Aptamer-based methods for biosensing of estrogen receptors and human epidermal growth factor receptor 2 in breast cancer

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Abstract

Breast cancer is a common malignancy and a leading cause of cancer related mortality among women. Early diagnosis and better prognosis are vital to improve breast cancer outcomes and survival rates. Clinical assessment of the expression levels of biomarkers including estrogen receptors (ERs) and human epidermal growth factor receptor 2 (HER2) had been beneficial in assisting the prognosis and deriving treatment regimes. Conventionally, these biomarkers were detected mostly using antibody-based methods. However, recent advances in identifying aptamers as antibody substitutes have favoured development of aptamer-based sensing platforms for rapid, cost-effective, and point-of-care testing applications. The last decade witnessed substantial use of aptamers in cancer diagnosis. This review provides a thorough update of recent developments in aptamer-based detection of human estrogen receptors (ERs) and epidermal growth factor receptor 2 (HER2) in breast cancer. The widely used tissue and serum-based biomarkers of breast cancer are introduced, and the conventional methods of ERs and HER2 detection are discussed. Electrochemical and optical methods of aptasensing strategies based on "on/off", "target sandwich", "nanomaterial beacons" and AuNP-based localized surface plasmon resonance (LSPR) approaches are then elaborated. Finally, a perspective for futuristic remote health care monitoring of breast cancer patients using smart wireless nano-aptasensor is provided.

Key words

Breast cancer; Estrogen receptors; Human epidermal growth factor receptor-2; Aptasensor

1. Introduction

Breast cancer, a heterogeneous and multifaceted disease is the most frequent malignancy among women throughout the world (Bray et al., 2018; Mathur et al., 2020). Multiple disease models have been proposed for carcinogenic growth of the breast tissue. Genetic and epigenetic alterations that accumulate in stem-like cells and progressively endow them with the capacity to replicate relentlessly and invade surrounding tissue to colonize at distant sites to form metastases is the most referred model (Hanahan & Weinberg, 2000). The immunosurveillance theory which suggest that tumours evolve and progress uncontrollably only from cells able to escape immune recognition via immunoediting, creating immunosuppressive environment or other unknown mechanisms is another model explaining the cause of breast carcinogenicity (Kroemer, Senovilla, Galluzzi, André, & Zitvogel, 2015). Also, various modifiable and non-modifiable risk factors have been also associated with the onset of occurrence and progress of carcinogenic growth as well as treatment outcomes (Maas et al., 2016). Further, recent advances in molecular biology and immunotherapy allowed tailoring targeted therapies for a more efficient and pathophysiology specific treatment of breast cancer patients (Masoud & Pagès, 2017). Despite the improving understanding of the disease and treatment options, breast cancer stands as a leading health issue and a major cause of cancer-related deaths among women and the second leading cause of death globally (Bray et al., 2018; Mathur et al., 2020; Society, 2019). Breast cancer was estimated to account for more than 2.1 million new cases and 0.6 million deaths globally in year 2018 (Bray et al., 2018). For instance, in US alone, over 0.268 million new cases of breast cancer (incidence rate 124.7) and over 41 thousand deaths (death rate 20.6) are estimated in the year 2019 whereas this burden is estimated to increase to 0.232 million by 2025 in India from the current 0.205 million breast cancer incidences (Mathur et al., 2020; Society, 2019).

Early diagnosis and better prognostic approaches are vital to improve breast cancer outcomes and survival rates, particularly in developing countries such as India where the breast cancer mortality is alarmingly high due to late presentation at advanced stage and incidence of cancer among young premenopausal women (Malvia, Bagadi, Dubey, & Saxena, 2017; Mathur et al., 2020). While the conventional methods of breast cancer screening and diagnosis using mammography, ultrasound imaging, magnetic resonance imaging and biopsy are successful in identifying carcinogenic lumps in the breast tissues, assessment of expression levels of breast cancer biomarkers (Table 1), particularly estrogen receptors (ERs) and human epidermal growth factor receptor 2 (HER2) is required to assist with prognosis (Kuhl et al., 2005; H. Liu, Zhan, Sun, & Zhang, 2020; Sturgeon et al., 2008; Weigel & Dowsett, 2010). Results from these tests helps in decision making on adopting a specific treatment regimen, for example use of tamoxifen (anti-estrogen drug) and transtuzumab for targeted treatment of ER and HER2 positive breast cancer, respectively (EBCTCG, 1998; Slamon et al., 2011).

Estrogen receptors are proteins belonging to the superfamily of the steroid hormone nuclear receptors, and exist as ERα and ERβ isoforms (Kumar et al., 2011). Structurally, they are composed of 3 independent interacting functional domains: the N-terminal domain that is involved in protein-protein interactions and transcriptional activation of target gene expression, a DNA binding domain which have two zinc finger structure and help in DNA sequence-specific receptor binding and receptor dimerization, and a C-terminal ligand-binding domain that mediates ligand binding, receptor dimerization, nuclear translocation, and transactivation of target gene expression (Tsai & O'Malley, 1994). Experimental and clinical evidence demonstrate these receptors playing critical role in both controlling mammary gland development as well as tumorigenesis and breast cancer development (McGuire, 1973; Platet, Cathiard, Gleizes, & Garcia, 2004). ERs has been documented for

role in ductal morphogenesis, and found expressed in almost 75% of breast cancers. Anti-estrogen treatment of metastatic breast cancer is evidenced responding better among patients with tumours expressing the ERs (Viale et al., 2007).

Human epidermal growth factor receptor 2 (HER2) is a transmembrane protein belonging to the family of tyrosine kinases involved in pathways that regulate proliferation, cell death, angiogenesis, and migration (Krysan et al., 2005; Normanno et al., 2006). HER2 signalling cascade, initiated by external growth factors affect the transcription of genes of a series of transmembrane proteins and intracellular signalling intermediates through phosphorylation or dephosphorylation (Krysan et al., 2005). HER-2/neu gene is normally expressed on the epithelial cells of numerous organs, including lung, bladder, pancreas, breast, and prostate, and has been found to be overexpressed in cancer cells. High levels of HER-2 expression were shown associated with decreased survival rate in breast cancer patients. The shredded extracellular domain of overexpressed HER2 is easily detectable in the blood, and is used for prognostic estimation and prediction of resistance to endocrine therapy or response to anti-HER2 therapies (L. C. Kim, Song, & Haura, 2009; Wolff et al., 2014).

