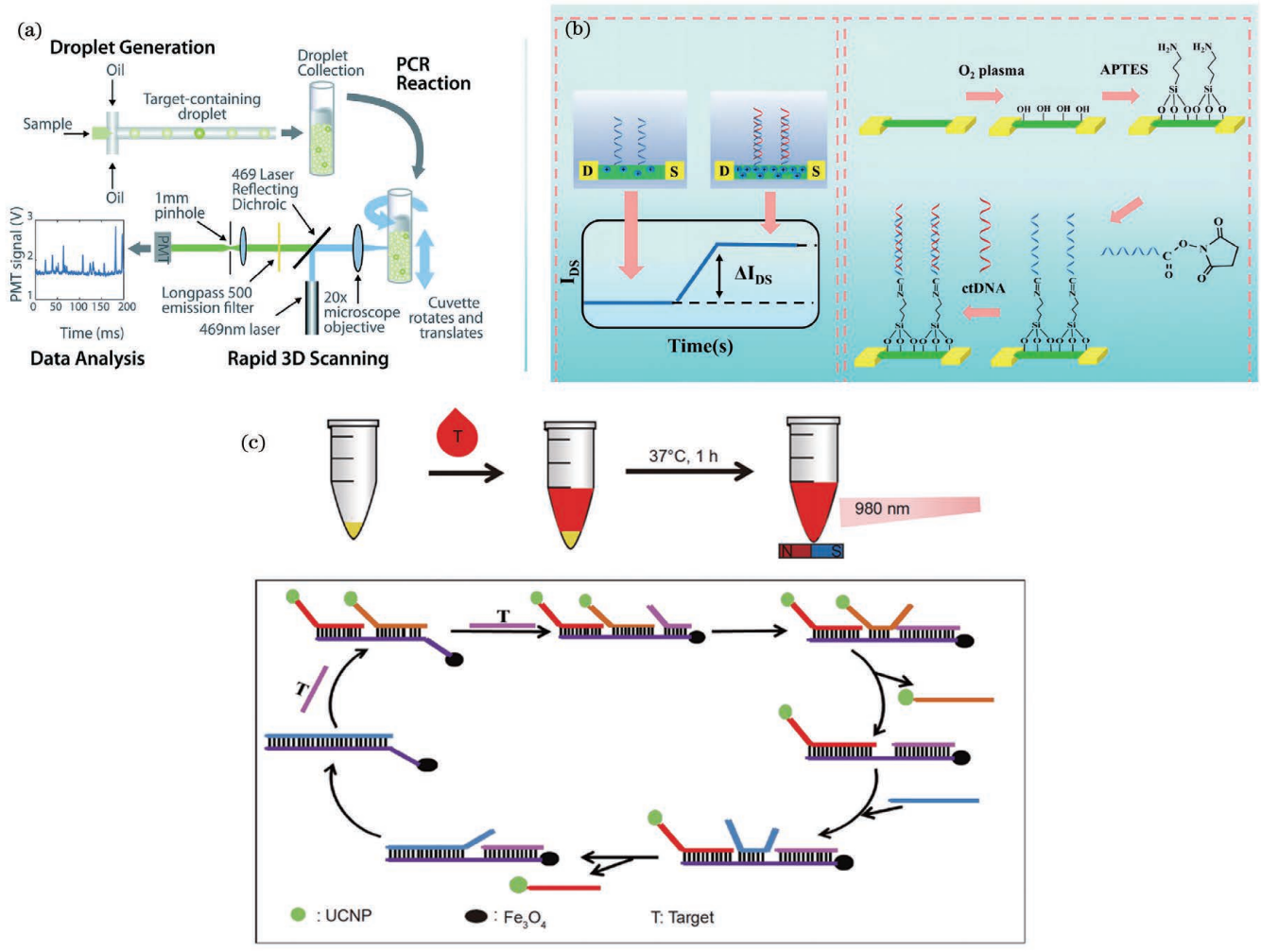

图5　ctDNA的检测。(a)基于综合液滴数字平台的ctDNA检测[114];(b)基于硅纳米线阵列生物传感器的ctDNA检测[86];(c)基于上转换纳米粒子的ctDNA检测策略[117]

Fig. 5　Detection of ctDNA. (a) ctDNA detection based on integrated comprehensive droplet digital system[114]; (b) ctDNA detection based on silicon nanowire array biosensor[86]; (c) ctDNA detection based on upconversion nanoparticles[117]

CTC的直接检测。Su研究团队[113]利用模式识别和结构选择性酶等温扩增技术，开发了一种基于双探针的快速荧光检测方法，用于检测ctDNA的缺失突变。该方法借助特殊设计的线性分子信标（LMBs）和双LMB探针，可在一组实验中同时检测纯合子和杂合子缺失构型，检测时间不到2 h，检出限可达到pmol量级。另外，该方法快速、简单，对表皮生长因子受体（EGFR）所有缺失突变构型的评估兼具特异性和敏感性，具有快速诊断癌症的潜能。

生物传感器是将生物信号转换为电、光或热力学信号的装置，可以测量整个细胞的生化信号，具有方便精确的优点，为ctDNA的检测提供了一种有效方法。Li等[86]设计了用于PIK3CA E542K ctDNA定量检测的生物传感器。如图5(b)所示，该传感器基于硅纳米线阵列场效应晶体管（FET），可以高选择性地识别复杂样品中的靶ctDNA，具有实时、无标记和超灵敏的特点，在0.1 fmol/L～100 pmol/L的浓度范围内具有良好的线性，检出限低至10 amol/L。Zhang等[115]设计了一种用于ctDNA检测的基于均匀钯纳米丝的荧光生物传感器，该传感器具有优异的荧光猝灭效率，对ssDNA和dsDNA的亲和力不同，与一对带有荧光团的DNA检测探针结合后无需信号放大即可高灵敏、高选择性地检测目标DNA。Miao研究团队[116]提出了一种新的比率荧光生物传感器，该传感器对基于DNA四路连接（FWJ）的ctDNA具有高灵敏度和高选择性检测。在系统中，3个发夹结构的DNA探针保持稳定，FWJ只有在ctDNA引发的链置换扩增产生DNA时才会形成；当两个荧光团的荧光共振能量转移（FRET）状态改变时，其荧光峰值强度在相反的方向上变化，通过比率荧光响应记录即可实现对ctDNA的高灵敏度检测，检出限为0.12 nmol/L。Ma等[78]开发了用于AKT2基因（乳腺癌相关ctDNA）直接无标记检测的生物传感器，该传感器基于全碳纳米管薄膜晶体管设计而成，具有6个数量级宽的线性检测范围和2 fmol/L的检出限。此外，该传感器具有良好的选择性和可重复性，在集成化应用和多重检测方面具有较大潜力。

