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Sensitive surface plasmon resonance detection of methyltransferase activity and screening of its inhibitors amplified by p53 protein bound to methylation-specific ds-DNA consensus sites

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ABSTRACT

Abnormal DNA methylation is closely related to cancer initiation and progression, and strategies to assay methyltransferase activity and screen its inhibitors are essential for cancer diagnosis and therapy. In this work, surface plasmon resonance (SPR)-based assay for real-time and sensitive monitoring of DNA methyltransferase activity and screening of its inhibitors was conducted via methylation of double-stranded (ds)-DNA consensus sites and the follow-up p53 protein recognition. The consensus ds-DNA possesses a specific sequence of 5′-CCGG-3′ in which the second C base can be methylated by M.*Sss*I methyltransferase (M.*Sss*I MTase) and the methylation process impedes the recognition and cleavage of the ds-DNA by *Hpa*II endonuclease, thus, the attachment of p53 protein leads to remarkable SPR signals. In contrast, inhibition of M.*Sss*I MTase activity by a potent inhibitor leaves the consensus ds-DNA unmethylated, and the cleavage of the ds-DNA by *Hpa*II prevents p53 protein from adsorbing onto the chip surface, leading to tiny SPR signals. The binding affinity (K_D) between p53 protein and the methylated consensus ds-DNA was deduced to be 3.04 nM, evidencing the strong binding capability. Two nucleoside inhibitors of 5-Azacytidine (5-Aza) and 5-aza-2′-deoxycytidine (5-Aza-dC), and a non-nucleoside inhibitor of procaine were examined, and their half-maximal inhibiting concentration (IC_{50}) values were highly comparable with those by other methods. The sensing protocol has been successfully utilized for the assay of M.*Sss*I MTase activity in normal and cancer cell lysates. The proof-of-concept experiments demonstrate that SPR serves as a viable means for sensitive detection of methyltransferase activity and screening of its inhibitors using p53 protein bound to methylation-specific ds-DNA consensus sites.

1. Introduction

As one of the most important epigenetic modifications, DNA methylation is characterized by the transfer of methyl groups from S-adenosyl-L-methionine to the carbon-5 position of cytosine within cytosine-guanine dinucleotides by methyltransferase (MTase) (Smith and Meissner, 2013). DNA methyltransferase not only influences the status of DNA methylation, but also is involved in a variety of cellular processes, such as transcriptional regulation, genome stability, and tumorigenesis (Flynn et al., 1996; Miranda and Jones, 2007; Frommer et al., 1992; Pradhan et al., 1999). By silencing the tumor suppressor genes, abnormal DNA methylation processes are associated with several genetic diseases and various types of cancers (Keith, 2001). Therefore, accurate monitoring of DNA methyltransferase activity and screening of its inhibitors are of great significance for unraveling abnormal DNA methylation-related disease initiation and progression.

Conventional methods for the assay of DNA MTase activity include, but are not limited to radioactive assay (Kim et al., 2004; Gros et al., 2013), bisulfite-based assay (Taylor et al., 2007), methylation-specific polymerase chain reaction (Herman et al., 1996), gel electrophoresis (Mohannath and Pikaard, 2016), mass spectrometry (Liu et al., 2007), and high-performance liquid chromatography (Reenilä et al., 1995).

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Among them, radioactive assay and bisulfite-based assay have been widely used as standard techniques. The radioactive assay relies on the radioactively labeled [methyl-^{3}H]-S-adenosyl-L-methionine, and involves radioactive reagents and tedious processes (Gros et al., 2013). The bisulfite-based assay is dependent on sodium bisulfite-mediated conversion of cytosine to uracil, and usually causes longer treatment time, sample DNA degradation, and false-positive results by the incomplete transformation (Kurita et al., 2015; Rauf et al., 2017; Nazmul Islam et al., 2017). In recent years, alternative electrochemical (Li et al., 2012), fluorescent (Scarabelli et al., 2017), and colorimetric (Haque et al., 2017) sensors for the assay of MTase activity have been well established, however, these methods usually suffer from false-positive results, cumbersome chemical pre-treatment, the use of antibodies, and complicated experimental procedures (Syedmoradi et al., 2016; Nazmul Islam et al., 2017).