Aberrant expression of these markers in breast tissues is quantified based on gene expression levels (determined through fluorescent in-situ hybridization) or protein expression levels (through radio immune assay, enzyme linked immunosorbent assay, immunohistochemistry, and flow cytometry) (Kriege et al., 2004; Panieri, 2012; Sauter, Lee, Bartlett, Slamon, & Press, 2009; Singh et al., 2016). These methods are sensitive, robust and accurate but require bulky sophisticated instruments and trained manpower. The search for simple, rapid and cost-effective methods to diagnose breast cancer at point-of-care setting acknowledge aptamer-based methods as effective alternate to conventional method of biomarker detection in breast cancer (Dehghani et al., 2018; Hassan & DeRosa, 2020; Razmi

et al., 2018; Şahin, Caglayan, & Üstündağ, 2020; Yousefi et al., 2019). Aptamers are custom synthesized short single-stranded oligonucleotides screened from oligonucleotides libraries through the process of systematic evolution of ligands by exponential enrichment (SELEX) (Ellington & Szostak, 1990; Stoltenburg, Reinemann, & Strehlitz, 2007). In the last three decades, multiple aptamers have been identified against myriad targets ranging from small molecules such as divalent cations to bulky and complex structured proteins and cellular markers (McKeague & DeRosa, 2012; Sefah, Shangguan, Xiong, O'Donoghue, & Tan, 2010; Stoltenburg et al., 2007). Aptamers have been reported an emerging tool in diagnostic and therapeutic applications (Hori, Herrera, Rossi, & Zhou, 2018; Kaur, Bruno, Kumar, & Sharma, 2018; K. Liu, Lin, & Lan, 2013; Ng et al., 2006; Pei, Zhang, & Liu, 2014), chromatographic separations (R. Ahirwar & Nahar, 2015a; Perret & Boschetti, 2018), food testing (R. Ahirwar & Nahar, 2015b; Huo, Hu, Gao, & Li, 2021; Ma et al., 2014), and environmental monitoring (Nguyen, Kwon, & Gu, 2017). Applying aptamers in biosensing applications inherit the advantages of high thermal stability, cheaper production, and easy chemical modification. Interestingly, multiple DNA and RNA-based aptamers identified against many breast cancer markers have been also used in fabricating a variety of low cost and rapid sensing strategies.

Benefiting the unique structure changing ability of aptamers, multiple sensing strategies based on electrochemical, optical (fluorimetric and colorimetric), and piezoelectric readout systems have been developed for different breast cancer biomarkers. Several reviews on the use of aptamers for detecting different cancer biomarkers common to multiple cancers are available in the literature (Dehghani et al., 2018; Hassan & DeRosa, 2020; Razmi et al., 2018; Şahin et al., 2020; Yousefi et al., 2019). The role of aptamer-based biosensors, particularly those fabricated on optical and microfluidic platforms in early detection and diagnosis of multiple cancer have been reviewed by Hassan et al. (Hassan & DeRosa, 2020).

Recent developments in aptamer-based optical and electrochemical biosensors for detection of platelet-derived growth factor, an important protein marker in the cancers of breast, prostate, gastro intestine, liver and blood and bone marrow have been reviewed by Razmi et al. (Razmi et al., 2018). Similarly, aptasensing strategies for detection and quantitative determination of MUC1 (a membrane-associated glycoprotein overexpressed in many human malignancies such as ovary, breast, colorectal, gall bladder, non-small cell lung and gastric cancer) and VEGF (a key regulator of vascular formation in cancer angiogenesis) based on optical and electrochemical platforms were reviewed by Yousefi et al., and Dehghani et al. (Dehghani et al., 2018; Yousefi et al., 2019). A recent review by Sachin et al. al presented updates on development of nanomaterials based aptasensors for breast cancer markers VEGF, HER2 and MUC1 (Şahin et al., 2020).

Aptamer-based sensing strategies for ER have not been reviewed previously. This review provides a comprehensive assessment of reported aptasensing strategies for ERs and HER2 based on literature review of past one decade and provides futuristic insight to smart wireless apta-nanosensors for remote health care monitoring. The review first provide an overview of various conventional methods of ER and HER2 detection in breast cancer, discussing key advantage and limitations of each method, followed by detailed discussion of various electrochemical and optical methods of ER and HER2 aptasensing. The last section envisages the application of breast cancer aptasensors in remote healthcare monitoring to improve quality of life and overall survival among breast cancer patients.

Table 1.

2. Conventional methods for ERs/HER2 profiling in breast tissues

Precise detection and quantitative estimation of low levels of these circulating and tissue based markers require the detection methods to be sufficiently specific and sensitive.

Detection of ERs and HER2 receptors in breast tumour cells have been carried out traditionally using radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA) and immunohistochemistry (IHC) methods (Fendly et al., 1990; Gutierrez & Schiff, 2011; Leclercq et al., 1986; Newby et al., 1995; Nicholson et al., 1986; Shafie & Brooks, 1979; Starkey & Orth, 1977).

RIA such as the dextran-coated charcoal assay (DCC) was the most primitive methods to quantify tissue-based ERα from tissue extracts of breast cancer patients. These methods were extremely sensitive and reproducible (Goussard, Lechevrel, Martin, & Roussel, 1986), however lacked the ability to locate the source of receptor (normal/neoplastic tissue) as well as faced health issues due to radioactivity hazards.

Development of antibodies against proteinous targets and subsequent advances in protein immobilization chemistries replaced the RIA by the enzyme linked immunosorbent assay (ELISA) and immunohistochemistry (IHC). The IHC is used as gold standard method for analysis of tissue-based ERs and HER2 expression (Hammond et al., 2010; Wolff et al., 2018). While the ELISA is preferred for soluble targets, e.g. in tissue extracts, the IHC is a method of choice for assessment of tissue imbedded targets. These methods allow analyses based on both frozen and fresh tissues. A generalized sandwich ELISA protocol for ER/HER2 involve coating of the ER/HER2 antibodies to a suitable solid support (e.g. microtitre plate) and its incubation with tumour tissue extract, followed by subsequent incubations with primary and secondary antibodies to translate the presence of target markers to a chromogenic or fluorescent signal. Among various commercial ELISA protocols developed for these two marker proteins, one automated and one manual ELISA protocols for HER2 developed by Siemens Healthcare Diagnostics, Tarrytown, NY were approved by the FDA for clinical diagnostics. An IHC assay is similar to the ELISA in its principle of detection, except it assess the ER/HER content within tissues and provide visual image of

ER/HER2 positive cells in tissue context. Unlike ELISA which is a quantitative method, IHC outcome is scored on a semiquantitative scale. For example, the HER2 expression is scored as IHC 0 (no staining or incomplete and barely perceptible staining and in ≤ 10% of tumour cells), IHC 1+ (incomplete and barely perceptible membrane staining, and in > 10% of tumour cells), IHC 2+ (weak to moderate complete membrane staining observed in > 10% of tumour cells), or IHC 3+ (circumferential membrane staining that is complete, intense, and in > 10% of tumour cells) where the score of IHC 0 or 1+, 2+ and 3+ are considered negative, equivocal and positive, respectively (Wolff et al., 2018).

Use of antibodies-based biosensors (i.e. immunosensors) for ER/HER2 detection is another area of antibody-based diagnosis. Next-generation bioanalytical systems based on immunosensors using antibodies as biorecognition element have been fabricated for multiple breast cancer markers including the ERs/HER2 (Table 2). Biosensors are analytical devices composed of a biorecognition unit (i.e. antibody in case of immunosensors) that sense the presence of an analyte, and a transducer that convert the biorecognition event into a measurable signal (Bhalla, Jolly, Formisano, & Estrela, 2016). A typical immunosensor has following components: (i) bioreceptor– antibody to selectively recognise and bind the ERs/HER2 in soluble phase, and generate signal in the form of light, heat, pH, charge or mass change, (ii) a transducer– electronic device that converts bioreceptor generated signal into measurable electrical or optical signals, (iii) a signal processing and display unit– the electronic circuitry for signal conditioning (amplification and analogue to digital conversion), and user interface to obtain results in an user-understandable format (e.g. real numbers, plot, or image). Immunosensors based on multiple detection strategies (electrochemical, optical, and piezoelectric) have been reported in literature for rapid detection of ERs/HER2. The electrochemical immunosensors measure electrons or the resistance in electron flow (amperometry, voltammetry, potentiometry and impedance) resulting from the biorecognition

event at electrode surface. Conversely, the optical immunosensors measure absorbance, reflectance or fluorescence emissions of photons interacting with the antigen-antibody pair or its metabolized products. Piezoelectric immunosensors record the changes in pressure or mass caused by binding of ERs/HER2 to its antibodies. A list of some immunosensors for ERs and HER2 is shown in Table 2.

