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Detection of mutant p53 using field-effect transistor biosensor

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ABSTRACT

We assessed the abilities of wild p53 and mutant p53 proteins to interact with the consensus DNA-binding sequence using a MOSFET biosensor. This is the first report in which mutant p53 has been detected on the basis of DNA–protein interaction using a FET-type biosensor. In an effort to evaluate the performance of this protocol, we constructed the core domain of wild p53 and mutant p53 (R248W), which is DNA-binding-defective. After the immobilization of the cognate DNA to the sensing layer, wild p53 and mutant p53 were applied to the DNA-coated gate surface, and subsequently analyzed using a semiconductor analyzer. As a consequence, a significant up-shift in drain current was noted in response to wild p53, but not mutant p53, thereby indicating that sequence-specific DNA–protein interactions could be successfully monitored using a field-effect-based biosensor. These data also corresponded to the results obtained using surface plasmon resonance (SPR) measurements. Taken together, our results show that a FET-type biosensor might be promising for the monitoring of mutant p53 on the basis of its DNA-binding activity, providing us with very valuable insights into the monitoring for diseases, particularly those associated with DNA–protein binding events.

1. Introduction

One of the most frequent genetic alterations in human cancers is the mutation of the p53 gene [1,2]. The activation of cell growth arrest and apoptosis induction by the p53 tumor suppressor under genotoxic stress stimuli enables the maintenance of genome integrity [3,4]. For this reason, p53 has been referred to as the "master switch" for the control of cell proliferation. In particular, p53 exerts its transactivational activity via binding to specific DNA sequences, thereby turning on the transcription of target genes in response to a variety of cellular stresses [5]. Previous studies have amply demonstrated that the p53 mutations found in all types of human cancers occur almost uniformly at the central core DNA-binding domain [6,7]. In this context, the failure of the DNA-binding capability of p53 thus contributes to the uncontrolled cell cycle, thereby resulting in cancer progression [8]. Considering the biological significance of the mutated p53 protein, it is worth evaluating the DNA-binding ability of wild p53 and mutant p53 proteins.

A metal oxide semiconductor field-effect transistor (MOSFET), generally referred to as an insulated-gate FET (IGFET), is a type of potentiometric biosensor consisting of four terminal devices—the source, the gate, the drain, and the substrate. The principle underlying the operation of a MOSFET depends on an electric field to control the size and shape of a channel from the source to the drain. Upon exposure to the analyte, a gate modulates the flow of electrons through the channel, thereby inducing changes in the drain current [9,10]. Owing to its special properties, which include sensitivity, specificity, rapidity, miniaturizability, and portability, there have recently been notable advances in FET-type biosensors for biosensing research, including the ISFET-based monitoring of protein conformational changes [11], the development of the enzyme-immobilized FET which detects H^+ ion concentration [12,13], the DNA-modified FET based on DNA hybridization detection [14,15], and the cell-based FET for cell metabolism sensing or the measurement of extracellular potential [16,17].

In this study, we attempted for the first time to evaluate the DNA-binding activity of wild p53 and mutant p53 using a FET-type biosensor. After the DNA-binding of wild p53 and mutant p53 on the MOSFET surface, the resultant current changes through the semiconductor channel were investigated. A significant increase in drain current was noted with wild p53, whereas no such response was detected with mutant p53. This result clearly demonstrates the apparent differences in DNA-binding activity between wild p53

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and mutant p53. From these results, it has also been demonstrated that the FET-type biosensor convincingly enables the monitoring of DNA–protein interactions on the basis of label-free electrical detection.

2. Experimental

2.1. Fabrication of MOSFET device

The n-type MOSFET biosensor was fabricated using CMOS (complementary metal oxide semiconductor) technology, and Au film was used as the gate electrode for thiol-modification. This semiconductor device is composed of a p-type (1 0 0) silicon-on-insulator (SOI) wafer (15–20 Ω cm) as a starting material, with a 150-nm thick top silicon layer and a 400-nm thick buried oxide (BOX) layer. The SOI wafer contains a carrier layer, which harbors an insulating intermediate layer, and the insulating intermediate layer contains an active semiconductor layer. The active region was formed via standard photolithography, followed by plasma etching to pattern the sacrificial layer. The n+ source/drain layers were then formed via phosphorous ion implantation, and the gate oxide was obtained via high-temperature dry oxidation for field oxide growth to a thickness of 45 nm. In the last process, the metal layer of gold (450 Å) and nickel–chromium alloy (50 Å) was patterned using a wet etching process.

