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Highly amplified detection of visceral adipose tissue-derived serpin (vaspin) using a cognate aptamer duo

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ABSTRACT

A cognate aptamer duo for visceral adipose tissue-derived serpin (vaspin) which distinctively bind to two different sites on vaspin with high affinity and specificity were successfully developed by using graphene oxide-based systematic evolution of ligands by exponential enrichment (GO-SELEX), which offers immobilization-free screening of aptamers. The specific and simultaneous bindings of this aptamer duo (V1 and V49 aptamers) to the different sites of vaspin were confirmed by circular dichroism (CD) analysis and both sandwich-type surface plasmon resonance (SPR) and quantum dot labelled fluorescence imaging analysis (V1 aptamer serves as primary capturing aptamer and V49 aptamer as secondary signalling aptamer or vice versa). With this vaspin cognate aptamer duo on SPR platform, the detection of the target vaspin were improved to the limit of detection down to 3.5 ng/ml in buffer and 4.7 ng/ml in human serum samples. This cognate aptamer duo based biosensor could be utilized in the early diagnosis of type-2 diabetes.

1. Introduction

Since the first oligonucleotide inhibitors that can bind to two different distinct epitopes of human thrombin was reported (Tasset et al., 1997), a broad adoption of thrombin dual aptamers has evolved in the successful development of novel analytical techniques, and molecular diagnostic applications (Lee et al., 2013; Liu et al., 2013a; Vinkenborg et al., 2011). Dual aptamers or even aptamer cocktails (Kim et al., 2014) offer an advanced biosensor and molecular system modifications, by mostly implementing one aptamer as capturing probe and another aptamer as signalling probe. Moreover, the use of dual aptamers was proved to provide a higher binding affinity and specificity to their target protein than the use of any of the individual aptamers alone (Jo et al., 2014; Rinker et al., 2008). By taking advantage of the superiority of aptamers towards antibodies, such as aptamers can be synthesized chemically and easily modified for wide applications, its thermostability, and low production cost, the production of dual aptamers are pressingly needed. For example, the use of dual aptamers that can bind to different sites of a target protein could be implemented to a significant sandwich platform with an improved performance over commonly used antibody pairs sandwich format, the enzyme-linked immunosorbent assay (ELISA) (Zhao et al., 2011). The sandwich format biosensor is widely adopted for the detection of targets due to its flexible modification to generate signal. Moreover, easy modification of aptamer over antibody, which is not reducing its affinity to target, the dual aptamer development is indispensable.

However, the approaches to generate dual aptamers remain as a big challenge, with only few studies reported, to date, that require special yet complicated engineering techniques, such as the use of other ligands (Shi et al., 2007) and SELEX-RNA pool (Gong et al., 2012) as competitive inhibitors to challenge aptamer selection to different surfaces of the target proteins. These approaches, however, need unpromising firstly produced high affinity aptamers pool for further screening of secondary aptamers, which requires additional selection rounds, time and cost, and low exposure of target surfaces due to immobilization of protein targets on separation carrier such as magnetic beads. Previously, an efficient immobilization-free screening of aptamers assisted by graphene oxide (GO-SELEX) method was successfully developed in our laboratory by taking advantage on the interaction between oligonucleotides and graphene (Park et al., 2012). Interestingly, this immobilization-free of target increases target's surface exposure and chances for binding between ssDNAs and the target protein, which therefore increases the possibility of more than one aptamer to bind to a target naturally, at a time.

Vaspin is an adipokine which is known as a marker for insulin resistance involved in obesity and type-2 diabetes (Kloting et al., 2007; Van Harmelen et al., 1998). This protein marker was first identified from visceral adipose tissue in a rat model of obesity and type 2 diabetes (Hida et al., 2005), found to play roles in obesity

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and obesity-related diseases (Li et al., 2008; Youn et al., 2008). However, the mechanisms behind the mode of action of vaspin and its molecular targets are still unknown (Bluher, 2012). Therefore, the production of aptamer targeting vaspin is required for further investigations on the mechanisms and also for the development of novel strategies in alleviating obesity and diabetes diseases in human. Hence, in this study, we successfully generate a cognate aptamer duo for visceral adipose tissue-derived serpin (vaspin) and further characterize the binding of this aptamer duo to two distinct vaspin sites with high affinity and specificity.