Table 2.

Mass spectrometry presents another promising approach in breast cancer diagnostics to improve cancer detection and enable earlier treatment. Proteomic assay formats based on targeted quantitative mass spectrometry involve selective digestion of proteins to component peptides using an enzyme such as trypsin, which are then separated, fragmented, ionised, and detected by mass spectrometers as quantitative stoichiometric surrogates for protein concentration in the sample (Gerber, Rush, Stemman, Kirschner, & Gygi, 2003; Schoenherr et al., 2012). A quantitative mass spectrometry-based peptide and protein assays (peptide immunoaffinity enrichment mass spectrometry) was implemented by Schoenherr et al. to detect ERs and HER2 in cell lysates and human surgical specimens which showed good correlation with ELISA and IHC assays (Schoenherr et al., 2012).

Table 3

3. Aptamer-based electrochemical methods for ER/HER2 detection

Biosensors measure biochemical interactions and reactions by generating electrical or optical signals proportional to the concentration of the analyte. Functionally, the biosensors consist of four parts: (i) a bioreceptor that specifically interacts with the analyte, (ii) a transducer that converts the bio-recognition event into measurable optical or electrical signal, (iii) an electronic circuitry to amplify and convert analogue signals into digital, and (iv) a

display unit to produce numbers, graphs, or tables understandable by the user (Bhalla et al., 2016). A variety of biosensors based on different methods used for signal transduction, such as the electrochemical, optical, thermometric, piezoelectric or magnetic, and distinct recognition elements like antibody, enzyme, DNA and aptamer have been developed for detection of breast cancer biomarkers. Aptasensors are constructed by combining analyte specific DNA or RNA aptamers with highly sensitive optical, electrochemical, mass-sensitive transducers.

Figure 1

3.1. Aptasensing strategies

Most of the aptasensors for breast cancer biomarkers are developed on two broad strategies: electrochemical and optical. Electrochemical aptasensors use aptamers as biological recognition elements coated onto electrodes (transducers) made of metals (platinum, gold, silver, stainless steel), carbon-based materials (graphite, carbon black, carbon fibre), or conducting composites (as in interdigited electrodes) for selective recognition and detection of a target analyte. Electrochemical aptasensors measure electrons or the resistance in electron flow, and based on detection principle, they are categorised to amperometric, potentiometric, conductometric, voltammetric, and impedimetric biosensors (Sun, Lu, Zhang, & Chen, 2019; Topkaya, Azimzadeh, & Ozsoz, 2016). The amperometric methods measure the electric current against time over a constant electric potential. Aptasensors based on amperometry are fabricated by mounting aptamer on the electrode's surface where the electric current, in proportion to target concentration, is produced as a result of oxidative or reductive action of aptamer. Voltammetric methods measure current response as a function of applied potential that is varied either step by step or continuously between a working and reference electrode. Three electrodes- working, auxiliary, and

reference are mostly used in voltammetric aptasensors for accurate and stable application of potentials and the current measurement. Cyclic voltammetry (CV) and pulse voltammetry (e.g. Differential Pulse Voltammetry) are two commonly used voltametric techniques. Cyclic voltammetry technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. The current generated is then plotted against potential to produce a CV graph that provides insights on the transducer material based on the anodic peak current (Ipa) from oxidation process and cathodic peak current (Ipc) from reduction process which occur on the WE. On the other hand, the DPV technique uses a series of potential pulses of fixed amplitude (10 to 100 mV) which is superimposed on a slowly changing base potential and measures current at two points for each pulse- just before the application of the pulse and at the end of the pulse. A conductometric aptasensor measures conductivity change from production or consumption of ionic species involved in the metabolic process. These biosensors require no reference electrode in the system, hence, can be miniaturized. However, as all charge carriers may lead to a change of conductivity, this directly affects the device selectivity. A potentiometric biosensor is based on the potential difference between working and reference electrodes. They measure the potential difference between two ion-selective electrodes under the conditions of no current flow, but at different analyte concentrations. The electric response depends on the activity of the species in comparison to the reference electrode, with the output signal recorded in voltage units. Layer-by-layer technique is widely used for surface modifications. Impedance is a measure of the ability of a circuit to resist the flow of electrical current (resistance concept is limited to ideal resistors only). Electrochemical impedance is usually measured by applying an AC potential to an electrochemical cell and then measuring the current through the cell.

3.2. Electrochemical aptasensors based on direct signal ON/OFF approaches

Direct sensing of biomarkers based on its molecular interactions with the bioreceptor (aptamer) present the common approaches reported for aptasensing of ERs/HER2 in breast cancer. These direct approaches are based on the fact that binding of an aptamer to its target often lead to changes in the structure and spatial conformation of the aptamer, which is immediately sensed as change in the electron flow. When the target binding to aptamer results in an increased electrochemical signal, it is termed as signal-ON assays, whereas the decrease in intensity of signal is called signal-OFF assays. Simply, the processing of electrochemical signal from weak to strong and vice-versa before and after target recognition is called signal ON/OFF approach. The literature suggest use of direct capturing of target or aptamer to electrodes surface or through an intermediate nanoparticle layer, and subsequent monitoring of the variations in electric signals upon binding of aptamer or target with or without soluble redox indicators (Figure 2, Table 4).

A simplest aptasensor for ERα protein was devised by (R. Ahirwar et al., 2019) via immobilizing the thiolated aptamer on to screen printed gold electrode, and measuring the change in the differential pulse voltammetric (DPV) signal upon binding of the target ERα. The screen printed aptamer-coated gold electrode depicted enhanced flow of electrons (high I), which start getting reduced as more and more ERα bind to the electrode coated aptamer (in a concentration dependent manner). This on/off approach enabled quantifying as low as 0.001 ng/ml of full−length ERα (66.2kDa) in a detection time of mere 10 minutes (R. Ahirwar et al., 2019).

A similar approach was adopted by the Bezerra et al. to detect HER2 in human serum (Bezerra et al., 2019). The immobilized HER2 aptamer (sequence specified in Table 4) on the poly-L lysine film modified carbon working electrode showed current peak of high amplitude in absence of target, but the increase in the concentration of HER2 on sensor resulted in a decrease in the current signal. This reduction in current flow with increasing concentrations

of HER2 was presumed resulting from confirmation changes in the aptamer in target presence that either hindered or impeded the access of methylene blue to the guanines (Bezerra et al., 2019). Another study reported the use of glassy carbon electrode which was modified with densely packed gold nanoparticles placed on a composite consisting of electrochemically reduced graphene oxide and single walled carbon nanotubes (ErGO-SWCNTs) to immobilize the HER2 aptamer that respond to the presence of target HER2 protein by an increased charge transfer resistance (Rct) of the electrode (Rostamabadi & Heydari-Bafrooei, 2019).

