Aptamers as Theragnostic Tools in Prostate Cancer

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Aptamers are DNA and RNA oligonucleotides that can adopt tridimensional structures that enable them to join specifically to any desired target. Aptamers are capable of binding to specific molecules including drugs, proteins, carbohydrates, cells, and viruses. Aptamers were first described in 1990, and since then several groups have used their binding properties to isolate a diversity of specific aptamers. Aptamers have been studied for treatment and detection of many diseases including cancer. In Prostate Cancer, numerous works have reported their use in the development of new approaches in diagnostics and treatment strategies. Aptamers have been joined with drugs or other specific molecules such as silencing RNAs (aptamer–siRNA chimeras) to specifically reduce the expression of oncogenes in prostate cancer (PCa) cells. These studies have shown good results in the early stages, more research is still needed to demonstrate the clinical value of aptamers in PCa.

Keywords: prostate cancer ; aptamers ; PCa diagnosis ; PCa treatment

1. Aptamers in Cancer

Since aptamers can recognize targets and modulate biological activities with higher specificity and affinity than antibodies, it is not surprising that these molecules are considered promising therapeutic tools for the treatment of different types of cancer. Importantly, unlike antibodies, aptamers have low toxicity, are non-immunogenic, and they can easily penetrate the tumor core because of their smaller size [1]. Therefore, a 3-fold increase in the number of articles on aptamers in cancer research has been reported in the last four years (**Figure 1**A). Between 2012 and 2013 the number of articles using aptamers was less than 100, whereas from 2019 to 2021 it was higher than 300. Notably, more than 22% of the articles reporting the use of aptamers are used in breast cancer, followed by aptamer applications in prostate cancer (PCa) and lung cancer (**Figure 1**B).

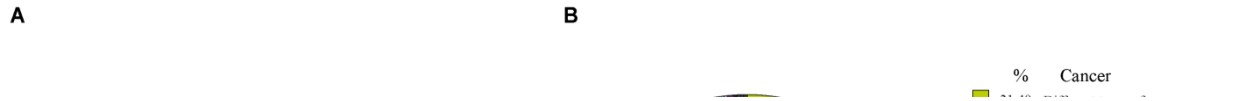

Figure 1. Aptamers in cancer research. (**A**) Yearly trend of published articles reporting the application of aptamers in cancer research. (**B**) Proportion of types of cancer in which aptamers are most frequently used for therapeutic or diagnostic purposes.

Numerous works have suggested that aptamers can be used in cancer therapy as inhibitors of growth factors or oncoproteins, as delivery methods for anti-cancer drugs or siRNAs into tumor cells, or even as immune stimulators to fight cancer [2][3][4]; however, most of them are still in a preclinical stage. Noteworthy, the aptamers AS1411 and NOX-A12 are the most advanced aptamer-based therapies for leukemia undergoing clinical research [5]. AS1411 is a guanine-rich 26-base quadruplex DNA aptamer targeting nucleolin (also called C23) [6]. Although nucleolin is found in the cell membrane,

it is mainly present in the nucleolus, and high expression of cell surface nucleolin is associated with poor prognosis and higher risk of metastasis [7]. The binding of AS1411 to nucleolin inhibits DNA synthesis that leads to destabilization of BCL2 mRNA and apoptosis [8]. AS1411 is currently undergoing phase II clinical trials for acute myeloid leukemia (AML) and metastatic renal cell carcinoma [9][8][10]. The NOX-A12 aptamer is capable of targeting CXCL12, a chemokine that promotes homing and retention of leukemia cells [10]. Treatment with NOX-A12 sensitizes leukemia cells to conventional therapies and, in combination with bendamustine/rituximab, it improves the therapeutic response in patients with chronic lymphocytic leukemia and multiple myeloma [10].

In addition, the aptamer A30, which binds to the extracellular domain of the human epidermal growth factor receptor-3 (HER3) and does reduce cell proliferation by inhibiting heregulin (HRG) signaling, is under investigation in breast cancer therapy. The combination of A30 aptamer with siRNAs against EEF2, PLK-1, GRK4, and SKIP5, induced specific gene silencing and suppressed cell proliferation. Since the aptamer–siRNA chimera was taken up specifically by HER3-expressing breast cancer cells [11], this aptamer is a promising candidate in breast cancer treatment.