荧光分子诊断技术以其灵敏度高、适用范围广等优点引起了人们的高度重视，并被逐渐应用于ctDNA的快速检测和快速响应。如图5(c)所示，Chen等[117]设计了基于上转换和Fe_3O_4纳米粒子的超灵敏无酶光学ctDNA检测系统。在该检测系统中，稀土上转换纳米粒子和Fe_3O_4通过DNA碱基互补配对原则自组装在一起，目标序列可以通过熵驱动链置换（ESDR）反应将连着上转换纳米粒子的核酸链释放出来。经磁性分离后，上清液中的上转换纳米粒子在980 nm激光激发下的荧光强度与目标序列在100 amol/L～1 nmol/L浓度范围内具有非常好的线性关系，检出限可低至1.6 amol/L。Wang等[118]设计开发了一种基于Yb^{3+}、Tm^{3+}掺杂上转换纳米粒子和金纳米粒子的近红外荧光探针，该探针可用于单碱基突变ctDNA的快速诊断。该荧光探针的信号在近红外波段传输，可有效避免生物样品中背景荧光的干扰，检测范围在5 pmol/L～1000 pmol/L之间，检出限为6.30 pmol/L。这种具有稳定性和高特异性的NIR探针可以直接用于血清环境，无须预先进行复杂的预处理和扩增过程。

3.4 外泌体检测

外泌体是细胞分泌到胞外的一种囊泡（EVs），其尺寸在40～150 nm之间，具有脂质双层膜[119]。与CTC、ctDNA、传统的血清蛋白质标记物相比，外泌体在丰度和稳定性方面具有独特的优势[120]。几乎所有类型的细胞都可以产生外泌体，正常人的血液中含有约2000万亿个外泌体，癌症患者的血液含有约4000万亿个外泌体[13]。外泌体是高度异质性的，与肿瘤的转移、血管生成、免疫和肿瘤生成等过程密切相关，可作为液体活检的非侵入性生物标志物，用于癌症和其他疾病的诊断、治疗、判断预后等[13,119-121]。此外，外泌体含有DNA、RNA、蛋白质、脂质、氨基酸和代谢物等多种生物成分，其内外物质不仅可以单独作为肿瘤检测的靶标，还可以通过多组分分析进行治疗监测和明确疾病的进展[13,121]。

尽管外泌体具有诊断和治疗潜力，但外泌体的体积小、与其他细胞外囊泡重叠、来源和生物环境复杂等特征严重阻碍了人们对它的研究。目前，外泌体检测分析方法包括超速离心、超滤、质谱、酶联免疫吸附、电化学发光、微流体法和流式细胞术等。此外，由于每种技术只能给出外泌体某一方面的信息，因此通常需要几种方法结合起来进行外泌体的综合分析[80,122-125]。

生物传感器具有方便、精确的优点，在外泌体检测中被广泛应用。如图6(a)所示，Chen等[126]基于磁性纳米粒子（MNP）和辣根过氧化物酶（HRP）设计了一种用于外泌体检测的夹心型荧光生物传感器。该传感器兼具高灵敏度和高特异性的特点，适用于各种来源的外泌体检测，检测范围为(576 ± 15)～$(5.76\times10^7\pm5.1\times10^5)$个/mL，检出限低至$(200\pm9)$个/mL。适体是一类短的单链核酸寡聚体，具有与蛋白结合的特异性、化学稳定性和低成本等特点，被视为最具吸引力的抗体替代品。Zhang等[127]将量子点与光子晶体的仿生纳米结构相结合，构建了一种高灵敏度、高特异性的外泌体诊断芯片。该芯片不需要酶的参与就可以提高荧光团的发射强度，并具有良好的动态检测范围（1.0×10^7～1.0×10^9个/mL），是一种灵敏度高、样品消耗少、重现性好、适用于复杂生物样品的检测方法。

最近，荧光材料标记的适体被广泛应用于外泌体的检测中。Wang等[128]设计了一种基于稀土掺杂上转换纳米粒子（UCNPs）和四甲基罗丹明（TAMRA）的发光共振能量转移的无洗涤适体传感器，该传感器利用荧光强度与外泌体浓度之间的线性关系对外泌体

进行定量和高灵敏度检测，检出限为80个/μL，也可以通过适体的筛选应用于其他靶标的检测。如图6(b)所示，Li等[129]用适体、聚集诱导发光材料和氧化石墨烯分别作为识别元件、荧光染料和猝灭剂，开发了用于外泌体蛋白检测的荧光适体传感器。该传感器的检测灵敏度低于其他荧光/比色适体传感器，目标外泌体的线性检测范围为0.68～30.4 pmol/L，检出限为0.57 pmol/L，可在30 min内通过单一步骤测量外泌体蛋白质，是一种方便且经济有效的检测平台。此外，该传感平台在对前列腺癌与健康个体的区分(area under curve，AUC：0.9790)以及乳腺癌高效诊断(AUC：0.9845)上表现出色。如图6(c)所示，Cheng等[130]开发了基于无酶信号放大和同步荧光技术的双色DNA纳米设备，该设备以CD63适体和MUC1适体作为识别元件，对外泌体表面蛋白进行灵敏检测和同步分析，结果显示，CD63和MUC1的检出限分别为67个/μL和67个/μL。

