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The interaction of azurin and C-terminal domain of p53 is mediated by nucleic acids

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ABSTRACT

Azurin is bacterial protein, which was been reported to promote cancer cell death in vitro. The interaction of azurin and p53 is important for the cytotoxic effect of azurin towards cancer cells. In this study, it was found that nucleic acids mediated the interaction of azurin and the C-terminal domain of p53 (residues 352–393). The results provide novel insight into the interaction, and raising the possibility that the allosteric regulation of C-terminus of p53 by nucleic acids play an important role in the interaction of p53 with azurin. Meanwhile an elongated expressed product of azurin was cloned and purified, which was found to have stronger interaction with C-terminal domain of p53. Cytotoxicity studies showed that the cytotoxic effect of this elongated expressed product of azurin was stronger than wild-type azurin. The difference found in the cytotoxic effect of azurin with various sequence may provide valuable insight for finding more effective anticancer peptides.

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Introduction

The tumor suppressor protein p53 is a transcription factor that plays a key role in an intricate regulatory network controlling cell growth, genomic stability and cell death. Dysregulation or loss of function of p53 has been shown to contribute to cancer development [1–3]. The levels and the activity of p53 are tightly controlled. It is generally present at a low level in mammalian cells due to its short half-life of a few minutes. In response to genotoxic stresses such as DNA damage, p53 is stabilized and accumulated in the nucleus [4,5]. The accumulation of p53 in cancer cells promotes apoptosis or cell-cycle arrest through expressing relevant proteins including Bax, p21, etc. [2]. The stabilization of p53 is thought to result primarily from disruption of the interaction between p53 and MDM-2, which negatively regulates p53 levels through ubiquitin–proteasome pathway [6].

p53 has two discrete DNA-binding domains. The one located at the core of p53 is a sequence-specific DNA-binding domain, while the other one located at C-terminus interacts with a wide variety of non-specific DNA structures [4]. This feature adds the complexity to the regulation of p53 by sequence-specific DNA. Allosteric regulation of the C-terminus by nucleic acids has been suggested to influence the binding of p53 core domain to specific sequences [7,8]. It is reported that the binding of DNA to the C-terminus changes the structure of p53, thus modulating the interaction of p53 with other proteins [9]. For example, the binding and acetylation of the C-terminal domain by p300 protein is DNA-dependent. The allosteric effect of DNA binding changed the conformation of p53, and thus removed the barrier to acetylation [10,11].

Azurin is a redox protein which acts as an electron transfer shuttle in *Pseudomonas aeruginosa* and other bacteria. It has been suggested that azurin may form a complex with p53 and stabilize it [12]. The stabilization of p53 leads to an increase of intracellular p53, which in turn promotes apoptosis by regulating expression of pro-apoptotic proteins [13–15]. In vitro studies have revealed that there is a direct interaction between azurin and p53 [16–18]. But it is not known whether nucleic acids play roles in the interaction of p53 and azurin. In the course of examining the interaction of azurin and p53, it was found that the interaction of azurin and the C-terminal domain of p53 was mediated by nucleic acids. We then characterized the effect of nucleic acids on the interaction, and proposed a putative model that nucleic acids might allosterically regulate the conformation of the C-terminal domain of p53 to promote its interaction with azurin.

Materials and methods

Materials

All the restriction enzymes, Pfu polymerase, and DNA ligase used in constructing plasmid were obtained from Fermantas (MBI, Germany). The primers and the single strand nucleic acid used in the GST pull-down assay were synthesized in Sangon (Shanghai, China) or Invitrogen (San Diego, CA). The Nickle Sepharose beads and

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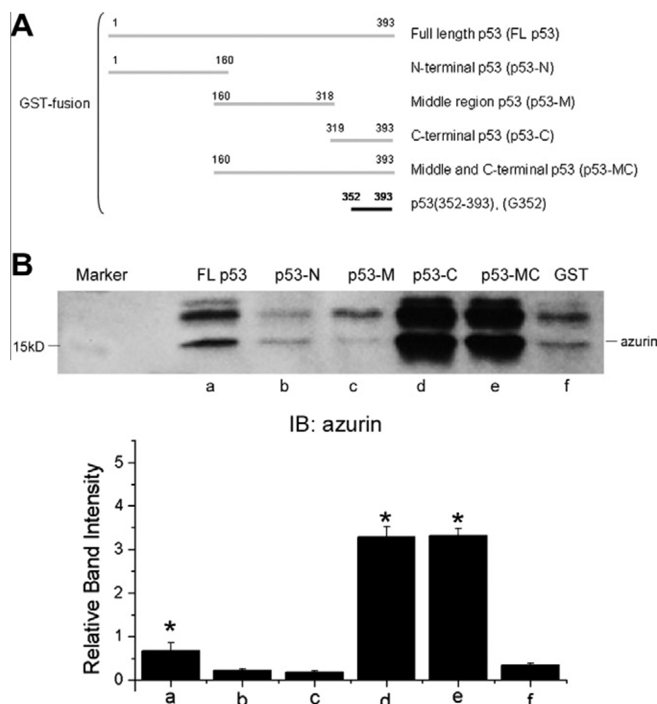