2.2. p53 core domain constructs

The core domains of wild p53 and the site-directed p53 mutant were generated via PCR (polymerase chain reaction) using synthetic oligonucleotides, and the mutation was confirmed via DNA sequencing. In brief, the p53 core domain (amino acids 83–293) was PCR-amplified using the 5′ primer (5′-GGAATTCCATATGGCGGCCCCTGCACCAG-3′) and the 3′ primer (5′-CGGGATCCAACCCTTTCTTGCGG-3′) with the NdeI and BamHI sites, respectively. In order to generate the site-directed p53 mutant (R248W), the 5′ primer (5′-GGGCGGCATGAACTGGAGGCCCATCCTCA-3′) and the 3′ primer (5′-TGAGGATGGGCCTCCAGTTCATGCCGCCC-3′) was utilized for PCR amplification. The resultant DNA fragments were then inserted into pET21a (Novagen, USA) using the NdeI and BamHI sites. The plasmids were then transferred to the expression host, *E. coli* BL21 (DE3) (Stratagene, USA), and the expression and purification of the wild and mutant p53 core domains were conducted as described previously [11].

2.3. MOSFET measurement

The I_D–V_D characteristics of NMOSFET were checked prior to DNA immobilization. For the cleaning gate electrode of the device, acetone, methanol, and distilled water were applied for 5 min with sonication. After cleaning, the surface of the gate electrode was activated via 120 s of O_2 plasma treatment at 120 W. 100 nM thiol-modified GADD45 promoter DNA (5′-SH-$(CH_2)_6$-GTACAGAACATGTCTAAGCATGCTGGGGAC-3′) in 0.1× PBS was immobilized onto the gate electrode for 90 min. As a blocking agent and spacer, 10 μM 6-mercapto-1-hexanol (Sigma–Aldrich, USA) in 0.1× PBS was added to the gate electrode for 60 min. The hybridization of GADD45 promoter complementary DNA (5′-GTCCCCAGCATGCTTAGACATGTTCTGTAC-3′) was also conducted in 0.1× PBS for 60 min. After DNA immobilization and hybridization, the p53 samples (100 nM) in 0.1× PBS were added to the gate electrode for 30 min. The drain current measurement was conducted in a shield box at 50% humidity.

2.4. SPR analysis

The instrument utilized for SPR analysis was the BIACORE 3000 (BIACORE AB, Sweden), and the carboxymethylated dextran chip (CM5 chip) was also purchased from BIACORE AB. The DNA immobilization was conducted as described previously [18]. After DNA immobilization, the SPR instrument was primed with 0.1× PBS for the protein binding experiment. The purified wild p53 and mutant p53 (R248W) were injected at a concentration of 100 nM and a flow rate of 5 μL min^{-1} for 6 min.

3. Results and discussion

3.1. Theory and experimental design concept

A NMOSFET (n-type metal oxide semiconductor field-effect transistor) device was fabricated in order to monitor the mutant p53 protein. In the MOSFET device, the gate voltage basically modulates the current flow from the source to the drain through an n-channel formed at the top of a p-type substrate located below the gate. Theoretically, the interrelation between the drain current and the gate potential of the MOSFET-based biosensor at the saturation region is elucidated in accordance with the conventional MOSFET principle, as follows:

$$I_d = \left(\frac{1}{2}\right)\mu_n C\left(\frac{W}{L}\right)(V_g - V_t + V_{bio})^2 \quad \text{(at saturation region)}$$

in which μ_n is the electron mobility, C is the total gate capacitance, W is the gate width, L is the gate length, and V_g, V_t, and V_{bio} are the gate, threshold, and biomolecular potential, respectively. In this experiment, the drain current, I_d, is related directly to the potential of the biomolecules, V_{bio}. Drain current decreases (increases) when negatively (positively) charged molecules bind to the gate surface in an n-type FET. When DNA molecules (p53 target binding sequences) bind to the gate surface, it is supposed to decrease the drain current because V_{bio} has a negative value due to the negative charge of DNA. When the binding event between the negatively charged DNA and the positively charged proteins (wild p53) occurs, the drain current increases above that of the DNA-bound gate, as the result of a lesser negative net charge (Fig. 1A).