Surface plasmon resonance (SPR) is a powerful optical technology, being highly sensitive to the tiny changes in the thickness or refractive index caused by the molecular interactions at the chip surface (Tao et al., 1999). A streptavidin-modified SPR sensor for methylation analysis of adenomatous polyposis coli gene promotor using methylated cytosine-guanine site-specific protein has been developed (Pan et al., 2010). The end-to-end Au nanorod assembly-enhanced SPR has been proposed for evaluation of Dam MTase activity and inhibitor screening by coupling with polymerization and nicking reactions (Li et al., 2015). Sensitive detection of M.*Sss*I MTase activity by SPR has been demonstrated via dual signal amplification by Au nanoparticles and DNA chain cyclic reactions (Li et al., 2018). Using highly specific molecular inversion probes, DNA methylation in bisulfite-treated genomic DNA from cancer cell lines was sensitively detected by SPR (Carrascosa et al., 2014). On the basis of a bifunctional linker molecule, SPR determination of DNA methylation has been demonstrated (Kurinomaru et al., 2017). Despite their numerous advantages, these methods involve either dual amplification strategies (Li et al., 2018), complicated modification of the nanoparticles (Li et al., 2015), bisulfite treatment (Carrascosa et al., 2014) or the use of anti-5-methyl-cytosine antibody (Kurinomaru et al., 2017) to achieve higher sensitivity. Furthermore, the feasibility of the methods for inhibitor screening has not been demonstrated (Pan et al., 2010; Carrascosa et al., 2014; Kurinomaru et al., 2017).

In this study, the rationally designed consensus ds-DNA, which is a specific recognizer for p53 protein (Miyashita and Reed, 1995; Zambetti et al., 1992), contains a sequence of 5′-CCGG-3′ in which the second C base can be methylated by M.*Sss*I MTase (Rauf et al., 2017; Zhu et al., 2016). The extent of the methylation reaction exerts a profound influence on the cleavage of the ds-DNA by *Hpa*II endonuclease, and the attachment of various amounts of p53 protein leads to different SPR signals. The proposed assay is simple, sensitive, and selective, providing a viable means for detection of methyltransferase activity and screening of its inhibitors.

2. Experimental

2.1. Materials

11-mercaptoundecanoic (MUA), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), ethanolamine hydrochloride (EA), Tris-HCl, EDTA, NaOH, NaCl, procaine, 5-Azacytidine (5-Aza) and 5-aza-2′-deoxycytidine (5-Aza-dC) were acquired from Sigma (St. Louis, MO). S-adenosyl-L-methionine, M.*Sss*I methyltransferase (M.*Sss*I MTase) and restriction endonucleases of *Hpa*II and *Hha*I were purchased from New England Biolabs (Ipswich, MA). Recombinant p53 protein was obtained from BD Biosciences Pharmingen (San Diego, CA). DNA with various sequences was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). For the formation of the consensus double-stranded (ds)-DNA, the sequences of the animated DNA probe and its complementary target are 5′-H_2N-$(CH_2)_6$-TTTTT GGA CATG CCC GGA CATG CCC-3′ and 5′-GGG CATG TCC GGG CATG TCC-3′, respectively (the recognition site of M.*Sss*I MTase in 5′-CCGG-3′ is underlined). The methylated DNA probe and its complementary strand have sequences of 5′-H_2N-$(CH_2)_6$-TTTTT GGA CATG CC^{m}C GGA CATG CCC-3′ and 5′-GGG CATG TC^{m}C GGG CATG TCC-3′, respectively (the underlined C base is methylated). The DNA probe and its complementary strand for the formation of the non-consensus ds-DNA possess sequences of 5′-H_2N-$(CH_2)_6$-TTTTT GTC GGCC GAG GTC GGCC GAG-3′ and 5′-CTC GGCC GAC CTC GGCC GAC-3′, respectively. All the reagents are of analytical purity and used as received. All the stock solutions were prepared daily with deionized water treated with a water purification system (Simplicity 185, Millipore Corp., Billerica, MA).

2.2. Instruments

The SPR measurements were conducted on a BI-SPR 3000 system (Biosensing Instrument Inc., Tempe, AZ), in which the diode lasers serve as the light source with an output of up to 1 mW of visible radiation at 670 nm. The carrier solution of TNE buffer (10 mM Tris-HCl + 50 mM NaCl + 1.0 mM EDTA, pH 7.4) was degassed through vacuum pumping for 30 min prior to the measurements. 15.3 nM p53 protein was preloaded into a 200 μL sample loop and then injected into the flow cell by a syringe pump (Model KDS260, KD Scientific, Holliston, MA) at a flow rate of 10 μL/min. For the kinetic measurement, p53 protein with various concentrations was injected onto the sensor chips at 40 μL/min.