Three studies reported aptasensing strategies for HER2 based on redox electric current generated by the DNA backbone on reacting with molybdate. Hu et al. showed that HER2 molecules captured on bare gold electrodes via a peptide (CKLRLEWNR) upon binding to its aptamer can generate redox electric current in presence of molybdate (Hu et al., 2017). The phosphate group the aptamer backbone can form molybdophosphate precipitate and generate proportionate (electrochemical) current. Authors reported that the current peak of bare electrode in the $[Fe(CN)_6]^{3-/4}$ solution decreased upon immobilization of peptide, followed by HER2 capture and aptamer binding in sequential and concentration-dependant manner (Fig. 2). In further attempts, the same group showed that initiating auxiliary DNA self-assembly on the aptamer bonded to the captured HER2 on gold electrode allow forming long one-dimensional DNA and result in achieving better sensitivity due to the extended length of DNA strand over the aptasensor without DNA self-assembly (Shen et al., 2017). Another study reported a dual signal amplification strategy for HER2 based on the reaction of phosphate and aptamer functionalized on MnO_2 nanosheets with molybdate, leading to the formation of redox-active molybdophosphate and consequent generation of electrochemical current (Chai, Li, & Yang, 2019).

In a similar approach, Tabasi et al., developed an aptasensor for HER2 detection via covalent coupling the amino-functionalized aptamer on the graphene oxide-chitosan (rGO-Chit) film-modified glassy carbon (GC/rGO-Chit) electrode, and measured the increase in the DPV signal in the presence of HER2 (Tabasi, Noorbakhsh, & Sharifi, 2017). Multiple signal amplification was achieved by methylene blue (electrochemical probe) through its accumulation to the modified electrode surface via both affinity interaction to aptamer molecules and electrostatic adsorption to the HER2 analyte as well as high charge transfer kinetic properties of the applied rGO-Chit film.

Another study reported photoelectrochemical (PEC) sensor for HER2 by utilizing hexagonal carbon nitride tubes (HCNT) as photoactive material (Luo, Liang, Qiu, & Yang, 2019). Gold nanoparticles (AuNPs) were deposited onto the surface of the HCNT to enhance the photocurrent intensity of the HCNT. The detection is based on suppression of the PEC current intensity of the sensor from deposition of molybdophosphate precipitate to the surface of HCNT. The molybdophosphate precipitate was produced from reaction of phosphate groups on HER2-bound aptamer with molybdate..

Figure 2.

3.3. Electrochemical aptasensors based on “target sandwich” approaches

Another routinely used approach of aptasensing is sandwich format that work by sandwiching a target marker between a capture probe coated onto electrode surface and a signal probe carrying moiety for electrochemical reaction. However, modifying the electrode surfaces with single stranded aptamers are prone to tangle and aggregation with each other due to its flexibility, and precise control on distance between coated aptamers is difficult. To overcome these, instead of immobilizing aptamer directly to the electrode surface, prior

coating of electrode surfaces with advanced nanomaterials having high surface-to-volume ratio or with DNA tetrahedron having well-organized spatial orientation allows immobilisation of much more capture probes on electrode surface and increases the sensitivity of the proposed aptasensor. Use of carbon nanotubes, graphene and graphene oxide, gold nanoparticles, silver nanoparticles, metal oxide nanomaterials and quantum dots have been reported in developing aptasensors with improved target sensitivity (Hernandez & Ozalp, 2012; Jo & Ban, 2016; Urmann, Modrejewski, Scheper, & Walter, 2017).

A simplest sandwich aptasensor for epidermal growth factor receptor (EGFR) detection was developed by (Ilkhani, Sarparast, Noori, Zahra Bathaie, & Mousavi, 2015). The biotinylated EGFR aptamer immobilized on streptavidin-coated magnetic beads (MB) was used as a capture probe, and a polyclonal EGFR antibody conjugated to citrate-coated gold nanoparticles was used as a signalling probe. The presence of EGFR causes formation of a sandwich structure on the MB surface that corresponds to a target-depended DPV signal.

Another aptasensor based on a sandwich format for HER2 analysis was introduced by Chen et al., using a DNA tetrahedron with pendant aptamer as recognition element recognize and bind to HER2, and the bioconjugate of gold nanorods-Pd carrying the HER2 aptamer as the signal nanoprobe for detection via sandwiching and signal amplification (Chen et al., 2019).

Salimian et al., developed an electrocatalytically amplified assay for HER2 by co-adsorbing thiolated HER-2 aptamer with C11alkanethiol bearing two ethylene-glycol (EG)2 head groups (surface blocker) on gold electrode and carrying out the electrochemical reduction of a ferricyanide redox indicator by methylene blue electrostatically interacting with negatively charged HER-2 (Salimian, Kékedy-Nagy, & Ferapontova, 2017). Similarly, Ou et al., devised a sandwich-type electrochemical aptasensor for HER2 based on tetrahedral DNA nanostructures (TDNs) - HER aptamer-1 capture probe, and flower-like Mn_3O_4

nanoparticles decorated with Pd-Pt nanozymes, horseradish peroxidase and HER2 aptamer-2 signal nanoprobes (Ou et al., 2019). The aptasensor was constructed by attaching the thiolated TDNs of HER2 aptamer-1 to the gold electrode surface blocked with 6-mercapto-1-hexanol. The captured HER2 on electrode surface was then sandwiched with a signal nanoprobe consist of HRP-decorated Mn_3O_4/Pd-Pt nanocarrier and a signal amplifier nanoprobe made of Pd-Pt/HRP/aptamer complementary DNA to catalyze the oxidation of hydroquinone to benzoquinone for amplified electrochemical signals that relates directly to the amount of captured HER2.

Figure 3.

3.4. Electrochemical aptasensors based on other approaches

Besides the commonly used direct and sandwich format assay platforms, few studies reported use of novel approaches that allow sensitive detection of target markers using different combination of nanomaterials and signal retrieval methods. One of such methods based on exonuclease recycling amplification and host-guest recognition was suggested by (Yang, You, Zhang, Wang, & He, 2018). The authors fabricated a sensitive electrochemical aptasensing platform for the detection of HER2 by hybridizing HER2 aptamer with ferrocene-labelled DNA/Au nanospheres (FcNS), and then bound with the target HER2. The released FcNS homogeneously hybridized with horseradish peroxidase-labelled DNA/Au nanospheres (HRPNS). Benefiting from the introduction of RecJf exonuclease, HER2 was recycled as the degradation of aptamer and bound another aptamer connected on FcNS. Thus, FcNS/HRPNS in large amounts was generated and captured by the modified Au electrode through the host-guest recognition between beta-cyclodextrin (β-CD) and ferrocene (Fc).

Horseradish peroxidase (HRP) catalyzed o-PD in presence of H_2O_2, producing a significantly amplified signal.

Figure 4.

Table 4.

