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肿瘤生物标志物的超灵敏检测研究进展

许爱君†, 王子月†, 张春阳*

山东师范大学化学化工与材料科学学院, 济南 250014
† 同等贡献
* 联系人, E-mail: cyzhang@sdnu.edu.cn

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摘要 肿瘤是严重威胁人类健康的高发病率和高死亡率疾病. 肿瘤生物标志物是反映肿瘤的发生和发展、监测肿瘤对治疗反应的一类物质. 肿瘤生物标志物的超灵敏检测有助于肿瘤的早期诊断和临床治疗. 近年来, 肿瘤生物标志物的检测方法研究迅猛发展, 成为国内外研究的热点. 本文对最新发展的肿瘤生物标志物检测方法进行了全面综述, 着重介绍了比色法、质谱法、荧光分析法、化学发光、表面增强拉曼散射、电化学分析法和单分子检测, 并对肿瘤生物标志物检测方法的发展方向做了展望.

关键词 肿瘤生物标志物, 癌症, 肿瘤细胞, 超灵敏检测, 分析方法, 发展方向

肿瘤是生物体内组织细胞异常增生分裂失控后而形成的新生物[1]. 在多种因素(如来自于外部的离子辐射、致癌化学试剂、病毒感染和来自内部的遗传因素、肿瘤免疫、内分泌因素等)的长期作用下[2,3], 机体局部组织的细胞在基因水平上失去对其生长的调控, 导致单克隆性异常增生而形成局部肿块, 以至破坏正常组织器官的结构并影响其功能[4,5]. 肿瘤严重危害人体健康, 是造成人类死亡的重要原因[6]. 大量研究证实, 早期诊断和早期治疗是防治肿瘤与降低死亡率的最有效办法. 因此肿瘤生物标志物的超灵敏检测成为癌症临床诊断和治疗的迫切要求[7].

肿瘤生物标志物是指在肿瘤发生和发展过程中, 由肿瘤细胞本身合成、释放, 或因机体对肿瘤细胞反应而产生或升高的一类物质[8]. 肿瘤生物标志物存在于肿瘤患者的细胞、血液、体液和组织中, 可反映肿瘤的存在和生长, 并应用于临床肿瘤的早期诊断、治疗监测和预后判断[9]. 自1963年Abelev发现甲胎蛋白[10]、1965年Gold和Freeman发现癌胚抗原后[11], 肿瘤生物标志物的检测开始应用于临床诊断. 常用的肿瘤生物标志物可分为两类: (1) 肿瘤细胞内生物标志物, 如miRNA[12]、端粒酶[13]和转录因子[14]等. miRNA是一类非编码小分子单链RNA, 参与转录后基因表达调控[15]. miRNA的异常表达与癌症等疾病的发生和发展密切相关[16,17]; 端粒酶是一种核蛋白逆转录酶, 负责将重复序列TTAGGG添加在染色体末端, 增强细胞的增殖能力[18]. 在肿瘤细胞内, 端粒酶活性被激活, 导致细胞无限分裂, 引发癌症[19,20,21]; 转录因子是与特定序列专一性结合的蛋白质, 可控制基因的转录. 转录因子的异常表达可导致癌症等重大疾病的发生[22]. (2) 肿瘤细胞膜上的生物标志物, 如上皮膜抗原(EMA)[23]和癌胚抗原(CEA)[24]等. EMA是从人乳中脂肪球膜上提取的一种糖蛋白, 可作为上皮源性肿瘤的标志物[25]; CEA[26]是从结肠癌中分离出来的一种酸性糖蛋白, 不受肿瘤分化程度影响, 作为上皮性肿瘤的重要标志物常用于胃肠道癌的检测[27].

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传统的肿瘤生物标志物检测方法主要基于免疫组织化学技术[28]和聚合酶链反应技术[29,30]. 这些方法存在成本高、检测灵敏度低、耗时长以及实验操作繁琐等问题. 近年来研究者将质谱[31~33]、恒温扩增、化学发光[34,35]、表面增强拉曼[36~38]、电化学[39~41]、单分子检测技术[42~45]和新型纳米材料(如纳米金和量子点)[46~48]及荧光染料[49~54]相结合, 发展了一系列新的检测方法, 为重大疾病的早期诊断、治疗监测和预后判断提供了新工具. 本文结合本课题组的工作, 对近年来肿瘤标志物检测方法的研究进展进行较为全面的评述.

1 肿瘤生物标志物的检测方法

1.1 比色法

比色法通过可视化的颜色变化对生物标志物进行定量分析, 具有简单、直观和成本低等优点. 常用的比色分析法利用纳米金凝聚可以变色的光学特性[55]. Zhang等人[14]将恒温指数扩增(exponential amplification reaction, EXPAR)和纳米金介导的比色法相结合, 用于检测宫颈癌细胞内的转录因子. 如图1所示, 在转录因子存在时, 双链DNA底物在核酸外切酶III(Exonuclease III, Exo III)的作用下变为单链DNA. 该单链DNA可与DNA模板结合, 作为引物启动聚合酶扩增反应, 产生具有切刻内切酶位点的双链DNA. 随后在切刻内切酶作用下启动循环链置换反应, 产生大量新DNA引物. 新DNA引物能与DNA模板结合, 启动新一轮聚合和酶切循环, 产生大量DNA引物. 该DNA引物可作为报告探针, 与相邻两个纳米金颗粒(AuNP)上的DNA互补序列杂交, 导致AuNP聚集, 溶液颜色由红色变为紫色. 该方法无需荧光标记, 肉眼可视, 检测限可达3.8 pmol/L, 灵敏度比基于AuNP的传统比色法提高4个数量级.

