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Review

Phage Display for Early Diagnosis of Prostate Cancer

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Abstract: Prostate cancer (PC) is the second most diagnosed cancer among men. It was observed that early diagnosis of disease is highly beneficial for survival of cancer patients. Therefore, extension and increasing quality of life of PC patients can be achieved by broadening the Cancer Screening programs that are aimed at the identification of cancer manifestation in patients at earlier stages before they demonstrate well-understood signs of the disease. Therefore, there is an urgent need in standard, sensitive, robust, and commonly available screening and diagnosis tools for identification of early signs of cancer pathologies. In this respect, "Holy Grail" of cancer researchers and bioengineers for decades have been molecular sensing probes that would allow diagnosis, prognosis, and monitoring of cancer diseases by their interaction with cell-secreted and cellassociated PC biomarkers, e.g. PSA and PSMA correspondinly. At present, most PSA tests are performed at centralized laboratories using high throughput Total PSA immune analyzers which are suitable for dedicated laboratories and are not readily available for the broad health screening. Therefore, the current trend in detection of PC is developments of the portable biosensors for mobile laboratories and individual use. Phage display since its conception by George Smith in 1985 has emerged as a premier tool in molecular biology with widespread applications. This review describes how the paradigm of molecular evolution and phage display revolutionized the methods of early diagnosis and monitoring of PC.

Keywords: phage display; landscape phage; molecular evolution; affinity selection; recombinant antibodies; PC; prostate specific antigen (PSA); prostate-specific matrix antigen (PSMA); Enzyme-linked immunosorbent assay (ELISA); phage ELISA; phage capture assay; electrochemical biosensor; total prostate-specific antigen (t-PSA); free prostate-specific antigen (F-PSA); electrochemical impedance spectroscopy; label-free immunosensor

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1. Introduction

Prostate cancer (PC) is the second most diagnosed cancer among men [1] It was observed that early diagnosis of disease is highly beneficial for survival of cancer patients [2,3]. Therefore, extension and increasing quality of life of PC patients can be achieved by broadening the Cancer Screening programs that are aimed at the identification of cancer manifestation in patients at earlier stages, before they demonstrate well-understood signs of the disease[2–7]. Significant social impact and economical effect of PC screening was estimated taking into consideration that the cost for treating the advanced disease is much higher than the scanning cost [8]. Therefore, there is an urgent need in standard, sensitive, robust, and commonly available screening tools for identification of early signs of cancer pathologies [9]. In this respect, "Holy Grail" of cancer researchers and bioengineers for decades have been molecular sensing probes that would allow diagnosis, prognosis and monitoring of cancer diseases by their interaction with tumor-associated cancer cells and/or blood-solubilized PC biomarkers, such as PSA, PCA3, sarcosine oxidase andothers [2,10–17]. At present, most PSA tests are performed at centralized laboratories using high throughput Total PSA immune analyzers. There are clear practical advantages of using these fully automated analyzers, including lower detection limits and high throughput of samples. Meantime, several authors noted a persistent disagreement among PSA results obtained by different commercial immunoassays [18,19,22]. This serious drawback of the PSA immunoanalizers can be attributed to the use of capture and tracer antibodies with different epitope specificities and affinities [20] Furthermore, , the important limitation of the PSA analyzers is that they are suitable for dedicated laboratories and

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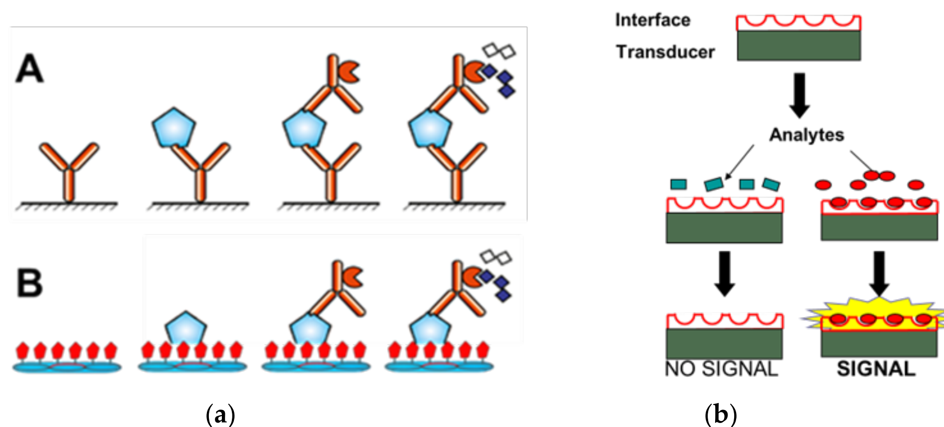
are not readily available for the broad health care community[19]. Therefore, the emerging trend in screening and detection of PC is using the portable biosensors for mobile laboratories and individual use [14,17,21–23]. Phage display since its conception by George Smith in 1985 has emerged as a premier tool in molecular biology with widespread applications. This review describes how the paradigm of molecular evolution and phage display revolutionized the methods of early diagnosis and monitoring of PC.