It is well-known that p53 as a transcription factor is mutated in more than 50% of all human cancers [19], and 90% of the corresponding mutations are detected within the DNA-binding domain [5]. Once p53 loses its transacting function to bind to DNA or to promote transcription, a marked decline concomitantly occurs in the expression of anti-proliferative proteins, thereby resulting in an acceleration of tumor growth. In this regard, it is important to note that the monitoring of variations in DNA-binding ability between wild p53 and mutant p53 may prove extremely valuable in the field of p53 cancer biology. In service of this goal, we sought to measure the DNA-binding activities of wild p53 and mutant p53 using a FET-type biosensor. The principal feature of this approach is that the gate sensing layer is coated with p53 target-binding DNA, then exposed to wild p53 and mutant p53 proteins, for measuring the DNA–p53 interaction (Fig. 1A).

It is also known that p53 mutations usually abolish DNA-binding activity. Particularly, one of the most common p53 mutations is a R248W mutant, which abrogates the DNA-binding ability of the p53 tumor suppressor [20,21]. Previously, the R248W mutant has been shown to be DNA-binding-defective, and thus inactive in transactivation [22]. For the reason, mutant p53 (R248W) has been widely used as a negative control for p53 DNA-binding analyses *in vitro* or *in vivo*. [23,24]. In order to generate the p53 mutation, a mutant p53 (R248W) gene was constructed via a site-directed mutagenesis technique, as described in the Experimental section. After the

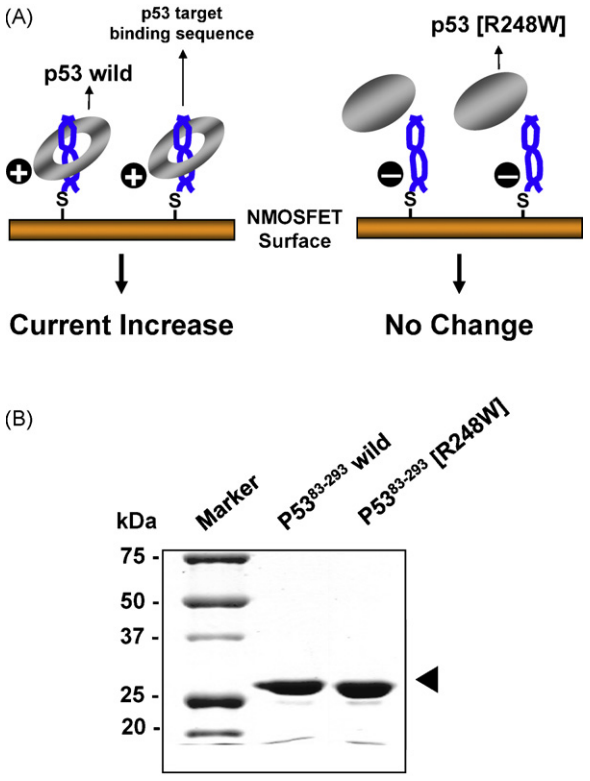

Fig. 1. Experimental design concept. Upon the binding of wild p53 onto the cognate DNA-modified MOSFET surface, drain current increases as a consequence of the charge effect. In contrast, no change in drain current is anticipated in response to mutant p53 (R248W), owing to a failure of DNA binding (A). The purification of the core domain of wild p53 (amino acids 83–293) and mutant p53 (amino acids 83–293, R248W) (B). Purified recombinant proteins were analyzed via 10% SDS PAGE, and visualized via Coomassie staining. The arrowhead indicates the expressed wild p53 and mutant p53 proteins.

expression and purification of wild p53 and mutant p53 (R248W) (Fig. 1B), the corresponding proteins at a concentration of 100 nM were further applied in the MOSFET and SPR experiments immediately following preparation.

3.2. Real-time electrical detection of DNA-modified MOSFET surface

After establishing the current baseline, the MOSFET gate surface was treated with 0.1× PBS buffer (pH 7.4) containing p53 target binding DNA. The immobilization of DNA onto the gate surface was evaluated by the detection of the drain current changes using a semiconductor parameter analyzer (KEITHLEY 4200). As shown in Fig. 2 (upper panel), upon the injection of thiol-modified ssDNA, the drain current of the MOSFET was decreased by approximately 13.0 μA under a fixed output of 2.0 V. Likewise, when the complementary DNA was hybridized with thiol-modified-DNA immobilized on an Au layer, the down-shift in drain current was also clearly observed; this is attributable principally to the DNA (−) charge effect (Fig. 2, lower panel). The data can be explained by the operation principle that the negative charges near the gate induce the drop in the drain current of the n-type MOSFET, as the negative charges stored on the gate result in a (−) gate potential of the device.