Fig. 1. (A) Genetic constructs of full-length p53 (FL-p53) and various truncated p53: N-terminal (p53-N), middle region (p53-M), C-terminal (p53-C), middle and C-terminal region (p53-MC), and a fragment of p53 (residues 352–393), namely G352. These GST-fused proteins were purified and used for GST pull-down assay. (B) GST pull-down assay to examine the interaction between azurin and full-length or various truncated p53. (IB: azurin): Antibody specifically recognizing His-tag was used to probe the His-tagged azurin in immunoblots. The pulled down azurin were significantly different in (* vs GST) ($P < 0.05$).

Glutathione Sepharose 4B beads were from Amersham Pharmacia Biotech Inc., Piscataway. The Heparin-Sepharose beads were obtained from Weishibohui Company (Beijing, China). Dulbecco's modified Eagle's medium (DMEM)[1] and fetal calf serum (FCS) were obtained from Invitrogen. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), isopropyl β-D-thiogalactopyranoside (IPTG) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) were purchased from Amresco Inc. (Solon, OH, USA). BCA Protein Assay Kits were purchased from Pierce (Rockford, IL). J774 cells were provided by the Cell Resource Center, Chinese Academy of Medical Sciences/Peking Union Medical College (CAMS/PUMC). All other chemicals were made in China and were of analytical grade.

Preparation of azurin

The azurin-encoding gene of *P. aeruginosa* was amplified and cloned into PQE30. Azurin was purified from recombinant *Escherichia coli* BL21 (DE3) cells according to a previously published method [19]. After harvested by centrifugation, the cells were resuspended in lysis buffer containing in 50 mM NaCl and 50 mM Tris–HCl (pH 8.0) and lysed by sonication on ice. After centrifugation, the supernatant containing the His-tagged azurin was loaded onto a Ni-affinity column and eluted using a step gradient of 0.05–0.5 M imidazole. The fraction containing azurin were pooled. Further purification was made by an anion exchange column. The purified azurin was tested by SDS–PAGE. And the protein concentration was determined using a BCA kit.

[1] *Abbreviations used:* DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IPTG, isopropyl β-D-thiogalactopyranoside; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide; PCR, polymerase chain reaction; DMSO, dimethyl sulfoxide; FL-p53, Full-length p53.

Preparation of full length and various truncated p53

GST-fused full-length p53 and truncated p53 (Fig. 1A) were kind gifts from Dr. Thomas Shenk. Expression of GST-fused p53 was induced according to a previously published method [20,21]. The purification was achieved by Glutathione Sepharose beads. The eluent from the beads was dialyzed against PBS. Further purification of the C-terminus of p53 (residues 352–393) was made by an anion exchange column (Q-sucrose from Amersham Biosciences).

The peptide of 352–393 of p53 was made by incubating the GST-tagged p53 (352–393) with Prescission Protease according to the manufacturer's instructions (Amersham Biosciences). The GST tag was cut-off and removed by Glutathione Sepharose beads. The peptide in the flow-through of the Glutathione Sepharose beads was further purified by the affinity chromatography with a Heparin-Sepharose column. The purity of the peptide 352–393 was examined by Tricine–SDS–PAGE [22].

GST pull-down assay and Heparin-Sepharose beads pull-down assay

Equal amounts of GST-tagged p53 or GST alone were mixed with the Glutathione Sepharose 4B beads (Amersham). After loaded with the GST-fused proteins, the beads were washed by PBS containing 0.05% NP-40 to removed unbound GST-fused proteins. The beads were then used to pull-down azurin. The unbound azurin was removed by washing the beads. The protein bound to the beads was eluted by 10 mM glutathione and 20 mM Tris–HCl (pH 8.0). The eluted proteins were subjected to SDS–PAGE and transferred onto a nitrocellulose membrane (Millipore). The antibody for His-tag was used to detect the His-tagged azurin which was pulled down and transferred onto the membrane.

To examine the effect of nucleic acids on the interaction of azurin and C-terminus of p53, the lysates of *E. coli* expressing GST protein or sequence-specific nucleic acids were added into the reaction solution. Two oligonucleotides that were reported to bind to the C-terminus of p53 were used in the experiment: 5′-ATCGAACTAGTT AACTAGTACGCAA-3′ and 5′-TTAAGGACATGCCCGGGCATGTCC-3′ [23].