2. Advanced Phage-Driven Analytical Tools for Diagnosis of PC

New urgent requirements for fast, sensitive, accurate, and inexpensive tools for early diagnosis of PC devalue the traditional PSA detection methods [17], such as enzyme-linked sorbent immunoassay (ELISA), radioimmunoassay immunoradiometric assay, and time-resolved immunofluorescence assay which require complex operation, hard miniaturization and can reveal limited sensitivity [24–27]. Modern immunoassays and biosensors require a biorecognition probe, which is attached to the interface of the analytical device, bind the target biological ligand and participate in generating a measurable signal [14,15,28,29], as illustrated in Figure 1. For example, in electrochemical biosensors the signal can be displayed in impedimetric, amperometric, or potentiometric formats.



(a) (b)

Figure 1. (a): Sandwich PSA ELISA (A) vs Phage PSA ELISA (B): The capture antibody (A), or phage (B) immobilized onto ELISA plates bind the analyte protein, and detection antibodies linked to enzyme are added to catalyze the appearance of a colored or fluorescent product. **(b): PSA biosensors.** Molecular interface linked to a transducer binds the analyte and generates a signal: changes in mass, capacitance, resistance, surface plasmon resonance, reflectometric interference, etc.

PC detection techniques can be divided into two major categories: 1) cancer cell imaging techniques and 2) cancer cells-secreted soluble biomarker detection techniques [16]. Most analytic platforms rely on the use of monoclonal antibodies as biorecognition probes, such as Fab′ 8G8F5 monoclonal antibody (mAb) shown in in Figure 2 as a complex with PSA. However, their broad application is limited by high cost, low specificity, less-than-op timal affinity, and sensitivity to components of body fluids [7,16,17,22,23,33–39]. Harnessing the power of molecular evolution, phage display technique offered a new way for generating a rich repertoire of binding probes for any protein ligand or receptor[40–44].. The idea of phage display as a molecular evolution tool lies in the genetic fusion of a foreign protein to the virion structural proteins and its preservation through the viral replication. Considering desirable characteristics of different display systems, filamentous bacteriophages M13 and fd were commonly preferred as suitable vectors for generating peptide phage-displayed libraries (Figure 3). As complementary to p3-type phage display vectors, which were designed to discover diagnostic peptides and antibodies, the p8-type phage technology was developed with the goal of creation of diagnostic and detection nanoprobes by resurfacing of the whole phage particles [31,45–54].

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Figure 2. Overall view of the complex structure of Fab' 8G8F5 (H- blue, L – red) with –PSA (cyan)-substrate (magenta). Adapted from [30]. PDB ID: 2ZCK.

Figure 3. Left: Electron microscopy image of the wild-type phage fd. Blue and red arrows depict the sharp and blunt ends of the phage capsid with attached minor coat proteins p3/p6 and p7/p9, respectively (five copies each). Major coat protein p8 (~2700 copies) forms the tubular capsid around viral single-stranded DNA (scale bar: 100 nm). **Right:** Peptide phage-displayed libraries. There are two essential types of phage display — display in the minor coat protein p3, and display in the major coat protein p8. . P3 type display is used when the desired product is the displayed peptide or antibody. In the alternative type 8 phage display, a fragment of foreign DNA is inserted into the gene VIII and a foreign peptide is fused to every pVIII subunit. In the p8 display, the whole phage virion is the goal of the discovery. Adapted with modifications from[31].