适配体与纳米材料相结合, 可用于肿瘤标志物

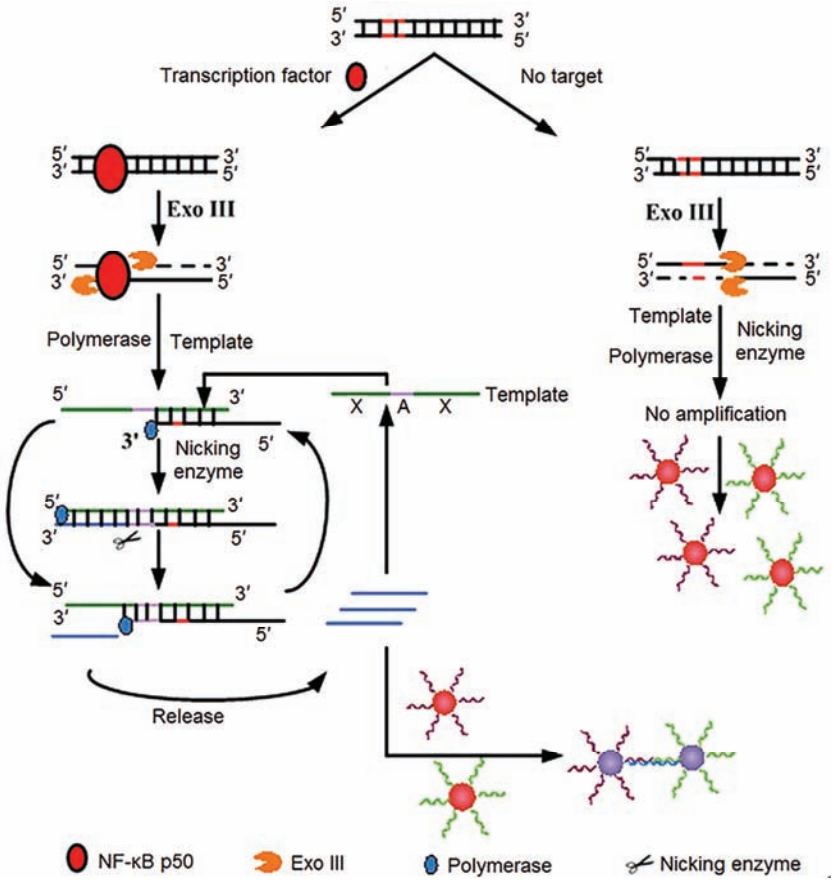

图 1 基于EXPAR的比色法检测转录因子示意图[14]

Figure 1 Schematic illustration of EXPAR-based colorimetric assay for the detection of transcription factors[14]

的超灵敏检测. Yu等人[46]利用信使DNA(mDNA)和磁珠(MB)修饰的适配体(Apt)制备mDNAs-Apt-MBss复合物，用于检测人子宫颈癌细胞(HeLa)和人乳腺癌细胞(MCF-7)等多种肿瘤标志物. 当肿瘤细胞存在时，mDNAs-Apt-MBss复合物构型发生改变，释放5种mDNA，实现第1次信号扩增. 释放的mDNA吸附在AuNP表面，有效避免凝聚沉降. 经磁性分离，mDNA和磁珠修饰的连接DNA(Linker-DNA-MBs)及限制性核酸内切酶混合，启动循环扩增反应，产生大量单链DNA. 单链DNA吸附在AuNP表面，导致AuNP无法聚集，溶液呈红色. 该检测方法具有可视化、操作简单和灵敏度高等优点，检测限可达4个细胞/mL. Hosseini课题组[47]利用基于适配体的比色法检测MCF-7细胞膜上的核仁蛋白. 当MCF-7细胞存在时，核仁蛋白与核仁蛋白适配体的结合能有效阻止AuNP表面上的单链DNA探针杂交形成二聚体，溶液呈红色. 当MCF-7细胞不存在时，适配体与AuNP上的单链DNA探针杂交，导致溶液变为紫色. 该方法的检测限可达10个细胞/mL. Ray课题组[48]利用抗体和适配体修饰纳米金，用于特异性识别MCF-7细胞. MCF-7细胞存在时，溶液由红色变为紫色，可实现MCF-7细胞的定量检测.

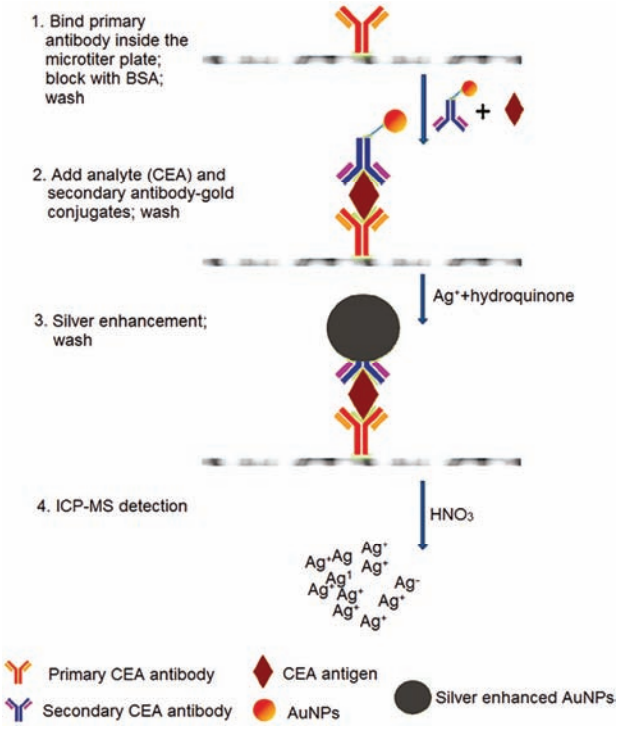

图 2 (网络版彩色)基于AuNP辅助的免疫反应和银介导的信号放大检测人类CEA原理图[31]

Figure 2 (Color online) Schematic illustration of sandwich immunoassay and silver amplification for the detection of human CEA using AuNP tags[31]

1.2 质谱法

质谱分析是常用的定量检测方法之一. 电感耦合等离子体质谱(inductively coupled plasma mass spectrometry, ICP-MS)因检测范围广、分辨率高和检测限低等优点被广泛应用于肿瘤标志物的定量分析[56]. AuNP常作为元素标记物用于ICP-MS分析[57]. Lv课题组[31]将ICP-MS和AuNP相结合，用于检测抗原CEA. 如图2所示，他们将CEA第一抗体固定在聚苯乙烯微量滴定板表面. AuNP修饰的CEA第二抗体和CEA第一抗体共同特异性识别抗原CEA，形成三明治复合体. 随后加入Ag^+和对苯二酚，AuNP能催化还原Ag^+为Ag，产生大量Ag，酸溶后可检测到增强ICP-MS信号. 该方法操作简单、成本低、灵敏度高，检测限可达0.15 pmol/L.

Hu课题组[32]将杂交链式反应(hybridization chain reaction, HCR)扩增和AuNP引发的双重信号放大与ICP-MS相结合，用于检测肿瘤细胞，检测限可达15个细胞. 另外，Hu课题组[33]将磁珠分离与ICP-MS相结合，将anti-CD2修饰的AuNP和anti-CD3修饰的磁珠分别作为检测探针和捕获探针，用于检测人外周血白血病T细胞标志物. 该检测方法快速且操作简单，检测灵敏度可达86个细胞.

1.3 荧光检测

荧光法可实现对肿瘤标志物的定量分析. 本课题组[49]将滚环扩增(rolling circle amplification, RCA)与SYBR Green II荧光染料相结合，用于检测肺癌miRNA. 如图3所示，靶miRNA可与发夹探针部分序列互补结合，展开发夹探针，露出与环形模板互补的序列. 在DNA聚合酶作用下，露出的互补序列可作为引物，引发RCA反应，产生大量单链DNA. SYBR Green II染料能与单链DNA结合，产生增强荧光信号，实现对靶miRNA定量检测. 该方法操作简单、灵敏度高，检测限可达10 fmol/L，可用于临床非小细胞肺癌样品分析.

