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Graphene-based biosensors for the detection of prostate cancer protein biomarkers: a review

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Abstract
Prostate cancer (PC) is the sixth most common cancer type in the world, which causes approximately 10% of total cancer fatalities. The detection of protein biomarkers in body fluids is the key topic for the diagnosis and prognosis of PC. Highly sensitive screening of PC is the most effective approach for reducing mortality. Thus, there are a growing number of literature that recognizes the importance of new technologies for early diagnosis of PC. Graphene is playing an important role in the biosensor field with remarkable physical, optical, electrochemical and magnetic properties. Many recent studies demonstrated the potential of graphene materials for sensitive detection of protein biomarkers. In this review, the graphene-based biosensors toward PC analysis are mainly discussed in two groups: Firstly, novel biosensor interfaces were constructed through the modification of graphene materials onto sensor surfaces. Secondly, ingenious signal amplification strategies were developed using graphene materials as catalysts or carriers. Graphene-based biosensors have exhibited remarkable performance with high sensitivities, wide detection ranges, and long-term stabilities.

Keywords: Prostate cancer, Protein biomarker, Graphene, Biosensor

Introduction
PC is one of the most common cancers in the world which causes a fatality of approximately 10% in all cancer patients [1–4]. PC is a type of malignant neoplasm of the prostate gland which is extremely prevalent among men of age 50 and older [5, 6]. The established risk factors for PC include advancing age, race, positive family history of PC and diet [7, 8]. Being asymptomatic, it is very difficult to detect PC at early stages [9]. In clinical practice, early screening and diagnosis of PC is the most effective approach for reducing mortality [9, 10]. Thus, there is a growing body of literature that recognizes the importance of new technologies for early screening and diagnosis of PC [11, 12].

Tumor markers for early clinical screening and rapid diagnosis cover a wide range of biochemical entities, including, proteins [13, 14], nucleic acids [15–17], small metabolites [18, 19], cytogetic and cytokinetic parameters [20], and entire tumor cells [21, 22] in body fluid [23]. So far, protein biomarkers are still recognized as a golden standard for PC diagnosis [24]. In the past few decades, a variety of promising biosensors have been developed based on the specific recognition of PC protein biomarkers, aiming at better performance of cancer diagnosis such as easy operation, portability, and real-time analysis [25–28]. Among them, the graphene-based biosensors have received considerable critical attention for the potential use in point-to-care (POC) testing devices, because of the unique properties of graphene such as large surface area, high electrical conductivity, excellent...
biocompatibility and convenient production/functionali-
ization [29–31].

This review highlights recent graphene-based biosen-
sors for PC protein biomarkers detection. As far as we
know, this is the first review that focuses on specific one
disease. We reviewed recent progress of graphene-based
biosensors for PC protein biomarker detection. Our
manuscript clearly stated the advantages and shortcom-
ings of most of the graphene-based when facing PC diag-
nosis, thus, the manuscript should be valuable for the
future application of graphene-based biosensors.

**Most commonly used protein biomarkers for PC
detection**

Protein biomarkers for cancer diagnosis are usually pro-
duced by either cancer cells or other cells in response
to cancer [32–34], which have been proved to be prom-
ising targets for early diagnosis, monitoring treatment
response, detecting recurrence or following up prognosis
of cancer [35–37]. Protein biomarkers are usually in low
abundance and unstable in body fluids, and thus, the spe-
cific detection of protein biomarkers is usually affected
by the crude or complex environment [33, 38]. Thus, sen-
sitivity, specificity, and accuracy are basic requirements
to consider for protein biosensor fabrication [39–41].

Prostate-specific antigen (PSA) [42], which is also called
human kallikrein 3 (hK3 or KLK3), has been widely rec-
ognized in clinical application as one of the earliest found,
serological PC biomarkers [43, 44]. The PSA value above
4.0 ng/mL is usually considered as abnormal [45], thus,
4.0 ng/mL of PSA is the internationally recognized thresh-
old value for PC occurrence [46, 47]. However, the specific-
ity of PSA is still limited [48], because higher PSA levels can
also be found in benign conditions, such as benign prostatic
hyperplasia (BPH) [49–51], and PSA could be produced by
normal breast and breast cancer cells [48]. These limita-
tions indicate that PSA alone is not an appropriate surrogate
marker for the diagnosis and screening of PC. Fortunately,
several other protein PC biomarkers are developed.