微流控技术具有高灵敏度、高纯度、高通量、低材料消耗等优点，结合微流控的各类荧光检测方法已被普遍地应用于外泌体检测。Bai等[120]开发了一种用

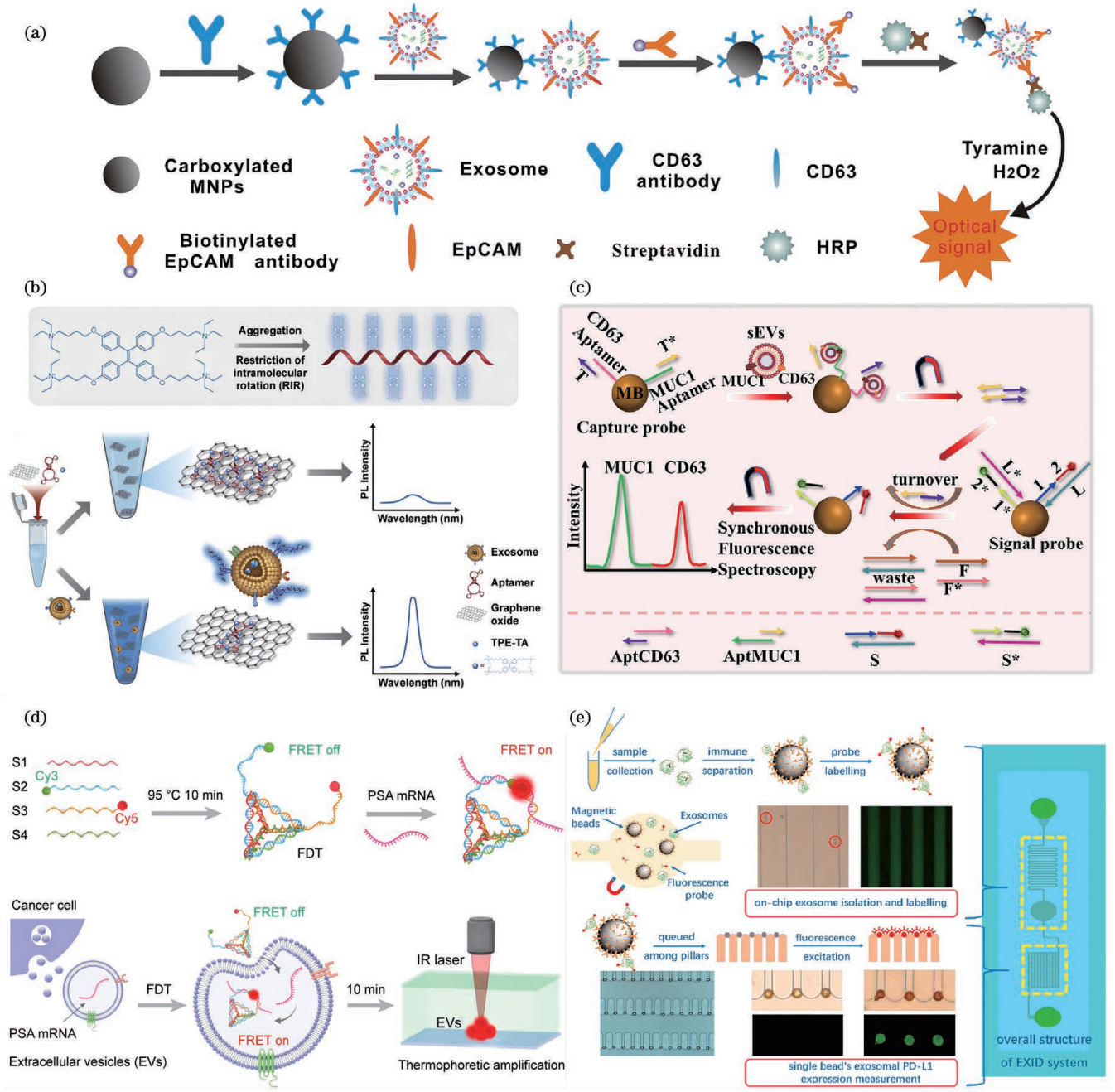

图6　外泌体检测。(a)基于荧光生物传感器的外泌体检测[126]；(b)基于荧光适体传感器的外泌体检测[129]；(c)外泌体表面蛋白检测[130]；(d)外泌体的原位测量[131]；(e)基于微流控的外泌体检测[133]

Fig. 6　Detection of exosomes. (a) Detection of exosomes based on fluorescent biosensor[126]; (b) detection of exosome based on fluorescent aptasensor[129]; (c) detection of exosome surface proteins[130]; (d) *in situ* measurement of exosomes[131]; (e) detection of exosomes based on microfluidic system[133]

于外泌体分离和多重肿瘤标志物检测的微流控芯片，该芯片通过结合珠子表面的抗体来分离外泌体，用量子点探针来检测肿瘤标志物。珠子被均匀地捕获并排列在芯片的微柱中，可以避免光学干扰，并使测试结果更准确。如图6(d)所示，孙佳姝研究团队[131]基于荧光共振能量转移(FRET)的DNA四面体(FDT)检测和DNA四面体热泳分析(DTTA)，开发了一种用于原位测量外泌体PSA和mRNA的超灵敏分析方法，该方法不需要RNA提取和酶扩增，可用于多重外泌体mRNA分析，极大地拓展了DNA纳米结构液体活检的应用范围。同时，该团队提出了一种λ-DNA和适体介导的方法[132]，该方法可对单个EV进行大小选择性分离和表面蛋白质分析，并可通过EV信号分析辨别乳腺细胞系和乳腺癌患者，为在单个EV水平上评估EV异质性以及确定EV亚群在其他类型癌症中的诊断提供了一个重要工具。如图6(e)所示，Lu等[133]开发了集成的外泌体分离和检测微流控系统，该系统通过磁珠阵列分离和荧光标记定量，对外泌体蛋白PD-L1在不同细胞系间的表达水平进行分析，单次分析耗时少于2 h，检出限可降至10.76个/μL，为个性化诊断和免疫治疗提供了有效工具。

3.5 癌胚抗原检测

癌胚抗原(CEA)是一种与细胞黏附密切相关的酸性糖蛋白，存在于由内胚层细胞分化形成的肿瘤细胞表面。CEA是一种广谱肿瘤标志物，它的升高是结直肠癌、肺癌、乳腺癌、胃癌、肝癌、胰腺癌等恶性肿瘤诊断中不可缺少的参考指标。作为世界上临床应用最广泛的肿瘤标志物之一，CEA虽然不能作为诊断某种恶性肿瘤的特异性指标，但在癌症的鉴别诊断、预测治疗、预后监测等方面，仍然具有重要的临床价值[134-137]。在人血清中，CEA的正常水平低于5 ng/mL，一些良性疾病可以将这一水平提高到10 ng/mL。癌症的发展或复发可能会使癌胚抗原的浓度增加，癌细胞转移状态下通常会出现高于20 ng/mL的CEA水平[134-135]。因此，快速、准确、灵敏的CEA检测对于癌症的早期诊断和治疗具有重要意义。

目前，CEA的检测方法几乎都是基于抗原-抗体或适体识别的免疫学方法，例如酶联免疫吸附测定、免疫胶体金技术、电化学免疫分析、荧光分析等。近年来，在荧光纳米材料光学和电学特性优良、生物相容性好、成本低的基础上，荧光纳米探针及荧光免疫传感器凭借超灵敏、操作简单的优势，备受研究人员的青睐[138-139]。Wang等[134]设计了一种借助镧系掺杂上转换纳米粒子和氧化石墨烯之间荧光共振能量转移进行CEA检测的超灵敏均相适配传感器，该传感器可以直接监测人血清中的CEA水平，具有优异的检测性能，在水溶液和人血清样本中的检出限分别为7.9 pg/mL和10.7 pg/mL。Zhan等[140]设计了一种用于检测CEA的开-关-开型双光子荧光免疫传感策略，可在降低检测成本的同时实现CEA的高灵敏度检测，检出限低至74.5 pg/mL，线性范围为0.1～80 ng/mL。如图7(a)所示，Wang等[135]基于CDs@SiO_2纳米棒制备了荧光-红外吸收双模纳米探针，用于CEA的准确灵敏检测，检出限可达到794.6 ag/mL。该检测策略在确保检测结果可靠性的同时大大提高了检测的整体灵敏度，并且具有优于商业试剂盒的回收率。

然而，尽管基于抗原-抗体的方法具有较高的特异性、敏感性和临床适用性，但抗体的制备比较复杂和昂贵，抗原-抗体反应需要较长时间，而且需要专业的检测设备，因此该方法不适用于人群中快速癌症筛查。如图7(b)所示，Sun等[137]根据荧光修饰的鸟嘌呤脱氧核糖核酸二级结构的改变与荧光信号变化的原理，提出了一种检测CEA的新方法。Sun等根据荧光强度的变化计算CEA的浓度，建立了一个简单、快速、廉价的生物标志物检测传感平台，该平台在人群中开展肿瘤筛查具有巨大潜力。