本课题组[50]还利用免淬灭荧光法检测肺癌组织中的miRNA. 靶miRNA可作为引物与2-氨基嘌呤修饰的分子探针结合，在DNA聚合酶协助下，启动聚

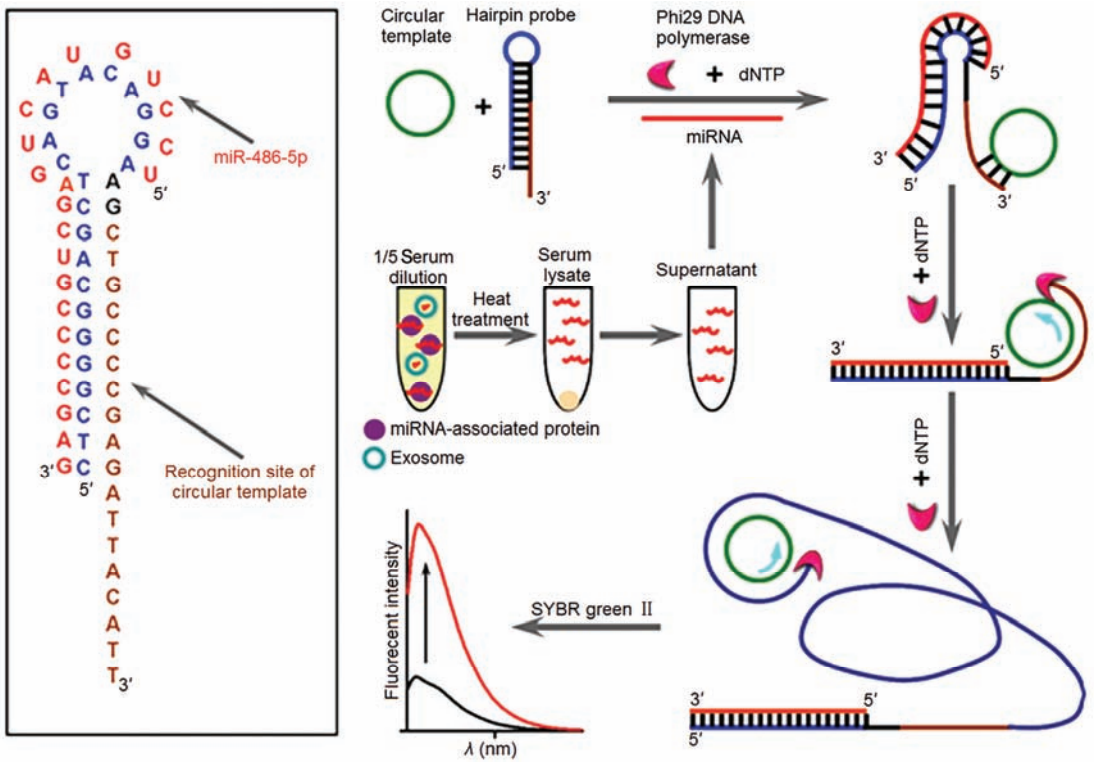

图 3 基于发夹探针的滚环扩增法检测miRNA示意图[49]

Figure 3 Schematic illustration of the hairpin probe-based rolling circle amplification (HP-RCA) for miRNA assay[49]

合延伸反应，置换掉靶miRNA，生成带有两个切刻内切酶识别位点的双链DNA. 在切刻内切酶和核酸外切酶作用下，2-氨基嘌呤修饰的分子探针被切割和消化，释放2-氨基嘌呤分子，产生荧光信号. 当靶miRNA不存在时，切刻内切酶和λ核酸外切酶不会对2-氨基嘌呤修饰的分子探针进行切割和消化，无荧光信号产生. 该方法无需引入淬灭剂，检测灵敏度高，可达0.3 fmol/L，可用于临床非小细胞肺癌样品中let-7a miRNA的分析.

荧光共振能量转移(fluorescence resonance energy transfer, FRET)是指激发供体荧光团，诱导受体发色团发光的非放射性过程[58]，可用于肿瘤标志物的检测. 本课题组[51]将恒温指数扩增和FRET相结合，用于HeLa细胞内端粒酶活性分析. 如图4所示，端粒酶能识别DNA引物序列，将大量端粒重复序列(TTAGGG)添加到引物序列的3′末端，形成一条长单链DNA. 随后加入的反向引物能与端粒酶的延伸产物互补结合，在DNA聚合酶作用下，引发聚合延伸反应，形成带有两个切刻内切酶识别位点的双链DNA，在切刻内切酶作用下引发循环链置换反应，释放大量DNA 酶. DNA 酶可与游离的引物序列结合，引发新一轮聚合、酶切和释放，触发指数扩增反应，

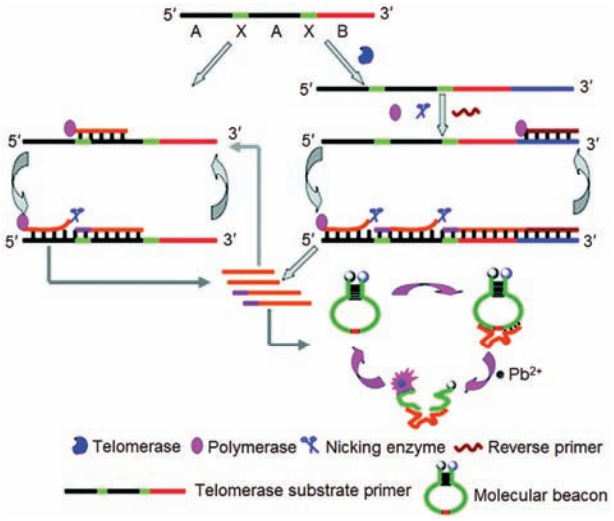

图 4 基于恒温指数扩增的DNA酶生物传感器检测端粒酶活性示意图[51]

Figure 4 Schematic illustration of a telomere-triggered isothermal exponential amplification-based DNAzyme biosensor for the detection of telomerase activity[51]

生成大量DNA酶. DNA酶与修饰有荧光分子和淬灭分子的发夹探针结合, 在辅因子Pb^{2+}催化下, 发夹探针被切割, 导致荧光分子和淬灭分子分离. 释放的DNA酶可结合新的发夹探针并被切割, 产生放大的荧光信号. 当端粒酶不存在时, 发夹探针不会被切割, 无荧光增强信号被检测. 由于引入两步信号扩增, 该方法检测限范围跨越11个数量级(0.1 amol/~10 nmol/L), 可检测到单个细胞中端粒酶活性. Xie等人[52]将DNA酶探针和分子信标相结合, 利用FRET实现了对肝癌细胞内mRNA的检测. 该方法具有信噪比高和特异性好等优点, 检测限达3.2 nmol/L.

Cai等人[53]基于Tb^{3+}结合单链DNA探针可增强荧光信号的原理, 利用DNA适配体识别细胞表面蛋白MUC1, 分析MCF-7细胞中MUC1含量. 该方法无需固定、标记和修饰, 成本低, 检测灵敏度可达70细胞/mL. Kuang课题组[54]利用手性等离子纳米金字塔结构探针分析HeLa细胞和MCF-7细胞中的miRNA, 检测限可达0.12 fmol/10 μg RNA.