Prostate-specific membrane antigen (PSMA) [52] is a
type II transmembrane protein, and PSMA expression
has been reported in benign prostatic hyperplasia and
increased to higher lever in high-grade prostatic intraepi-
thelial neoplasia and prostatic adenocarcinoma [53].
Further, stronger PSMA expression correlates to mali-
gnancy [54, 55]. The available research results suggest the
potential clinical use for PSMA in PC patients. So far, the
major PSMA clinical application has been in therapeu-
tics and imaging [56–58]. Prostate stem cell antigen [59]
is another recently discovered PC biomarker [60], which
is highly expressed by a large number of human prostate
tumors, such as metastatic and hormone-refractory, but
barely expressed in normal tissues [60–62]. Engrailed-2
(EN2) protein is found in the urine sample of prostatic
cancer patients and showed a specificity of 88.2% and a
sensitivity of 66% [63, 64]. Therefore, the EN2 in urine is
widely recognized as a potential biomarker of PC.

**Properties of graphene materials in biosensor study**

Graphene is a two-dimensional (2D) nanomaterial, which
plays an important role in the biosensor field [64–66].
The use of graphene in biosensing platform offers remarkable
physical, optical, electrochemical and magnetic properties
[67–70]. Different kinds of graphene materials are researched
in biosensors including pristine graphene and functionalized
graphene such as graphene oxide (GO), reduced graphene
oxide (rGO), and graphene-based quantum dots (GQDs),
etc. [71–74]. Pristine graphene is identified as the array of
a 2D hexagonal lattice of sp²-bonded carbon atoms. GO is
chemically produced by oxidation and exfoliation of gra-
phene, causing extensive oxidative modification of the basal
plane [31, 75–77]. The rGO is prepared through reductive
process of GO, for this purpose, different methods have been
developed to reduce its oxygen content, including thermal,
chemical, microwave, photochemical, microbial/bacterial,
and photo-thermal methods [78–80]. GQDs consist of single
to tens of layers of graphene with a size of a few nanometers
which exhibit quantum phenomena [81, 82].

Development of protein biosensors based on graphene
could be classified into two main groups (Fig. 1): Firstly,
functioned graphene materials including GO, rGO and
GQDs [72] were assembled onto the biosensor surface
[electrode, field-effect transistors (FET) channel, etc.] to
construct novel biosensor interfaces for improved assem-
bling of molecular receptors [83]. In this group, excellent
biosensor performance was achieved mainly based on the
increased specific surface area and the unique π–π orbital
interaction on the interface. Secondly, many recent stud-
ies applied graphene materials as excellent carriers for
the construction of novel nanocomposites [84], and in
this group, interesting biosensor signal amplification and
unique catalytic/chemical activity was realized for sensi-
tive protein biomarker analysis [85].

**Biosensor interfaces based on graphene**

Graphene and its derivatives are studied for the construc-
tion of novel biosensor interface [67], which is critical
for interface-based biosensors including electrochemical
biosensors, electrochemiluminescent (ECL) biosensors
and FET biosensor [86]. Many recent studies reported
that nanocomposites based on graphene showed improved capability of combining different biomolecules,
with higher surface area [87] and excellent biocompat-
ibility [88].
Construction of antibody-graphene biosensor interface

Traditionally, antibodies are physically adsorbed onto the immune-assay surfaces, such as classic 96-well plates and colloidal gold test strips. However, one of the main obstacles is the affinity and capacity, because the hydrophobic and hydrophilic interaction is relatively weak and the orientation of the antibody molecules is random [89]. As several recent studies reported, the strong cross-linking between carboxylic acid groups on graphene materials and the amine groups of antibodies (COOH-NH2) was used for the assembling of antibody on novel biosensor interfaces [90, 91]. In their work, the application of graphene materials increased the loading amount, orientation controllability as well as binding capability of the antibodies or antibody fragments. For example, Li et al. developed a graphene modified sensor platform with increased surface area, and then assembled antibody onto the surface through COOH–NH2 combining, with the assistant of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), and they finally achieved a low detection limit of 2 pg/mL [92].

In order to realize better-oriented assembling of antibody, Mao et al. applied chitosan as the dispersant to construct an immuno-interface on a glassy carbon electrode (Fig. 2A), which provided much more amino groups for PSA antibody bonding. They finally developed a simple, label-free electrochemical immunosensor on graphene-methylene blue composite modified electrode [93]. More recently, Jang et al. developed a novel 3D graphene-Au composite (Fig. 2B), toward increased accessible surface area for antibody combination than 2D graphene sheet. More importantly, the crumpled graphene could produce higher capacitances, which is crucial for the following electrochemical immunoensing [94].