2.1. Selection of Phage Probes against PC Cell-Associated Antigens

2.1.1. Selection of PC Cell-Binding Phages from f8-Type (landscape) Libraries

Since it was proven that malignant transformation of cells is linked with expression of cell antigens, the tumor cell-specific phage-displayed peptides and antibodies were considered as prospective versatile, diagnostic and therapeutic reagents[55,56]. The first PC cell-targeting landscape phages were discovered by Victor Romanov with colleagues [57]. It was shown that the phage displaying N-terminal 8-mer peptide DPRATPGS inserted in all 4000 domains of major coat protein p8, selected from p8-type (landscape) library f8/8 by it's affinity selection (biopanning) against PC cells LNCaP (Figure 3,4) blocked spreading of LNCaP cells and their relatives C4-2 and C4-2b [45]. Later, the major principles and methods of phage sselection have been used in publications of other groups, but some details of the protocol were modified with the purpose of increasing specificity and selectivity of discovered phage probes towards the target cancer cells.

Figure 4. Vectors and libraries. In the nucleotide sequences corresponding to the part of recombinant gene *gpVIII* encoding the N-terminal part of the major coat protein, randomized structures are designated as nnk, where n = A, T, G, or C, and k = G or T. Restriction sites for PstI and BamHI are underlined. N-terminal amino acid structures of mature recombinant pVIII proteins in libraries indicated by capital single letters according to amino acid abbreviations. Randomized amino acids are designated in small letters (a-h in the f8/8 library and a-i in the f8/9 library). Amino acids are numbered as in vector phage f8-5 .[32].

Figure 5. Selection of landscape phage interacting with PC cell-associated antigens.. The most common phage survey strategy is affinity selection, called 'biopanning' which enriches for phage particles whose displayed peptides bind the target cells in culture or whole tissues in living animals. To use biopanning for selection of landscape phages against a variety of different PC, the researchers add the library to the immobilized target cells, wash away non-bound phage, elute bound phage particles, and amplify them. After 2-4 rounds of selection, propagate individual clones, and analyze them. This procedure was named biopanning, because it reminds panning — the process of extraction of gold particles from sand – panning.

In continuation of this pioneering work, Prashanth Jayanna et al. used PC3 cells as target cells as they imitate the profile of advanced prostate tumors[58]. To increase repertoire of binding phages, f8/9 (9-mer) library was used in addition to f8/8 (8 -mer) library [52,59]. To isolating phages high selectivity towards PC-specific antigens, the libraies were depleted against plastic, serum and normal fibroblast cells before being allowed to interact with the target cells. Relative affinity of selected clones towards targeted and control cells was estimated using selectivity assay is based on interaction of phage particles with PC-3M cells in comparisons with other control cells The affinity of **DTDSHVNL** to PC3 cells was ~9 times higher than to either of the control cells and 32 times higher than to serum(cell-free media) whereas the affinity of **DTPYLDTG** to PC3 cells was ~8 times higher than to either of the control cells and 15 times higher than to serum. The other clones analyzed

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showed high affinity to target cells as well control cancer cells but not to normal epithelial cells or serum leading us to asume that these probes may be directed against a universal cancer receptor. A single clone **DVVYALSDD** isolated from 9-mer library demonstrated an affinity to PC3 cells that was almost eighty times higher than to the control cells and 600 times than to serum (cell-free media). Surprisingly, the other clones analyzed showed high affinity to target as well as normal epithelial cells but not to control cancer cells or serum indicating that they may directed towards a receptor that is common to both tumor and normal cells. A phage bearing an un-related streptavidin-avid peptide (**VPEGAFSS**) was used a control to demonstrate specificity of our phage probes.

Figure 6. Selectivity and specificity of phage probes. Phage probes selected from preliminary screening assays were incubated with target PC3 cells, control cells or serum treated wells of a 96-well cell culture plate. Phage associated with cells or serum were titered in bacteria and the ratio of phage output to phage input was expressed as recovery % to obtain the measure of the selectivity of a particular clone. The % recovery of the control phage bearing unrelated peptide relative to selected phage probe was indicative of the probe's specificity. Results are the average of three replicates. A – f8/8 library, B – f8/9 library. zdapted from [58,99].