1.4 化学发光检测

化学发光分析法是根据化学反应产生的光辐射来确定物质含量的痕量分析方法, 具有灵敏度高和无需激发等优点[59]. 本课题组[34]将EXPAR与化学发光相结合, 用于检测HeLa细胞和人乳腺癌细胞(MDA-MB-231)中端粒酶活性. 如图5所示, 端粒酶催化端粒重复序列(TTAGGG)加到引物3′末端, 形成恒温扩增模板. 在反向引物、DNA聚合酶共同作用下, 启动聚合延伸反应, 生成含有3个切刻内切酶识别位点的双链DNA. 随后在切刻内切酶作用下, 双链DNA被切割, 引发循环链置换反应, 释放大量触发物、DNA酶和富含G的端粒重复序列. 触发物可与端粒酶引物结合, 引发新一轮聚合、酶切、释放, 触发EXPAR, 生成大量DNA酶和富含G的端粒重复序列. DNA酶和富含G的端粒重复序列与氯高铁血红素(hemin)作用, 形成氯高铁血红素-G-四联体复合物. 在鲁米诺(luminol)和过氧化氢(H_2O_2)共同作用下, 氯高铁血红素-G-四联体复合物产生化学发光信号. 当端粒酶不存在时, 两级恒温扩增反应无法启动, 无化学发光信号产生. 该方法可超灵敏检测端粒酶活性, 检测范围跨越10个数量级(0.1 amol/L~1 nmol/L), 检测限达0.1 amol/L, 能在单细胞水平上检测端粒酶活性, 且无需标记、热循环和分离等繁琐操作步骤.

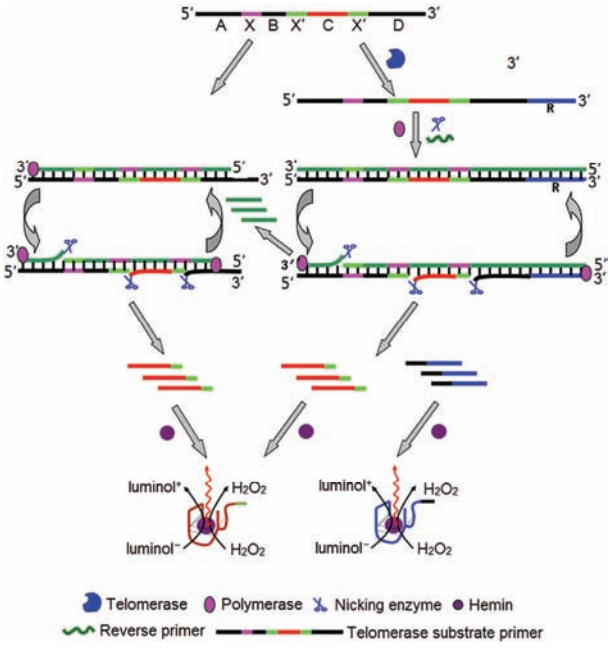

图 5 由端粒酶诱导的两级恒温扩增介导的化学发光法检测端粒酶活性示意图[34]

Figure 5 Schematic illustration of telomeres-induced two-stage isothermal amplification-mediated chemiluminescence assay for sensitive detection of telomerase activity[34]

Wu课题组[35]将石墨烯半导体量子点(graphene quantum dots, GQDs)与化学发光相结合, 用于检测口腔癌细胞的生物标志物CA-125. 当抗原CA-125不存在时, 辣根过氧化物酶(HRP)标记的抗体Ab-HRP和GQDs上的捕获抗体(cAb)之间存在静电排斥, 导致Ab-HRP远离GQDs. HRP酶催化H_2O_2产生活性氧化鲁米诺, 生成激发态电子, 当电子跃迁回基态时产生化学发光信号. 当CA-125抗原存在时, 形成GQDs-cAb-CA-125-Ab-HRP三明治结构, HRP酶催化产生的二价阴离子和GQDs之间发生能量共振转移, 化学发光淬灭. 该方法灵敏高, 检测限可达0.05 U/mL.

1.5 表面增强拉曼散射检测

表面增强拉曼光谱(surface enhanced Raman spectroscopy, SERS)具有光谱带窄、光褪色慢等优点[60]. 本课题组[36]将EXPAR和SERS技术相结合, 用于非小细胞肺癌中miRNA的定量分析. 如图6所示, 靶miRNA与EXPAR模板上X序列互补结合, 在Vent(exo-)DNA聚合酶和切刻内切酶作用下进行聚合延伸、酶切, 释放大量DNA触发物. DNA触发物可与EXPAR模板T序列结合, 启动新一轮聚合、酶切, 引

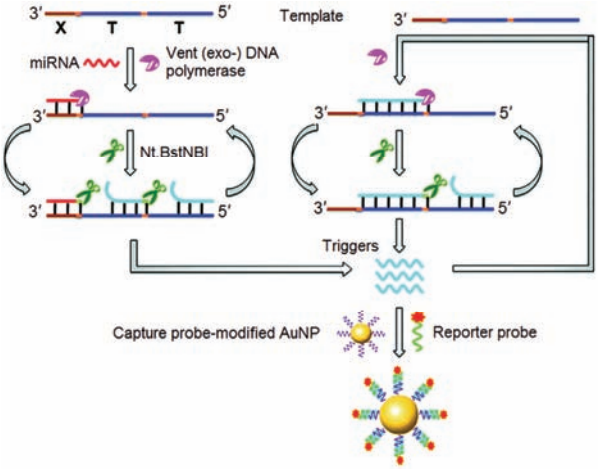

图 6 基于EXPAR的SERS法检测miRNA示意图[36]

Figure 6 Schematic illustration of EXPAR-based SERS for miRNA assay[36]

发EXPAR反应，最终产生大量DNA触发物．DNA触发物与报告探针和AuNP修饰的捕获探针形成三明治结构，产生增强SERS信号．该方法具有良好选择性和较高灵敏度，检测限可达0.5 fmol/L，可进一步应用于细胞内多种miRNA的同时检测．

Kim课题组[37]设计了一种新型纳米探针，用于表面增强拉曼散射(surface-enhanced resonance Raman scattering，SERRS)分析MCF-7细胞膜上的生物标志物．该纳米探针由Ag纳米壳(Ag nanoshell，AgNS)和氧化石墨烯构成．Ag纳米壳为拉曼活性纳米结构，氧化石墨烯可作为拉曼报告分子．该探针无细胞毒性，能够在单个微粒水平上检测SERRS信号．Yang课题组[38]将纳米银颗粒包裹的单层碳纳米管复合材料(SWNT@AgNPs)与SERS技术相结合，用于检测MCF-7细胞提取物中的miRNA，检测限可达5 pmol/L．

1.6 电化学检测

电化学传感器可实现对肿瘤生物标志物的快速、低成本、高灵敏检测[61]．Kelley课题组[39]利用DNA离合器探针(DNA clutch probes，DCPs)对黑色素瘤和肺癌患者的循环肿瘤DNA(ctDNA)进行超灵敏检测．如图7所示，双链DNA高温退火形成两条单链DNA，其中一条单链DNA与DCPs杂交，阻止重新聚合成双链DNA．随后肽核酸(PNA)与野生型单链DNA杂交，导致只有靶突变单链DNA呈单链状态．靶突变单链DNA能与PNA探针杂交，引发芯片产生电化学信号．该方法检测时间短、特异性强、灵敏度高，能够分辨100 pg/μL野生型DNA中1 pg/μL突变体．