2. Material and methods

2.1. Selection of vaspin specific ssDNA aptamers

The GO-SELEX process was performed by initially heating 200 pmoles of random library consisting a randomized region of 30 (N30) nucleotides flanked by two constant regions allowing primer annealing and PCR amplification (primer sequences: FP 5′-CGTACGGAATTCGCTAGC-3′ and RP 5′-GGATCCGAGCTCCACGTG-3′), at 95 °C for 15 min and then cooling for 5 min on ice for the best conformational structure of oligonucleotides. First round of SELEX started with the denatured ssDNA library, which then was incubated with a counter-target mixture in binding buffer (100 mM NaCl, 20 mM Tris–HCl, 2 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, pH 7.6) that included four other adipokine proteins which are adiponectin, visfatin, retinol-binding protein 4 (RBP4) and resistin, and human serum albumin (HSA). The counter selection was performed at every selection round to remove ssDNA that bound to the counter targets, which are very similar to the target in terms of molecular structure and properties. After 30 min incubation on slow rotator, 4 mg of GO was added and incubated for 2 h (final volume 1 mL) in order to separate the oligonucleotides that did not bind to the counter targets. The solution was centrifuged at 14,680 rpm for 10 min to precipitate the GO and the supernatant was discarded. Then, the ssDNA was recovered from GO with the addition of target vaspin (45 kDa). The supernatant had undergone ethanol precipitation and the DNA concentration was measured by nanodrop (Nanodrop, ND-1000, spectrophotometer). The obtained library pool was amplified by PCR (for PCR amplification, FP 5′-fluorescein-CGTACGGAATTCGCTAGC-3′ and RP 5′-GGATCC-GAGCTCCACGTG-3′ were used) and ssDNA was again generated by PAGE separation and purification. The ssDNA obtained from each round were used as initial ssDNA pool for the following round. All procedures described previously were repeated until the recovery of ssDNA reached saturation. Next, cloning and sequencing were performed to obtain the aptamer sequences. All oligonucleotides sequencing and systhesis services were purchased from Genotech, Korea, and all chemicals used in this study were purchased from Sigma Aldrich (U.S.A). All adipokines used in this study were purchased from Adipogen, Korea.

2.2. Characterization of selected aptamers by SPR

The selected aptamers obtained from GO-SELEX were characterized using SPR-based analysis. First, SPR gold chip was prepared following previously reported studies (Lee et al., 2008; Liu et al., 2013b). The SPR gold chip (K-MAC, Korea) was washed with ethanol and distilled water. The clean chip was then immersed in 50 mM DTPA solution (diluted in 100% ethanol) at room temperature overnight. Then, carboxyl functional groups on chip surface were activated with 200 mM of N-ethyl-N′-(dimethylaminopropyl) carbodiimide (EDC) and 50 mM of N-hydroxysuccinimide (NHS) for 30 min. After that, chip was incubated with 100 μg/ml streptavidin for 90 min on ice. The non-reacted functional groups were blocked by addition of 50 mM ethanolamine solution for 30 min. Then 5′-T_{10}-biotin labelled aptamers were incubated (0.5 μM) for 60 min at room temperature. Finally the chip was blocked with 50 μg/ml BSA solution for 30 min and washed with