In the Heparin-Sepharose beads pull-down assay, equal amounts of GST-tagged p53 (352–393), GST, and the peptide 352–393 were loaded onto the beads. After removing the unbound proteins, the beads were used to pull-down azurin similarly as GST pull-down assay.

Identify the elongated expression of azurin

The gene of the elongated expression of azurin was amplified by a polymerase chain reaction (PCR) using primers 5′-CGGGATCCA TGGCCGAGTGCTCGGTG-3′ and 5′-CGCGAGCTCTTATCATCCGGGGTC AGCACC-3′ designed based on the sequence of azurin in PQE30. The PCR product was purified with the Gel Extraction kit and cloned into PQE30. The positive clones were identified by restriction enzyme digestion. The termination codon in the plasmid constructed above was then replaced by the codon for glycine. The site-directed mutagenesis of the azurin gene was performed using a QuickChange Site-directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. A single set of oligonucleotides was designed for mutation as follows: 5′-CCCTGACCCTGAAGGGAGGGAAGCTTCAC GC-3′ and 5′-GCGTGAAGCTTCCCTCCCTTCAGGGTCAGGG-3′. Mutation was confirmed by DNA sequencing. And the elongated expression of azurin was purified similarly as azurin.

J774 cell culture and cytotoxicity assay

J774 cells were cultured in DMEM supplemented with 10% fetal bovine serum, at 37 °C in a humidified incubator with 5% CO_2. The

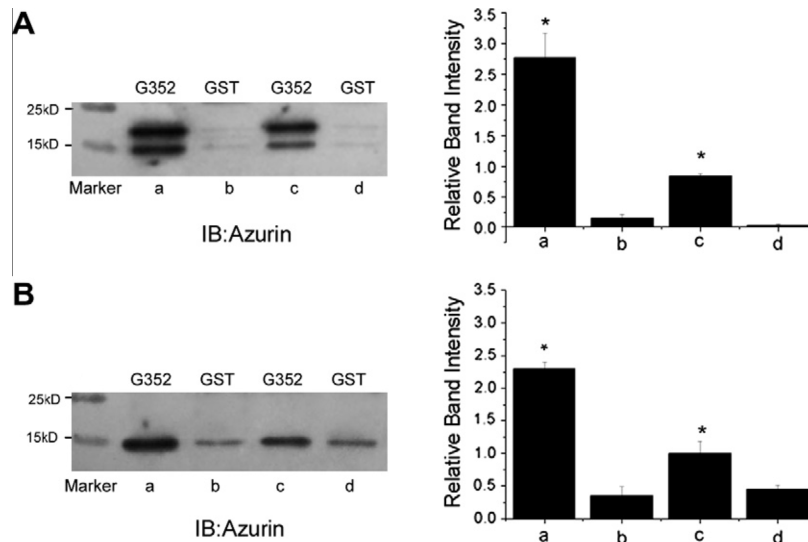

Fig. 2. (A) GST pull-down assay was performed to demonstrate the interaction of C-terminus of p53 (residues 352–393) and azurin purified by Ni-Sepharose affinity column. Different concentration of azurin (8.5 μM in a, b) and (1.7 μM in c, d) were used in the pull-down assay. (B) The interaction of C-terminus of p53 (residues 352–393) and azurin which was further purified using anion exchange column. Different concentration of azurin (8.5 μM in a, b) and (1.7 μM in c, d) were used in the pull-down assay. The pulled down azurin were significantly different in (* vs GST) ($P < 0.05$).

cytotoxicity of azurin was measured by MTT assay according to a previously published method [12] with modifications. About 5×104 cells per well were seeded onto 96-well culture plates in 120 μL of DMEM. After overnight culture, the medium was replaced with fresh medium containing different concentrations of azurin. Cells were incubated with azurin for 24 h and the medium was then replaced with fresh medium free of serum containing 0.5 mg/mL MTT solution. After incubation for 3 h at 37 °C, MTT was removed, and cells were lysed with dimethyl sulfoxide (DMSO). The absorbance at 595 nm was measured using a Bio-Rad 3350 microplate reader.

Statistical analysis

One-way ANOVA was used to estimate overall significance followed by post hoc Tukey's tests corrected for multiple comparisons [24]. Data are presented as mean ± SEM. A probability level of 5% ($P < 0.05$) was considered significant.

Results

GST-tagged full-length p53 and various truncated p53 pulled down azurin

GST pull-down assay was used to examine the interaction between azurin and p53. The various p53 versions (Fig. 1A) had different interaction with azurin (Fig. 1B). Full-length p53 (FL-p53) had weak interaction with azurin. And the versions of p53-C (residues 319–393) and p53-MC (residues 160–393) had the stronger binding activity with azurin than the FL-p53. The N-terminal domain and the middle region of p53 did not seem to interact with azurin. In the GST pull-down result, there is a bigger molecular weight protein which was pulled down along with azurin.