Meanwhile, assuming that the DNA charge is distributed evenly in a manner similar to that of a planar electrode, the surface charge density of DNA immobilized onto the gate can be estimated as reported previously [15]. Changes in the DNA surface

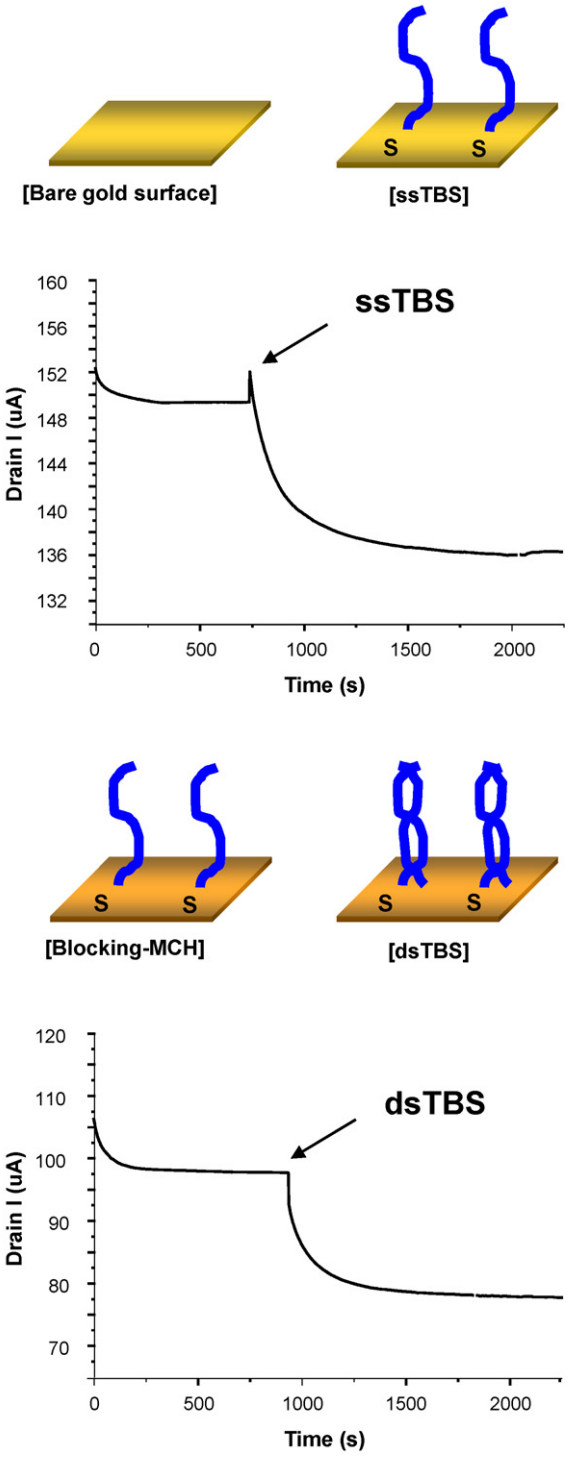

Fig. 2. Real-time detection of DNA-modified MOSFET surface. Changes in drain current versus time were measured when DNA was immobilized (ssDNA) and hybridized (dsDNA). TBS, p53 target binding sequence.

charge density induce an altered gate potential and channel conductance, which are connected with variations in the drain current. On the basis of this principle, the number per unit area of thiol-modified DNA on the gold thin film was calculated as approximately 3.28×10^{12} [$N\,m^{-2}$, number of DNA per unit area]. This DNA adsorption value is broadly consistent with the previously reported molecular surface density [15].