除了最为普遍的血清样本检测外，唾液检测作为一种替代方法，具有易获取、安全无创的显著优势。由于唾液的成分约99.5%是水，肿瘤标志物的表达浓度远低于人血清中的浓度，因此，实现唾液中肿瘤标志物的高灵敏度和高特异性检测是一个艰巨的挑战[141-142]。陈学元研究团队[136]提出了基于镧系纳米探针发光放大的策略，为唾液肿瘤标志物的检测提供了一种简便、超灵敏的方法。如图7(c)所示，该策略巧妙地将溶解增强型发光生物检测技术与小型化检测装置相结合，在酸性增强剂溶液中使用易溶解、可转化为大量Eu^{3+}胶束的高发光Eu_2O_3纳米晶作为生物探针，采用装有硝酸纤维素膜的一次性注射器作为生物检测平台，可在10 min内完成检测过程，对CEA的检出限可达1.47 pg/mL。该团队将该探针的测试结果与商业试剂盒的检测结果进行了对比，验证了其可靠性和实用性。

3.6 甲胎蛋白检测

甲胎蛋白(AFP)是一种在卵黄囊中发现的糖蛋白，主要来源于肝脏。在健康人血清中，AFP的质量浓度通常低于25 ng/mL，在近75%的肝细胞癌(HCC)患者中，其血清内的AFP平均水平可增至400 ng/mL。成人血清中的AFP浓度可作为多种肿瘤的阳性检测指标。此外，AFP也是临床上原发性肝癌的血清标志物[143-146]。开发高灵敏度、高选择性的AFP检测方法对于HCC的早期诊断、治疗预测、预后监测和长期生存评估具有重要意义。人们已经探索了多种AFP的检测策略，包括酶联免疫分析、放射免疫分析、荧光免疫分析、电化学发光免疫分析、生物传感等在内的常规技术，由于具有良好的灵敏度而被广泛用于生物流体中AFP的测定[147-149]。

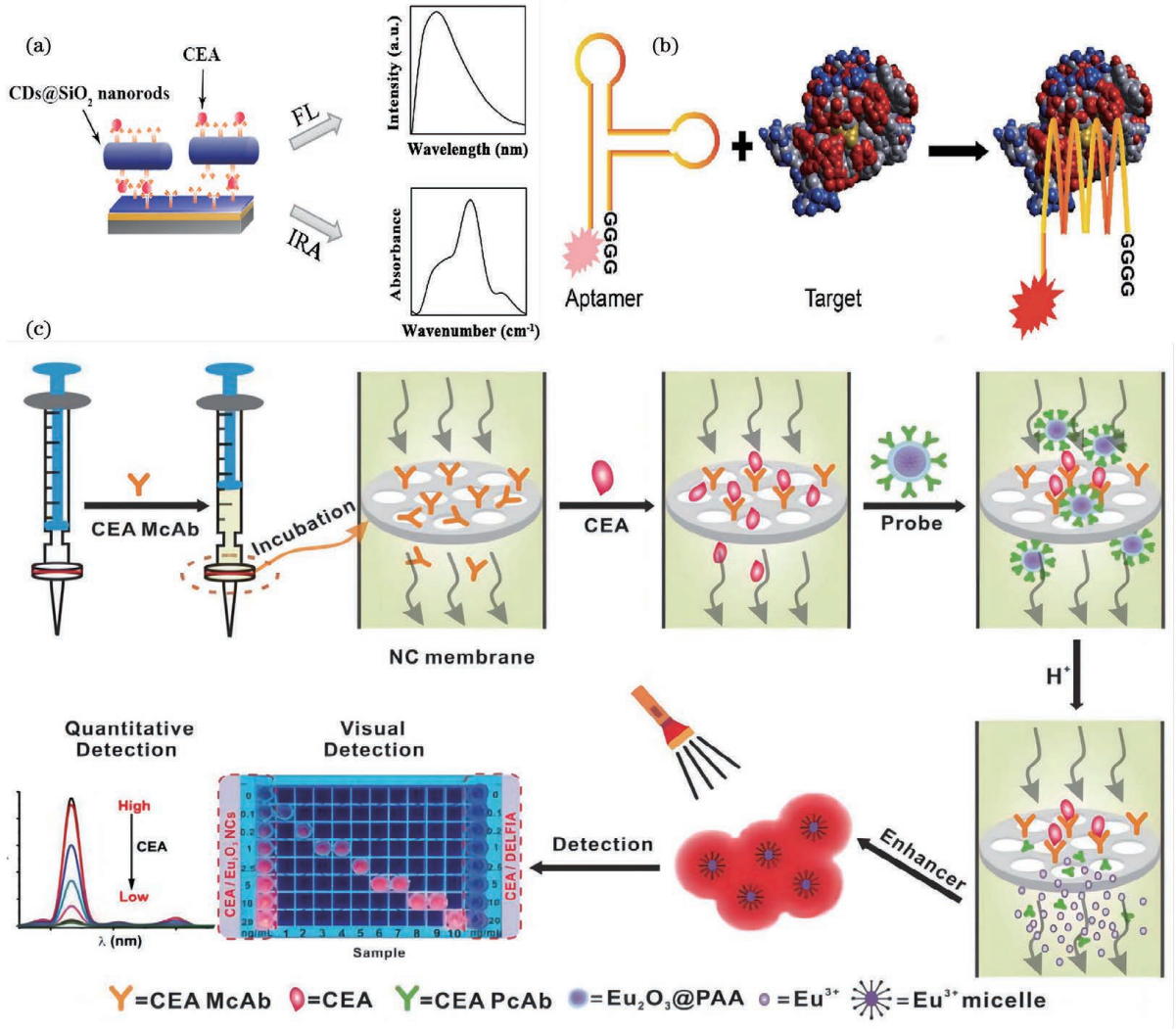

图7　CEA的检测。(a)荧光红外吸收双模纳米探针检测CEA[135]；(b)CEA的快速筛查策略[137]；(c)唾液样品中CEA的检测[136]

Fig. 7　Detection of CEA. (a) Detection of CEA by fluorescencee-infrared absorption dual-mode nanoprobes[135]; (b) rapid tumor screen strategy for CEA[137]; (c) CEA detection in saliva samples[136]

荧光生物传感器具有灵敏度高、反应速度快、操作简单和成本低的特点，近年来被广泛用于AFP检测。如图8(a)所示，Li等[144]利用5-羧基荧光素标记的甲胎蛋白适体(FAM)与钯纳米颗粒(PdNPs)之间的荧光共振能量转移开发了用于AFP检测的高灵敏度荧光适体纳米探针，该探针的检出限低至1.38 ng/mL。Zhou等[150]利用荧光共振能量转移原理，以AFP适体标记的CdTe量子点为供体，同时以抗AFP单克隆抗体功能化金纳米粒子为受体，设计了一种简单、超灵敏的AFP均相荧光传感器。该传感器的荧光强度随着AFP浓度的降低而变化，检测线性范围为0.50～45 ng/mL，检出限为400 pg/mL，具有简单可靠的特点，在现场快速检测和癌症筛查中有很大的应用潜力。此外，Wang等[145]以扁豆凝集素衍生物和胆固醇为原料，将凝集素聚合物脂质磁性球和时间分辨荧光免疫分析相结合，实现了临床样本中甲胎蛋白及其变体(AFP-L3)的简单、准确、快速检测。