The C-terminus of p53 (residues 352–393) pulled down azurin in GST pull-down assay

The C-terminus of p53 (residues 319–393, p53-C) contained the oligomerizated domain [25], causing p53-C to form oligomer in the gel filtration. Then the oligomerizated domain was cut-off, and a fragment of p53 between residues 352–393 was constructed. The fragment of p53 (352–393), tested on a Superdex200 column, was monomer. The interaction between p53 (352–393) and azurin is as strong as that between p53-C (residues 319–393) and azurin (Fig. 2), indicating that the oligomerizated domain of p53 is not involved in the interaction.

In the GST pull-down result shown in Fig. 2A, there is an elongated expression of azurin (demonstrated below), which interacts with p53 (352–393) strongly. The azurin used in Fig. 2A was purified by Ni-affinity column and dialyzed against PBS. When azurin was further purified by anion exchange chromatography, the bigger protein disappeared in the pull-down result (Fig. 2B).

Nucleic acids mediated the interaction of p53 (352–393) and azurin

The GST-tagged p53 (352–393) fragment was further purified by the anion exchange column. It was eluted by a NaCl gradient from the column (Fig. 3A). The first eluent is p53 (352–393) which was examined by SDS–PAGE (Fig. 3B) and designated as "#G352" to distinguish from "G352", which was purified by the Glutathione Sepharose beads only. The second eluent at a higher salinity was non-protein, tested by the SDS–PAGE. And the second eluent has a strong absorbance at OD_{260}, which indicated that the second eluent might be nucleic acid.

Surprisingly the purified "#G352" weakly interacted with azurin than "G352" (Fig. 4A). When the lysate of *E. coli* expressing GST protein was added into the pull-down interaction, the intensity of interaction of "#G352"and azurin was resumed, similar as the interaction of "G352" and azurin. In control group, the same lysate did not make GST protein to pull-down azurin. Furthermore sequence-specific nucleic acids which were reported to bind to the C-terminus of p53 were used [23]. In the GST pull-down result the two oligonucleotides especially the second one increased the intensity of the interaction between "#G352" and azurin significantly (Fig. 4B).

Purification of the fragment of p53 (352–393) without GST tag and examining the interaction by Heparin-Sepharose pull-down assay

The GST tag was cut-off from the fused p53 (352–393) by the Prescission Protease. As the peptide 352–393 has a DNA-binding domain, the further purification was made by the Heparin-Sepharose

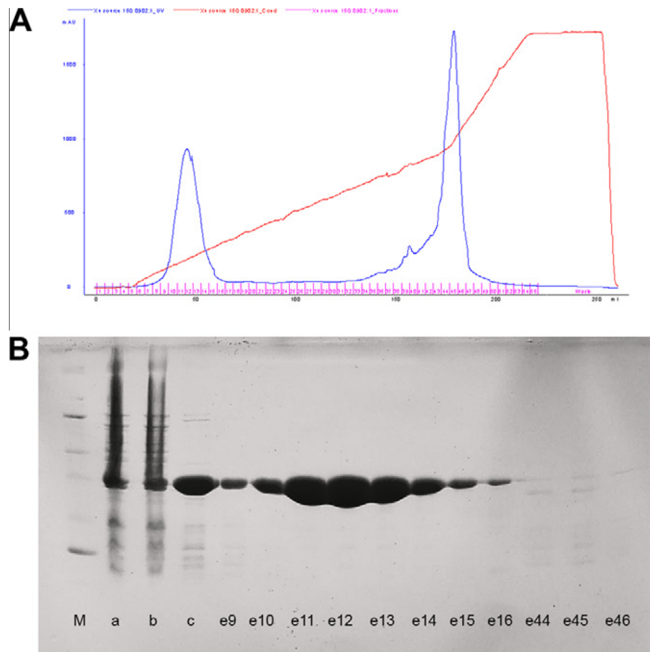

Fig. 3. (A) Purification of C-terminus of p53 (residues 352–393) through anion exchange chromatography. The blue line indicated the UV absorbance in OD280. The red line indicated the increase concentration of salinity, and the final salinity for elution is 1 M NaCl. (B) The eluent samples were tested by SDS–PAGE. a and b, the lysate of *E. coli* expressing G352; c, the purified G352 by Glutathione Sepharose; e9–e16, the first eluent samples in the lower salinity; e44, e45, e46, the second eluent samples in the higher salinity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