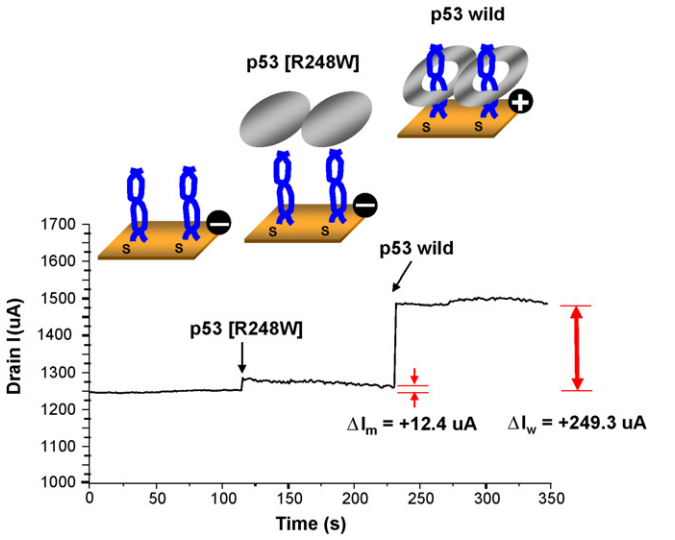

Fig. 3. Real-time monitoring of mutant p53 using MOSFET. Plot of drain current versus time in response to mutant p53 (R248 W) and wild p53 on a single MOSFET device at an applied voltage of 2.0 V. The measurement was conducted on a probe station using a semiconductor parameter analyzer (KEITHLEY 4200).

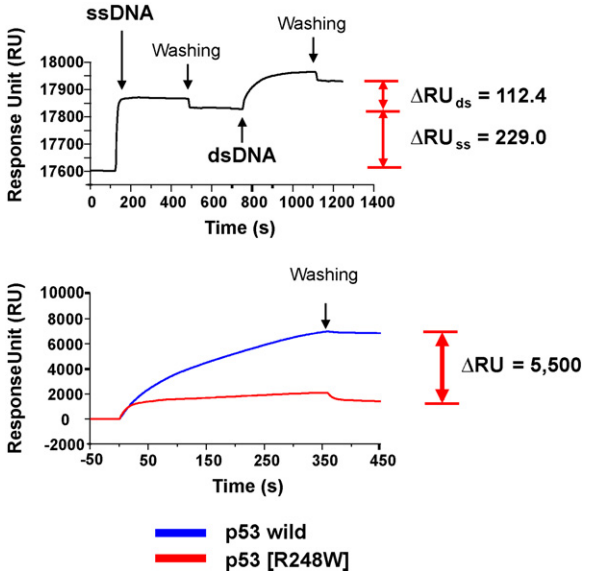

Fig. 4. SPR analysis. The SPR sensorgram illustrates the resonance angle shift in accordance with DNA injection (upper panel). The SPR sensorgram shows the interaction of wild p53 with the cognate DNA, but not mutant p53 (R248W) (lower panel). The SPR signal intensities were expressed in resonance units (RU).

3.3. MOSFET measurement

In order to evaluate the sequence-specific binding of the p53 tumor suppressor using a FET-type biosensor, the saturation drain current was monitored in response to the wild p53 and mutant p53 proteins. In this study, the GADD45 cis-acting p53-responsive element used as a p53 target binding DNA was particularly adopted to the detection of the human p53 protein, due to its extensive use as a model sequence for studies of p53 binding. In an effort to evaluate the performance of MOSFET-based p53 monitoring, the human p53 core domain (amino acids 83–293), rather than full-length p53, was purified with an in-house protein purification system, as described in the Experimental section. There are two reasons why the human p53 core domain was selected for use in this study. The first reason is that this domain specifically binds to the p53 target binding DNA *in vitro*, as reported previously [25]. Another reason for this is that the isoelectric point (p*I*) value of the core domain is approximately 8.8, which will result in a net positive charge under our experimental buffer conditions at pH 7.4. In the case of the full sequence of human p53, we measured a p*I* value of 6.3, at which the surface harbors a net negative charge at a given pH. As DNA is negatively charged, the use of a protein with net negative charge was deemed inappropriate for use in our experimental setting.