A graphene-modified electrode was also reported in ECL biosensor [95] for PSA detection. More recently, Wu et al. developed an electrode surface modified with Au/Ag–rGO (Fig. 3A), and then, a large amount of amminated GQDs and carboxyl GQDs were combined onto the electrode surface. In their work, Au and Ag nanoparticles were used for the adsorption of PSA antibody, and meanwhile, GQDs were for the ECL signal amplification. Finally, they constructed a label-free PSA ECL biosensor with a detection limit as low as 0.29 pg/mL [96].

Graphene materials were also applied in FET biosensors, for the construction of 2D nano-FET biosensors [97–101], with unique advantages like more receptor biomolecules, low noise, and high sensitivity, compared with 1D FET biosensors [102–104]. As a successful example, Kim and coworkers [105] developed an rGO-based FET biosensor for label-free and ultrasensitive analysis of PSA/α1-antichymotrypsin (PSA-ACT) (Fig. 3B). The FET biosensor was produced by combining rGO onto an aminated glass surface, and then, functionalized with PSA antibody. When PSA-ACT was captured by the antibodies on FET substrate, a linear shift of the gate voltage ($\Delta V_{g,\text{min}}$) was achieved, indicating the minimum conductivity. Finally, they successfully performed detection of PSA-ACT of femtomolar level.

Construction of aptamer-graphene biosensor interface

For interface-based PC biosensors, the DNA capture probe plays a key role, which could recognize and...
Fig. 2  Schematic illustration of Label-free electrochemical immunosensors for PC protein biomarkers based on: A graphene-methylene blue nanocomposite, Reprinted with permission from [93], Copyright 2011 Elsevier. B graphene-Au nanocomposite (Reprinted with permission from [94]. Copyright 2014 Elsevier)
capture the target molecules [106]. The very famous DNA probe in PC biosensor is DNA aptamer [10, 107–109], which is a special single-strand DNA (ssDNA) isolated from DNA/RNA libraries of random sequence, by using an in vitro selection process called systematic evolution of ligands by exponential enrichment (SELEX) [110–113].

As the first step toward an aptasensing platform, scientists developed several different strategies to assemble the DNA aptamer onto the electrode as the key recognition element [114–117]. In many reported studies, graphene-based nanocomposites were firstly prepared consisting of graphene and another combing material. For example, Bafrooei et al. modified the electrode with rGOmulti-walled carbon nanotube (MWCNT) nanocomposite and then produced a layer of gold nanoparticles (AuNPs) through electrochemical reduction under $-0.2 \text{ V}$ in HAuCl$_4$, then SH-labeled DNA aptamer was combined to Au on the electrode surface. Finally, their aptasensor achieved 1.0 pg/mL limit of detection (LOD) by using both DPV and ESI methods. Different chemical reactions were applied for the assembling of DNA onto graphene.

Fig. 3 Illustration of PSA immunosensor fabrication process. A An ECL immunosensor on the electrode surface modified with Au/Ag-rGO. Reprinted with permission from [96]. B FET immunosensor on an rGO channel (Reprinted with permission from [105]. Copyright 2012 Elsevier)
Branched polyethylenimine (PEI) was applied by Pan et al. to connect thiol-mediated ssDNA onto carboxylated GO for PSA detection [118]. Recently, EDC-NHS coupling was applied by Settu et al. to combine DNA probe onto a screen-printed carbon-graphene-modified electrode of the detection of EN2 protein [119].

**Graphene-based composites for signal-amplification**

**Peroxidase-like activity of GO**

In 2010, Qu’s group firstly reported the peroxidase-like activity of GO (Fig. 4a) [120]. Before long, Yang and coworkers found GO was capable of catalyzing the oxidation of dihydroxy benzene in a glucose oxidase-dependent way and achieved the colorimetric detection of glucose.

![Fig. 4](image_url)

**Fig. 4** a Schematic illustration of peroxidase-like activity of GO for the colorimetric detection of glucose. Reprinted with permission from [120], Copyright 2010, John Wiley and Sons. b Schematic representation of the immunoassay procedure (Reprinted with permission from [134]. Copyright 2010 Elsevier)
of hydroquinone with the assistant of H$_2$O$_2$, producing a brown color solution. Thus, they produced an antibody-functionalized GO as the signal tag and developed a sandwich-type colorimetric immunoassay for the detection of PSA. In their work [121], an immunocomplex was established when PSA combined GO with secondary anti-PSA (GO-Ab$_2$) and magnetic bead (MB) with primary anti-PSA antibody (MB-Ab$_1$). After the separation in a magnetic field, the color signal was detected corresponding to the concentration of PSA. Their simple immunoassay can be detected by naked eyes (Fig. 4b).