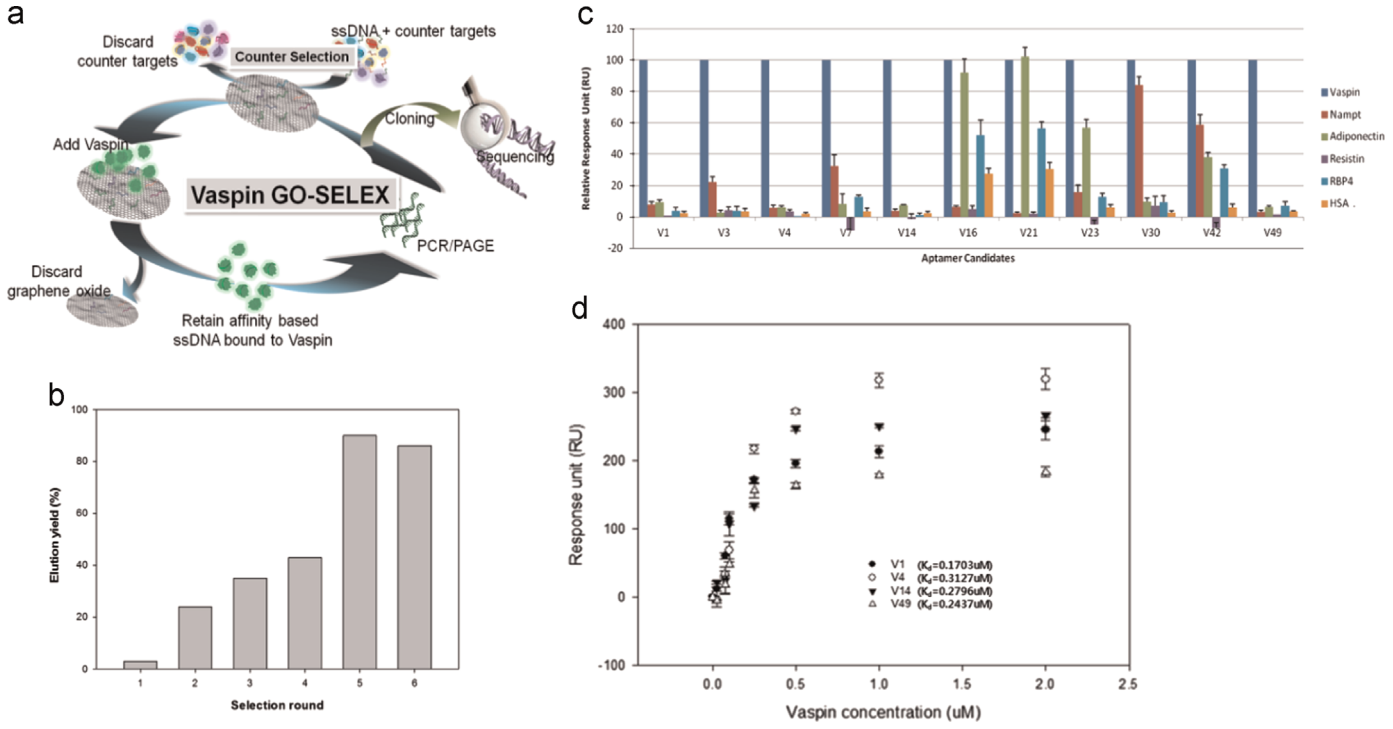

Fig. 1. Selection of ssDNA aptamers for vaspin by GO-SELEX. (a) The schematic diagram of advanced screening method of vaspin by GO-SELEX. (b) Enrichment of vaspin-specific aptamers during GO-SELEX. (c) The specificity of the selected aptamers V1, V3, V4, V7, V14, V16, V21, V23, V30, V42 and V49 for vaspin by surface plasmon resonance (SPR) analysis. (d) Dose-dependent saturation plots for vaspin binding to aptamers V1, V4, V14 and V49 obtained by surface plasmon resonance SPR analysis.

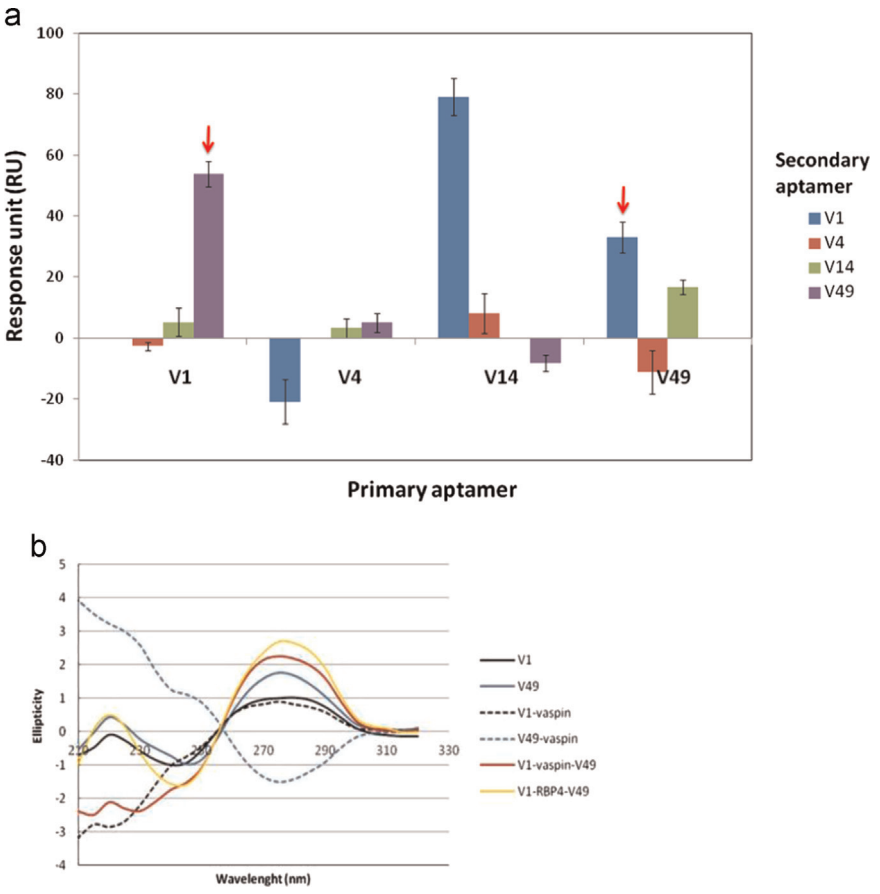

Fig. 2. Characterization of cognate aptamer duo for vaspin detection (a) Screening of aptamer duo for vaspin detection using sandwich-type SPR based assay. 0.5 μM of biotin-labelled primary aptamer was immobilized on SPR gold chip. 0.5 μM of vaspin was immobilized on primary aptamer followed by washing step to remove unbound vaspin (baseline after washing step was measured [1]) (Fig. S1). Then, 1 μM of bare secondary aptamer was added and unbound secondary aptamer was washed out [2]. Response unit of baseline [2]–[1] was measured. (b) Circular dichroism (CD) analysis of vaspin aptamer duo with and without vaspin, and with and without vaspin aptamer duo combination.

distilled water. By using this aptamer modified gold chip, analysis was performed using Eco Chemie Autolab SPR instrument (Netherlands) by generating baseline (using same binding buffer used in SELEX process) for 5 min and binding by injection of 50 μl of each counter targets and main target for 30 min with a 5 min dissociation time at room temperature. After specificity analysis for all 11 aptamer candidates, 4 selected aptamers with high specificity to vaspin were further analyzed with dose dependently and dissociation constant (Kd value) was determined with nonlinear regression analysis.