To note, the capacity of aptamers as drug carriers for cancer cells is also a matter of extensive research. Thus, the A10 aptamer that binds to Prostate-Specific Membrane Antigen (PSMA) has been conjugated with doxorubicin to confer both high affinity and specificity against prostate cancer cells. This efficient drug-delivery aptamer significantly inhibited cell proliferation of PSMA-positive cells [12]. Similarly, for AML, aptamer–drug conjugates have been developed. Specifically, aptamer–drug conjugates with methotrexate (Apt-MTX) were able to inhibit AML cell growth, trigger cell apoptosis, and induce cell cycle arrest in the G1 phase in a highly specific manner. Trials with human bone marrow specimens demonstrated that this aptamer–drug conjugate induced selective growth inhibition of primary AML cells without toxicity in normal marrow cells after Apt-MTX exposure. Overall, these findings demonstrate the potential clinical value of Apt-MTX for targeting AML [13]. Another chimeric aptamer siRNA targeting BCL-2 was bound to doxorubicin (siRNA-Dox). siRNA-Dox increased sensitivity of cells to apoptosis and, in turn, decreased cell viability in multi-drug-resistant MCF-7 breast cancer cells [14].

Moreover, aptamers can be used as biosensors for cancer detection. For example, an electrochemical apta-sensor against mucin-1 (MUC1) was recently developed. MUC1 is a surface glycan highly expressed in cancer cells. Voltage changes induced by the chemical reaction between the aptamer conjugated to magnetic beads and gold reduction allow cancer detection by electrochemical analysis [15]. Aptamers can also be used in the field of circulating tumor cells (CTCs). The aptamer BC-15 has been used to specifically identify rare CTCs out of background nucleated cells. This aptamer showed high affinity for nuclei of different human cancer cell lines as well as CTCs isolated from pancreatic cancer patients. The target of the BC-15 aptamer is the heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1). Overexpression of hnRNP A1 has been reported in breast, small cell lung, ovarian, colorectal carcinoma, and pancreatic cancer [16][17][18]. Such results establish a novel way to identify CTCs by using a synthetic aptamer probe [19]. In addition, aptamers can be used in microfluidic systems to capture cells with high affinity. In ovarian cancer, the CX-BG1-10-A aptamer captures CTCs faster than with antibodies in whole blood [20]. Overall, these works show that aptamers are promising candidates to be used in cancer diagnosis and therapy.

2. Aptamers in Prostate Cancer (PCa)

Prostate cancer (PCa) is the most frequent cause of cancer-related death in men [21]. The main factors involved in disease etiology are age, lifestyle, and diet. As aging populations worldwide are increasing, the development of new tools to achieve quick and safe diagnosis and treatment in PCa has become highly relevant [22]. The diagnosis and treatment of PCa is a challenge due to the lack of biomarkers and therapeutic targets specific to the disease. For the diagnosis, the PSA-specific prostate antigen is the biomarker used for excellence in the clinic, but it lacks specificity [23][24].

Although most clinicians agree with a PSA threshold of 4.0 ng/mL for men over 50 years old as normal, several factors can produce PSA fluctuations, for example, prostatitis and benign prostatic hyperplasia (BPH) increase PSA levels [25]. Thus, men with PSA levels of 4–10 ng/mL have a 1 in a 4 chance of having PCa, whereas in the cases that PSA is superior to 10, the probability increases to 50% [26][27][28]. Whereas when elevated PSA levels are found, but no symptoms of PCa are present, another PSA test may be recommended to confirm the original finding. If the PSA level is still high, the test must be supplemented with digital rectal exams, imaging tests, and/or prostate biopsy; highlighting the importance of searching for more precise and specific markers in PCa. Hence, other molecules are being studied for the diagnosis of PCa, such as the lncRNA PCA3, a set of kallikreins including klk2 and klk3, and fusion genes such as TMPRSS2-ERG [29][30][31]. Regarding the treatment of PCa, classic chemotherapy consists of blocking androgen receptor activity also called chemical castration. This therapy has good effects at the beginning of the treatment; however, PCa cells become resistant to castration as time progresses [32].

PCa represents a heterogeneous variety of tumors, and numerous studies have reported the use of aptamers in PCa to target these different types of tumors. The yearly average of reported works using aptamers in PCa research has been 28 in the last 10 years (**Figure 2**B). In these articles, more than 20 proteins have been used as targets in aptamer research in either diagnostic or therapeutic approaches (**Figure 2**B). In the field of aptamer-based diagnostics, PSA is the most frequently used protein as the target in aptamer technology, whereas PSMA is highly used as the target in therapeutic approaches (**Figure 2**B).