**Graphene materials being applied as the carrier of signal tags**

Many recent studies applied graphene-related materials as excellent carriers for the construction of novel nanocomposites for biosensor signal amplification [122–124]. These graphene-based composites were developed by combining graphene or its derivates with metal oxides, metal nanoparticles, or conductive polymers, etc., and this kind of composites showed unique catalytic/chemical activity [86], that has been widely applied in PC biosensors [125].

Han et al. developed a novel signal tag for PSA and free PSA (fPSA) detection, by using onion-like mesoporous graphene sheets (O-GS) as the carrier of different AuNP-based nanohybrids [126]. As the novel O-GS have multilayer lamellar structure, large surface-to-volume ratio, and excellent electronic transport properties, two kinds of redox nanocomposites were attached to the surface of O-GS, which could accelerate the electron transfer rate and enhance the immobilization amount of enzyme and detection antibodies. Sun et al. reported a signal label by combining bovine serum albumin (BSA)-stabilized silver nanoparticles onto ZnO nanorods modified rGO, and the AgNPs in the composite showed super catalytic performance toward hydrogen peroxide (H$_2$O$_2$), generating a current signal [127]. Feng et al. developed a sandwich-type electrochemical immunosensor for the detection of PSA. In their work, a GO platform (Au@Th/GO) was used to immobilize primary antibodies and accelerate the electron transfer on the electrode interface. An

![Graphene materials being applied as the carrier of signal tags](image-url)

**Fig. 5** Protein capture and detection mediated by Fe$_3$O$_4$@GO sheets. Proteins captured by Fe$_3$O$_4$@GO decorated with detection antibodies. Composite with biomarker was then captured on the sensor surfaces coated with graphene and capture antibodies. Amperometric signal was generated by injecting 100 μL 5 mM H$_2$O$_2$ (Reprinted with permission from [129]. Copyright 2016 Elsevier)
rGO-based nanocomposite (PtCu@rGO/g–C₃N₄) with large surface area, good biocompatibility, and excellent conductivity were used as labels for combining secondary antibodies and amplifying signals. Then secondary antibodies were combined onto this platform and signals were amplified from H₂O₂ reduction [128].

Sharafeldin et al. [129] assembled Fe₃O₄ nanoparticles together with antibody onto GO sheets to produce a multi-function nanocomposite (Fig. 5). When the GO-antibody-Fe₃O₄ nanocomposite specifically combined to PSA and PSMA proteins, the resulted complex could be isolated in a magnetic field and delivered in microfluidic channel to an electrochemical detection cell. The Fe₃O₄–GO particles subsequently catalyze H₂O₂ reduction, generating a current signal. Improved LOD of 15 fg/mL of PSA and 4.8 fg/mL of PSMA was achieved, which was 1000-times better than previously reported PSA biosensors using Fe₃O₄ only, probably because GO carried more Fe₃O₄ particles and thus dramatically increased the electrochemical signal.

Conclusion and future perspectives
Biosensors for cancer biomarker detection opened a new avenue for the POC PC detection. In spite of their very short history, graphene-based materials have successfully demonstrated their unique advantages in biosensors for PC protein biomarkers. This review has summarized recent advances, challenges, and trends in the application of graphene-based materials for biosensing of PC protein biomarkers. In this review, the commonly used PC protein biomarkers for biosensor, the unique properties of graphene and the roles of graphene-based materials for biosensing were introduced. Among various PC protein biomarkers, PSA was the most frequently selected target for PC detection biosensor construction. Most studies focused on single biomarker detection and studies on detection of multiple biomarkers are limited. A variety of graphene-based materials such as pristine graphene, functionalyzed graphene (GO, rGO, GODs) were used in PC biosensor development and most of them were combined with other nanomaterials like nanoparticles. We have also summarized various strategies and approaches which can be used for graphene-based biosensor development. Graphene-based materials were used not only for novel biosensor interfaces construction but also as excellent carriers for the construction of novel nanocomposites for signal amplification. In most of the cases, graphene-based biosensors have exhibited satisfactory biocompatibility towards the bioactive species and remarkable performance with high sensitivities, wide linear detection ranges, low detection limits and long-term stabilities (Table 1). As other 2D materials have now been explored, we believe that more 2D