2.3. Preparation of AuNPs-reporter aptamer conjugates for signal enhancement

10 μl of 5′-thiolated reporter aptamer with C6 spacer (50 μM) was stabilized with 10 mM of Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) (2 μl), and incubated 1 h at room temperature. 1 mL of prepared AuNPs was transferred to NaOH-treated tube and 10 μl of thiolated reporter aptamer which had been stabilized with TCEP was added. AuNPs–aptamer mixture was incubated for at least 16 h at room temperature. Buffer containing 500 mM Tris acetate pH 8.2 and 1 M NaCl were added dropwise with gentle hand shaking. The samples were incubated for at least 24 h. Free-thiolated aptamer was removed by centrifugation at 16,000 rcf for 25 min. Precipitates were washed with 5 mM Tris–HCl pH 7.4 for three times. 6-Mercapto-1-hexanol (MCH) was used for efficient blocking of unoccupied region on AuNPs surface. MCH with final concentration 10 μM was added to aptamer-modified AuNPs conjugates and incubated at RT for 30 min and stored at 8 h in 4 °C. After blocking, the conjugates were washed with 5 mM Tris–HCl (2 times) and stored in 4 °C. Several concentrations of prepared AuNPs were tested on SPR gold chip for non-specific adsorption test and full recovery of AuNPs were obtained at concentration of 1 nM. The concentration of aptamer modified-AuNPs conjugates used in this study was fixed at 0.5 nM with 1:1000 ratio to aptamer concentration immobilized on gold chip (0.5 μM), following previously reported study (Park et al., 2014).

2.4. Immobilization of cognate aptamer duo on magnetic bead and quantum dot for confocal microscopy analysis

Immobilization of the biotinylated probes on the surface of the streptavidin coated QDs and MBs was achieved using the avidin–biotin interaction, following previously reported protocol (Kim et al., 2006). Qdots® 525 streptavidin conjugate (Molecular Probes, Life Technologies Corp., Carlsbad, CA, USA) and beads (Dynabeads MyOne Streptavidin C_1, diameter 1 μm), were purchased from Dynal Biotech Inc. (Lake Succes, NY, USA). First 10 μl of QD 525 streptavidin conjugates (0.1 μM) were incubated with 10 μL 5′-T_{10}-biotinylated V49 aptamer (0.5 μM) and 5′-T_{10}-biotin-BVDV34 aptamer (5′-CGTACGGAATTCGCTAGCCGCTCGGGGCGCTGCACGTAGGGTGGGGTGGGATCCG-3′) in 200 μl of borate buffer (50 mM, pH 8.3) for 1 h at room temperature. The BVDV34 aptamer was chosen by randomly picked as a random aptamer sequence in a negative control. The molar ratio of the aptamer/QDs was 5:1. The magnetic beads (MB) coated with streptavidin were functionalized with 5′-biotinylated ssDNA (V1 aptamer)

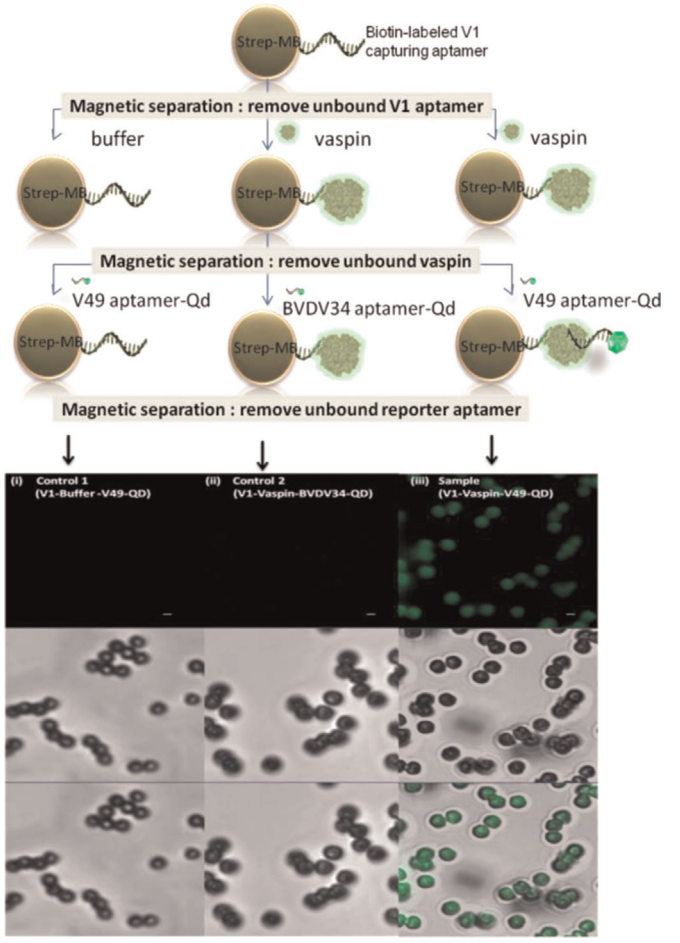

Fig. 3. Vaspin cognate aptamer duo binding assay with QD-labelled V49 reporter aptamer for confocal laser scanning microscopy (CLSM) analysis. (i) In the absence of target vaspin, and (ii) V49-Qd reporter aptamer, no fluorescent could be seen on the streptavidin coated magnetic bead. The scale bar is a 1 μm.