2.3. Procedures

2.3.1. Solution preparation

DNA probe and target were prepared with TNE buffer. The ds-DNA was formed by heating the mixed solution of 2 μM DNA probe and 2 μM target to 90 °C and then cooling down to room temperature. M.*Sss*I MTase, S-adenosyl-L-methionine, *Hpa*II, *Hha*I, and p53 protein were prepared or diluted with TNE buffer. MUA and EA were dissolved in ethyl alcohol and water, respectively. EDC/NHS solution was prepared by mixing 0.4 M EDC and 0.1 M NHS in water before activation of the MUA films.

2.3.2. SPR chip modification

The bare Au chips were annealed in a hydrogen flame to eliminate surface contaminants. MUA-covered gold films were formed by immersing the chips in 0.5 mM MUA solution for 24 h, followed by activation by 0.4 M EDC and 0.1 M NHS for 20 min. The pre-formed ds-DNA was cast onto the MUA-modified surface for 3 h at room temperature. After washing thoroughly with TNE buffer and water, the sensor chips were soaked in 0.5 M EA for 15 min to block the empty sites. Finally, the chips were thoroughly rinsed with water and dried under nitrogen.

2.3.3. SPR assay of M.SssI MTase activity

To methylate the specific site in ds-DNA, the sensor chips pre-immobilized with consensus ds-DNA were treated with 160 μM S-adenosyl-L-methionine and M.*Sss*I MTase with various concentrations for 3 h at 37 °C. M.*Sss*I MTase could catalyze the transfer of a methyl group from S-adenosyl-L-methionine to the second C base in 5′-CCGG-3′ (Rauf et al., 2017; Deng et al., 2014). The methylated ds-DNA-covered chips were then treated with 25 U/mL *Hpa*II for 3 h at 37 °C, followed by rinsing three times with water and drying under nitrogen. The SPR signals were attained upon injection of 15.3 nM p53 protein.

2.3.4. Inhibitor screening

The mixed solutions containing 80 U/mL M.*Sss*I MTase, 160 μM S-adenosyl-L-methionine and the candidate inhibitors were dropped onto the sensor chips pre-immobilized with consensus ds-DNA for 3 h at 37 °C, and this is followed by treatment with 25 U/mL *Hpa*II for 3 h at

37 °C. The inhibition potency was estimated by examining the SPR signals upon injection of 15.3 nM p53 protein.

2.3.5. M.SssI MTase activity assay in cell lysates

HeLa and MCF-7 cancer cell lines and MCF-10A normal cell line were obtained from Xiangya School of Medicine, Central South University (Changsha, China), and cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2. After the cells were cultured for 48 h, the culture media were discarded and the cells were washed with ice-cold PBS (10 mM phosphate, pH 7.2) for three times. The cells were suspended in 200 μL lysis buffer on ice for 20 min and then transferred to the centrifuge tube, followed by sonication with four 5-s pulses. The resulting supernatant was collected by centrifugation at 12,000 rpm for 10 min and then immediately used for the assay of M.*Sss*I MTase activity in cell lysates. For the inhibition of 5-Aza-dC toward M.*Sss*I MTase activity in cell lysates, 10 μM 5-Aza-dC was added to the cell lysates and the activity of M.*Sss*I MTase was examined.

3. Results and discussion

3.1. Principle of M.SssI MTase activity assay and inhibitor screening

The schematic of SPR assay of M.*Sss*I MTase activity and screening of its inhibitors is illustrated in Fig. 1. The consensus ds-DNA was pre-immobilized onto the MUA-covered chips through amide bond formation. The consensus ds-DNA is capable of binding to the central domain of p53 protein and contains a specific sequence of 5′-CCGG-3′ in which the second C base can be methylated by M.*Sss*I MTase (Nichols and Matthews, 2001; Li et al., 2012). Typically, the ds-DNA consensus site consists of two repeats of 10-base-pair half-site with the sequence of 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′ (where Pu and Py represent purines and pyrimidines, respectively) (Cho et al., 1994; Bian and Sun, 1997). Deletion of the consensus site has dramatically reduced the binding of the consensus ds-DNA to p53 protein (Cho et al., 1994; Wang et al., 1995). As a widely used restriction enzyme, *Hpa*II endonuclease could recognize and catalyze the cleavage reaction between the cytosine residues within 5′-CCGG-3′ of ds-DNA (Rauf et al., 2017; Deng et al., 2014). In the absence of a potent inhibitor, the methylation of the consensus ds-DNA by M.*Sss*I MTase impedes the subsequent cleavage reaction of *Hpa*II, and the recognition of the methylated ds-DNA by p53 protein produces larger SPR signals (top scheme). However, in the presence of a potent inhibitor, the activity of M.*Sss*I MTase is suppressed, and the consensus ds-DNA is recognized and cleaved by *Hpa*II, detaching the ds-DNA fragments from the chip surface. Note that the remaining DNA fragments left on the chip surface could not be recognized by p53 protein, leading to tiny SPR signals (bottom scheme). Thus, assay of M.*Sss*I MTase activity and screening of its inhibitors could be achieved via examining the SPR signals arising from the binding of p53 protein to the methylated consensus ds-DNA.