| Technique | Receptor system | Target proteins | LOD | Detection ranges | References |
|-----------|----------------|-----------------|-----|-----------------|------------|
| ECHEM     | rGO-MWCNT/AuNPs | PSA             | 1.0 pg/mL | (0.005–20) ng/mL for DPV, (0.005–100) ng/mL for EIS | [132]     |
| ECHEM     | rGO/Ag@BSA      | HCG, PSA, CEA   | 0.0007 mU/mL for HCG, 0.35 pg/mL for PSA, and 0.33 pg/mL for CEA | (0.002–120) mU/mL for HCG, (0.001–110) ng/mL for PSA, (0.001–100) ng/mL for CEA | [127]     |
| ECHEM     | Au@Th/GO, PtCu@rGO/graphitic carbon nitride | PSA         | 16.6 fg/mL | 50 fg/mL–40 ng/mL | [128]     |
| ECHEM     | GO/ssDNA/PLLA NPs | VEGF, PSA      | –     | (0.05–100) ng/mL for VEGF, (1–100) ng/mL for PSA | [118]     |
| ECHEM     | Fe₃O₄/PDDA/GO   | PSA, PSMA      | 15 fg/mL for PSA, 4.8 fg/mL for PSMA | (61 fg/mL–3.9 pg/mL) for PSA, (9.8 fg/mL–10 pg/mL for PSMA | [129]     |
| ECHEM     | Au@gPNPs/O-GS, Au@gNPs/O-GS | fPSA, PSA     | 6.7 pg/mL for fPSA, 3.4 pg/mL for PSA | (0.02–10) ng/mL for fPSA, (0.01–50) ng/mL for PSA | [126]     |
| ECHEM     | GS/OA/Fe₃O₄/FC  | PSA             | 2 pg/mL | (0.01–40) ng/mL | [92]       |
| ECHEM     | Carbon-graphene/aptamer | EN2 protein | 38.5 nM | (35–185) nM | [119]     |
| ECHEM     | GS-MB-CS        | PSA             | 13 pg/mL | (0.05–5.00) ng/mL | [93]       |
| ECHEM     | 3D graphene/Au  | PSA             | 0.59 ng/mL | (0–10) ng/mL | [94]       |
| FET       | rGO            | PSA-ACT         | 100 fg/mL | (10−1−1) μg/mL | [105]     |
| Fluorescence | GQDs–NR   | ACP             | 28 μU/mL | (0–1500) μU/mL | [133]     |
| Fluorescence | GQD/peptide/FITC | PSA           | 0.3 nM | (0–20) nM | [64]       |
| ECL       | Au/Ag-rGO/aminated-GQDs/carboxyl-GQDs | PSA         | 0.29 pg/mL | 1 pg/mL–10 ng/mL | [96]       |
| ECL       | graphene       | PSA             | 8 pg/mL | 10 pg/mL–8 ng/mL | [95]       |
| Colorimetric | GO/MB    | PSA             | –     | – | [134]       |
materials like MoS$_2$ could be employed and integrated into biosensors for PC biomarker detection in the upcoming future.

Although tremendous progress has been made in the past a few years of graphene-based biosensors for PC detection, there still remain some challenges. Firstly, PSA has been demonstrated not a specific biomarker in prostate cancer early screening. As a result, detection of multiple biomarkers is crucial for precise diagnosis and prognosis of PC [130, 131]. More attention should be paid to studies on the simultaneous detection of multiple biomarkers in the future. In addition, there are only a few studies on PC biomarker detection in different body fluid. To improve the accuracy and practicability of the diagnosis, more studies are expected to perform biomarker detection in different body fluid.

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Authors’ contributions
LYW and SP contributed to the data collection. VRSSM contributed to the publication preparation. IM helped revising the manuscript. YL, MD, SZR and LX contributed to the conception of the review and manuscript writing. The authors declare that they have no competing interests.

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Availability of data and materials
Not applicable