To extend the panel of PC imaging phage probes, Olusegun Fagbohun et al. have screened landscape phage library f8/8 against metastatic PC cells PC-3M [60]. The most selective for PC-3M cells Phage **EPTHSWAT** was able to penetrate the PC-3M cells as revealed by immunofluorescence microscopy (Figure 6). Selectivity of the PC-specific phages **EPTHSWAT** towards PC-3M cells was studied by phage capture assayand demonstrated 35-fold greater binding than the non-relevant control phage **VPEGAFSS**. Furthermore, phage **EPTHSWAT** showed a statistically significant higher interaction with PC-3M cells than with other cells RWPE-1, HT-29, and serum. This high interaction of the phage particles with PC-3M cells might be due to pspecific hage interaction with an overexpressed PC cell antigen.

Figure 7. immunofluorescence microscopic demonstration of phage **EPTHSWAT** interaction with PC-3M cells at 15 min and 1 h in comparison with the control non-relevant phage **VPEGAFSS**. Adapted with modifications from [60].

2.1.2. Selection of PC Cell Binders from p3-Type Phage-Displayed Antibody Libraries

Polyclonal antibodies purified from the serum of an immunized animal (i.e., mouse, rabbit, goat, lama, etc.) and monoclonal antibodies (mAb) secreted by immortalized B cells from the spleen of an immunized animal are commonly used in immunological assays. Their dominant role in immunochemical applications was faded after appearance of phage-displayed antibodies which are currently commonly used for discovery and detection of cancer-specific antigens and biomarkers [29,43,61,63]. To isolate antibodies with desired specificities, phage library selections must be performed on tumor-derived antigen sources. The phage display strategy for the selection of rabbit monoclonal antibodies that recognize PC tumor-associated antigens was reported by Mikhail Popkov et al[63]. Researchers immunized rabbits with either human PC cell line LNCap or DU145. Chimeric rabbit/human Fab libraries were generatedthrough oligo(dT)-primed, reverse transcription of RNA from animal's spleen and bone marrow[64]. The antibody variable domains VL and VH were amplified, fused to human constant domains CL and CH1 and cloned into the phagemid vector pComb3X. Constructed by this way phage-displayed chimeric rabbit/human Fab libraries were screened against human PC cells DU145 using a novel whole-cell panning protocol resulting in discovery of clones bound selectively to DU145 cells but not to primary human prostate epithelial cell line PrEC, as detected by flow cytometry. In summary, this work first demonstrated the potential of immune antibody libraries for identification of imaging phage probes interacting with tumor-associated cell surface antigens.

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2.2. Selection of Phage Probes against Prostate Specific Antigen (PSA)

2.2.1. PSA as a PC Biomarker

PSA is a serum marker that is commonly used for the diagnosis of prostatic diseases. Normally, It is produced by epithelial cells of the prostate and exist mostly in two molecular forms: free PSA (f-PSA), 10%–30% of PSAs, and the PSA-α1-antichymotrypsin complex (PSA-ACT), 70%–90% of PSAs [65–68]. The sum of f-PSA and PSA-ACT is called as the total PSA (t-PSA) is regarded in clinical medicine as the important index for early diagnosis of PC, evaluation of curative effect and monitoring of post operation [7,69–73]. Thus, specific detection of certain subforms could permit discrimination between benign and malignant cases. In general, the content of t-PSA in serum of healthy people is lower than 4 ng/mL, the level accepted as a threshold value in the clinical test of PC. When the content of t-PSA in serum is more than 10 ng/mL, the risk of PC is high, thus the accuracy rates of diagnosis of PC can reach 70 − 80% [74–77]. Therefore, joint detection of the ratio of f-PSA/t-PSA and the level of t-PSA can more accurately discriminate PC. and prostate diseases.