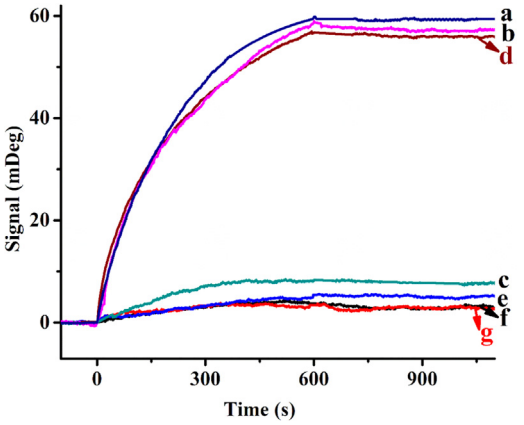

Fig. 2. SPR sensorgrams acquired upon injection of 15.3 nM p53 protein onto the chips pre-immobilized with (a) consensus ds-DNA, (b) consensus ds-DNA after treatment with 80 U/mL M.*Sss*I MTase, (c) consensus ds-DNA after treatment with 25 U/mL *Hpa*II, (d) consensus ds-DNA after treatment with 80 U/mL M.*Sss*I MTase and then 25 U/mL *Hpa*II, (e) non-consensus ds-DNA, (f) non-consensus ds-DNA after treatment with 80 U/mL M.*Sss*I MTase, (g) non-consensus ds-DNA after treatment with 80 U/mL M.*Sss*I MTase and then 25 U/mL *Hpa*II. Duration of the injection of p53 protein was 600 s at 10 μL/min.

3.2. SPR detection of M.SssI MTase activity

As shown in Fig. 2, an obvious SPR signal of 59.36 mDeg was observed via injection of 15.3 nM p53 protein onto the chip surface pre-immobilized with the consensus ds-DNA (curve a). After methylation of the consensus ds-DNA with M.*Sss*I MTase, the slightly decreased SPR signal in curve b (57.15 mDeg) indicates that the methylation process does not affect the binding affinity between the consensus ds-DNA and p53 protein. It has been demonstrated that p53 protein could make contact with the bases in the major and minor grooves of consensus ds-DNA through hydrogen bonds (Cho et al., 1994). In the absence of M.*Sss*I MTase, a smaller SPR signal of 7.64 mDeg in curve c was attained because half of the ds-DNA consensus site had been cleaved off by *Hpa*II, which prevents the interaction with p53 protein. In the presence of M.*Sss*I MTase and then *Hpa*II, similar SPR response (56.40 mDeg, curve d) with that in curve b suggests that the methylation of consensus ds-DNA could effectively prevent the cleavage reaction by *Hpa*II. Tiny or negligible SPR signals were obtained upon injection of 15.3 nM p53 protein onto the chip surfaces pre-immobilized with non-consensus ds-DNA (4.84 mDeg, curve e), followed by treatment with M.*Sss*I MTase (3.12 mDeg, curve f), and then *Hpa*II (2.48 mDeg, curve