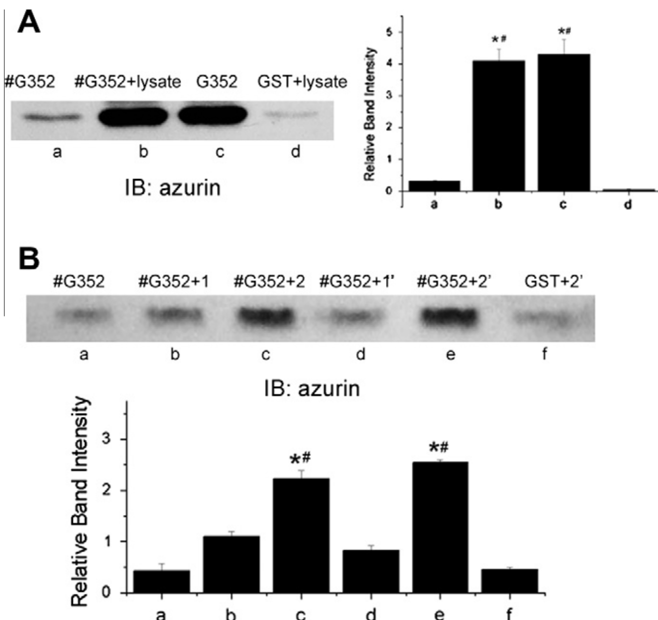

Fig. 4. (A) GST pull-down assay was performed to examine the interaction of azurin and #G352 or G352. The first eluent of p53 (352–393) is designated as "#G352", to distinguish from "G352" which was purified by the Glutathione Sepharose beads only. "#G352" only or "#G352" in the presence of the lysate of *E. coli* expressing GST protein pulled down azurin separately in a, b. The "G352" only or GST in the presence of the same lysate (as in b) pulled down azurin in c, d. (B) Effect of sequence-specific nucleic acids on the interaction of "#G352" with azurin examined by GST pull-down assay. The sequences of the two oligonucleotides used in the assay are: 5′-ATCGAACTAGTTAACTAGTACGCAA-3′ (1 synthesized in Sangon and 1′ synthesized in Invitrogen) and 5′-TTAAGGACATGCCCGGGCATGTCC-3′ (2 synthesized in Sangon and 2′ synthesized in Invitrogen). The pulled down azurin were significantly different in (∗ vs GST) and (# vs #G352) ($P < 0.05$).

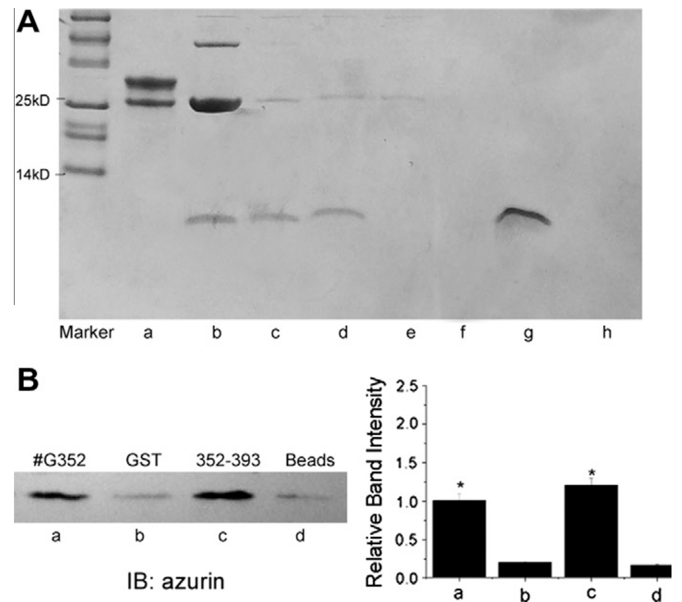

Fig. 5. (A) Characterization of the purified peptide 352–393 from the C-terminus of p53 by Tricine–SDS–PAGE. (a) the purified GST-tagged p53 (352–393); (b) the digested product of GST-tagged p53 (352–393) by Prescission Protease; (c) the flow-through protein from the Glutathione Sepharose beads on which the digested product were loaded; (d) the diluted sample in (c) by the same volume of double distilled water; (e) the flow-through protein from the Heparin-Sepharose column; (f) There was no eluted protein in the equilibrated Heparin-Sepharose column by 20 mM Tris–HCl (pH 8.0); (g) the peptide of 352–393 was eluted by the buffer of 20 mM Tris–HCl (pH 8.0) containing 0.5 M NaCl; (h) there was no protein by further elution with the buffer containing 1 M NaCl. (B) Heparin-Sepharose beads loaded with different proteins were used to pull-down azurin. Heparin-Sepharose beads loaded with GST-tagged p53 (352–393) (a), GST protein (b),or the peptide 352–393 (c), and the Heparin-Sepharose beads themselves (d) pulled down azurin. The pulled down azurin were significantly different in (∗ vs GST) ($P < 0.05$).

chromatography. The purified peptide is about 4kD, which was shown in Tricine–SDS–PAGE (Fig. 5A). Immobilized heparin, a negatively charged sugar-containing macromolecule, is often used to purify the RNA and DNA binding proteins. We took advantage of this character of Heparin-Sepharose beads to examine the interaction of the p53 peptides with azurin, just like the GST pull-down assay. The Heparin-Sepharose beads loaded with different protein pulled down azurin in different manners (Fig. 5B). The beads loaded with GST-tagged p53 (352–393) and the peptide 352–393 could pull-down azurin strongly, while the beads loaded with GST protein and the beads themselves only weakly pull-down azurin.