4. Aptamer-based optical methods for ER/HER2 detection

Optical sensing is the most widely used method for measurement of analytes in simple transparent liquid samples. The optical biosensors are very common due to their real-time detection characteristic along with advantages of high specificity, sensitivity, small size and cost-effectiveness. However, they are used mostly in transparent liquids and analysis of analytes in complex and non-transparent samples preferably require electrochemical and mass-sensitive aptasensors. Optical aptasensors are constructed by compacting aptamers with optical transducer like interferometers, resonators, gratings, refractometers, and surface plasmon resonance (SPR) to measure absorbance, reflectance or fluorescence emissions that correlates with analyte concentrations (Damborský, Švitel, & Katrlík, 2016). Many optical methods of aptasensing ER and HER2 have been reported in the literature. These include fluorometric and colorimetric methods based on enzyme linked immunosorbent assay (ELISA), lateral flow assay, imaging, nanoparticle beacons, lateral surface plasmon resonance (LSPR) and other for the detection of different cancer biomarkers.

4.1. Fluorometric methods of aptasensing

High sensitivity of fluorescent based methods has attracted extensive attention in the field of aptasensing. Methods based on fluorophore labels and label-free (based on novel nanomaterials) strategies have been used widely in aptasensing of diverse biomolecules

including the breast cancer markers. Fabricating fluorescence based methods require choosing an appropriate fluorophore amongst organic dyes, metal ions and noble metal nanoclusters. Various fluorescent dyes such as fluorescein, fluorescein isothiocyanate, cyanine dyes Cy3 and Cy5, Rhodamine B, and 5-carboxyfluorescein are reported in development of aptasensors due to its convenience of labelling with the aptamers and choices of different pair of fluorophores and quencher. Among the key strategy reported for converting aptamer–ligand binding into fluorescent signals for ER and HER2 detection is the nanomaterials beacons constructed using carbon, gold, silver, graphite oxide and silica based nanoparticles, nanoclusters and/or nanosheets (Figure 5) (Jo, Her, & Ban, 2015; Wu, Li, & Liu, 2015; Jiayao Xu et al., 2019; Zhang et al., 2019). These nanoparticle beacons use fluorescence resonance energy transfer (FRET) or autofluorescence based approaches to allow sensitive detection by fluorescence quenching or fluorescence recovery/enhancement. In the FRET based nanoparticle beacon, the close proximity of the fluorophore and quencher in absence of target analyte kept the fluorescence intensity at minimal level, until the addition of analyte which results in change in aptamer configuration, displacing fluorophore and quencher far apart to inhibit FRET, allowing recover in fluorescence intensity (Wu et al., 2015; Jiayao Xu et al., 2019). In autofluorescence based approaches, the proximity of nanoparticles to DNA sequences affects the natural fluorescence of nanoparticles to allow sensitive detection (Zhang et al., 2019).

Xu et al., demonstrated sensitive aptamer-based fluorometric method (FRET based) for simultaneous detection of multiple breast cancer markers using graphene oxide (GO) nanomaterials having excellent fluorescence quenching properties (Xu, Chen, Shi, & Huang, 2019). The GO-based four-colour nanoprobe aptasensor for HER2 and other markers including alpha-fetoprotein, vascular endothelial growth factor-165, and carcinoembryonic antigen was developed by adsorbing target-aptamers labelled with different dyes (FAM, Cy5,

Cy3, AF405) onto GO sheets, where the fluorescence of all dyes was very weak (OFF state). Treating the timorous cells with this nanoprobe internalizing it into cells, causing the labelled aptamers to dissociate from GO and bind individual target proteins, producing target-specific fluorescence (ON state) (J. Xu et al., 2019).

As the modification of aptamers with organic fluorescent dyes is expensive and time-consuming processes, which also suffers low quantum yield (low fluorescence intensity) and rapid photo bleaching, and labelling with fluorophores and quenchers may also affect the aptamers affinity and sensitivity for its target, the use of nanomaterials made of silver, gold, carbon, and grapheme oxide have been shown to overcome most of the limitations of conventional organic dye based fluorescence measurement.

Silver nanoparticles or nanoclusters (AgNPs/ AgNCs) have also attracted great attention in biosensing and bioimaging applications due to its good biocompatibility, sub-nanometre size, high stability and photostability, low toxicity, and strong fluorescence (Lin et al., 2009). Though the brightness of DNA conjugated AgNCs is combatively weak than other commonly used fluorescence probes, few reports suggest drastic improvement in the fluorescence emission of DNA conjugated AgNCs by bringing them in proximity to guanine bases or G-rich sequence (Yeh et al., 2012). Using the same phenomenon, Zhang et al., developed a label-free aptasensor using AgNC-coated aptamer/G-rich sequences for the fluorescence “turn on” detection of HER2 (Zhang et al., 2019). The AgNCs and template G-rich sequence were used as fluorescent probe and signal enhancer in the developed HER2 aptasensor that may allow point-of-care monitoring of HER in patient serum samples for disease progression monitoring.

Dye-doped silica nanoparticles (SiNPs) offer high photostability, cost effective synthesis, great fluorescent signal, and good biocompatibility required for in vivo imaging and biosensing applications (Wang, He, Yang, & Shi, 2013). SiNPs doped with a variety of

dyes, such as Tris(bipyridine)ruthenium(II) chloride ($[Ru(bpy)_3]^{2+}$), fluorescein isothiocyanate (FITC), and Rhodamine B-d have been reported used for live cell and in vivo imaging. $Ru(bpy)_3$-doped SiNPs which have been previously shown to resist photo bleaching of $Ru(bpy)_3$ dye based on coulombic interaction between positively charged dye and negatively charged SiNPs was used to construct a dual aptamer-modified SiNP- based sensing method for simultaneous detection of HER2 and MUC1 (Jo et al., 2015). The nanoparticle based aptasensing methods was designed by co-immobilizing biotinylated HER2/MUC1 aptamers onto dye-doped PEG coated SiNPs (dual SiNP) and magnetic streptavidin beads (dual-MB). The dual-MB were used to first separate HER2/MUC1 positive and negative breast cancer cells, and then incubated with dual-SiNPs, wherein fluorescence intensities of each type of magnetic bead-cell-SiNP complex were measured to detect and quantify the HER2/MUC1 breast cancer cells.

Besides, multiple direct aptasensing approaches based on fluorescent activated cell sorting, immunocytochemistry and immunohistochemistry and ELISA to detect or visualize the ERs/ ER+ve cells in breast cancer samples using the fluorescence labelled or biotinylated aptamer have also been reported by multiple groups (R. Ahirwar et al., 2016; He et al., 2015; Sett, Borthakur, Sharma, Kataki, & Bora, 2017).

Figure 5.