2.2.2. Selection of p3-Type Phage Displayed Peptides against PSA

To obtain peptide ligands specificly recognizing different forms of PSA , phage-displayed linear and cyclic peptide libraries were screened against PSA-coated microplate wells or PSA supported by immobilized anti-total PSA mAbs[78]. In the pioneering work, Ping Wu et all. [79–81] discovered PSA-binding peptides by screening p3-type cyclic and linear peptide phage display libraries. A p3-fused cyclic peptide with four bridged cysteine residues showed the highest affinity for PSA. The binding specificity was characterized by competition with monoclonal anti-PSA antibodies of known epitope specificities. The peptides bound to the same region as mAbs specific for free PSA indicating that they bind close to the active site of the enzyme. These results demonstrated that peptides binding to PSA and modulating its enzyme activity can be developed by phage display technique. However, when discovered peptides were tested in sandwich capturing PSA assays with the anti-PSA 5D5A5 mAb the lowest concentrations of detectable PSA were 0.2-2 mg/ml, not sensitive enough to allow PSA to be quantified in sera from patients with prostatic diseases where the concentration of PSA was superior to 2–4 ng/ml. In the recent study of Wang et al.[82] the elution strategy in the biopanning of p3-type phage displayed peptide library Ph.D.–12 (New England Biolabs) against PSA was optimized by by additional BSA pre-screening and serum interference. PSA-specific phage expressing peptide TSIANYIGLALR showed the best affinity and specificity against PSA, was conjugated through C-terminal GGGGSK-biotin linker to streptavidin and used this construct as an signal amplifier in the, sandwiched ELISA system. The system could detect total prostate-specific antigen (tPSA) with the linear range of 0.25-200 ng/mL and the detection limit of 0.18 ng/ml demonstrating a good prospect of using peptide-streptavidin conjugates as substitute signaling antibodies in t-PSA.

2.2.3. Development and Affinity Maturation of p3-Type Phage Displayed Antibodies against PSA

The power of directed-evolution and phage display was succeeded by Muller et al. in enhancing affinity and sensitivity of immunoassay while maintaining its selectivity [83]. The original f-PSA assay based on the use of the high off rate 4D4 Mab as a tracer was less than ideal regarding sensitivity and low-end robustness of the assay. Attempts to use the 4D4 Mab for capturing was also not successful. Using phage-display library-derived mutant L3-2 Fab with reduced off-rate dissociation constant, both configurations were possible and improved assay performances. In comparison with the wild-type scFv, the best binders showed an enhancement of sensitivity in sandwich immunoassay.

2.2.4. Selection of PSA-Binding p8-Type Multivalent Landscape Phage Probes

In the p8-type phage display system, called Landscape Phage, the dense array of foreign peptides on the body of the phage composes a unique organic landscape, in which the structure and

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function of individual peptides can be influenced by interactions with neighboring proteins (Figure 3). Therefore, each landscape phage can be treated as a unique nanomaterial with novel and emergent properties that cannot be observed by use of an individual synthetic peptide alone. In many applications, including detection and imaging of PC cells, the extreme multivalency of landscape phages is a great advantage . It was proved that the landscape libraries represent an inexhaustible rich source of substitute antibodies—filaments that bind protein and glycoprotein antigens with nanomolar affinities and high specificity [26,46,48,50,51,84–90]. The foreign amino acids that form the bispecific `active site' of a landscape phage comprise up to 25% by weight of the particle and subtend up to 50% of its surface area which can accommodate hundreds of bound protein antigens. The phage structure is extraordinarily robust, being resistant to heat, organic solvents, urea, acid, or alkali, and can tolerate different modifications that increase efficacy of their use as detection and diagnostic probes [91]. Purified phages can be stored indefinitely at moderate temperatures without losing their infectivity and binding activity [92–95]. More detailed information regarding the evolution of Landscape Phage Detector paradigm, starting from its appearance in 1996 as a distinct part of the Phage Display concept[40,96], use the landscape phage as Phage Substitute Antibodies in the first Phage Biosensor and development of the landscape phage-based biosensors for liquid biopsy of PC can be found in the references[46,48,50,57,60,84,88,93,97–112]

Figure 8. Schematic illustration of bio panning for t-PSA (f-PSA and PSA-ACT). The f8/8 landscape phage library was added to the dishes with different immobilized forms of PSA. Unbound phages were washed away, and bound phages were eluted and used as a sub-library in the next round of biopanning. After three rounds, the individual phage clones were propagated, and their DNA segments corresponding to gpVIII were sequenced to determine corresponding phage-displayed peptide sequences. Detailed procedures can be found in [26,51,62].