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References
1. Healy DA, Hayes CJ, Leonard P, McKenna L, O’Kennedy R (2007) Biosensor developments: application to prostate-specific antigen detection. Trends Biotechnol 25:125–131
2. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67:7–30
3. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
4. Kim EH, Andriole GL (2018) Prostate cancer review. Mo Med 115:131
5. Pettersson A, Robinson D, Garmo H, Holmberg L, Stattin P (2018) Age at diagnosis and prostate cancer treatment and prognosis: a population-based cohort study. Ann Oncol 29:377–385
6. Lemanska A, Dearnaley DP, Jena R, Sydes MR, Fallowfield L (2018) Older age, early symptoms and physical function are associated with the severity of late symptom clusters for men undergoing radiotherapy for prostate cancer. Clin Oncol (R Coll Radiol) 30:334–345
7. Bashir MN (2015) Epidemiology of prostate cancer. Asian J Cancer Prev 16:5137–5141
8. Pernar CH, Ebot EM, Wilson KM, Muco LA (2018) The epidemiology of prostate cancer. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a030361
9. Sammon JD, Serrell EC, Karabon P, Abdollah F, Weissman J, Han PKJ, Hansen M, Menon M, Trih QD (2018) Prostate cancer screening in early medical expansion states. J Urol 199:81–88
10. Carroll PR, Parsons JK, Andriole G, Bahnsen RR, Castle EP, Catalona WJ, Dhillon JS, Davis JW, Epstein JL, Etzioni RB, Farrington T, Hemstreet GP, Kawachi MH, Kim S, Lange PH, Lowrance W, Marucci P, Mohler J, Morgan TM, Moses KA, Nadler RB, Poch M, Scales C, Shanefield TM, Smallbone MC, Sonn G, Spindler P, Vickers AJ, Wake R, Sheehan DA, Freedman-Cass DA (2016) Prostate cancer early detection, version 2.2. J Natl Compr Canc Netw 14:509–519
11. Partin AW (2013) Early detection of prostate cancer continues to support rational, limited screening. J Urol 190:427–428
12. Cremers RG, Eiles RA, Bankoet EK, Ringelberg-Borsboom J, Jasen HF, Van Asperen CJ, Committee IS, Schalken JA, Verhaegh GW, Kierneyme LA (2015) The role of the prostate cancer gene 3 urine test in addition to serum prostate-specific antigen level in prostate cancer screening among breast cancer, early-onset gene mutation carriers. Urol Oncol 33:202 e19-e28
13. Rifai N, Gillette MA, Carr SA (2006) Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat Biotechnol 24(8):971–983
14. Ordonez NG (2014) Value of podoplanin as an immunohistochemical marker in tumor diagnosis: a review and update. Appl Immunohistochem Mol Morphol 22:331–347
15. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O’Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M (2008) Circulating miRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105:10513–10518
16. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang YX, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonaraki ES, Aza ND, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Galia GL, Gibb P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao YC, Juhi HH, Kim JJ, Siravegna G, Laheur DA, Laurcella C, Lim M, Lipson EJ, Marie SKN, Netto GJ, Olliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmitt K, Shih IM, Obia-Shinmo S, Siena S, Theudecosc D, Tiej NK, Harkins TT, Verones S, Wang TL, Weingart JG, Wolfgang C, Wool DQ, Xing DM, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Lus AJ (2014) Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6(224):224ra24
17. Isbell JM, Jones DR, Li BT (2018) Circulating tumor DNA: A promising biomarker to guide postoperative treatment and surveillance of non-small cell lung cancer. J Thorac Cardiovasc Surg 153:2628–2631
18. Wu LL, Chiou CC, Chang PT, Wu JT (2004) Urinary 8-OhdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. Clin Chim Acta 339:1–9
19. Monteiro M, Moreira N, Pinto J, Pres-Luis AS, Henrique R, Jeronimo C, Bastos ML, Gomes AM, Carvalho M, Cardoso P, Silva CR, Cardoso FM, Carvalho MM, Brito ML, Ferreira MA, Silva AL, Varela-Lopes P (2018) Non-commercial use: an approach for the identification of a potential VOC-biomarker panel in the urine of renal cell carcinoma patients. J Cell Med 210:2090–2105
20. Natarajan H, Kumar L, Bakhshi S, Sharma A, Velandapan T, Kabra M, Gogia A, Ranjan Biswas N, Gupta YK (2018) Imatinib trough levels: a potential biomarker to predict cytogenetic and molecular response
in newly diagnosed patients with chronic myeloid leukemia. Leuk Lymphoma 60:1–8