Figure 2. Aptamer publications in PCa research. (**A**) Yearly trend of published articles reporting the use of aptamers in PCa. (**B**) Proteins used as targets of the aptamers in PCa research. Blue bars represent the number of articles using aptamers directed against each protein in diagnosis, whereas yellow bars refer to articles using aptamers in PCa therapy. PCA3 = prostate-cancer-associated 3; Muc1 = mucin 1; EpCAM = epithelial cellular adhesion molecule; PAP = prostatic acid phosphatase; SIRT-6 = sirtuin 6; CD133 = prominin-1; ATP5B = ATP synthase F1 subunit beta; HRE = hormone response element; STAT5 = signal transducer and activator of transcription 5A.

3. Aptamers against Prostate-Specific Membrane Antigen (PSMA)

PSMA is a type II membrane protein expressed in all forms of prostate tissues, including carcinoma [33]. PSMA is coded by *FOLH1*, a gene located at chromosome 11 in a region that is not commonly deleted in prostate cancer. PSMA has peptidase and hydrolase activity and digests dietary folates [34]. As PSMA expression is increased in PCa [35], it has been assayed as a predictor of disease recurrence by using anti-PSMA monoclonal antibodies [36]. In addition, PSMA peptides have been used in PCa treatment for stimulating the immune response by infusing dendritic cells pulsed by these PSMA peptides [37]. Altogether, these works highlight the potential value of PSMA as a target in the development of new approaches to diagnose and treat PCa.

3.1. PSMA Aptamers in PCa Diagnosis

Aptamers have been also used as tools for the development of effective diagnostic methods in PCa. For example, an aptamer against PSMA has been used to improve the diagnosis from images by using a nano-crystal semiconductor known as quantum dots (QD's). Bagalkot et al. developed a targeted QD imaging system with the A10 RNA aptamer capable of differential uptake and imaging of PCa cells expressing PSMA. Some of the advantages of this type of system are wide absorption with narrow photoluminescence, high quantum performance spectra, low photobleaching, and resistance to chemical degradation [38]. Although PSMA is a valuable marker in PCa, two types of cell lines, PSMA (+) and PSMA (−) cells, can be found in the prostate of patients with PCa. Thus, a new approach based on an RNA/peptide dual-aptamer probe was developed by Min et al. (2010) to detect both PSMA (+) and PSMA (−) prostate cancer cells [39]. Herein, two aptamers specific to prostate cancer cells, the A10 RNA aptamer (for the PSMA (+) cell line) and the DUP-1 peptide aptamer (for the PSMA (−) cell line), were conjugated to streptavidin to build the dual-aptamer probe and synchronously detected both prostate cancer cells with a high specificity by electrochemical impedance spectroscopy. Another approach uses lipid nanobubbles functionalized with the A10-3.2 aptamer that, when injected in the abdominal area of mice, the abdominal color Doppler blood flow imaging was significantly improved [40].

In addition, the detection of differentially expressed antigens (biomarkers) has proven to be important for PCa diagnosis and therapy. The main advantage of the use of aptamers over common techniques such as ELISA and tissue staining is that it does not require substantial amounts of starting material. For example, Pai and Ellington adapted the proximity

ligation assay (PLA) to cell surface protein targets using modified RNA aptamers detecting and differentiating between cells that distinctively express PSMA tumor antigen [41]. Another application of PSMA aptamers in diagnosis is as biosensors, which are characterized by being ultra-sensitive. Farzin et al. (2017) developed an aptamer-based biosensor (aptasensor) to detect the tumor marker MUC 1 in serum from human samples [42].