Target-specific landscape-phage probes can be prepared as described in commonly available protocols [26,51], as illustrated in (Figure 8). Thus, the specific phage probes against t-PSA were selected from the f8/8 landscape phage library[88,113]. Through three rounds of bio panning and phage capture assay, a novel phage clone P1 and P5 displaying octamers ERNSVSPS and ATRSANGM with the best affinity and selectivity for t-PSA was successfully obtained and then used as the capture probe to establish both ELISA and DPV assay systems. (Figure 10)

3. Development of Phage-Driven Biosensors for Detection of Different forms of PSA

The critical factor that determines the efficacy of early cancer detection is the analytical platform that converts invisible molecular binding events into the optical or electrical signal (Figure 1).. The common methods used for the detection of cancer-specific antigens (bio-markers) include immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and different types of immunosensors [84,114,115]. To date, most analytical platforms for detection of cancer biomarkers use monoclonal antibodies (mAb) as sensing probes [10,15,21,35,37,116–123] There is an urgent need in robust inexpensive highly sensitive and easily available sensing probes, such as landscape phage substitute antibodies [40,46]. It was shown that due to presentation of diagnostic peptide in ~4,000 copies on the surface of the phage particles, that form a dense interface in detection platforms, significantly increase their affinity and selectivity towards the analyte markers[48,49,124–126], as illustrated in Figure 9.

Figure 9. The phages can be conjugated to the gold electrode surface using carbodiimide chemistry (A), or immobilized to the gold sensor through physical adsorption [50] (B) and analyzed by atomic force microscope (A) or electron microscopy (B). As shown in Fig. 1B, a mass of filamentous phages were observed on the gold surface. The phages or phages bundles were covalently attached to the gold surface to generate an intercrossing random network. Adapted with modifications from [50,88].

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3.1. Landscape Phage-Driven Enzyme-Linked Immunosorbent Assay (Phage ELISA)

ELISA is normally designed in two different formats, broadly categorized as direct, for the detection of antigens, and indirect, for the detection of antigen-specific antibodies [127,128]. Within these broad categories, there are multiple variants, including the sandwich direct ELISA, in which the immobilized capture antibody binds the water-soluble target antigen to form a complex, which is detected by detector antibody that binds the captured antigen and produce a visible signal after adding a chromogenic substrat**.** Landscape phage can be used in direct ELISA as substitutes both for capture and detection antibodies [62].. The phage-based **substitute** antibody leverages the multivalency of the p8 display system for enhanced analyte capture, whereas the landscape phage-based signaling antibody benefits from phage multivalency to achieve ignal amplification**, as illustrated in** Figure 1. The details about the affinity selection of t-PSA- and f-PSA-specific octapeptide-fusion phages can be found in [26,106,113]. **Shortly, p**hage**s** selected in biopanning procedure**s** against immobilized recombinant t-PSA **and f-PSA** showed the best affinity and selectivity **as** capture probe**s** in a model ELISA and demonstrated good sensitivity and reliability in the t-PSA **and f-PSA** analysis in real serum samples. This work first proved that phage-based immunoassay can be applicable to clinical diagnosis of prostatic cancer. It is prospective that the obtained specific landscape phage would be used as a novel probe to replace traditional antibodies in practical PSA immunoassay.

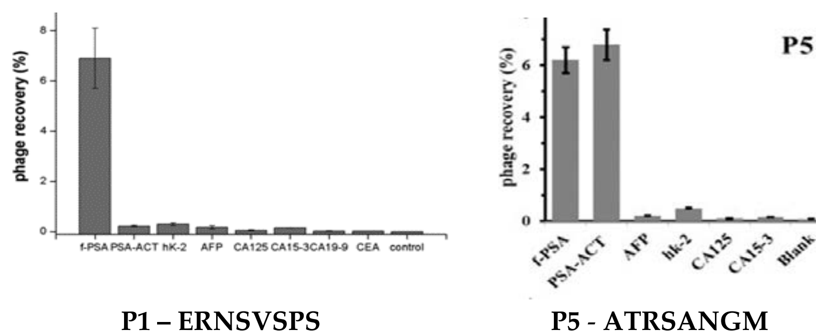

Figure 10. Specificity of Phage Binding to f-PSA and t-PSA. The specificity (or selectivity) of discovered phages, as their ability to distinguish f-PSA from t-PSA and other cancer biomarkers, was established using phage capture assay. The protein samples were incubated separately in the wells of the 96-well plate. After blocking and washing of the wells, candidate phages were added and incubated with the biomarkers at 4 °C overnight. Wells were washed to remove unbound phages. Then bound phages were eluted and tittered, and the phage recovery was calculated to compare the captured phages by different target Specificity of different phage binding to different targets.: Adapted from [26,84].