21. Yu M, Stott S, Toner M, Maheswaran S, Haber DA (2011) Circulating tumor cells: approaches to isolation and characterization. J Cell Biol 192:373–382
22. Inoue M, Otsuka K, Shibata H (2016) Circulating tumor cell count as a biomarker of specific gastric cancer subgroup characterized by bone metastasis and/or disseminated intravascular coagulation—an early indicator of chemotherapeutic response. Oncol Lett 11:1294–1298
23. Wu L, Qu X (2015) Cancer biomarker detection: recent achievements and challenges. Chem Soc Rev 44:2963–2997
24. Severi G, FitzGerald LM, Muller DC, Pedersen J, Longano A, Southey MC, Hopper JL, English DR, Giles GG, Mills J (2014) A three-protein biomarker panel assessed in diagnostic tissue predicts death from prostate cancer for men with localized disease. Cancer Med 3:1266–1274
25. Shui B, Tao D, Florea A, Cheng J, Zhao Q, Gu Y, Li W, Jaffrezic-Renault N, Mei Y, Guo Z (2018) Biosensors for Alzheimer’s disease biomarker detection: a review. Biochimie 147:13–24
26. Lin PY, Cheng KL, McGuffin-Cawley JD, Shieu FS, Samia AC, Gupta S, Parra-Cabrera C, Samitier J, Homs-Corbera A (2016) Multiple biomarkers biosensor with just-in-time functionalization: application to prostate cancer detection. Biosens Bioelectron 77:1192–1200
27. Narwal V, Kumar P, Joon P, Pundir CS (2018) Fabrication of an amperometric sacrosine biosensor based on sacrosine oxidase/chitosan/CuNP/Ag-MWCNT/Au electrode for detection of prostate cancer. Enzyme Microb Technol 113:44–51
28. Geim AK, Novoselov KS (2007) The rise of graphene. Nat Mater 6:183–191
29. Pena-Bahamonde J, Nguyen HN, Fanourakis SK, Rodrigues DF (2018) Recent advances in graphene-based biosensor technology with applications in life sciences. J Nanobiotechnol 16:75
30. Kim J, Park SJ, Min DH (2017) Emerging approaches for graphene oxide biosensor. Anal Chem 89:232–248
31. Tarro G, Perera A, Esposito C (2005) Early diagnosis of lung cancer by detection of tumor liberated protein. J Cell Physiol 203:1–5
32. Borrebaeck CA (2017) Precision diagnostics: moving towards protein biomarker signatures of clinical utility in cancer. Nat Rev Cancer 17:199–204
33. Sunniva S, Radova L, Choi M, Stovall J, Brenner H, Vitek O, Hujduch M, Aebi Rüedi L (2015) Non-invasive prognostic protein biomarker signatures associated with colorectal cancer. EMBO Mol Med 7:1153–1165
34. Morin PJ (2005) Claudins in human cancer: promising new targets for diagnosis and therapy. Cancer Res 65:9603–9606
35. Tang T, Yang C, Brown HE, Huang J (2018) Circulating heat shock protein 70 is a novel biomarker for early diagnosis of lung cancer. Dis Markers 2018:1874646
36. Jung YJ, Kittlaus E, Ostroff RM, Kim Y, Seok M, Lee S, Jang S, Kim WS, Choi CM (2017) Development of a protein biomarker panel to detect non-small-cell lung cancer in Korea. Clin Lung Cancer 18:e96–e107
37. Yang Z, Li DM, Xie Q, Dai DQ (2015) Protein expression and promoter methylation of the candidate biomarker TCF21 in gastric cancer. J Cancer Res Clin Oncol 141:211–220
38. Zhuravskij A, Patsik P, Zed R, Tully S, Champoux JP, Onghena P, Estrella P (2018) Sensitive and selective Affimer-functionalised interdigitated electrode-based capacitive biosensor for Her2 protein tumour biomarker detection. Biosens Bioelectron 108:1–8
39. Arsenault F, Beauregard JM, Pouliot F (2018) Prostate specific membrane antigen as therapy target: tissue expression and in vivo efficacy of an engineered protein hormone antagonist. Mol BioSyst 8:1441–1445
40. Sadlowski C, Balderston S, Sandhu M, Hajian R, Liu C, Tran TP, Conboy MJ, Paredes J, Murphy N, Conboy IM, Apar A (2018) Graphene-based...
107. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346:818–822
108. Robertson DL, Joyce GF (1990) Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature 344:467–468
109. Zhang LQ, Wan S, Jiang Y, Wang YY, Fu T, Liu QL, Cao ZJ, Qu L, Tan WH (2017) Molecular elucidation of disease biomarkers at the interface of chemistry and biology. J Am Chem Soc 139(7):2532–2540
110. Zhang J, Li S, Liu F, Zhao L, Shao N, Zhao X (2015) SELEX aptamer used as a probe to detect circulating tumor cells in peripheral blood of pancreatic cancer patients. PLoS ONE 10:e0121920
111. Chen C, Zhou S, Cai Y, Tang F (2017) Nucleic acid aptamer application in diagnosis and therapy of colorectal cancer based on cell-SELEX technology. NPJ Precis Oncol 1:37