4.2. Colorimetric aptasensors based on LSPR and chemiluminescence

Colorimetric methods allow biomarker detection based on changes in color which is visible to naked eyes or can be quantitatively measured by simple portable optical detectors. Conventional methods based on ELISA and IHC, and recent nanomaterials based methods such as localized surface plasmon resonance (LSPR) are prominent colorimetric approaches

used in colorimetric aptasensing of ERs and HER2. LSPR is the response of surface electrons on AuNPs to an external electromagnetic excitation. Localized surface plasmon resonance based aptasensors utilize the inter-particle distance-dependent color transition property of AuNPs. The AuNPs at nanomolar concentrations exhibits vibrant colours in visible region due to very high molar extinction coefficients (107–1011), for example, 10–50 nm AuNPs are bright ruby red with absorption maxima at 520 nm, and the same AuNPs upon aggregating appear pale blue or purple, with absorption maxima above 700 nm. This change in physical state as well as colour of AuNPs can be effectively brought about by capping the AuNPs with aptamers that prevent AuNP aggregation via electrostatic repulsion, but in presence of the ligand, it detaches from nanoparticles and allows nanoparticle aggregation in the presence of an inducer such as salt. A colorimetric aptasensors based on LSPR-based color transition of AuNPs was reported for ERα detection in cellular extracts of breast cancer cells (Rajesh Ahirwar & Nahar, 2016). The aptasensor was constructed by electrostatic-coating of AuNPs with a 17-nt RNA aptamer that prevented the aggregation of AuNPs at high salt concentrations (150 mM) until availability of target ERα protein that binds the aptamers detaching them from nanoparticles, and making the nanoparticles susceptible to salt induced aggregation. The spontaneous change in color of AuNPs from wine red to purple allowed the necked eyes-based assessment of assay outcome. The same approach was used by Ranganathan et al. to detect HER2 in a plate-based and a lateral-flow assay format (Ranganathan, Srinivasan, Singh, & DeRosa, 2020).

Figure 6.

Table 5.

Conclusions and future perspective

Identification of aptamers to a vast variety of targets such as multiple cancer biomarkers has fuelled rapid development of aptamer-based methods such as ELISA, lateral flow assays, blotting techniques, immunocytochemistry, immunohistochemistry, and targeted drug delivery vehicles for molecular diagnostics, research and therapeutic applications. These methods represented alternatives to the conventional antibody-based methods for the functional similarity of aptamers to the antibodies. Recently, the focus of aptamer-based diagnostics has turned to biosensing applications because of substantial progress in miniaturisation and micro fabrication techniques, and use of novel nanomaterials in lab-on-chip devices. Biosensors for routine biomedical diagnosis and point-of-care monitoring of treatment and disease progression are pivotal for a proactive healthcare system where an individual can monitor his/her health status on a real-time basis to detect possible diseases at an early stage in a comfortable environment outside of clinical setting. This review summarized the key developments in aptamer-based methods for rapid, sensitive and hassle-free detection of ERs and HER2 in breast cancer samples. Most of the reported electrochemical methods of ER/HER2 aptasensing are based on (i) ON/OFF approaches based on processing of electrochemical signal from weak to strong and vice-versa before and after target recognition, and (ii) target sandwich approach in which the target (biomarker) is sandwiched between a capture probe coated onto electrode surface and a signal probe carrying moiety for electrochemical reaction. The capture and signal probe often use either antibody or a peptide with an aptamer. On the other hand, the key optical approaches of aptasensing are nanoparticle beacons, LSPR, and aptamer-assisted ELISA and IHC assays. The nanomaterial beacons are nanoparticles, nanoclusters or nanosheets made of materials like carbon, silica, gold, silver, and graphene oxide which use fluorescence resonance energy transfer (FRET) or autofluorescence based approaches for sensitive detection by fluorescence quenching or fluorescence recovery/enhancement. The AuNPs LSPR is based on the

size/aggregation dependent response of surface electrons on AuNPs to an external electromagnetic excitation. Colorimetric assays based on AuNPs LSPR use color transition property (wine red in dispersed form, violet/blue upon aggregation) to monitor or quantify a target. The aptamer-assisted ELISA and IHC uses biotin/fluorophore labelled aptamers in place of antibodies in the conventional assays. Despite the reported approaches in this review provides efficient means to detect breast cancer biomarker ERs and HER2 in the patient serum or biopsied tissues, the real challenge is to derive and develop methods that allows early diagnosis of the disease before clinical appearance of the disease symptoms. The bottleneck for this is the inability of available biomarkers, due to invasive collection of biomarker, to allow detection of breast cancer at an early stage. Secondly, testing the aptamers on next generation biosensors, i.e. smart wireless nanosensors such as the "diabetes patch" developed by Lee et al, for continuous sweat-based diabetes monitoring and feedback therapy. This wearable patch (skin-mounted) of graphene-hybrid device has humidity, glucose and pH sensors (for sweat-based glucose and pH monitoring), a heater, temperature, and polymeric microneedles (for controlled transcutaneous drug delivery through bioresorbable temperature responsive microneedles), and wireless connectivity to a portable devices like Smartphone for continuous monitoring and control (Lee et al., 2016). Testing biorecognition capabilities of aptamers on smart nanosensors empowered with impeccably small dimensions, biocompatibility, wireless connectivity and memory, and automated decision-making ability would allow true representation of their clinical utility for futuristic remote healthcare monitoring applications. To improve the quality of life and overall survival among breast cancer patients through real-time monitoring of disease parameters via smart nanosensors would require combined efforts of multiple disciplines, particularly molecular biology, nanotechnology and electrical engineering to develop impeccable devices for futuristic aptamer-based remote health care monitoring.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

R.A. conceptualized the study, collected data from literature and wrote manuscript. SK reviewed and edited the manuscript. KA helped in data collection from literature. All authors made a substantial, direct and intellectual contribution to the work.

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Tables

Table 1. List of widely investigated tissue-based and serum-based tumours markers for breast cancer (Inwald et al., 2013; Kabel, 2017; Sturgeon et al., 2008; Weigel & Dowsett, 2010).

Biomarker	Biomarker description	Proposed Use
ERs	Steroid hormone (estrogen) nuclear receptor protein	Prognosis (in combination to other factors) and predicting response to hormone therapy
PRs	Steroid hormone (progesterone) nuclear receptor protein	Predicting response to hormone therapy
HER2	Transmembrane receptor tyrosine kinase protein	Prognosis (most useful in lymph node-positive cases), and predicting resistance to tamoxifen and CMF and response to anthracyline-based therapy in early breast cancer
uPA and PAI-1	Serine protease (plasminogen → plasmin) and its inhibitor	Determining prognosis, and predicting resistance to hormone therapy in advanced cancer and response to chemotherapy in early cancer
p53	Tumour suppressor nuclear protein	Prognosis and predicting response to chemotherapy or hormone therapy
Nestin	Intermediate filament protein	Prognosis, diagnosis and management of aggressive forms of breast cancer
Cyclin D1 and cyclin E	Regulatory protein of the G1–S transition (cell cycle) in somatic cells	Prognosis
Cathepsin D	An aspartic endo-protease found in lysosomes	Prognosis
MicroRNAs	Small noncoding RNA nucleotide (18 – 28 long) regulating protein expression at posttranscriptional level	Prognosis
Multigene signatures	Cancer-related genes	*Oncotype DX* (21 genes): Predicting recurrence in node-negative, ER-positive patients receiving tamoxifen and predicting chemotherapy benefit in node-negative ER-positive patients. *MammaPrint* (70 genes): predicting recurrence within 5 years in all node-negative and node-positive patients. *Genomic-grade index* (97 genes): Prognosis and prediction of relapse in

		endocrine treated ER-positive breast cancer. *Rotterdam signature* (76 genes): Prognostic for development of distant metastases within 5 years.
Ki-67	Non-histone nuclear protein	Prognosis
CA 15-3 and 27.29	Glycosylated transmembrane proteins (mucins)	Prognosis, monitoring therapy in advanced disease and post-operative surveillance
CEA	Cell surface glycoproteins	Prognosis, monitoring therapy in advanced disease (especially if CA 15-3/27.29 is not elevated) and post-operative surveillance
BRCA1 and *BRCA2*	Human tumour suppressor gene responsible for repairing DNA	Identifying individuals who are at high risk of developing breast cancer in high risk families
Circulating tumour cells		Prognosis, staging and monitoring therapy in advanced disease

ER: estrogen receptors, PR: progesterone receptors, HER2: human epidermal growth factor receptor 2, uPA: urokinase plasminogen activator, PAI: plasminogen activator inhibitor, CEA: carcinoembryonic antigen, BRCA: Breast Cancer gene.