using the protocol suggested by manufacturer. Before immobilization, 50 μl of the streptavidin modified magnetic beads (10 mg/mL, approx. 7–12 × 109 beads) were washed two times with 100 μL of 2 × Binding & Washing (B&W) buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 2.0 M NaCl) to remove any preservatives and then resuspended in 100 μl of 2 × B&W buffer. Next, 100 μl of the MB-probes (10 μM), in distilled water, were mixed with the washed MBs suspension and incubated for 10 min at room temperature with gentle agitation. The magnetic beads, now coated with MB-probes, were separated from the weakly bound MB-probes and washed three times with 1 × B&W buffer using a magnet.

2.5. Circular dichroism spectroscopy analysis

The CD spectrums of the 1 μM DNA aptamer and 2 μM of vaspin were analyzed from 320 to 210 nm wavelengths using circular dichroism spectrometer (Applied Photophysics, UK). The data obtained were the average of four scans at a scanning rate of 100 nm/min. The scan of the binding buffer recorded at room temperature was subtracted from the average scans.

2.6. Detection of vaspin in human serum samples using cognate aptamer duo-based SPR analysis

The dose dependent binding assay was performed using 0–2.5 nM of final concentration of vaspin spiked in 10-fold diluted human serum samples. Human serum (from human male AB plasma, Sigma Aldrich, USA) was diluted to reduce the ion strength and interference from abundant complex in the serum (Zhang et al., 2009). The binding assays were performed using 0.5 μM V1 aptamer conjugated on SPR gold chip and 0.5 nM AuNPs–V49 aptamer conjugates. All of binding assays were performed in triplicate and the detection limit was calculated by using blank sample (buffer)+3 standard deviations (SDs).

3. Results and discussion

3.1. Selection of vaspin specific ssDNA aptamers

The vaspin-binding aptamers selection was performed by using the GO-SELEX method as illustrated in Fig. 1a. The ssDNA library was firstly incubated with a counter target mixture consisting four other adipokine proteins which are adiponectin, visfatin, retinol-binding protein 4 (RBP4) and resistin, and human serum albumin (HSA). These counter targets were chosen based on their similar structure to vaspin and belongs to the same adipokine group. The counter selection step was performed at every selection round to increase the selectivity of ssDNA to target vaspin by the efficient removal of ssDNA non-specific binding. Then, the ssDNAs which bound to the counter targets were removed by centrifugation (in supernatant), leaving the unbound ssDNAs that adsorbed onto GO through phi–phi stacking interactions. After vaspin target was added, the ssDNAs detached from GO and bound to vaspin by target-induced affinity-based detachment. This SELEX procedure was briefly described in the method section. The fraction of ssDNAs eluted from each selection round was increased from round 1 to round 5 with recovery yield up to 90% of the inlet ssDNA pool and reached saturation state as can be seen in Fig. 1b. As a result, 42 sequences of ssDNA aptamer candidates were obtained after cloning and sequencing of 5th round ssDNA pool. Furthermore, we selected 11 sequences of aptamer candidates ranked by Gibb's free energy for further analysis (Table S1). Fig. 1c shows the specificity of 11 selected aptamer candidates, V1, V3, V4, V7, V14, V16, V21, V23, V30, V42 and V49 for vaspin based on SPR analysis. From the specificity analysis of the selected 11 aptamer candidates with five counter targets, four aptamers (V1, V4, V14 and V49) were found to have highly specific binding only to vaspin with no or very low binding to other five counter targets (nampt, adiponectin, resistin, RBP4 and human serum albumin (HSA)). Thus, the binding affinities of these four aptamers were evaluated and their dissociation constant (*Kd*) values were measured to be 0.17, 0.31, 0.28 and 0.24 μM, for V1, V4, V14 and V49, respectively (Fig. 1d), analyzed by SPR.

3.2. Characterization of cognate aptamer duo for vaspin detection

The binding of four single aptamers with vaspin was found to be concentration dependent in a range from 25 nM to 2 μM of target. We further investigated the possibilities of having secondary interactions amongst four selected aptamers, to develop novel cognate aptamer duo for vaspin, which can bind to two different epitopes on vaspin. All 12 possible combinations of primary and secondary aptamers for 4 aptamers were tested and we found that the combination of V1 and V49 aptamers were shown to have binding interactions for both primary and secondary positions, indicating that these aptamer duo bind distinctively to different site on vaspin surfaces and the combination is in harmony (Fig. 2a). In addition, V1 aptamer was preferred to be the primary aptamer and V49 as the secondary aptamer, because this cognate aptamer duo combination produced higher SPR response unit (RU) signal compared to the reverse combination. Thus, V1