In general, device-to-device variations exist in almost all types of FET biosensors, as this type of inter-wafer variations have proven a drawback in terms of the acceptable fidelity of the device. For this reason, in this study we conducted our electrical measurements using a single MOSFET device in an effort to circumvent the issue of device-to-device variation. As mutant p53 (R248W) does not interact with the cognate DNA, the mutant p53 protein will move away from the DNA-coated sensing layer after washing, thereby resulting in no changes in drain current—however, this is not the case with the wild p53 protein. With this consideration, immediately after the detection of the mutant p53 protein, the sequential measurement of wild p53 protein was achieved on the same MOSFET device. As shown in Fig. 3, almost no comparable changes (ΔI = +20.0 μA) in the drain current of the MOSFET device were observed, if any, when treated with mutant p53 (R248W) on a DNA-modified sensing layer, thereby implying the absence of an interaction between mutant p53 protein and the cognate DNA. However, following a thorough washing process in order to eliminate residual mutant p53 protein on the gate surface, a significant increase in drain current was measured approximately ΔI = +250.0 μA in response to the wild p53 protein, which is 12.5 times higher than that of mutant p53 (R248W). Above all, surface charge has been identified as a crucial determinant for the FET-based measurement of biomolecular interactions, as the drain current is associated strongly with biomolecular potential on the gate surface. Along this line, these results can be elucidated by assuming that the positively charged p53 protein shields the consensus DNA on the gate surface, thereby directing the up-shift in the drain current. The notion that positive charges on the n-type MOSFET surface induce the current increase is theoretically predicated on the principle underlying the operation of a FET-type biosensor, that when the (+) charged molecules exist on the gate surface, the channel conductance of the MOSFET device increases, thereby resulting in an augmentation of the drain current, a phenomenon referred to as the "charge dominant effect". The results acquired from the MOSFET measurements can be interpreted in accordance with the rationale of this approach that both the shielding of negatively charged DNA and the advent of positively charged protein as the consequence of DNA–p53 interaction are synergistically responsible for the dramatic increase in saturation drain current. Although the sensitivity obtained from this study was relatively poor under our experimental conditions, our findings clearly demonstrated the feasibility of MOSFET biosensor for detecting the sequence-specific DNA-binding activity of wild p53 and mutant p53 proteins. In terms of sensitivity, the low total surface area in thin-film MOSFET may be responsible for the lower concentration of analytes. Thus, further studies targeting toward an improvement of detection limit including the development of FET device for better performance will be required.

3.4. SPR analysis for the detection of p53 binding to the cognate DNA

The interaction behaviors of wild p53 and mutant p53 (R248W) with the cognate DNA were analyzed using a real-time SPR under the same experimental conditions. As shown in Fig. 4, the upper panel illustrates the changes of SPR angle in response to ssDNA and subsequent hybridization with the complementary DNA on the gold chip surface. The difference of RU value prior to/after ssDNA

injection (ΔRU_{ss}) was approximately 229.0, and after the treatment of the complementary DNA, ΔRU_{ds} was approximately 112.4, which is smaller than that of ssDNA injection. This may be caused by the feasibility of the incomplete hybridization of the complementary DNA. As shown in Fig. 4 (lower panel), the SPR sensorgrams of two protein samples evidenced significantly different binding profiles. The RU value for the wild p53 protein was approximately 7000 in the plateau region of the SPR sensorgram, whereas the mutant p53 protein evidenced a value of 1500 RU (Fig. 4). In general, the SPR angle shift is expressed in terms of resonance units (RU), where an RU value of 1000 corresponds to approximately 1 ng mm^{-2} of bound protein. Thus, the total amount of the bound p53 protein can be calculated as 7.0 ng mm^{-2} (7×1 ng mm^{-2}). The SPR data showed that the RU value of the mutant p53 protein was 4.7 times lower than that of wild p53 protein in response to 100 nM of each protein. Based on the notion that the SPR response is directly proportional to the amount of p53 protein interacted with the consensus DNA coated on the gold thin film, the results obtained from SPR sensorgrams demonstrated that the wild p53 protein could bind to the cognate DNA sequence, but that the mutant p53 protein could not, thereby providing a solid confirmation of the MOSFET analysis.

4. Conclusions

Here we attempted for the first time to assess the DNA-binding activity of wild p53 and mutant p53 using a MOSFET biosensor. Upon treatment with mutant p53 applied on a DNA-modified MOSFET surface, the current change could not be distinguished, indicating a failure of binding between mutant p53 and the cognate DNA element, whereas apparent up-shift in drain current was observed with wild p53 as a consequence of sequence-specific binding. Thus, our data clearly indicated that the MOSFET biosensor can be used to detect the mutant p53 protein by virtue of the electrical properties, as well as possibly to measure the DNA-binding ability of a particular protein from nuclear extracts obtained from diseased and control individuals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2010.03.006.

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