目前，许多低成本、快速、无酶的检测策略被开发出来用于肿瘤标志物检测，解决了酶基检测方法对基础设备依赖性强以及低稳定性和高成本的抗体对复杂酶标的特异性识别不适用于更为广泛及资源匮乏环境中使用等问题。如图8(b)所示，Zhu等[151]提出了通过荧光测定法和比色法对血清AFP进行检测的无酶双模超灵敏检测方法。该方法采用的是CdTe量子点修饰的ZnS纳米球，其荧光测定的检出限为10 pg/mL，比色法的检出限为7 pg/mL，检测灵敏度远高于商业酶联免疫吸附检测灵敏度，并且双重检测的互补性降低了假阳性或假阴性的概率。如图8(c)所示，Li等[146]基于适体识别和错配催化发夹(MCHA)自组装成功开发了一种无酶、高灵敏度的AFP荧光传感检测策略。该策略具有较强的抗干扰能力，检测范围为0.1 ng/mL～10 mg/mL，检出限为0.033 ng/mL，检测时间约为60 min。Tawfik等[152]基于荧光分子印迹共轭聚噻吩(FMICPs)纳米纤维，开发了一种无酶、低成本且超灵敏的肿瘤生物

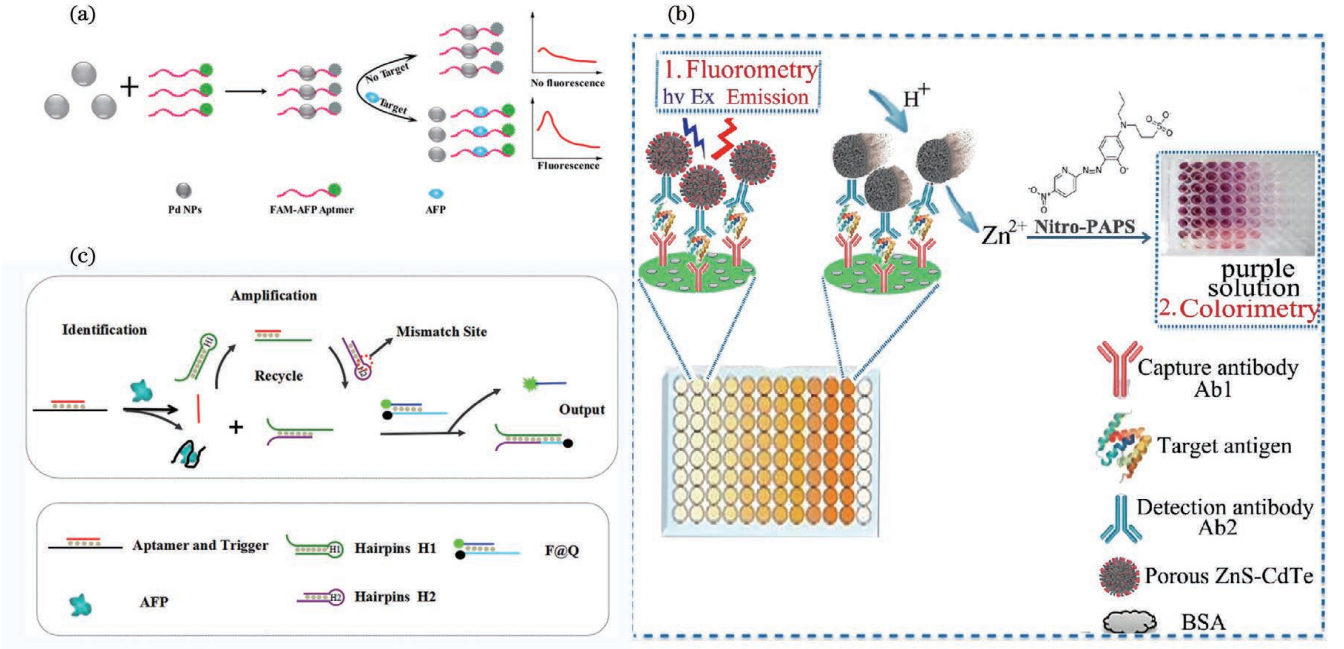

图 8　AFP 的检测。(a)荧光生物传感器检测 AFP[144]；(b)AFP 的无酶双模荧光检测策略[151]；(c)基于适体的 AFP 无酶检测策略[146]

Fig. 8　Detection of AFP. (a) AFP detection by fluorescence biosensor[144]; (b) enzyme-free and dual-mode fluorescence detection of AFP[151]; (c) enzyme-free detection of AFP based on aptamer[146]

标志物检测方法。该方法对 AFP 与 CEA 的检出限分别低至 15 fg/mL 和 3.5 fg/mL，灵敏度比标准酶法高出 3 个数量级，且响应时间仅为 15 min。此外，该方案还集成了基于纸基的打印技术、便携式原型测试设备和经济高效的智能设备，并将集成检测系统用于快速肝癌诊断。

3.7　前列腺特异性抗原检测

前列腺特异性抗原(PSA)是一种受雄激素调节的丝氨酸蛋白酶，也是组织激肽释放酶家族的一员，主要由前列腺导管和腺泡上皮细胞产生，大量存在于精液中并参与精液的液化过程[153-154]。血液中的 PSA 是游离态 PSA(fPSA)与复合态 PSA(cPSA)的总和，也称为总 PSA(tPSA)。前列腺癌患者的血清 PSA 水平通常大于或等于 10 ng/mL，PSA 水平小于或等于 4 ng/mL 时出现前列腺癌的概率往往被认为很低，PSA 水平介于 4～10 ng/mL 之间则被称为“灰色地带”。此外，fPSA 与 tPSA 的比值也可以作为癌症诊断的参数[155-157]。