3.2. Phage-Driven Electrochemical Immunosensors for Detection of PSA

Electrochemical immunosensors attracted attention of bioengineers as bioanalytical platforms for PC detection because of their high sensitivity, specificity, simple operation and easy miniaturization. Among numerous electrochemical methods, the electrochemical impedance spectroscopy (EIS) not only inherits the normal advantages of electrochemical immunosensor, but also shows ultra-high sensitivity [37,129–134]. Like other immunoassays, the EIS technology are based on the specific immunological recognition of the ligand with the antigen [135,136]. Considering the unique properties of landscape phages, Lei Han et al. constructed first phage-based dual f-PSA/t-PSA ratio assay [88]. As sensing probes for construction of the sensor's interface (Figure 9) the researchers used landscape phages fused with octapeptides **ERNSVSPS** and **ATRSANGM** that have been discovered through screening the f8/8 landscape phages library against f-PSA and t-PSA respectively (Figure 10) [26,113]. The fabricated immunosensors showed high specificity, ultra-low limit of detection, wide linear detection range, excellent reusability, high reproducibility, and good stability that corresponds to high stability and biocompatibility of fibrous phage interface, which is determined by multivalency of surface-displayed octapeptides. Specifically, the phage-driven

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sensors demonstrated wide linear ranges (0.02 pg mL−1 – 200 ng mL-1 for f-PSA; 0.02 pg mL−1 – 200 ng mL−1 for t-PSA. Moreover, the immunosensors showed remarkably lower limits of detection (3 fg mL−1 f-PSA, S/N = 3; 4 fg mL−1 t-PSA, S/N = 3) than antibody- and aptamer-based methods reviewed in [113]. The results of this study provided a novel avenue to construct the phage-based sensors for the dual detection of f-PSA and t-PSA and analysis of f-PSA/t-PSA ratio in human blood. Authors suggested that phage clones for different epitopes of PSA can be obtained by biopanning and more phage-based immunosensors would be developed.

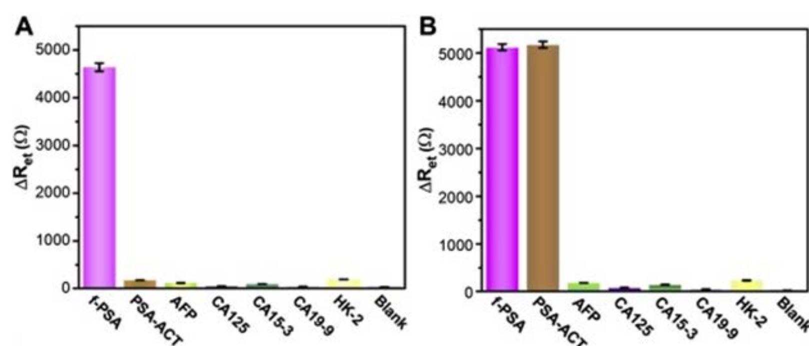

Figure 11. The selectivity assay of P1- **ERNSVSPS** -immunosensor (A) and P5- **ATRSANGM** immunosensor (B) for f-PSA and t-PSA (total of f-PSA and PSA-ACT) in comparison with other common cancer biomarkers (AFP, CA125, CA15-3, CA19-9 and hK-2) in the serum as controls. The samples were dropped onto the phage-covered immunosensors., and incubated for same time. EIS assay was performed for the above immunosensors as described [88].

4. Conclusions

To summarize, the fabrication of the specific phage-based immunosensors for ultrasensitive detection of f-PSA and t-PSA in human sera is a encouraging example of effective harnessing the power of molecular evolution and phage display for creation of smart materials that can be used for extension and improving the quality of life of millions PC patients.

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