Identification of the elongated expression product of azurin and its interaction with p53 (352–393)

In the GST pull-down result shown in Fig. 2, there is a bigger molecular weight protein which was pulled down along with azurin. Although this bigger protein was hardly to see on the gel of SDS–PAGE, it was strongly pulled down by p53 (352–393). When azurin expressed from another plasmid vector was purified using the same procedure and used in GST pull-down assay, the bigger protein was not detected (result unshown). Thus, it was speculated that the bigger protein might be a product of read-through in expression.

The plasmid PQE30 expressing azurin was then sequenced. It was surprised to us that the sequence after the termination codon of TGA is different from the sequence of the original plasmid. The mostly expressed proteins by the changed plasmid were the wild-type azurin. Small quantities of elongated expression were produced by read-through the first termination codon, ending at

the second termination codon. The change might be a result a genetic recombination, but how it happened is still a mystery to us. As a result of the changes in the sequence of PQE30, we failed to construct the elongated expression by direct mutagenesis of the first termination codon. The gene of the elongated expression of azurin had to be amplified first and ligated into an original plasmid (relative to the changed plasmid PQE30). Then the termination codon in the plasmid constructed above was replaced by others, here we used the codon for glycine to substitution. Thus we got the elongated expression of azurin (Fig. 6A). The elongated expression product is about 4 kDa bigger than azurin. And the elongated expression had stronger interaction with p53 (352–393) than azurin (Fig. 6B).

The cytotoxic effect of azurin and its interaction with p53 (352–393)

The cytotoxicity of azurin and the elongated expression was examined using J774 cancer cells. The viability of J774 cells was examined by MTT assay. It was found that the elongated expression had stronger cytotoxic effect than wild-type azurin (Fig. 7A).

A double mutant of azurin (M44K/M64E) was constructed. In the mutant M44K/M64E the two hydrophobic Met residues were replaced by two polar amino acids (Lys and Glu) around the hydrophobic patch, which was indicated to be involved in the interaction with p53 [15]. The mutant M44K/M64E was found to have stronger cytotoxic effect than wide-type azurin (Fig. 7A). As shown in the GST pull-down result (Fig. 7B), M44K/M64E interacted with p53 stronger than wide-type azurin.

Discussion

The interaction of p53 and azurin is suggested to be important for the cytotoxic effect of azurin towards cancer cells. Therefore, thoroughly understanding the mechanism of the interaction between azurin and p53 may lead to engineering more effective cytotoxic peptides from the sequence of azurin. Previous studies have shown the interaction between azurin and p53 [16–18]. The complex-forming interaction was determined based on glycerol-gradient ultracentrifugation and GST pull-down experiments.

In studies by Punj et al. [16], it was shown by GST pull-down assay that azurin formed a complex with N-terminal domain and DNA-binding domain of p53, but only had weak binding with C-terminal of p53. This result seemed to contradict our results. However, it is unknown from their report how the p53 was purified and used in the GST pull-down assay. The differently purified p53 might have different interaction with azurin as indicated in our result.

The GST pull-down result (Fig. 1) indicated that the fragments of p53 (p53-C, p53-MC) had stronger binding with azurin than the FL-p53. Furthermore the C-terminus of p53 (residues 352–