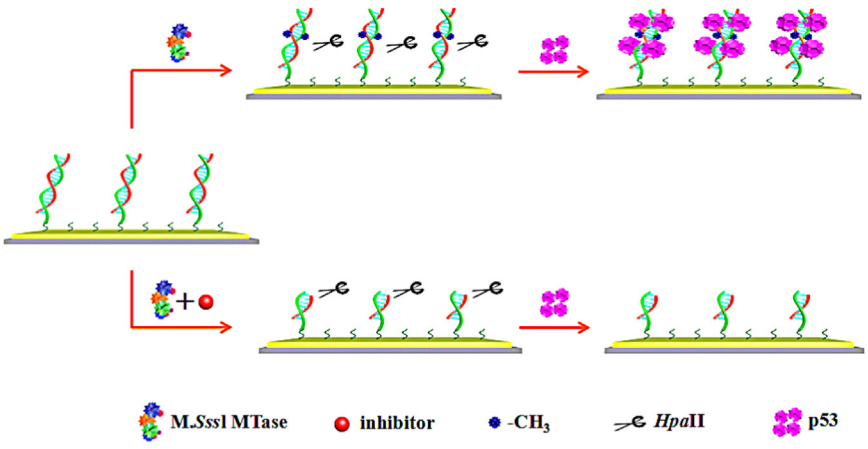

Fig. 1. Schematic of SPR assay of M.*Sss*I MTase activity (top) and screening of inhibitory compounds for M.*Sss*I MTase (bottom). The methylation of the surface-confined consensus ds-DNA catalyzed by M.*Sss*I MTase impedes the cleavage of ds-DNA by *Hpa*II, and the recognition of the methylated ds-DNA by p53 protein produces larger SPR signals (top). In the presence of an inhibitor, the activity of methyltransferase is suppressed, and the cleavage of the consensus ds-DNA by *Hpa*II prevents p53 protein from attaching to the chip surfaces, leads to tiny SPR signals (bottom).

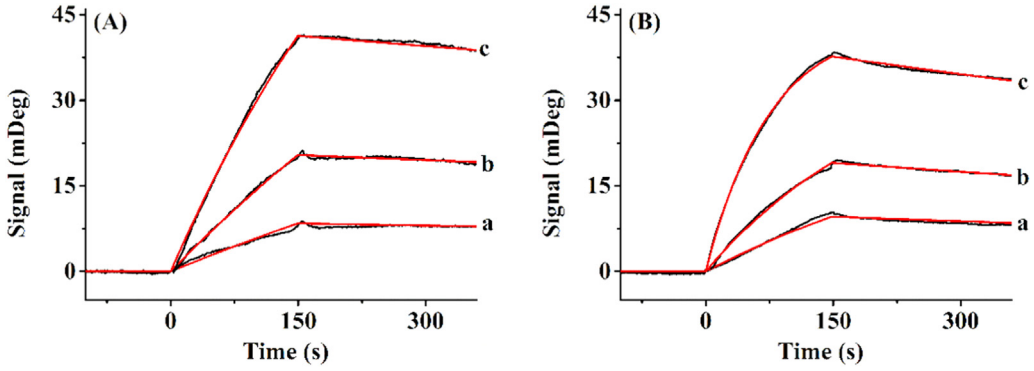

Fig. 3. Overlaid experimental (black) and simulated (red) SPR sensorgrams acquired upon injection of (a) 2.5, (b) 7.6 and (c) 15.3 nM p53 protein onto the sensor chips pre-immobilized with (A) consensus ds-DNA and (B) methylated consensus ds-DNA. Duration of the injection of p53 protein was 150 s at 40 μL/min (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

g). The proposed method thus largely eliminates the non-specific adsorption of p53 protein, serving as a viable means for enzymatic activity assay.

3.3. Kinetic measurement of the binding between methylated consensus ds-DNA and p53 protein

To further verify the strong interaction between methylated consensus ds-DNA and p53 protein, kinetic measurement of the binding of p53 protein to consensus ds-DNA (Fig. 3A) or methylated consensus ds-DNA (Fig. 3B) was performed. The background signal was subtracted by serially flowing p53 protein into the reference channel pre-immobilized with non-consensus ds-DNA. The dissociation constant (K_D) estimated from the kinetic simulation was 1.08 ± 0.3 nM for consensus ds-DNA and p53 protein (Fig. 3A), which is consistent with those by isothermal calorimetric technique (1.6 nM) (Nichols and Matthews, 2001), electrophoretic mobility shift assay (2 nM) (Heffler et al., 2012), and fluorescence correlation spectroscopy (1.1 nM) (Wolcke et al., 2003). The calculated K_D for methylated consensus ds-DNA and p53 protein was 3.04 ± 0.2 nM (Fig. 3B), and the weaker binding affinity than that between consensus ds-DNA and p53 protein might be ascribed to the steric hindrance imposed by the methyl groups located in the middle of the consensus ds-DNA (Carvin et al., 2003; Yu et al., 2010; Lazarovici et al., 2013). However, the nanomolar K_D values between p53 and methylated or unmethylated ds-DNA suggest the strong binding capability.

3.4. Optimized HpaII concentration and selectivity of the assay

The concentration of *Hpa*II used for effectively cleaving the pre-immobilized consensus ds-DNA was optimized (Fig. 4A). The cleavage of the consensus ds-DNA by *Hpa*II leads to the detachment of half of the ds-DNA consensus sites from the chip surface and a decreased SPR signal. The SPR signals decreased with the increased concentrations of *Hpa*II from 0 to 50 U/mL and began to level off beyond 25 U/mL. Thus, 25 U/mL was chosen as the optimal *Hpa*II concentration. As an alternative, assay of *Hpa*II activity and screening of its inhibitors could be accomplished by the proposed method. In addition to *Hpa*II, *Hha*I was also attempted for the cleavage of the consensus ds-DNA (Fig. 4B). *Hha*I is also a restriction endonuclease that recognizes and cleaves the similar sequence of 5'-GCGC-3' (Zacharias et al., 1984). As compared to the remarkably decreased SPR signal by *Hpa*II, *Hha*I could not recognize ds-DNA consensus sites and a larger SPR signal was obtained. The sensing protocol possesses high specificity toward *Hpa*II and can differentiate between similar restriction endonucleases.