Table 2. List of selective conventional and emerging methods for ER and HER2 detection in breast cancer samples

Analyte	**Method**	**Linearity** (ng mL^{-1})	**LOD** (ng mL^{-1})	**Reference**
ER	RIA	NR	50 fmol/mg	(Gehrig, Van Le, Olatidoye, & Geradts, 1999)
ER	ELISA	10 – 1000 fmol mg^{-1}	NR	(Leclercq et al., 1986)
ER	ELISA	Up to 400 fmol mg^{-1}	NR	(Nicholson et al., 1986)
Serum HER2	ELISA	2.2 – 29.2	NR	(Tchou et al., 2015)
Tissue HER2	ELISA	0 – 10.4	NR	(Tchou et al., 2015)
HER2	ELISA	0.2 –12.50	0.75	(Agnolon et al., 2020)
ERs	Immunosensing	0.1–200 nM	0.034 nM	(Li, Hu, Zhang, Zhang, & Yang, 2019)
HER2	Immunosensing	10-110	7.4	(Arkan, Saber, Karimi, & Shamsipur, 2015)
HER2	Immunosensing	0.0005 –50	2×10^{-5}	(Shamsipur, Emami, Farzin,

				& Saber, 2018)
HER2	Immunosensing	1–100	0.28	(Lah, Ahmad, Zaini, & Kamarudin, 2019)
ER	Immunosensing	0.5 – 100 nM	0.38 nM	(Zhu, Cao, Xu, Yin, & Li, 2013)
HER2	Mass spectrometry	0.012 – 100 fmol	0.155 fmol	(Steiner et al., 2015)
ER	Mass spectrometry	7 – 50000 fmol mg^{-1}	7 fmol mg^{-1}	(Schoenherr et al., 2012)
HER2	Mass spectrometry	6 – 50000 fmol mg^{-1}	6 fmol mg^{-1}	(Schoenherr et al., 2012)
ERs/HER2	Aptasensing	*See Table 4 and Table 5*		

Table 3. Comparison of various conventional and emerging methods of ERs/HER2 sensing .

Method	**Advantages**	**Disadvantages**
Radioimmunoassay	Highly sensitive, highly accurate	Hazard to health from exposure to harmful radiation from radioactive materials, requirement of large amount of fresh tissues.
Enzyme-linked immunosorbent assay	FDA approved, minimally invasive, quantitative assessment, allow monitoring changes in HER2 status following treatment	Results not reliable in patient receiving transtuzumab treatment, costly and time consuming.
Blotting techniques (Southern, Northern and Western)	Northern and Southern blot are cost effective methods, Western blot have high sensitivity	Not applicable in routine diagnostics due to time consuming procedures, does not allow morphologic preservation of tissue required for histological feature evaluation, source cells (normal vs. neoplastic) cannot be differentiated.
Immunohistochemistry	easy to perform and relatively inexpensive, allow visualizing tissue morphology as well location of ER/HER positive cells	Assay accuracy can be affected by fixative, duration of fixation, antigen retrieval method, and antibody batch-variability; semi quantitative scoring based interpretation produce equivocal results.
FISH, RT-PCR, and microarray	RT-PCR allow rapid, accurate and quantitative analysis,	RT-PCR not FDA approved, but FISH approved as diagnostic tool, FISH is time consuming protocol, costly methods.
LC-MS	Allow multiplexed analysis, good performance, and can be easily transferred	Not useful for biomarkers whose diagnostic utility is based not on total protein abundance but rather on mislocalization. May not distinguish

	across laboratories	distinguish between tumours containing a small subpopulation of biomarker-positive cells versus tumours showing low level biomarker expression in 1all the cells.
Immunosensing	No need of aseptic working space or trained personnel; Very fast; Inexpensive; Easy to perform; Very low reagent consumption; Portable; Multiplexing possible	High cost of antibodies and susceptibility to denaturation.

Table 4

Comparison of various electrochemical aptasensors for detection of ER/HER2 biomarkers in breast cancer tumour

Transducer	Analyte	Aptamer sequence	Detection method	Linearity (ng/mL)	LOD (ng/mL)	Assay time	Reference
Au electrode/TDN-Mn_3O_4/Pd-Pt/HRP	HER2	gcagcggtgtgggg	DPV	0.1–100	0.08	1.5 h	(Ou et al., 2019)
ID-Au micro electrode	HER2	aaccgcccaaatccctaagagtctgcacttgtcattttgtatatgtatttggtttttggctctcacagacacactacacacgcaca	Capacitance	0.1–1000	0.1	2 h	(Arya et al., 2018)
Glassy carbon-rGO–Chit-GLA	HER2	tttttaattaagccgcgaggggagggatagggtagggcgcggct	DPV	0.5–2; 2–75	0.21	1 h	(Tabasi et al., 2017)
ID-Au micro electrode	HER2	gggccgtcgaacacgagcatggtgcgtggacctaggatgacctgagtactgtcc	Capacitance	0.2–2	0.2	1 h	(Qureshi, Gurbuz, & Niazi, 2015)
AuNPs	HER2	tggggcctggatacggattggtaaggattagtagggggcatagct	CV, EIS	10^{-5}–100	5	15 min	(Chun et al., 2013)
Gold electrode / HER2p	HER2	gcagcggtgtggggtatcgttaattcggtcg	EIS	0.001–0.1	$4.7x10^{-5}$	2 h	(Shen et al., 2017)
Gold electrode/ HER2p/ MCH/ molybdate	HER2	gcagcggtgtggggtatcgttaattcggtcg, andgcagcggtgtgggg	CV	0.01–5	0.005		(Hu et al., 2017)
Gold nanorods@Pd/	HER2	ttttttaaccgcccaaatccctaagagtctgcacttgtcattttgtatatgtatttggtttttggctct	CV, DPV, EIS	10–200	0.15	1 h	(Chen et al., 2019)