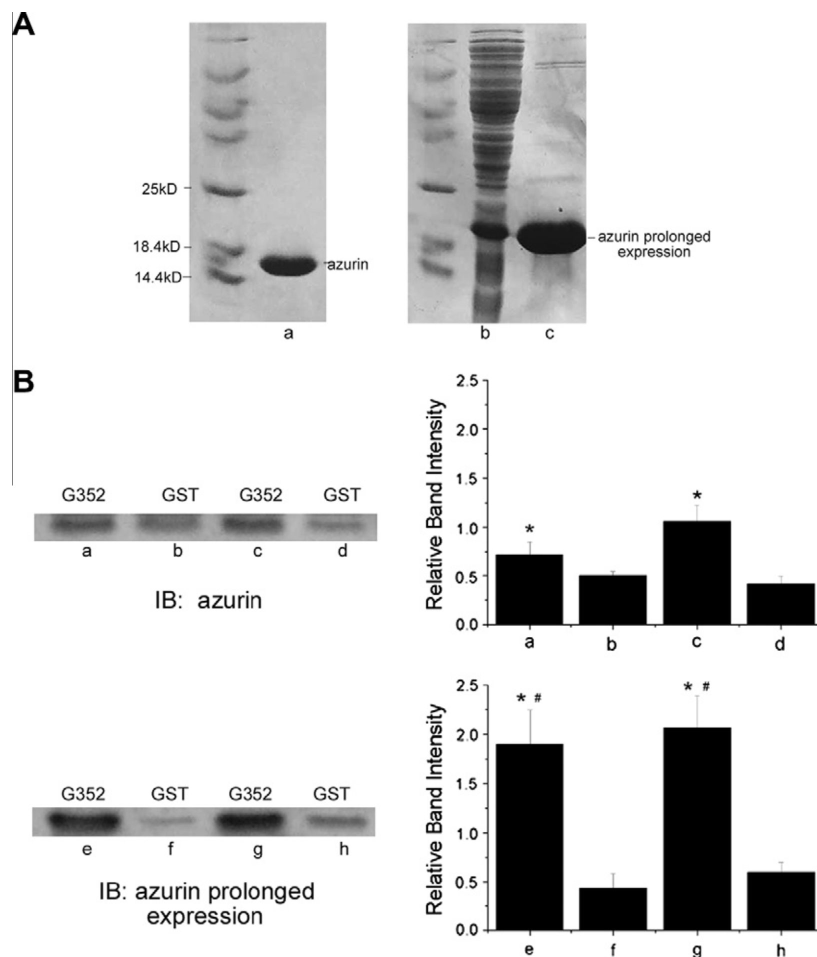

Fig. 6. (A) Identification of the elongated expression of azurin. Azurin (a) and the elongated expression (c) were purified. Lysate from the positive clone of *E. coli* expressing the elongated expression product was shown in (b). (B) GST pull-down assay to examine the interaction of p53 (352–393) with azurin or the elongated expression. Lower concentration of azurin and the elongated expression product (a, b, e, f 0.8 μM), or higher concentration (c, d, g, h 4 μM) were used in the GST pull-down assay. IB: Immunoblotting with antibody against His-tag to probe the His-tagged azurin and the His-tagged elongated expression of azurin. The pulled down azurin were significantly different in (∗ vs GST), ($P < 0.05$). Azurin and the elongated expression were pulled down differently by G352 (#, $P < 0.05$).

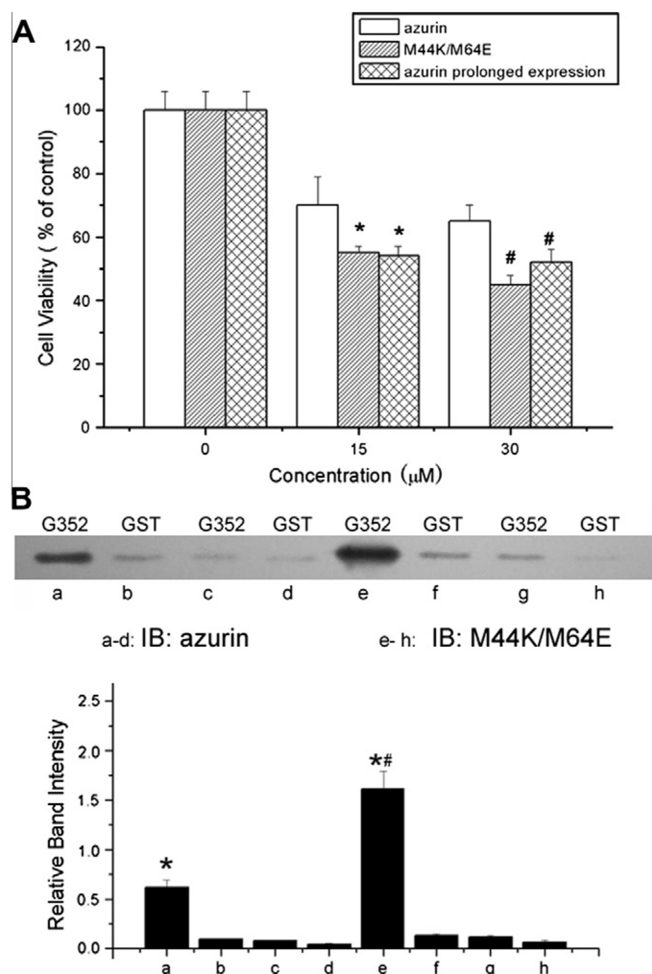

Fig. 7. (A) Cell viability of J774 cells treated with azurin, the double mutant (M44K/M64E), and the elongated expression product was examined. The cells were treated with various concentrations of proteins for 24 h and the cell viability was measured with the MTT assay. Each data point is the mean of six independent trials. * (15 μM) vs wild-type azurin and # (30 μM) vs wild-type azurin ($P < 0.05$). (B) GST pull-down assay to examine the interaction of p53 (352–393) with azurin or the double mutant M44K/M64E. Higher concentration of azurin and M44K/M64E (a, b, e, f 8.5 μM), or lower concentration (c, d, g, h 1.7 μM) were used in the GST pull-down result. IB: Immunoblotting with antibody against His-tag to probe the His-tagged azurin and the His-tagged double mutant M44K/M64E. The pulled down azurin were significantly different in (* vs GST), ($P < 0.05$). Azurin and the mutant M44K/M64E were pulled down differently by G352 (#, $P < 0.05$).