Qu课题组[40]设计了一种基于芘四羧酸(PTCA)功能化的石墨烯电化学传感器，用于区分HeLa细胞、MDA-MB-231细胞和正常细胞．PTCA的疏水性阻止石墨烯聚合[62]，表面带负电的羧基基团维持石墨烯的共轭体系[63]．氨基修饰的AS1411适配体通过碳二亚胺介导的湿化学法[64]与PTCA联合，形成适配体-PTCA纳米复合物，可特异性捕获肿瘤细胞．该法操作简单，无需任何标记，电极经简单水洗后可重复使用，检测灵敏度可达1000细胞．Chen课题组[41]发展了一种基于适配体信号放大的电化学传感器，用于MCF-7细胞膜上生物标志物的检测．该方法利用DNA步行者(DNA walker)进行检测，操作简单，无需标记、无需蛋白酶和DNA酶，检测灵敏度高，可达47个细胞．

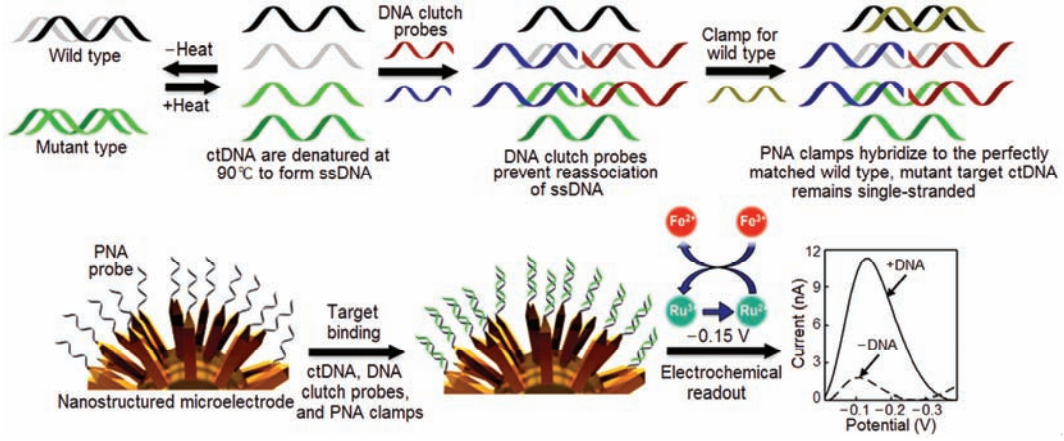

图 7 离合器探针法检测ctDNA示意图[39]

Figure 7 Schematic illustration of clutch probe strategy for ctDNA assay[39]

1.7 单分子检测

单分子检测技术能够在单分子水平上对肿瘤标志物进行超灵敏检测，具有信噪比高、样本需求量少等优点[65]. 量子点(QD)荧光量子产率高，稳定性好，抗漂白能力强，广泛应用于单分子检测[66,67]. 本课题组[42]发展了基于单个量子点的生物传感器，用于检测HeLa细胞内端粒酶活性. 如图8所示，端粒酶将重复序列(TTAGGG)添加到引物3′末端，同时将Cy5分子组装到DNA链上形成一条Cy5标记的单链DNA. Cy5标记的单链DNA与生物素修饰的捕获探针形成双链DNA，自组装到链霉亲和素修饰的QD表面，形成Cy5-双链DNA-QD纳米复合体，导致Cy5和QD之间发生FRET，产生Cy5荧光信号. 该方法检测灵敏度高，所需样品量少，无需复杂的热循环，检测限可达7细胞/μL.

本课题组[43]进一步将基于单个QD的生物传感器用于检测肺癌细胞中DNA点突变. 点突变与癌症的发生和发展密切相关[68]. 该方法利用聚合延伸反应和核酸内切酶的切刻特异性，准确区分靶突变体与野生型序列，检测限可达5.3 amol/L. 本课题组[44]还将适配体与单分子检测相结合，用于同时检测不同种类肺癌细胞. 该方法具有灵敏度高、样品需求量少、无需扩增等优点，可应用于临床上肺癌的分型研究[69]. 另外，Nie课题组[45]将特异性抗体标记的不同颜色纳米探针与单分子检测相结合，用于检测Raji-B细胞. 该方法无需扩增，无需洗涤和分离步骤，可进一步用于超灵敏检测病毒.

2 结论和展望

肿瘤生物标志物是由肿瘤细胞产生、与肿瘤性质密切相关的物质，它的存在或含量的变化可反映肿瘤的发生和发展状况. 肿瘤生物标志物的超灵敏检测有助于肿瘤的早期诊断、疗效监测和预后判断[9]. 目前，常用于临床早期诊断的肿瘤标志物包括miRNA[12]、端粒酶[13]、CEA[24]等. 近年来，肿瘤标志物检测的方法研究取得了较大进展，各种方法各具特点. 比色法操作简单，结果直观[14]；荧光分析法灵敏度高，应用范围广[49]；电化学分析法简单快速，成本低[39]；单分子检测法信噪比高，样品需求量少[42~45]. 表1对本文概述的肿瘤生物标志物检测方法进行了比较.

目前肿瘤生物标志物的检测正逐步向简单、快速、超灵敏和活细胞原位实时检测方向发展，并呈现以下趋势：(1) 纳米材料的应用. 与传统的有机染料相比，量子点等纳米颗粒在可调谐光发射、信号强度、光稳定性以及生物相容性等方面存在优势[70,71]. 由纳米颗粒与生物识别分子(如多肽、抗体和核酸等)构建新型荧光分子探针[72,73]，成为肿瘤生物标志物检测研究的新方向. 另外，纳米结构具有规则的几何形状、稳定的结构和较好的旋光性[74]，在发展智能生物传感器方面存在巨大潜能[75,76]. (2) 核酸适配体的应用. 与抗体探针相比，核酸适配体存在方便合成、特异性强、稳定性好和毒性小等优点[77]. 基于核酸适配体的生物传感器有望为肿瘤生物标志物检测提供便携经济的设备[40,78,79]. (3) 扩增技术的应用. 滚环扩

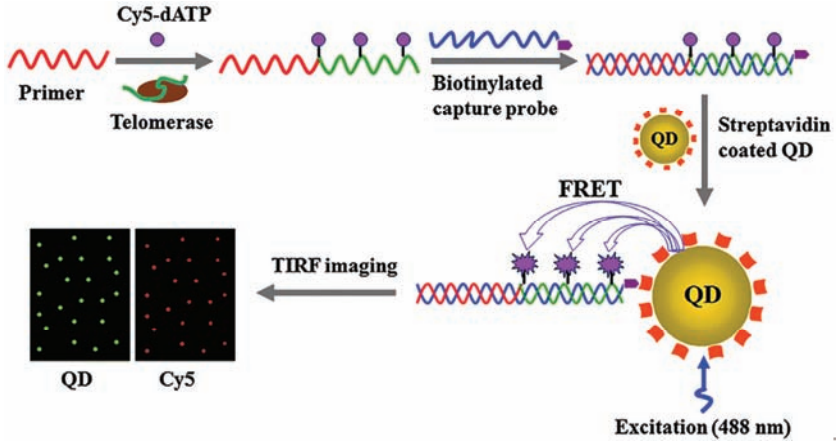

图 8 (网络版彩色)基于单个QD的生物传感器检测端粒酶活性示意图[42]