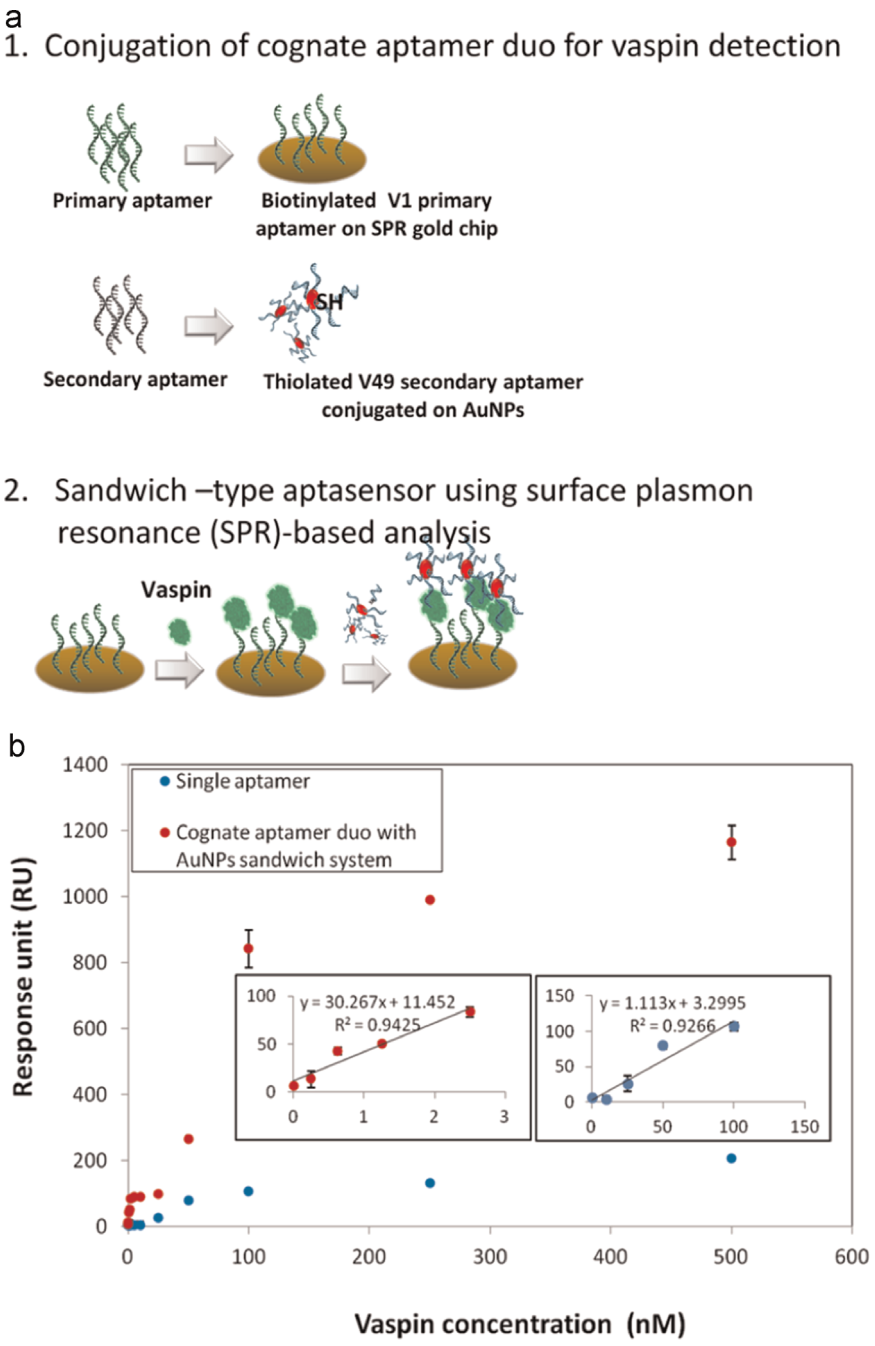

Fig. 4. Vaspin cognate aptamer duo enhanced vaspin detection via gold-nanoparticle (AuNPs) conjugation using SPR-based assay. (a) Schematic diagram of sandwich-type application of the developed vaspin aptamer duo. After the development of vaspin cognate aptamer duo, primary aptamer (V1 aptamer) was used as capturing probe and the secondary aptamer was used as reporting probe in the signal enhanced SPR-based biosensor platform. (b) Dose-dependent binding assays by using single and cognate aptamer duo for vaspin detection measured by SPR-based analysis. The inset figures showed enlarged spectrum analysis of lower vaspin concentration for single and cognate aptamer duo from 0 to 100 nM and 0 to 2.5 nM, respectively. The error bars represent average standard errors for three replicates. Each binding reaction were carried out by using 0.5 μM V1 aptamer conjugated on SPR gold chip and 0.5 nM AuNPs–V49 aptamer conjugates.

aptamer will serve as capturing aptamer and V49 aptamer as reporting aptamer for vaspin detection in further investigations.