目前，血液 PSA 检测方法主要有放射免疫测定法、表面增强拉曼光谱法、酶联免疫测定法、电化学法、化学发光免疫测定法、压电法等。但这些方法大多需要昂贵的实验室仪器和熟练的操作人员花费大量的时间来完成，不能满足巨大的应用需求[158-160]。因此，开发具有操作简单、成本较低、测定准确、灵敏度高、方便快捷的诊断系统仍是亟待完成的目标。本课题组[156]利用稀土掺杂的上转换纳米粒子($NaYF_4$：Yb^{3+}，Er^{3+})制备了具有双光子禁带的新型双层聚甲基丙烯酸甲酯蛋白石光子晶体生物芯片，如图 9(a)所示。该芯片具有处理样品时间短、使用灵活、可用于家庭自我筛查等优点，检测线性范围在 0.1～10 ng/mL 之间，检出限可低至 0.01 ng/mL，实现了 PSA 的高灵敏检测，为肿瘤标志物的早期诊断和生物材料传感器的设计提供了有潜力的工具。王升启研究团队[155]基于双色磁性量子点设计了荧光侧流免疫分析测试条，如图 9(b)所示。该测试条可同时对游离型和复合型 PSA 进行检测，便携式智能读出设备能够进行双色测试条的成像和数据处理，fPSA 和 cPSA 检出限分别为 0.009 ng/mL 和 0.087 ng/mL，具有现场诊断的潜力。如图 9(c)所示，Turan 等[157]将分子印迹聚合物的高选择性和表面增强拉曼光谱的高灵敏度融于一体，设计了 PSA 的高灵敏检测方法。该方法的 PSA 检出限为 0.9 pg/mL，定量限为 3.2 pg/mL，回收率高达 98.0%～100.1%(健康人)和 99.0%～101.3%(患者)，且血清 PSA 值的标准偏差低于 4.3%(健康人)和 3.3%(患者)，相较于传统免疫分析方法表现出成本低、检测步骤少、响应速度快、灵敏度和选择性高等优点。

4　肿瘤生物标志物的多重检测

多个生物参数和多个生物信号同时检测一直是生物医学研究人员开发多重分析方法、实现临床简单快速诊断的首要目标。单一生物标志物在许多疾病诊断和预后监测中的特异性和灵敏度较低，易产生假阳性和假阴性的风险；而多个生物标志物携带更多信息，能够减少检测过程中的样品消耗，提高检测效率和诊断准确性，为日益增长的疾病诊断和预后需求提供实用的解决方案。比色法、荧光法、电化学传感器法、质谱法、表面增强拉曼散射等检测方法具有多重分析潜力，

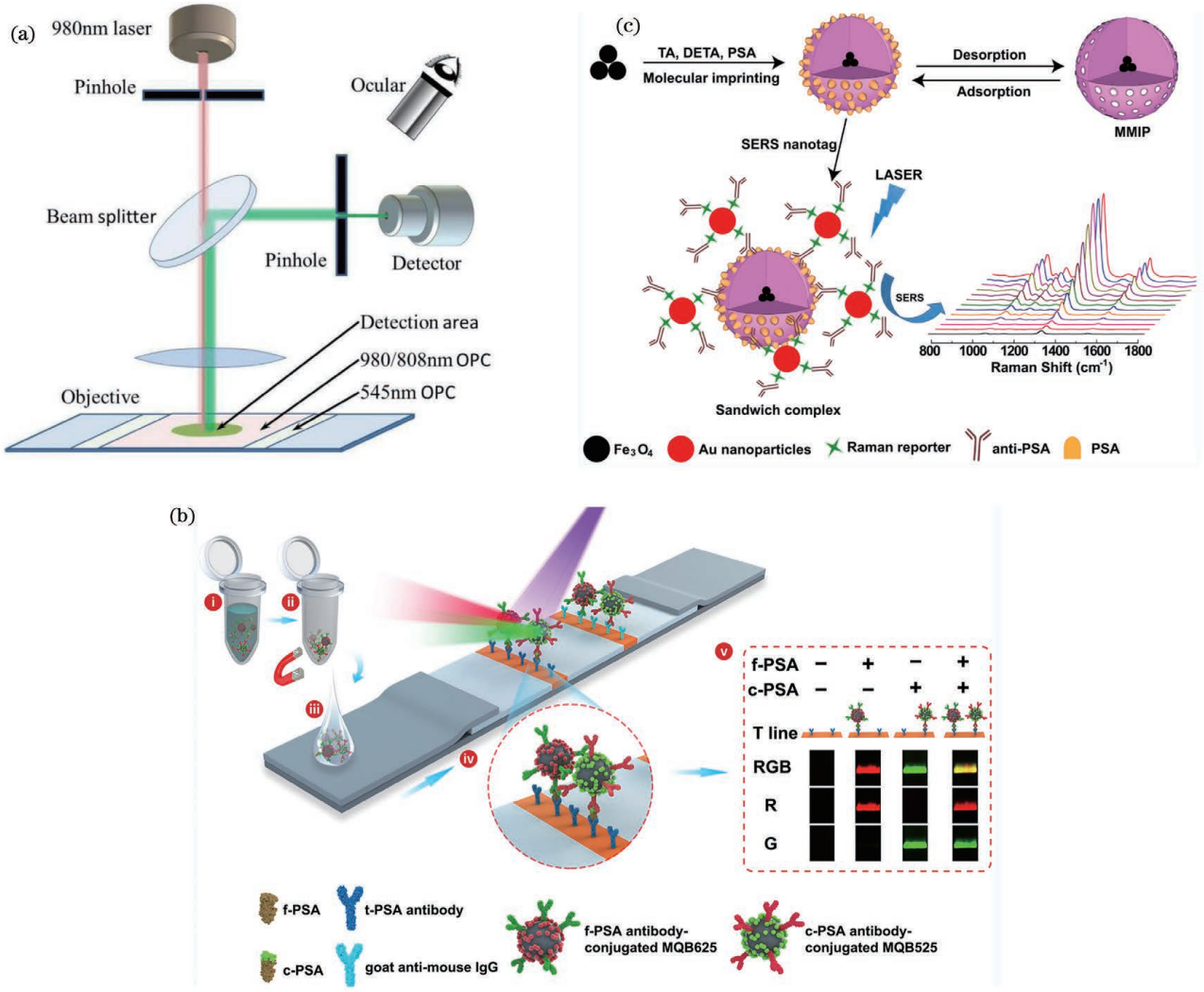

图 9　PSA 检测。(a)基于上转换纳米粒子的 PSA 检测[156]；(b)基于量子点的 PSA 检测[155]；(c)基于分子印迹聚合物的 PSA 检测[157]

Fig. 9　Detection of PSA. (a) Detection of PSA based on upconversion nanoparticles[156]; (b) detecyion of PSA based on quantum dot[155]; (c) detection of PSA based on molecularly-imprinted polymer[157]

在结构分析、环境监测、疾病诊断等领域得到了广泛应用[3-4,161-162]。

基于荧光纳米材料的多种检测方法具有高灵敏度、高特异性和原位实时检测的特点，在多个生物标志物的同时检测上备受青睐。Wu 等[163]采用多色量子点设计开发了能够定量检测三种肺癌肿瘤标志物的荧光生物传感器，如图 10(a)所示。该传感器完全消除了不同颜色量子点间的能量转移，具有较高的临床可靠性，并极大地提高了检测灵敏度。该传感器对鳞状细胞癌抗原(SCCA)、细胞角蛋白 19 片段(CYFRA21-1)和 CEA 的单重检出限分别为 0.079、0.175、0.014 ng/mL，三重检出限分别为 0.534、4.329、1.417 ng/mL。Chen 等[71]以光稳定性好的上转换纳米粒子为标记，通过多个宽视场显微镜计数对 4 种血液循环抗原(磷脂酰肌醇蛋白聚糖 1、瘦素、骨桥蛋白和血管内皮生长因子)进行检测，检出限分别为 0.0123、0.2711、0.1238、0.0158 ng/mL。Li 等[84]将熵驱动放大与银纳米团簇结合，开发了用于 MicroRNA 检测的传感平台。该平台具有出色的选择性、灵活性和窄带激发，在缓冲液和人血清样本中实现了 MicroRNA-141 和 MicroRNA-155 的多重同步检测，检出限分别为 6.1 pmol/L 和 8.7 pmol/L。