Figure 8 (Color online) Schematic illustration of single QD-based biosensor for telomerase assay[42]

表 1 肿瘤生物标志物检测方法的比较

Table 1 Comparison of the methods for the detection of tumor-related biomarkers

方法	优势	检测物	检测限	文献
比色法	通过可视化的颜色变化对生物标志物进行定量分析，简单、直观、成本低	HeLa细胞内转录因子	3.8 pmol/L	[14]
		HeLa和MCF-7细胞膜上的肿瘤生物标志物	4个细胞/mL	[46]
		MCF-7细胞膜上的核仁蛋白	10个细胞/mL	[47]
质谱法	检测范围广、分辨率高、检测限低	CEA	0.15 pmol/L	[31]
		肿瘤细胞膜上的肿瘤生物标志物	15个细胞	[32]
		人外周血白血病T细胞标志物	86个细胞	[33]
荧光检测法	灵敏度高、应用范围广	肺癌miRNA	10 fmol/L	[49]
		肺癌组织中的miRNA	0.3 fmol/L	[50]
		HeLa 细胞内端粒酶	0.1 amol/L	[51]
		肝癌细胞内mRNA	3.2 nmol/L	[52]
		MCF-7细胞内MUC1	70细胞/mL	[53]
		HeLa和MCF-7细胞内miRNA	0.12 fmol/10 μg	[54]
化学发光检测法	灵敏度高、无需激发	HeLa和MDA-MB-231细胞内端粒酶	0.1 amol/L	[34]
		口腔癌细胞CA-125	0.05 U/mL	[35]
表面增强拉曼散射检测法	光谱带窄、特异性好	非小细胞肺癌内miRNA	0.5 fmol/L	[36]
		MCF-7细胞膜上的肿瘤生物标志物	单个微粒	[37]
		MCF-7细胞提取物中的miRNA	5 pmol/L	[38]
电化学检测法	快速、成本低、灵敏度高	黑色素瘤和肺癌患者的循环肿瘤DNA	1 pg/μL	[39]
		HeLa和MDA-MB-231细胞膜上的肿瘤生物标志物	1000个细胞	[40]
		MCF-7细胞膜上的肿瘤生物标志物	47个细胞	[41]
单分子检测法	信噪比高、样本需求量少、灵敏度高	HeLa细胞内端粒酶	7细胞/μL	[42]
		肺癌细胞内DNA点突变	5.3 amol/L	[43]

增(RCA)、杂交链反应(HCR)和EXPAR等核酸扩增技术的引入，显著提高了检测灵敏度. 尤其HCR技术无需热循环，无需各种聚合酶或内切酶，稳定性好[80]，在肿瘤生物标志物检测研究中具有潜在应用价值. (4) 光学传感和成像技术的应用. 光学传感和成像技术具有无污染、灵敏度高的优点，能够实现多种生物标志物的同时检测，在肿瘤标志物的超灵敏检测研究中具有广阔应用前景[81,82].

参考文献

1 Hanahan D, Weinberg R A. The hallmarks of cancer. Cell, 2000, 100: 57–70

2 Nakachi K, Imai K, Hayashi S, et al. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res, 1993, 53: 2994–2999

3 Menke J F. A preliminary note on the extraction of a carcinogenic factor from primary human mammary cancer. Science, 1940, 27: 290–291

4 Hanahan D, Weinberg R A. Hallmarks of cancer: The next generation. Cell, 2011, 144: 646–674

5 Nowell P C. Mechanisms of tumor progression. Cancer Res, 1986, 46: 2203–2207

6 Jemal A, Bray F, Center M M, et al. Global cancer statistics. CA Cancer J Clin, 2011, 61: 69–90

7 Ferrari M. Cancer nanotechnology: Opportunities and challenges. Nat Rev Cancer, 2005, 5: 161–171

8 Ludwig J A, Weinstein J N. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer, 2005, 5: 845–856

9 Zheng G, Patolsky F, Cui Y, et al. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. Nat biotechnol, 2005, 23: 1294–1301