HRP/BSA		cacagacacactacacacgcaca					
Screen printed gold electrode	ERα	ataccagcttattcaattcgttgcatttaggtgc attacgggggttatccgctctctcagatagtat gtgcaatca	DPV	10^{-3}–1000	0.001	10 min	(R. Ahirwar et al., 2019)
Dopamine-coated gold nanorods/ mgPDMS	HER2	t*cct*ggcat*gt*t*cgat*ggaggcct* t*t*gat*t*acagcccaga	SPR	5–30	5	1– 2 h	(J.-H. Kim, Suh, & Yang, 2020)
Screen-printed carbon electrode/ poly l-lysine	HER2	gggagauaccagcuuauucaauuugga uggggagauccguugaguaagcgggcg ugucucucugccgccuugcuaugggga gauaguaagugcaaucu	DPV	10–60	3	4 min	(Bezerra et al., 2019)
Gold electrode/ PEG/MB	HER2	gcagcggtgtgggggcagcggtgtggggg cagcggtgtggggttttt	CV	10^{-12}–10^{-8} M	10^{-12} M	30 min	(Salimian et al., 2017)
Carbon dots@ZrHf-MOF	HER2	gggccgtcgaacacgagcatggtgcgtgg acctaggatgacctgagtactgtcc	EIS, DPV	10^{-4}–10; 10^{2}–10^{5} cell/mL	19 x10^{-6} ; 23 cell/ml	10 min	(Gu et al., 2019)
WS_2 NW/TM electrode	HER2	gcagcggtgtgggg	EIS	0.5–10	0.36	4.5 h	(Guo, Liu, Yang, Du, & Qu, 2019)
Glassy carbon/ ErGO-SWCNT/ AuNPs electrode	HER2	aaccgcccaaatccctaagagtctgcacttg tcattttgtatatgtatttggtttttggctctcaca gacacactacacacgcaca	CV, DPV, EIS	0.0001–1	0.00005	40 min	(Rostamabadi & Heydari-Bafrooei, 2019)
Gold electrode/ MCH/ MnO_2 nanosheet	HER2	gcagcggtgtgggg	CV	0.0001–0.5	0.00005	3–4 h	(Chai et al., 2019)
Au electrode / Au NPs/β-CD/ HT	HER2	aaagtaaaagaactgatcagcacgggatgg gatagggaggggagtgttgaaaa	DPV	10–150	4.9	5 h	(Yang et al.,

							2018)
Gold electrode/ MnFePBA@Au NP	HER2/ MCF-7	gggccgtcgaacacgagcatggtgcgtgg acctaggatgacctgagtactgtcc	EIS	0.001–1; 500–5x10^4 cells/ mL	0.00025; 36 cells/ mL	2–4 h	(Zhou et al., 2019)
Glassy carbon electrode/ HCNT/ AuNP/ MCH/ HER2	HER2	gcagcggtgtgggg	CV	0.5–1	0.00008	4 h	(Luo et al., 2019)
ZnO/graphene/ ITO electrode/ AuNP	HER2 (SK-BR-3)	uggaugggagauccguugaguaagcg ggcgugucucucugccgccuugcuaug ggg	EIS	10^{-2}–10^{-6} cells/mL	58 cells/mL	1 h	(F. Liu et al., 2014)
MB-Apt/EGFR/ Ab-AuNP	EGFR	ggcgcuccgaccuuagucucugugccgc uauaaugcacggauuuaaucgccguaga aaagcaugucaaagccggaaccguguag cacagcaga	DPV	1–40	0.05	1 h	(Ilkhani et al., 2015)

ID-Au micro electrode: Interdigitated gold microelectrode, rGO–Chit: Reduced graphene oxide-chitosan, TDN: tetrahedral DNA nanostructure, DPV: differential pulse voltammetry, CV: cyclic voltammetry, EIS: electrochemical impedance spectroscopy, HER2p: HER2 specific peptide; mgPDMS: micro-grooving PDMS substrates; ZrHf-MOF: metal–organic framework; Zr: Zirconium; Pd: Palladium; WS2 NW/TM: tungsten sulphide nanowire array on Ti mesh; Ad-De: adsorption-desorption; β-CD: beta-cyclodextrin; HT: hexanethiol; CTC: circulating tumour cells; HCNT: hexagonal carbon nitride tubes; AFP: alpha-fetoprotein; $VEGF_{165}$: vascular endothelial growth factor-165; CEA: carcinoembryonic antigen; EGFR: Epidermal growth factor receptor; t^*indicates 5-(N-napthylcarboxyamide)-2'-deoxyuridine, modified thymine.

Table 5. Comparison of various optical methods of ER/HER2 aptasensing

Fluorescence/ color source	Analyte	Aptamer sequence	Detection method	Linearity range (ng/mL)	Sensitivity (ng/mL)	Assay time	Reference
GO nanoprobe	HER2	aaccgcccaaatccctaagagtctgcacttgtcattttgtatatgtatttggtttttggctctcacagacacactacacacgcaca	Fluorescence	1.2–240 nM	0.96 nM	8 h	(Jiayao Xu et al., 2019)
AgNC	HER2	ggggtgtggcgacg	Fluorescence	8.5–225 fM	0.09 fM	20 min	(Zhang et al., 2019)
SiNPs	HER2	aaccgcccaaatccctaagagtctgcacttgtcattttgtatatgtatttggtttttggctctcacagacacactacacacgcaca	Fluorescence	1–10^3 cells/100 µL	1 cell/100 µL	2 h	(Jo et al., 2015)
CNPs/ UCP	CEA	ataccagcttattcaatt	FRET	0.1–40	NR	3 h	(Wu et al., 2015)
AuNPs	ERα	ggggucaaggugacccc	Spectrophotometry, Naked Eye	10–5000	0.64	20 min	(Rajesh Ahirwar & Nahar, 2016)
AuNPs	HER2	tctaaaaggattcttcccaaggggatccaattcaaacagc; AND gcagcggtgtgggggcagcggtgtgggggcagcggtgtgggg	Spectrophotometry, Lateral flow, Naked Eye	12–49 nM	10 nM	60 min	(Ranganathan et al., 2020)
Fluorescein, biotin	ERα	ataccagcttattcaattcgttgcatttaggtgcattacggggttatccgctctctcagatagtatgtgcaatca	Fluorescence (FACS, IF-ICC, IHC)	NA	NA	30 min – 2 h	(R. Ahirwar et al., 2016)
Fluorescein, biotin	ERα	cccggcatggttgcggagcaggagtataacactaccattg	FACS, ICC, IHC	NA	NA	30 min-2 h	(Sett et al., 2017)
Biotin	ERα	gtcaggtcacagtgacctgatcaaagttaatg	IHC	NA	NA	12 h	(He et al.,

	2015)

GO: gold nanoparticles, AgNC: silver nanoclusters, SiNPs: silica nanoparticles, CNP: carbon nanoparticles, UCP: upconversion phosphors, AuNP: gold nanoparticles, FACS: fluorescence activated cell sorting, IF-IHC: immunofluorescence based immunohistochemistry

Figure Legends

Figure 1. Schematic representation of various components of a biosensor. Biorecognition (the process of signal generation in form of light, heat, pH, charge or mass change), signalisation (conversion of received biorecognition signal into optical or electronic signal), and quantification (convert processed signal into user understandable values) are the three major processes of biosensing. The figure was obtained with permissions from (Bhalla et al., 2016).

Figure 2. Electrochemical approaches based on the ON/OFF strategy reported for aptasensing of ERs and HER2 biomarkers.

Figure 3. Electrochemical sensors developed for HER2 based on sandwich format approach. Image was obtained with permission from (Chen et al., 2019; Ilkhani et al., 2015).

Figure 4. The exonuclease recycling amplification and host-guest recognition based electrochemical aptasensing platform. Image was obtained with permission from (Yang et al., 2018).

Figure 5. Fluorescence-based optical aptasensors for ERs/HER2. Reproduced from (Jo et al., 2015; Wu et al., 2015; Jiayao Xu et al., 2019; Zhang et al., 2019).

Figure 6. Colorimetric aptasensing strategies based on AuNP LSPR for ERs/HER2 detection. The images were produced with permission from (Rajesh Ahirwar & Nahar, 2016; Ranganathan et al., 2020).