荧光共振能量转移作为最常用的传感机制，在生物标志物的多重检测中具有广泛应用。Zhang 等[164]设计了多重荧光共振能量转移微流控系统，如图 10(b)所示，该系统可在液滴中同时定量检测多种肿瘤标志物。该系统的样品消耗量少，在 33 nL 的液滴中可以同时检测 CEA、PSA 和血管内皮生长因子(VEGF165)，且检测过程快速灵敏，数据可靠，对 CEA、PSA 和 VEGF165 的检出限分别为 0.15、0.035、0.11 ng/mL。He 等[165]基于碳点和氧化石墨烯之间的荧光共振能量转移设计了一种无酶荧光信号放大策略，并将该策略成功应用于 PSA、CEA 和 ATP 的检测，检出限分别为 0.22 ng/mL、0.22 ng/mL 和 80 nmol/L。Zhang 等[166]开发了新型多信号比率荧光探针，该探针通过分子内电荷转移和荧光共振能量

转移的协同作用机制，实现了谷胱甘肽（GSH）及其代谢物二氧化硫（SO_2）的同时检测和实时监测，不会发生光谱交叉干扰，并且具有很高的选择性和灵敏度。

此外，免疫层析测定作为一种现场快速检测（POCT）技术，具有经济高效、简单便携、高通量且响应迅速的特点，被广泛应用于生物标志物的多重检测、快速疾病筛查和即时临床治疗[81,167]。Fang 等[167]基于聚合物点光稳定性好、易于功能化、单一激发和多色发射的特点，开发了用于微量样本和肿瘤标志物检测的荧光免疫层析测试条。该测试条可在 10 min 内完成 CEA、AFP、PSA 的快速筛查，检测灵敏度分别为 4.92、3.30、2.05 pg/mL，其快速灵敏和允许多个标志物同时检测的特点促进了体外和早期检测的发展。Ao 等[168]基于近红外发射的 PbS 量子点开发了荧光侧流免疫分析平台，如图 10(c)所示，并通过便携式 NIR-Ⅱ条形扫描仪在 12 min 内实现了肺癌生物标志物（CYFRA21-1、神经元特异性烯醇化酶、CEA）的同时检测，检出限分别为 0.18、0.28、0.11 ng/mL，灵敏度和特异性分别为 92.7%和 92.0%，对癌症早期筛查和快速诊断具有重要意义。

5　结束语

近年来，基于荧光方法的肿瘤标志物检测取得了飞速进步。多种具有高灵敏度、高特异性的检测策略

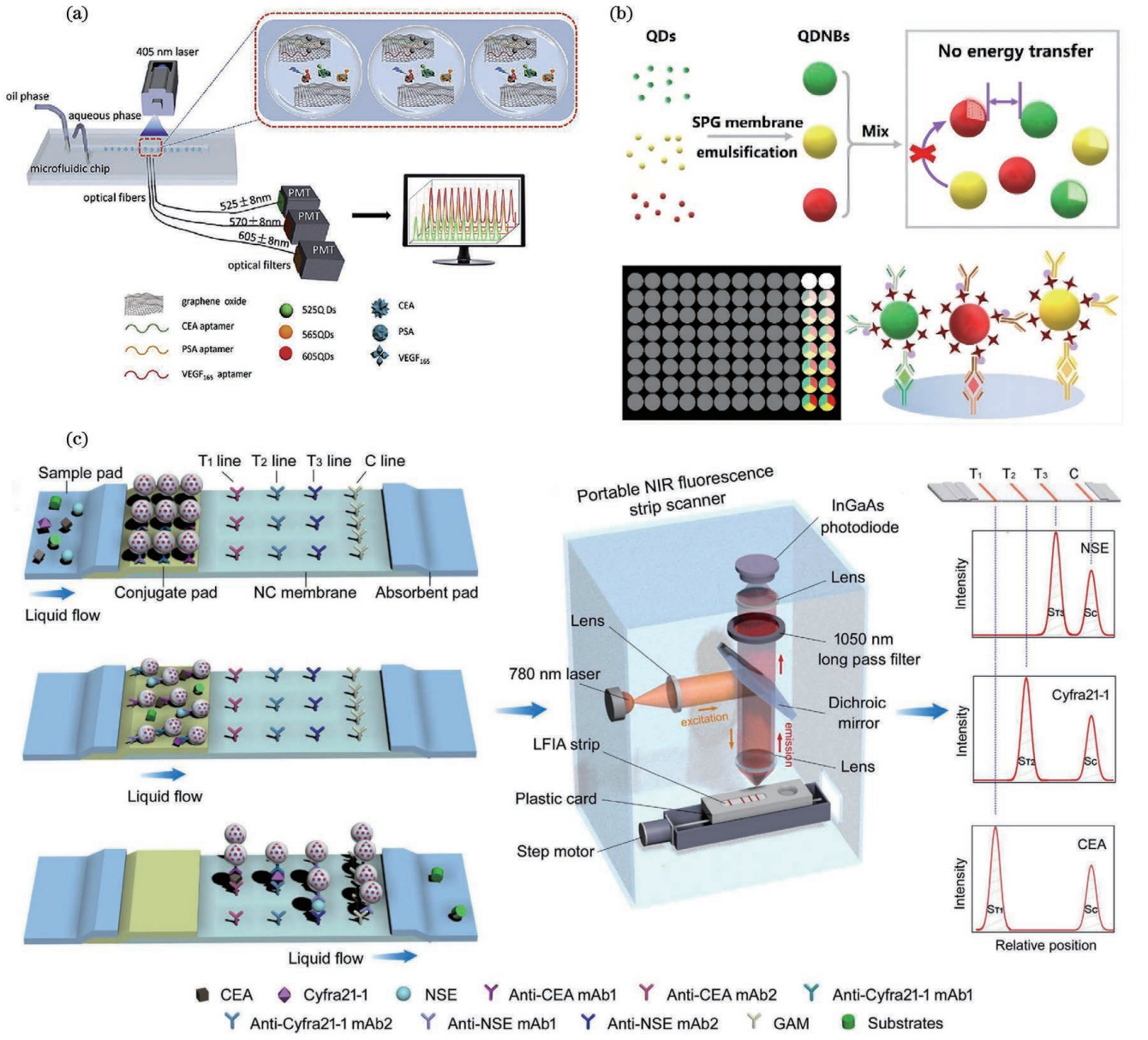

图 10　肿瘤标志物的多重检测。(a)基于荧光材料的生物标志物检测[163]；(b)基于荧光共振能量转移系统的生物标志物检测[164]；(c)基于现场快速检测技术的生物标志物检测[168]