3.5. Performance analysis of M.SssI MTase

The feasibility of the method for assay of M.*Sss*I MTase activity was demonstrated in Fig. 5. The consensus ds-DNA was treated with M.*Sss*I MTase from 0.5 to 120 U/mL and then 25 U/mL *Hpa*II, followed by injection of 15.3 nM p53 protein. The methylation of more ds-DNA consensus sites by M.*Sss*I MTase severely impeded the cleavage reaction by *Hpa*II, and the attachment of more p53 protein yields larger SPR signals. The SPR responses gradually increased with the increasing concentrations of M.*Sss*I MTase and began to level off beyond 80 U/mL. The SPR signals were proportional to the concentrations of M.*Sss*I MTase between 0.5 and 50 U/mL, and the linear regression equation was expressed as Signal (mDeg) = 10.8 + 0.67 [M.*Sss*I MTase] (U/mL) (R^2 = 0.98). The limit of detection was estimated to be 0.09 U/mL, which is lower than or comparable with those by circular dichroism spectroscopy using the probes of DNA-driven self-assembled gold nanoparticle dimers (0.27 U/mL) (Liu et al., 2015) and electrochemical immune assay based on specific conjugation of anti-5-methyl-cytosine antibody to the methylation sites (0.1 U/mL) (Wang et al., 2012).

3.6. Inhibitor screening

The abnormal methylation process is closely related to cancer initiation and progression, and the inhibition of methyltransferase

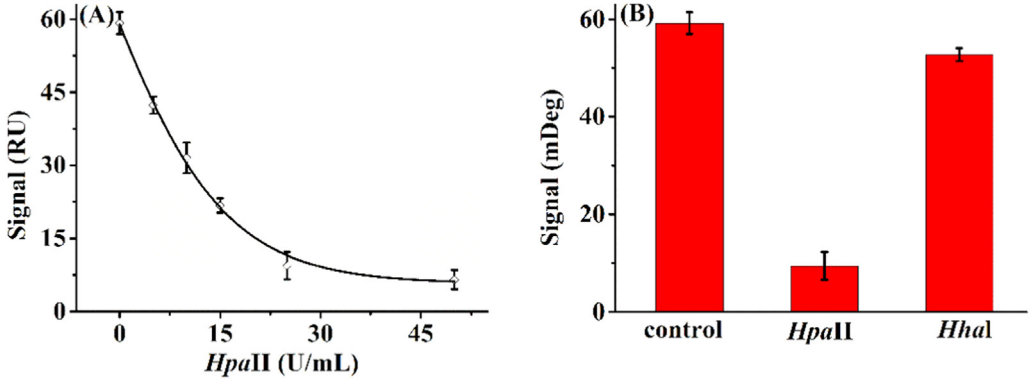

Fig. 4. (A) Dependence of the SPR signals on the concentrations of *Hpa*II. The consensus ds-DNA-modified chips were exposed to 0, 5, 10, 15, 25, and 50 U/mL *Hpa*II, followed by injection of 15.3 nM p53 protein. (B) Selectivity of the SPR assay. The SPR signals were obtained upon injection of 15.3 nM p53 protein onto the consensus ds-DNA-covered chips after treatment with 25 U/mL *Hpa*II or 25 U/mL *Hha*I. The control shows the SPR signal in the absence of *Hpa*II.

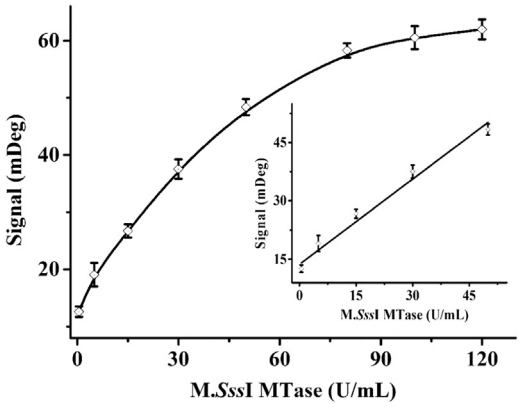

Fig. 5. Dependence of the SPR signals on the concentrations of M.*Sss*I MTase. The SPR signals were obtained upon injection of 15.3 nM p53 protein onto the consensus ds-DNA-modified chips after treatment with M.*Sss*I MTase and then 25 U/mL *Hpa*II. The concentrations of M.*Sss*I MTase are 0.5, 5, 15, 30, 50, 80, 100, and 120 U/mL. The inset shows the linear portion of the curve. The relative standard deviations for three replicate experiments are less than 6.0%.