3.2. PSMA Aptamers in PCa Therapy

The standard care for PCa treatment is prostatectomy or chemical castration to reduce the circulating levels of testosterone and induce the apoptosis of androgen-dependent tumor cells [43]. Although prostate cancer cells are highly sensitive to androgen ablation, prostate cancer tumors contain a population of cells resistant to the treatment, for instance, cells resistant to chemical castration, and prone to both maintenance and progression of the tumor toward metastatic events [44]. Actually, ^{177}Lutetium-PSMA-617 has been approved in PCa therapy by the FDA. Although this radioligand therapy has been shown to be safe in patients resistant to chemotherapy, the median progression-free survival is still limited (3.8 months) [45]. Therefore, therapy with aptamers is an advantageous tool since it would allow the design of molecules capable of specifically recognizing various types of tumor populations to achieve an effective pharmacological treatment. Several examples of drug conjugates with aptamers exist in the scientific literature for PCa. Dhar et al. (2011) demonstrated enhanced in vivo pharmacokinetics (PK), tolerability, and efficacy of the cisplatin aptamer (Pt-PLGA-b-PEG-Apt-NP) when compared to cisplatin alone administered in a PSMA-expressing LNCaP subcutaneous xenograft mouse model of PCa [46]. In addition, docetaxel (Dtx), that is the drug of choice in PCa therapeutics, has also been used in aptamer technology to improve its pharmacological properties (administration route, solubility) and decrease its toxicity and side effects. Hence, docetaxel (Dtx)-encapsulated nanoparticles formulated with a biocompatible and biodegradable poly(D, L-lactic-co-glycolic acid)-block-poly (ethylene glycol) (PLGA-b-PEG) copolymer and surface functionalized with the A10 RNA aptamer improved the targeted delivery and uptake of drugs [47]. An additional work by Chen et al. (2016) also showed that the aptamer coupled to nanoparticles and Dtx (Dtx-apt-NPs) improved the antitumor effect in vivo on an LNCaP cell xenograft tumor model and was more effective in inducing LNCaP cell apoptosis or death through G2/M phase cell cycle arrest compared to Dtx-free nanoparticles [48].

Another application of aptamers in PCa has been as vehicles to direct drugs or simply to improve their bioavailability. This approach allows not only a better bioavailability but also a more specific recognition of cancer cells. For example, the use of unimolecular micelles coupled to aptamers as vehicles for transporting doxorubicin to tumor cells of prostate cancer. This type of conjugated molecule induced a high accumulation in the tumor tissue when compared to those without the aptamer in their system [49]. The conjugation of aptamers with liposomes for PCa treatment has been a widely used tool in research. Bandekar et al. (2014) evaluated targeted liposomes loaded with Ac-225 to selectively kill prostate-specific membrane antigen (PSMA)-expressing cells with the aim to assess their potential as targeted antivascular radiotherapy [50].

Moreover, using gold nanoparticles for imaging and therapy of PCa coupled to aptamers has been tested. The latter is based on functionalization of the surface of gold nanoparticles (GNPs) with an RNA aptamer targeting PSMA. The resulting PSMA aptamer-conjugated GNP produced a 4-fold increase in the computed tomography (CT) intensity for targeted LNCaP cells in comparison to non-targeted PC3 cells. Furthermore, the conjugated aptamer was more potent against targeted LNCaP cells than non-targeted PC3 cells when doxorubicin was added to the system [51].

Another interesting application of aptamers is the sensitization of cancer cells to radiotherapy because radio-sensitization can occur by coupling physical agents that allow better absorption of radiation. In this regard, Ni et al. (2011) achieved radio-sensitization of cancer cells with aptamer–shRNA chimeras directed to PSMA for silencing the DNAPK protein [52]. It is well known that approximately 50% of PCa tumors do not express PSMA, some of them because ERG, a common overexpressed transcription factor in PCa, suppresses PSMA expression in tumors containing the TMPRSS2-ERG fusion [29]. To overcome the lack of PSMA, Jing et al. (2016) designed a dual recombinant adenovirus-aptamer system. The viral peptide DUP-1 is capable of recognizing the PSMA-negative cells, while the aptamer A10-3.2 recognizes the PSMA-positive cells. This system decreased the cell growth for both LNCaP (PSMA-positive) and PC3 (PSMA-negative) cells in vitro and in vivo [53].

Although it will be discussed in the aptamer–siRNA chimeras section, it is worth mentioning some examples of these conjugates in PCa therapy. Two anti-PSMA aptamers were designed by Wullner et al., in 2008, with specific cytotoxicity against PCa using siRNA-induced silencing of EEF2, resulting in enhanced cytotoxicity against cancer cells [54]. Dassie et al. (2009) showed that optimized aptamer–siRNA chimeras resulted in regression of PSMA-expressing tumors in athymic mice after systemic administration. This anti-tumor activity was enhanced by increasing the chimera's half-life using polyethylene glycol [55]. In addition, RNA nanoparticles were constructed by bottom-up self-assembly containing the anti-

PSMA aptamer as the targeting ligand and anti-miR17 or anti-miR21 as therapeutic systems. This conjugate was able to strongly bind to the tumors and repressed the tumor growth in mice that received low doses of the conjugate by systemic injection [56]. Finally, an approach that integrates several systems in one is a novel prostate surface membrane antigen (PSMA) aptamer-cationic liposome-double siRNA complex that targets prostate cancer cells to inhibit cell proliferation. The delivery system showed synergism in inhibiting the growth of tumor cells indicating the potential application of the double functional siRNA delivery system for gene therapy in PCa [57].