Fig. 10　Multiple detection of tumor marker. (a) Detection of biomarker based on fluorescent material[163]; (b) biomarker detection based on fluorescence resonance energy transfer system[164]; (c) biomarker detection based on point-of-care testing[168]

被提出，用于通过快速高效且准确的方式实现肿瘤标志物检测和肿瘤早期筛查诊断。由于单一标志物存在局限性，荧光检测策略也被用于多个生物标志物的检测，并且该方法具有较高的准确度。同时，基于现场快速检测设备的肿瘤标志物检测方案被不断提出。此外，已经报道了几种检测策略用于非血液样品（唾液、尿液）中肿瘤标志物的高灵敏度检测。

尽管基于荧光的肿瘤生物标志物检测技术快速发展，且这些技术在灵敏度、准确度和可靠性方面都有新的突破，但仍面临诸多挑战。一方面，生物标志物大多浓度较低，并且不能像核酸那样通过自我复制而“扩增”，需要对其进行富集。因此，研究人员需要不断改进及开发新的生物标志物检测方案，在提高检测灵敏度和特异性的同时，减少由检测造成的生物标志物的破坏。此外，许多潜在的肿瘤生物标志物不能兼顾特异性、敏感度及临床实用性，而部分具备应用潜力的生物标志物的生理机制尚未完全明确，单一生物标志物难以实现癌症相关组织、器官的高特异性和高灵敏度检测，这使得标志物的生理机制研究、新型标志物的开发、多种标志物在临床检测上的综合运用等成为亟待解决的需求。

另一方面，目前的荧光方法大多基于荧光纳米材料的荧光特性和电化学特性进行检测，但金属纳米团簇、碳点等材料的发光机制和结构特性尚未研究透彻，上转换纳米粒子、荧光碳纳米材料等没有理想的量子产率，许多低毒性材料的体内外生物安全性、临床适用性、潜在机理等仍有必要进行深入研究。另外，人们对生命健康的需求日益增加，个性化医疗和家庭检测逐渐兴起。这就要求研究人员在提高生物标志物检测灵敏度和准确度的同时，密切关注市场和应用需求，进行小型化、多功能、经济便携、简单快速的生物标志物检测平台的开发。肿瘤生物标志物检测技术的每一次突破都凝结着科学家的智慧与汗水，都需要多领域、多学科的融合与全面发展。未来，荧光方法在肿瘤筛查、诊断、治疗和预后中将会发挥越来越大的作用。

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Progress in Tumor Biomarker Detection Based on Fluorescence Method

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Abstract

Significance Cancer, characterized by abnormal cell proliferation, is one of the most important chronic and complex diseases. Not only is it a serious threat to people's health and life, but also it has been a leading cause of death in every country in the world for a long time. It has caused huge loss to society, including the negative influence on the emotions and economies of patients and their families, the burden on medical resources and treatment investments, and the loss of human resources. The cancer survival rate is usually low, which may be related to a lack of timely and effective detection, which leads to late diagnosis and missed opportunities for targeted and standardized treatment. Early detection and effective diagnosis of cancer can significantly reduce the death rates of patients through early and effective prevention and treatment.

Tumor markers are defined as substances that are synthesized and released by tumor cells themselves or that are produced by the body in response to the development and proliferation of tumors, and which can be identified in blood, urine, saliva, tissue, and other body fluids. Detection of tumor markers is an important indicator for cancer diagnosis, treatment, and efficient monitoring. It complements other clinical examination methods, such as imaging, endoscopic, and pathological examinations. The early detection of tumor markers has the advantages of being minimally or noninvasive, rapid, and convenient, and it has great application potential in cancer screening, diagnosis, treatment, prognosis, and other aspects.

In the past few decades, advances in the early detection of cancer have led cancer research to a minted stage. Based on the specific recognition of intracellular and extracellular biomarkers, several promising detection methods have been developed, including the polymerase chain reaction, enzyme-linked immunosorbent assay, electrophoresis, surface plasmon resonance, surface-enhanced Raman spectroscopy, electrochemical sensing, mass spectrometry, flow cytometry, and other technologies. However, some of these methods are limited by being expensive, time-consuming, and complicated operate. In most cases, the relatively low sensitivity and accuracy of these methods are not adequate to meet new clinical requirements, and they cannot be used in an environment with few resources and extensive point-of-care detection. It is, therefore, urgent to develop a new detection technology with the characteristics of high efficiency, sensitivity, accuracy, stability, and economic friendliness.

Fluorescence methods have attracted the attention of researchers in recent years because of their advantages of high sensitivity, low cost of instrumentation, and ease of operation, and tremendous advances have been made. The fluorescence method has been widely used for ultrasensitive and rapid detections of tumor markers, and the exploration of this method for the accurate detection of new markers is still being implemented in the laboratory. In addition, it is being used both to improve old methods and to create new methods for detecting tumor markers. Consequently, it is both important and necessary to summarize existing research in order to predict and guide the future development of the fluorescence method for tumor marker detection.

Progress This paper first introduces tumor biomarkers and methods for detecting them and then compares the principles and characteristics of different detection methods (Table 1). Next, novel fluorescent-probe materials—such as carbon dots, upconversion nanoparticles, and polymer dots—are briefly introduced and their applications in the detection of biomarkers are summarized (Table 2). Various methods of capturing and detecting circulating tumor cells (CTCs) are then introduced. Based on traditional methods for enhancing the capture efficiency and detection sensitivity for CTCs, the effective introduction of near-infrared light (Fig. 3) and the one-step method for detecting CTCs has gradually become a

new research focus (Fig. 4). Improvements in the traditional polymerase chain reaction for detecting circulating tumor DNA (ctDNA) and the development of fluorescence biosensor technology are next introduced (Fig. 5). Biosensors and emerging diagnostic technologies based on various fluorescent materials have greatly facilitated the development of ctDNA detection. Several methods for the comprehensive detection of exosomes are subsequently introduced (Fig. 6); they mainly include the combined application of microfluidics, nanotechnology, and fluorescent nanomaterials. Finally, the detection of carcinoembryonic antigen, alpha-fetoprotein, prostate-specific antigen, and simultaneous multiple detections of several biomarkers (Fig. 10) are briefly introduced. In addition to the basic requirements of high sensitivity and high specificity, fast, affordable, and portable detection platforms—such as biochips, immunochromatographic test strips, and point-of-care detection devices—are emerging.

Conclusions and Prospects In summary, the fluorescence method has a wide range of applications to many diseases, including tumor detection. Although great advancements have already been made, many challenges remain in tumor-marker detection. Detection technology based on the fluorescence method still needs to be explored and improved constantly to provide high detection sensitivity and accuracy, so that it can meet application demands more widely and more simply and adapt to the constantly updated understanding of disease mechanisms and test requirements.

Key words medical optics; fluorescence; tumor biomarker; nanomaterials; liquid biopsy