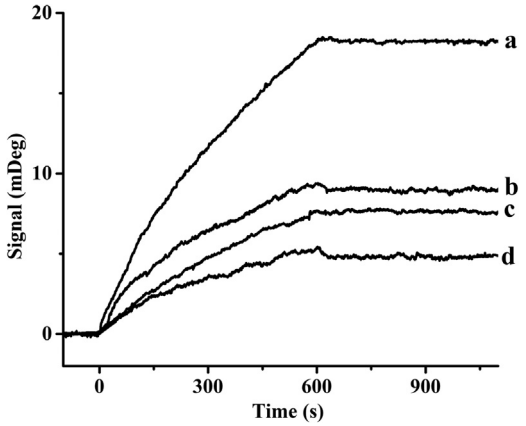

Fig. 7. SPR sensorgrams acquired upon injection of 15.3 nM p53 protein onto the consensus ds-DNA-covered chips after treatment with the mixed solution containing 160 μM S-adenosyl-L-methionine and (a) HeLa cell lysate, (b) HeLa cell lysate + 10 μM 5-Aza-dC, (c) cell lysis buffer, followed by cleavage with 25 U/mL *Hpa*II. Curve d was obtained upon injection of 15.3 nM p53 protein onto the non-consensus ds-DNA-modified chips after treatment with the mixture of 160 μM S-adenosyl-L-methionine and HeLa cell lysate, followed by cleavage with 25 U/mL *Hpa*II. Duration of the injection of p53 protein was 600 s at 10 μL/min.

activity has proved to be an efficient alternative for demethylation and reactivation of apoptotic pathways (Wang et al., 2017; Kang et al., 2014). The practicability of the proposed method for screening of potent inhibitors of M.*Sss*I MTase was evaluated (Fig. 6). The SPR signals decreased gradually with the increasing concentrations of 5-Aza (ribose nucleoside) and 5-Aza-dC (deoxyribose nucleoside), and the half-maximal inhibiting concentration (IC_{50}) of 5-Aza and 5-Aza-dC was deduced to be 2.4 μM and 0.67 μM, respectively. The IC_{50} values by our method are highly comparable with those by electrochemical assay (4.2 μM and 3.2 μM) (Deng et al., 2014), chemiluminescence immunoassay (2.26 μM and 0.48 μM) (Li et al., 2016), nicking endonuclease-mediated rolling circle amplification strategy (3.7 μM and 0.8 μM) (Huang et al., 2017), and electrochemiluminescence assay (3.2 μM and 2.0 μM) (Zhou et al., 2016). The lower IC_{50} value of 5-Aza-dC than that of 5-Aza suggests that 5-Aza-dC is a more effective inhibitor, because 5-Aza-dC could be directly incorporated into DNA, while 5-Aza needed to be converted to deoxyribonucleoside triphosphate before incorporation into DNA (Lyko and Brown, 2005; Zhou et al., 2016). Procaine is a non-nucleoside inhibitor of DNA methylation, specifically binding to CpG islands in DNA sequences and abolishing DNA methylation (Lyko and Brown, 2005). The IC_{50} value of procaine was deduced to be 199.5 μM by the present method, being comparable with that by the electrochemical assay (244 μM) (Zhang et al., 2016). Note that the IC_{50} value of procaine is much higher than those of 5-Aza and 5-Aza-dC, suggesting that the nucleoside inhibitors which could be incorporated into DNA are more effective in inhibiting M.*Sss*I MTase activity. Relying on the methylation-specific ds-DNA consensus sites for recognition of p53 protein, SPR assay of M.*Sss*I MTase activity and screening of its inhibitors were realized.