3.3. Chimeras of PSMA

In addition to the traditional use of PCa aptamers in either target recognition or inhibition, aptamers have also been used to mediate targeted delivery of small interfering RNA (siRNAs). This system is called the aptamer–siRNA chimera and allows the delivery of siRNAs in a cell-type-specific manner. McNamara et al. (2006) developed a system based on an aptamer–siRNA chimera whose aptamer portion mediated the binding to PSMA in prostate cancer cells, and the siRNA portion targeted the expression of two survival genes (PLK1 and BCL2) overexpressed in most human tumors [58]. Lupold et al. (2002) characterized two aptamers that bind the extracellular membrane fraction of the PSMA membrane protein [59]. After this, the use of aptamer–siRNA chimeras has been extended to recognize more than one molecular target on PSMA. Mathieu et al. (2017) designed an aptamer–siRNA capable of simultaneously inhibiting EGFR and survivin [60]. The use of chimeras allows not only the fusion of aptamers with other types of molecules such as siRNAs, liposomes, or viruses but also aptamer–aptamer chimeras are possible. This is the case of the RNA aptamer–aptamer chimera designed for transporting both paramagnetic iron oxide and doxorubicin simultaneously, this chimera being more cytotoxic to the targeted cells [61].

In recent years, the gene editing approaches have gained great importance in the field of cancer research. Besides the use of the well-known CRISPR-Cas system, aptamers both activate and repress gene expression. Li and Li (2017) succeeded in designing a system that fuses a PSMA aptamer with a small activation RNA known as saRNAs [62]. This model promoted the expression of the DPYSL3 protein, decreasing cell migration in cancer cells. Another report shows that pre-treatment of animals bearing PSMA-positive tumors with chemically synthesized and systemically administered aptamer–siRNA chimeras (two days before ionizing radiation therapy) can significantly enhance tumor response to IR [63]. This type of system can be used for different applications as the new multifunctional probe comprising a cell-specific internalization aptamer, fluorescent silver nanoclusters (Ag Ncs), and therapeutic siRNA encompassing one system [62].

Although aptamer–siRNA chimeras have effectiveness in the cell-specific delivery of siRNAs, improvements can be made. One example could contemplate the binding to other peptides that are known to be a fundamental part of the cell or that intervene in some important cell proliferation processes. A biotinylated PSMA-specific aptamer A10 and *SURVIVIN*-siRNA were linked to a Streptavidin-Trans-Activator of Transcription- Double strand RNA binding domain fusion protein (STD protein) to form a therapeutic complex. This complex demonstrated higher efficiency in delivering siRNA into target cells and increasing apoptosis compared to lipofectamine and A10–siRNA chimera [64]. Furthermore, Jiao et al. (2022) developed a ^{99m}Tc–Aptamer–siRNA chimera to both diagnose and treat PSMA-positive PCa in vivo [65]. This chimera was composed by the PSMA aptamer A10 to specifically deliver the siRNA against the Mouse double minute 2 homolog (MDM2) in PCa cells. The ^{99m}Tc-A10 Aptamer–MDM2 siRNA chimera decreased MDM2 expression in PSMA-positive PCa cell lines. The inclusion of the technetium radionuclide (^{99m}Tc) in the chimera allowed a good labeling rate and targeting to the tumor, indicating that the ^{99m}Tc-labeled MDM2 siRNA-Apt chimera can be used not only as a nucleic acid treatment drug, but also as an imaging probe [65]. Although further research is necessary, it was demonstrated that the potential of aptamers to develop new approaches that integrate diagnosis and treatment of PSMA-positive PCa to provide clinical support to PCa patients.