10 Farinati F, Marino D, De Giorgio M, et al. Diagnostic and prognostic role of α-Fetoprotein in hepatocellular carcinoma: Both or neither. Am J Gastroenterol, 2006, 101: 524–532
11 Roda G, Jianyu X, Park M S, et al. Characterizing CEACAM5 interaction with CD8α and CD1d in intestinal homeostasis. Mucosal immunol, 2014, 7: 615–624
12 Schwarzenbach H, Hoon D S, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer, 2011, 11: 426–437
13 Blackburn E H. Structure and function of telomeres. Nature, 1991, 350: 569–573
14 Zhang Y, Hu J, Zhang C Y. Sensitive detection of transcription factors by isothermal exponential amplification-based colorimetric assay. Anal Chem, 2012, 84: 9544–9549
15 Ambros V. The functions of animal microRNAs. Nature, 2004, 431: 350–355
16 Croce C M. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet, 2009, 10: 704–714
17 He L, Thomson J M, Hemann M T, et al. A microRNA polycistron as a potential human oncogene. Nature, 2005, 435: 828–833
18 Shippenlentz D, Blackburn E H. Functional evidence for an RNA template in telomerase. Science, 1990, 247: 546–552
19 Mergny J L, Riou J F, Mailliet P, et al. Natural and pharmacological regulation of telomerase. Nucleic Acids Res, 2002, 30: 839–865
20 Meyerson M. Role of telomerase in normal and cancer cells. J Clin Oncol, 2000, 18: 2626–2634
21 Kim N W, Piatyszek M A, Prowse K R, et al. Specific association of human telomerase activity with immortal cells and cancer. Science, 1994, 266: 2011–2015
22 Papavassiliou A G. Transcription factors. N Engl J Med, 1995, 332: 45–47
23 Vargas A J, Harris C C. Biomarker development in the precision medicine era: Lung cancer as a case study. Nat Rev Cancer, 2016, 16: 525–537
24 Gerhard M, Juhl H, Kalthoff H, et al. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol, 1994, 12: 725–729
25 Weikert S, Papac D, Briggs J, et al. Engineering Chinese hamster ovary cells to maximize sialic acid content of recombinant glycoproteins. Nat Biotechnol, 1999, 17: 1116–1121
26 Benchimol S, Fuks A, Jothy S, et al. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell, 1989, 57: 327–334
27 Ona F V, Zamcheck N, Dhar P, et al. Carcinoembryonic antigen (CEA) in the diagnosis of pancreatic cancer. Cancer, 1973, 31: 324–327
28 Singh S K, Hawkins C, Clarke I D, et al. Identification of human brain tumour initiating cells. Nature, 2004, 432: 396–401
29 Schamhart D, Swinnen J, Kurth K H, et al. Numeric definition of the clinical performance of the nested reverse transcription-PCR for detection of hematogenous epithelial cells and correction for specific mRNA of non-target cell origin as evaluated for prostate cancer cells. Clin Chem, 2003, 49: 1458–1466
30 Nolan T, Hands R E, Bustin S A. Quantification of mRNA using real-time RT-PCR. Nat Protoc, 2006, 1: 1559–1582
31 Liu R, Liu X, Tang Y, et al. Highly sensitive immunoassay based on immunogold-silver amplification and inductively coupled plasma mass spectrometric detection. Anal Chem, 2011, 83: 2330–2336
32 Zhang X, Chen B, He M, et al. Gold nanoparticles labeling with hybridization chain reaction amplification strategy for the sensitive detection of HepG2 cells by inductively coupled plasma mass spectrometry. Biosens Bioelectron, 2016, 86: 736–740
33 Zhang Y, Chen B, He M, et al. Immunomagnetic separation combined with inductively coupled plasma mass spectrometry for the detection of tumor cells using gold nanoparticle labeling. Anal Chem, 2014, 86: 8082–8089
34 Wang L J, Zhang Y, Zhang C Y. Ultrasensitive detection of telomerase activity at the single-cell level. Anal Chem, 2013, 85: 11509–11517
35 Al-Ogaidi I, Gou H, Aguilar Z P, et al. Detection of the ovarian cancer biomarker CA-125 using chemiluminescence resonance energy transfer to graphene quantum dots. Chem Commun, 2014, 50: 1344–1346
36 Ye L P, Hu J, Liang L, et al. Surface-enhanced Raman spectroscopy for simultaneous sensitive detection of multiple microRNAs in lung cancer cells. Chem Commun, 2014, 50: 11883–11886
37 Yim D, Kang H, Jeon S J, et al. Graphene oxide-encoded Ag nanoshells with single-particle detection sensitivity towards cancer cell imaging based on SERRS. Analyst, 2015, 140: 3362–3367
38 Zheng J, Bai J, Zhou Q, et al. DNA-templated *in situ* growth of AgNPs on SWNTs: A new approach for highly sensitive SERS assay of microRNA. Chem Commun, 2015, 51: 6552–6555
39 Das J, Ivanov I, Sargent E H, et al. DNA clutch probes for circulating tumor DNA analysis. J Am Chem Soc, 2016, 138: 11009–11016
40 Feng L, Chen Y, Ren J, et al. A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells. Biomaterials, 2011, 32: 2930–2937
41 Cai S, Chen M, Liu M, et al. A signal amplification electrochemical aptasensor for the detection of breast cancer cell via free-running DNA walker. Biosens Bioelectron, 2016, 85: 184–189

42 Zhu G, Yang K, Zhang C Y. A single quantum dot-based biosensor for telomerase assay. Chem Commun, 2105, 51: 6808–6811
43 Tang W, Zhu G, Liang L, et al. A single quantum dot-based biosensor for DNA point mutation assay. Analyst, 2015, 140: 5936–5943
44 Hu J, Zhang C Y. Multiplex detection of lung cancer cells at the single-molecule level. Chem Commun, 2014, 50: 13581–13584
45 Agrawal A, Zhang C Y, Byassee T, et al. Counting single native biomolecules and intact viruses with color-coded nanoparticles. Anal Chem, 2006, 78: 1061–1070
46 Yu T, Dai P P, Xu J J, et al. Highly sensitive colorimetric cancer cell detection based on dual signal amplification. ACS Appl Mater Interfaces, 2016, 8: 4434–4441
47 Borghei Y S, Hosseini M, Dadmehr M, et al. Visual detection of cancer cells by colorimetric aptasensor based on aggregation of gold nanoparticles induced by DNA hybridization. Anal Chim Acta, 2016, 904: 92–97
48 Lu W, Arumugam S R, Senapati D, et al. Multifunctional oval-shaped gold-nanoparticle-based selective detection of breast cancer cells using simple colorimetric and highly sensitive two-photon scattering assay. ACS Nano, 2010, 4: 1739–1749
49 Li Y, Liang L, Zhang C Y. Isothermally sensitive detection of serum circulating miRNAs for lung cancer diagnosis. Anal Chem, 2013, 85: 11174–11179
50 Zhu G, Liang L, Zhang C Y. Quencher-free fluorescent method for homogeneously sensitive detection of microRNAs in human lung tissues. Anal Chem, 2014, 86: 11410–11416
51 Zhang Y, Wang L J, Zhang C Y. Highly sensitive detection of telomerase using a telomere-triggered isothermal exponential amplification-based DNAzyme biosensor. Chem Commum, 2014, 50: 1909-1911
52 Xie N, Huang J, Yang X, et al. A DNA tetrahedron-based molecular beacon for tumor-related mRNA detection in living cells. Chem Commun, 2016, 52: 2346–2349
53 Cai S, Li G, Zhang X, et al. A signal-on fluorescent aptasensor based on single-stranded DNA-sensitized luminescence of terbium (III) for label-free detection of breast cancer cells. Talanta, 2015, 138: 225–230
54 Li S, Xu L, Ma W, et al. Dual-mode ultrasensitive quantification of microRNA in living cells by chiroplasmonic nanopyramids self-assembled from gold and upconversion nanoparticles. J Am Chem Soc, 2016, 138: 306–312
55 Mirkin C A, Letsinger R L, Mucic R C, et al. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature, 1996, 382: 607–609
56 Zheng C B, Yang L, Sturgeon R E, et al. UV Photochemical vapor generation sample introduction for determination of Ni, Fe, and Se in biological tissue by isotope dilution ICPMS. Anal Chem, 2010, 82: 3899–3904
57 Baranov V I, Quinn Z, Bandura D R, et al. A sensitive and quantitative element-tagged immunoassay with ICPMS detection. Anal Chem, 2002, 74: 1629–1636
58 Clapp A R, Medintz I L, Mauro J M, et al. Fluorescence resonance energy transfer between quantum dot donors and dye-labeled protein acceptors. J Am Chem Soc, 2004, 126: 301–310
59 Roda A, Guardigli M, Michelini E, et al. Analytical bioluminescence and chemiluminescence. Anal Chem, 2003, 75: 463–470
60 Kneipp K, Wang Y, Kneipp H, et al. Population pumping of excited vibrational states by spontaneous surface-enhanced Raman scattering. Phys Rev Lett, 1996, 78: 2444–2447
61 Li D, Song S P, Fan C H. Target-responsive structural switching for nucleic acid-based sensors. Acc Chem Res, 2010, 43: 631–641
62 Ghosh A, Rao K V, George S J, et al. Noncovalent functionalization, exfoliation, and solubilization of graphene in water by employing a fluorescent coronene carboxylate. Chemistry, 2010, 16: 2700–2704
63 Wang X R, Tabakman S M, Dai H J. Atomic layer deposition of metal oxides on pristine and functionalized graphene. J Am Chem Soc, 2008, 130: 8152–8153
64 Zelada-Guillen G A, Rius J, Duzgun A, et al. Immediate detection of living bacteria at ultralow concentrations using a carbon nanotube based potentiometric aptasensor. Angew Chem Int Ed, 2009, 48: 7334–7337
65 Ma F, Li Y, Tang B, et al. Fluorescent biosensors based on single-molecule counting. Acc Chem Res, 2016, 49: 1722–1730
66 Medintz I L, Uyeda H T, Goldman E, et al. Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater, 2005, 4: 435–446
67 Somers R C, Bawendi M G, Nocera D G. CdSe nanocrystal based chem-/bio-sensors. Chem Soc Rev, 2007, 36: 579–591
68 Syvänen A C. Accessing genetic variation: Genotyping single nucleotide polymorphisms. Nat Rev Genet, 2001, 2: 930–942
69 Tang Z, Shangguan D, Wang K, et al. Selection of aptamers for molecular recognition and characterization of cancer cells. Anal Chem, 2007, 79: 4900–4907
70 Alivisatos P. The use of nanocrystals in biological detection. Nat Biotechnol, 2004, 22: 47–52
71 Michalet X, Pinaud F F, Bentolila L A, et al. Quantum dots for live cells, *in vivo* imaging, and diagnostics. Science, 2005, 307: 538–544
72 Dubertret B, Skourides P, Norris D J, et al. *In vivo* imaging of quantum dots encapsulated in phospholipid micelles. Science, 2002, 298: 1759–1762