393) was found to be involved in the interaction. When p53 (352–393) was further purified by anion exchange column, it interacted weaker with azurin than the p53 (352–393) purified by the Glutathione Sepharose beads only. There were two eluents found from the anion exchange. The second eluent at a higher salinity is non-protein tested by SDS–PAGE (Fig. 3B), and its high absorbance at OD_{260} indicated that the second eluent might be nucleic acid. It is known that the C-terminal of p53 contained positive charged residues [26], which could bind with nucleic acids. Anion exchange purification got rid of nucleic acid, which might be required for the interaction. Therefore, it is possible to resume the interaction ability of the further purified p53 (352–393) by adding nucleic acids.

In fact, the lysate from *E. coli* cells expressing GST protein contained a lot of nucleic acid, which resumed the intensity of interaction between the anion exchange purified p53 and azurin (Fig. 4A). This result indicated that the nucleic acids might be necessary for the interaction of p53 (352–393) and azurin. Considering the complexity of the lysate of *E. coli*, we synthesized sequence-specific nucleic acids, which were reported to bind to the C-terminus of p53 [23]. These sequence-specific nucleic acids especially the second one increased the intensity of interaction between the anion exchange purified p53 and azurin significantly (Fig. 4B). Maybe the second nucleic acid efficiently induced the structural formation of the C-terminus of p53 (further discussed below). The resumption effect of the *E. coli* lysate or the synthesized nucleic acids indicated that the kinds of nucleic acids needed in the interaction might be non-specific.

In this study, we also examined whether azurin could bind to nucleic acids through electrostatic interactions. Heparin, which has similar structure and electronic charge as nucleic acids, was used to pull-down azurin. It was found that the Heparin-Sepharose beads loaded with GST-tagged p53 (352–393) and the peptide 352–393 could pull-down azurin strongly. While the beads loaded with GST protein and the Heparin-Sepharose beads only weakly pull-down azurin (Fig. 5B), which indicated that heparin itself with negative charge like nucleic acids did not bind to azurin. This result excluded the possible interaction between nucleic acids and azurin.

Azurin itself have weak interaction with nucleic acids and also have weak interaction with anion exchange purified p53 (352–393). How the added nucleic acid increased the intensity of the interaction between azurin and p53? We speculated that the specific nucleic acids might allosterically regulate the conformation of the C-terminal domain of p53, promoting the interaction with azurin. Many evidences have shown the allosteric effects of DNA on transcriptional regulators. For example, specific DNAs can act as allosteric ligands whose binding alter the regulator's affinity for other ligands (such as coactivators or corepressors) [27]. As far as the C-terminal domain of p53 is concerned, it also acts as a DNA-binding domain in the transcriptional function of p53. And this domain is characterized by unstructured [9], which structure is induced to form a similar fold as helix when binding to nucleic acid [23]. We speculated that the added nucleic acids induced the unstructured C-terminal domain of p53 to form a spatial complementary structure for docking azurin.

The interaction of azurin and p53 is important for the cytotoxic effect of azurin, which might regulate the stability of p53 as reported in [12]. The C-terminal domain of p53 is important for its stability, the mutants in this domain have been shown to interfere with the ubiquitination of p53 and thereby its degradation [28,29]. Our results showed that the elongated expression of azurin had better cytotoxic effect than wide-type azurin (Fig. 7A). The elongated expression also had stronger interaction with the C-terminal domain of p53 than wide-type azurin (Fig. 6B), suggesting that the interaction is important for the cytotoxic effect of azurin. However, it should also be noted that the intensity of the interaction is not always closely associated with the cytotoxicity. Other factors, such as the stability of azurin and the ability of azurin to entry cancer cells, may also influence the cytotoxicity of azurin. Although the factors involved in the cytotoxicity of azurin are complicated, it may still possible to develop new anticancer peptides based on the interaction of azurin with the C-terminus of p53.

In conclusion, our results indicated that azurin could interact with the C-terminal domain of p53, and this interaction was mediated by nucleic acids. The nucleic acids might allosterically regulate the conformation of the C-terminal domain of p53, thus influencing the interaction of p53 with azurin. The difference found in the cytotoxic effect of azurin with various sequence may provide valuable insight that leads to the development of new anticancer peptides.

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