112. Sedighian H, Halabian R, Amani J, Heiat M, Amin M, Fooladi AAI (2018) Staggered Target SELEX, a novel approach to isolate non-cross-reactive aptamer for detection of SEA by apta-qPCR. J Biotechnol 286:45–55
113. Nair J, Zhang LQ, Wang J, Zhang Y, Li Z, Sun G, Zhang Y, Wu Z, Jiang Y, Wang YY, Fu T, Liu QL, Cao ZJ, Qu L, Tan WH (2017) Recent progress in nanomaterial-based electrochemical biosensors for cancer biomarkers: a review. Molecules 22:1048
114. Han J, Zhuo Y, Chai YQ, Yuan R, Zhang W, Zhu Q (2012) Simultaneous electrochemical detection of multiple tumor markers based on dual catalysis amplification of multi-functionalized onion-like mesoporous graphene sheets. Anal Chim Acta 746:70–76
115. Wang X, Li W, Li Z, Li H, Xu D (2015) A highly sensitive fluorescence turn-on platform with silver nanoparticles aptasensing for human platelet-derived growth factor-BB. Talanta 144:1273–1278
116. Liu J, Zeng J, Tian Y, Zhou N (2017) An aptamer and functionalized nanoparticle-based strip biosensor for on-site detection of kanamycin in food samples. Analyst 143:182–189
117. Eissa S, Zourob M (2017) Aptamer-based label-free electrochemical biosensor array for the detection of total and glycated hemoglobin in human whole blood. Sci Rep 7:1016
118. Aliakbarinodahi N, Jolly P, Bhalla N, Miodek A, De Micheli G, Estrela P, Carrara S (2017) Aptamer-based field-effect biosensor for tenofovir detection. Sci Rep 7:44409
119. Pan LH, Kuo SH, Lin TY, Lin CW, Fang PY, Yang HW (2017) An electrochemical biosensor to simultaneously detect VEGF and PSA for early prostate cancer diagnosis based on graphene oxide/ssDNA/PALLA nanoparticles. Biosens. Bioelectron 89:598–605
120. Settu K, Liu JT, Chen CJ, Tsai JZ (2017) Development of carbon-graphene-based aptamer biosensor for EN2 protein detection. Anal Biochem 534:99–107
121. Song Y, Qu K, Zhao C, Ren J, Qu X (2010) Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. Adv Mater 22:2206–2210
122. Qu FL, Li T, Yang MH (2011) Colorimetric platform for visual detection of cancer biomarker based on intrinsic peroxidase activity of graphene oxide. Biosens. Bioelectron 26:3927–3931
123. Liu M, Chen Q, Dai C, Zhang Y, Deng J, Li H, Yao S (2013) A double signal amplification platform for ultrasensitive and simultaneous detection of ascorbic acid, dopamine, uric acid and acetaminophen based on a nanocomposite of ferrocene-thiolate stabilized Fe3O4@Au nanoparticles with graphene sheet. Biosens Bioelectron 48:75–81
124. Wang B, Akiba U, Arzaiji J (2017) Recent progress in nanomaterial-based electrochemical biosensors for cancer biomarkers: a review. Molecules 22:1048
125. Sharafeldin M, Bishop GW, Bhakta S, El-Sawy A, Suib SL, Rusling JF (2017) Fe3O4 nanoparticles on graphene oxide sheets for isolation and ultrasensitive amperometric detection of cancer biomarker proteins. Biosens Bioelectron 91:359–366
126. Zheng Z, Wu L, Li L, Zong S, Wang Z, Cui Y (2018) Simultaneous and highly sensitive detection of multiple breast cancer biomarkers in real samples using a SERS microfluidic chip. Talanta 188:507–515
127. Nie Y, Zhang P, Zhuo H, Chai YQ, Yuan R (2017) Ultrasensitive electrochemical biosensing platform for detection of multiple types of biomarkers toward identical cancer on a single interface. Anal Chem 89:12821–12827
128. Heydar-Bafrooei E, Shamszadeh NS (2017) Electrochemical bioassay development for ultrasensitive aptasensing of prostate specific antigen. Biosens Bioelectron 89:1736–1742
129. Cui Y, Zhang P, Zhong F, Li Q, Yuan R (2017) Highly sensitive detection of multiple breast cancer biomarkers based on intrinsic peroxidase activity of graphene oxide. Biosens Bioelectron 89:1736–1742
130. Ma C, Ge S, Yan M, Yu J, Song X (2015) Multiplexed enzyme-free electrochemical immunosensor based on ZnO nanorods modified reduced graphene oxide-paper electrode and silver deposition-induced signal amplification strategy. Biosens Bioelectron 71:30–36
131. Na W, Liu Q, Sui B, Hu T, Su X (2016) Highly sensitive detection of acetylcholinesterase using a graphene quantum dots-based Förster resonance energy transfer. Talanta 161:469–475
132. Qu F, Li T, Yang M (2011) Colorimetric platform for visual detection of cancer biomarker based on intrinsic peroxidase activity of graphene oxide. Biosens. Bioelectron 26:3927–3931

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