3.7. Detection of M.SssI MTase activity in cell lysates

The feasibility of the method for the detection of M.*Sss*I MTase activity in HeLa cell lysates has been demonstrated (Fig. 7). A much higher SPR signal of 18.21 mDeg was obtained after treatment of the consensus ds-DNA-covered chips with the mixed solution containing cell lysate and S-adenosyl-L-methionine (curve a), and 11.1 ± 0.1 U/mL M.*Sss*I MTase was found in the cell lysate. Upon incorporation of the inhibitor of 5-Aza-dC, the much lower SPR signal of 8.64 mDeg (curve b) confirms that the SPR response in curve a was derived from the methylation of ds-DNA consensus sites by M.*Sss*I MTase in the HeLa cell lysate. Due to the almost complete cleavage of the ds-DNA consensus sites by *Hpa*II, similar SPR signal in the case of cell lysis buffer (7.59 mDeg, curve c) with that of curve b was attained. In addition, the tiny SPR signal of 4.79 mDeg was expected upon injection of 15.3 nM p53 onto the non-consensus ds-DNA-modified chips after treatment with HeLa cell lysate, and then *Hpa*II (curve d). The above results indicate the capability of the method for assaying intracellular M.*Sss*I MTase activity.

To further test the feasibility of the method for clinical applications, MCF-7 cancer cell lysate and MCF-10A normal cell lysate were assayed, and 5.5 ± 0.6 and 1.1 ± 0.3 U/mL M.*Sss*I MTase were determined, respectively. The elevated M.*Sss*I MTase levels in cancer cell lysates

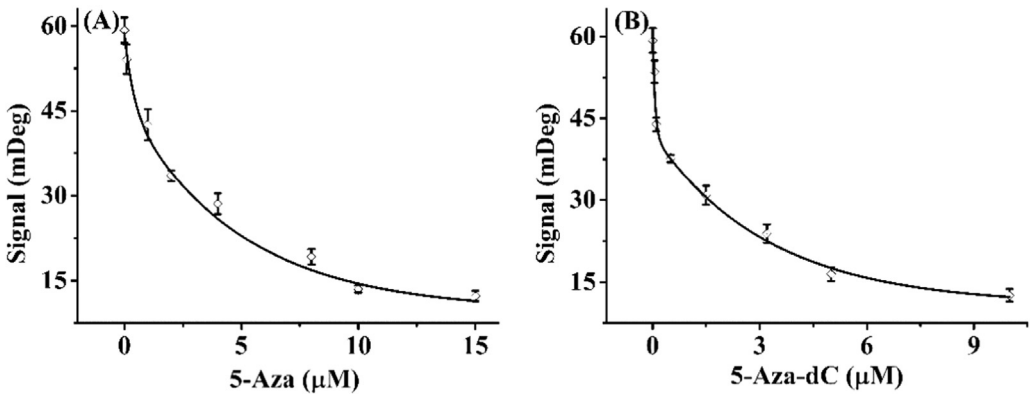

Fig. 6. Dependence of the SPR signals on the concentrations of (A) 5-Aza and (B) 5-Aza-dC. The SPR signals were attained upon injection of 15.3 nM p53 onto the consensus ds-DNA-modified chips after treatment with the mixed solutions containing 160 μM S-adenosyl-L-methionine, 80 U/mL M.*Sss*I MTase and its inhibitors (5-Aza or 5-Aza-dC), and then 25 U/mL *Hpa*II. The concentrations of 5-Aza examined are 0.1, 1, 2, 4, 8, 10 and 15 μM, and those of 5-Aza-dC are 0.05, 0.1, 0.5, 1.5, 3.2, 5 and 10 μM.

with respect to those in normal cell lysates are consistent with those measured by personal glucose meter (Deng et al., 2016) and electrochemiluminescence assay (Zhou et al., 2016). The recovery of 91–106.5% suggests that the method is largely free from the matrix effect of the complex cell lysate samples.

4. Conclusions

Real-time and sensitive SPR assay of methyltransferase activity and screening of methylation inhibitors have been performed based on p53 protein bound to methylation-specific ds-DNA consensus sites. The sensing protocol relies on the strong binding of methylated consensus ds-DNA to p53 protein and the dissociation constant (K_D) was determined to be 3.04 nM. The high binding capability between the methylated ds-DNA and p53 protein leads to the assay of M.*Sss*I MTase with a limit of detection down to 0.09 U/mL. The presence of potent inhibitors inhibits the activity of M.*Sss*I MTase, and the cleavage of the consensus ds-DNA by *Hpa*II leads to smaller SPR signals. Potent M.*Sss*I MTase inhibitors containing two nucleoside inhibitors of 5-Aza and 5-Aza-dC, and a non-nucleoside inhibitor of procaine were screened, and the nucleoside inhibitors are more effective in inhibiting M.*Sss*I MTase activity due to the ability to be incorporated into DNA. The clinical applications of the method for the assay of M.*Sss*I MTase activity in normal and cancer cell lysates have been demonstrated. The proposed method holds great promise for unraveling abnormal DNA methylation-related cancer initiation and progression.

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