The binding of this cognate aptamer duo combination to vaspin was also confirmed by circular dichroism (CD) analysis. As shown in Fig. 2b, upon addition of vaspin, V1 and V49 aptamers changed to different peak patterns compared to their own CD spectrum without vaspin. Moreover, in the presence of vaspin, the intense negative peak at 275 nm from the individual CD spectrum of V49 aptamer and a negative peak at 220 nm from the V1 aptamer CD spectrum, proving that both V1 and V49 aptamers have different structural conformations upon binding to vaspin. Hence, these evidences strongly supported that this aptamer duo has a significantly different secondary structure (also was shown by M-fold software prediction in Fig. S2), showing that these two aptamers naturally bind to different epitopes of vaspin, in consistent with previously reported study (Luo et al., 2013) for antitoxyplasma IgG dual aptamers case.

3.3. *Vaspin cognate aptamer duo binding assay with QD-labelled V49 reporter aptamer*

By having this cognate aptamer duo for vaspin detection, the

sandwich format biosensor could be easily applied. As can be seen in Fig. 3, by using the same sandwich format as SPR-based assay, we further visualized this aptamer duo binding on vaspin by using quantum dot labelled-V49 reporter aptamer. The biotin labelled V1 aptamer was used as primary aptamer conjugated on streptavidin coated magnetic bead and biotin labelled V49 secondary aptamer was then conjugated on streptavidin coated quantum dot. From the confocal laser scanning microscopy (CLSM) analysis, high signal of fluorescent could be seen only with the presence of vaspin (0.5 μM) together with the aptamer duo combination, while no fluorescence could be observed in control 1 (with the absence of vaspin) and control 2 (using other aptamer replacing V49 reporter aptamer).

3.4. Enhanced vaspin detection using vaspin cognate aptamer duo via gold-nanoparticle (AuNPs) conjugation

The biggest advantage of having cognate aptamer duo is an easy and broad modification of capturing and reporting aptamers in order to enhance the detection of target in sensing platform. Since the sandwich-type SPR-based assay using bare V49 reporting aptamer showed only a small increment in the SPR signal, we therefore performed signal enhancement approach by conjugating AuNPs to the V49 reporting aptamer (Fig. 4a). As can be seen in Fig. 4b, the aptamer conjugated with AuNPs sandwich system showed highly enhanced response unit on SPR sensor platform compared to the use of single aptamer. From the plotted SPR response unit in the inset Fig. 4b, detection limit was calculated by using blank sample (buffer)+3 standard deviations (SDs) for single and cognate aptamer duo systems which were found to be 399.7 ng/ml (8.9 nM) and 3.5 ng/ml (78 pM), respectively. Thus, the sensitivity was improved for about 114-fold increased for vaspin detection. In addition, the detection limit of vaspin in this study was within vaspin physiological range of interest (0.1–7 ng/ml).

Table 1
The comparison of vaspin detection limit from each aptasensing platform for the detection of vaspin.

Platform	Limit of detection (ng/ml)	Note
Single aptamer	399.7	In this study
Cognate aptamer duo sandwich with AuNPs	3.5 (in buffer) 4.7 (in human serum)	In this study
Single aptamer	1150	Kim (2010)
Vaspin binding aptamer-antibody sandwich (ELAAS)	39	Lee et al. (2012)

3.5. Detection of vaspin spiked in human serum samples using cognate aptamer duo-based SPR analysis

We analyzed the detection of vaspin spiked in human serum samples to demonstrate the ability of the cognate aptamer duo-based SPR system to detect vaspin in the biologically complex matrices. The same range of vaspin concentrations was used for the detection of vaspin spiked in human serum samples as in the binding buffer condition. From Fig. 5, the results show that the detection limit of vaspin spiked in human serum was found to be 0.1 nM (4.7 ng/ml) of vaspin, which is slightly higher than in the buffer condition but still within the physiological range of interest. This result supports that the vaspin cognate aptamer duo