3.4. PSMA Aptamers as Vehicles in PCa

Aptamers can also be used to selectively deliver drugs to PCa cells and enhance their effects at lower concentrations. The use of PSMA aptamers has resulted very efficient for this application because many PCa cells and tissues have high expression of this protein. Dhar et al. (2008) developed cisplatin(IV)-encapsulated nanoparticles with targeting aptamers to effectively deliver cisplatin to PCa cells [66]. These PSMA aptamers showed greater effectiveness (one order of magnitude higher than free cisplatin) of cisplatin on the PCa cell line, LNCaP [66]. This same system has been used to deliver a variety of anticancer drugs such as docetaxel using biocompatible and biodegradable co-polymers such as poly (D, L-lactic-co-glycolic acid)-block-poly (ethylene glycol) (PLGA-b-PEG) functionalized with the A10 aptamer that binds to PSMA [67]. In addition, Singh et al. (2020) encapsulated the A10 RNA aptamer in polysaccharide nanoparticles containing the natural compound thymoquinone (TQ) to inhibit the Hedgehog signaling pathway [68]. The resulting aptamer-based

nanoparticles carrying TQ were more effective in both inhibiting the Hh signaling in low drug concentrations and delivering the agent to the PCa cells [68].

PSMA aptamers can further improve their capacity as vehicles when conjugated to liposomal complexes, known as aptosomes. An example for this is an RNA micelle aptamer-conjugated liposome that specifically binds to LNCaP cells expressing PSMA. This aptamosome demonstrated in vivo an anticancer efficacy of the doxorrubicin-encapsulating PSMA-aptamosomes on tumor size regression in LNCaP xenograft mice [69].

The generation of aptamers conjugated to drugs that target PSMA is a growing area of interest in PCa therapy. To mention some seminal research, some involve the co-delivery of shRNAs against Bcl simultaneously with the delivery of doxorubicin. Another example is the encapsulation of cisplatin in positive nanoparticles with PSMA targeting aptamers on the surface of the nanoparticles to specifically deliver cisplatin to PCa cells [70]. The concomitant diagnostic and therapeutic advantages of aptamers have been reflected in the work by Wu et al. (2017), where poly (lactide-co-glycolic acid) nanobubbles (PLGA) modified with the aptamer A-10-3.2 were loaded with paclitaxel, producing tumor regression and diminishing neoplasic characteristics of cells in vitro and in vivo [71].

4. Other Aptamers in PCa

Although aptamers targeting PSMA are the second most used aptamers in PCa research (**Figure 2**B), there are others under study and have presented interesting results. **Figure 2**B summarize reported aptamers in diagnosis and treatment of PCa whose targets are different to PSMA. In the field of aptamers targeting cells, a pluri-targeting DNA aptamer called DML-7 was first designed for recognizing the human PCa cell line DU145. This aptamer internalized into the target cells and exhibited high binding affinity with dissociation constants (Kd) in the nanomolar range. Interestingly, DML-7 bound to DU145 and PC-3 cells but not to LNCaP or 22Rv1 cells [72].

There are many examples of aptamers used as delivery tools. The MUC1 aptamer was explored as a vehicle for delivering doxorubicin to cancer cells. This 86-base DNA aptamer (MA3) bound to the epitope of MUC1 with a Kd= 38.3 nM. The cancer cell lines, A549 (lung) and MCF-7 (breast) express MUC1, the aptamer MA3 preferentially bound to MUC1-positive but not MUC1-negative cells, suggesting that the MUC1 aptamer may have a potential utility as a targeting ligand for selective delivery of cytotoxic agents to MUC1-expressing tumors including prostate [73].

In diagnosis, aptamers have the potential to improve PSA-based tests as they show more sensibility and specificity and are less expensive than ELISA-based tests. The AS1411 aptamer, a 26-base guanine-rich oligonucleotide aptamer, has high affinity to nucleolin on tumor cell surfaces. The AS1411 aptamer labeled with (99m)Tc was stable in normal saline, human serum, and cellular experiments demonstrating specific binding. Since tumors had higher accumulation of radioactivity with this labeled aptamer, it could be a potential tool for use in molecular imaging of PCa [74]. In this line, aptamers have been modified to serve in diagnosis, for example, the mA4 aptamer was modified in the 2′ hydroxyl groups of RNA and poly-T in the 5′ sequence was added to increase its resistance to degradation by nucleases [75]. Other types of aptamers used in the diagnosis of PCa are based on electrochemical detection of PSA. For example, labeled free DNA aptamers coupled to gold particles showed to be useful in PSA detection after electrochemical impedance spectroscopy (EIS) with a range of 1–200 pg mL^{-1}. A similar study using a screen-printed carbon electrode (SPCE) showed improved results with a remarkably lower limit of detection of 0.077 pg/mL [76].

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