73 Dahan M, Levi S, Luccardini C, et al. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. Science, 2003, 302: 442–445
74 Kim P Y, Oh J W, Nam J M. Controlled co-assembly of nanoparticles and polymer into ultralong and continuous one-dimensional nanochains. J Am Chem Soc, 2015, 137: 8030–8033
75 Chou L Y, Zagorovsky K, Chan W C. DNA assembly of nanoparticle superstructures for controlled biological delivery and elimination. Nat Nanotechnol, 2014, 9: 148–155
76 Chinen A B, Guan C M, Ferrer J R, et al. Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence. Chem Rev, 2015, 115: 10530–10574
77 Tan W H, Donovan M J, Jiang J. Aptamers from cell-based selection for bioanalytical applications. Chem Rev, 2013, 113: 2842–2862
78 Li J, Mo L, Lu C H, et al. Functional nucleic acid-based hydrogels for bioanalytical and biomedical applications. Chem Soc Rev, 2016, 45: 1410 –1431
79 Zhang Z, Chen N, Li S, et al. Programmable hydrogels for controlled cell catch and release using hybridized aptamers and complementary sequences. J Am Chem Soc, 2012, 134: 15716–15719
80 Wang Y M, Wu Z, Liu S J, et al. Structure-switching aptamer triggering hybridization chain reaction on the cell surface for activatable theranostics. Anal Chem, 2015, 87: 6470–6474
81 Stich M I J, Fischer L H, Wolfbeis O S. Multiple fluorescent chemical sensing and imaging. Chem Soc Rev, 2010, 39: 3102–3114
82 Xu J J, Zhao W W, Song S, et al. Functional nanoprobes for ultrasensitive detection of biomolecules: An update. Chem Soc Rev, 2014, 43: 1601–1611

Summary for “肿瘤生物标志物的超灵敏检测研究进展”

Advance in development of methods for sensitive detection of tumor-related biomarkers

XU AiJun†, WANG ZiYue† & ZHANG ChunYang*

College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China
† Equally contributed to this work
* Corresponding author, E-mail: cyzhang@sdnu.edu.cn

Tumors are formed by the unlimited proliferation of normal cells in living body and pose a serious threat to human health with high morbidity and mortality. Tumor-related biomarkers are a class of substances resulting from the tumor cell formation, release, or the body’s response to tumor cells, which are generated and increase during tumor development and proliferation. Tumor-related biomarkers may be divided into two categories: one is inside cancer cells (e.g., microRNA (miRNA), telomerase and transcription factor) and the other is on the cancer cell membranes (e.g., epithelial membrane antigen (EMA) and carcino-embryonic antigen (CEA)). Tumor-related biomarkers may reflect tumorigenesis, progression, and the responses to drug treatment. Consequently, the sensitive detection of tumor-related biomarkers is essential to early clinical diagnosis and therapy. Polymerase chain reaction (PCR) and immunohistochemistry technique have frequently been used to detect tumor-related biomarkers, but they are usually laborious and time-consuming. In recent years, sensitive detection of tumor-related biomarkers has become a hot area, with great progress having been made. A variety of methods including colorimetric, mass spectrometry, fluorescent, chemiluminescent, surface enhanced Raman scattering (SERS), electrochemical and single-molecule detection approaches have been developed for sensitive detection of tumor-related biomarker. Colorimetric assay has significant advantages of visualization, simple operation, high sensitivity, and no need for delicate instruments. The advantages of inductively coupled plasma mass spectrometric (ICPMS) include low detection limits, low matrix effects, large dynamic ranges and high spectral resolution for elements and isotopes. Fluorescent method allows for the homogeneous assay of biomarkers in solution with significant advantages of simplicity, low cost, high sensitivity, and safety without the requirement of radioisotopes. Chemiluminescent assay does not require an excitation laser, and the signal interferences from the background can be efficiently avoided. SERS is characterized by its capability to identify target analytes with an information-rich vibrational spectrum, and its narrow well-resolved peaks allow for simultaneous detection of multiple targets. Electrochemical method has the advantage of simplicity, low cost, and high sensitivity. In comparison with conventional ensemble measurements, single-molecule detection possess distinct advantages of ultrahigh sensitivity, good selectivity, rapid analysis time, and low sample-consumption. These methods have been successfully applied for sensitive detection of DNA, miRNA and proteins with high sensitivity and good selectivity.

In addition, we give a new insight into future direction of tumor-related biomarker assay including: (1) the construction of novel fluorescent molecular probes which consist of nanoparticles (e.g., quantum dots) and biological recognition molecules (e.g., peptides, antibodies, and nucleic acids) for the detection of tumor-related biomarkers by taking advantage of the unique optical properties of nanoparticles such as tunable light emission, high brightness and multicolor excitation; (2) development of aptamer-based portable biosensors for the detection of tumor-related biomarkers by taking advantage of unique characteristics of aptamers such as convenient synthesis and controllable modification, high binding affinity and specificity, good stability, low toxicity, rapid tissue penetration, and low variability between different batches; (3) improvement of detection sensitivity through the introduction of amplification approaches such as isothermal nucleic acid amplification and hybridization chain reaction; and (4) the use of optical sensing and imaging techniques for simultaneous detection of multiple biomarkers by taking advantage of their unique properties such as high sensitivity, non-use of radioactive isotopes and multiplex detection capability.

tumor-related biomarkers, cancer, cancer cells, sensitive detection, analytical methods, future direction

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