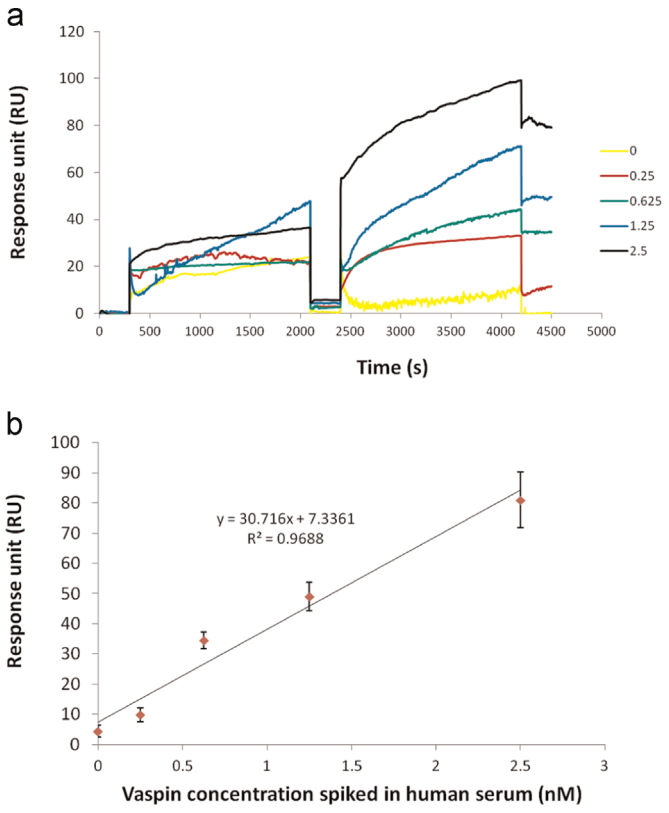

Fig. 5. Detection of vaspin spiked in human serum samples by using cognate aptamer duo-based SPR analysis. (a) SPR spectra and (b) response unit plot of dose dependent vaspin concentration spiked in human serum samples ranging from 0 to 2.5 nM. The binding assays were performed using 0.5 μM V1 aptamer conjugated on SPR gold chip and 0.5 nM AuNPs–V49 aptamer conjugates. The error bars represent average standard errors for three replicates.

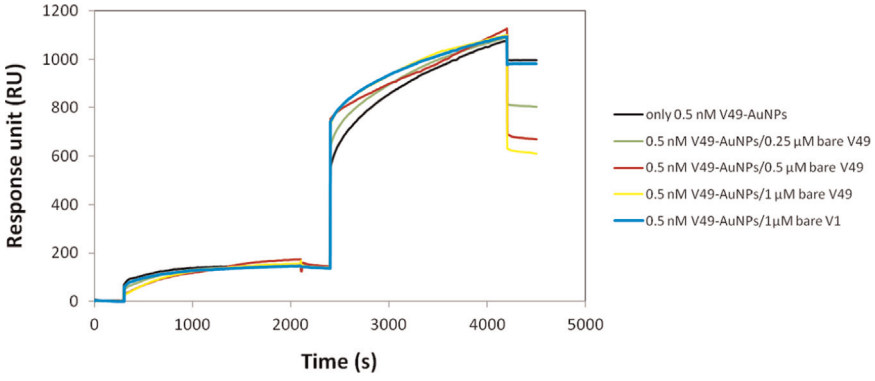

Fig. 6. Competitive binding assay of cognate aptamer duo to two different sites of vaspin. The binding assays were performed using 0.5 μM V1 aptamer conjugated on SPR gold chip and 0.25 μM of vaspin.

performance was not disturbed by the presence of complex matrices in human serum. Moreover, as shown in table 1, the use of vaspin cognate aptamer duo in this study was found to be competitive and possess the best sensitivity for vaspin detection with the use of aptamer. More importantly, this cognate aptamer duo is more flexible to be used with wide modification techniques on sandwich manners, and cost effective compared to the use of enzymes or antibodies.

3.6. Competitive binding assay of cognate aptamer duo on two different sites of vaspin

We performed competitive binding assays which targeted V49 aptamer binding site on vaspin. V49 bare aptamer was used to compete with V49–AuNPs aptamer conjugates binding on vaspin which caused reduction of SPR signal due to the decreasing amount of V49–AuNPs aptamer conjugates bound to vaspin. From Fig. 6, the reduction in the signals was observed when higher ratio of V49–AuNPs aptamer conjugates to V49 bare aptamer was added, but no reduction was observed in both cases that V49–AuNPs aptamer conjugates only or V49–AuNPs aptamer conjugates and V1 aptamer bare were added.

4. Conclusion

In summary, an advanced immobilization-free SELEX method was successfully adopted for screening of a novel cognate aptamer duo for vaspin detection. GO-SELEX was used due to the immobilization-free screening of aptamers which increases the surface exposure of vaspin target and chances for ssDNA binding, which therefore, increases the possibility of having more than one cognate aptamers binding to the different sites of a single target distinctively at one time. V1, V4, V14 and V49 aptamers were found to have high specificity to vaspin, while discriminating five other counter targets (adiponectin, visfatin, retinol-binding protein 4, resistin and human serum albumin), based on the surface plasmon resonance (SPR) analysis. The dissociation constant (Kd) value for the four aptamers were found to be 0.17, 0.31, 0.28 and 0.24 μM for V1, V4, V14 and V49 aptamers, respectively, analyzed by SPR-based assay. For the first time, a cognate aptamer duo was developed using immobilization free-GO SELEX for a protein target. V1 and V49 aptamers were found to display vaspin cognate aptamer duo, confirmed by CD and both sandwich-type SPR and quantum dot labelled fluorescence imaging analysis. Vaspin detection was also improved using gold-nanoparticles (AuNPs) conjugation on the V49 reporter aptamer, with enhanced limit of detection down to 3.5 ng/ml (114-fold increased) compared to the detection by using single aptamer. The detection limit of vaspin spiked in human serum was found to be 0.1 nM (4.7 ng/ml) of vaspin, which is slightly higher than in the buffer condition but still within the physiological range of interest. This novel cognate aptamer duo can be applied to various modifications in improving aptasensor, helps in the generation of new nanomaterials, as well as development of novel strategies in alleviating obesity and diabetes diseases in human.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.03.042.

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