A novel engineered interferon-α hybrid molecule increases anticancer efficacy of doxorubicin in breast cancer chemotherapy

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Abstract. Breast cancer is the most common carcinoma among Chinese women. Interferon α (IFNα) has been used to treat various types of cancer, including breast cancer, but its antitumor activity is relative low, which significantly hinders its clinical application. In this study, we utilized a Ph.D.-12 peptide library screening system to identify a short peptide that specifically binds to MCF-7 breast cancer cells. By fusing the MCF-7 binding peptide (MBP) to the C-terminus of IFNα, we constructed an engineered IFNα-MBP fusion molecule (IMBP), and applied this novel fusion protein to the treatment of breast cancer. We found that IMBP exhibited significantly higher activity than wild-type IFNα in inhibiting cell growth and inducing cell apoptosis. Additionally, IMBP potentiated the therapeutic efficacy of doxorubicin-based breast cancer chemotherapy via the activation of cell cycle arrest and cell apoptosis pathway genes including p53, p21, CDK2, cyclin A, caspase 9, Bcl-2 and Bax. The enhanced activity of the synthetic IMBP was also associated with the activation of signal transducer and activation of transcription 1 (STAT1) pathway target genes (STAT1, IFIT1, IFITM1 and MX1). This study evaluated the potential value of the synthetic IMBP as a novel anti-breast cancer agent.

Introduction

In recent years, cytokine research has been at the forefront of cancer research and cytokine approaches are involved in the treatment of various carcinomas. Cytokine approaches for cancer therapy have three potential mechanisms of action. They can i) directly induce cell death programs in tumor cells, ii) increase the number or activity of immune effector cells, or iii) increase the recognition of tumor cells by the immune system (1).

Interferons (IFNs) are one of the most important cytokines. They are naturally secreted glycoproteins produced by almost every cell type as a mechanism of host defense in response to microbial attack (2). The IFN family includes three different groups. IFNα belongs to the type I IFN group, and was discovered 50 years ago. It was the first cytokine to be produced by recombinant DNA technology, and it is used as an important regulator of cell growth and differentiation, affecting cellular communication and signal transduction pathways as well as immunological control (3). More recently, IFNα has been applied in the treatment of multiple carcinomas including leukemia, hepatocellular carcinoma, bladder cancer, and osteosarcoma (4-7).

Breast cancer is a leading cause of cancer-related death in women worldwide. According to GLOBOCAN 2012, there are approximately 1.67 million newly diagnosed breast cancer patients every year (8). In China, breast cancer is the most commonly diagnosed cancer, and the number of breast cancer patients has been increasing annually (9). Surgery, chemotherapy, radiotherapy, endocrinotherapy and molecular targeting therapy are the major treatment modalities for breast cancer. However, challenges remain in the treatment of breast cancer, and novel therapeutic approaches are urgently needed.

Although the use of IFN in clinical practice is widely recommended, its antineoplastic activity and clinical efficacy for breast cancer is still unclear and controversial (10,11). In this study, an NEB Ph.D.-12 peptide library was employed to select a short peptide that specifically binds to the cell membrane of MCF-7 cells, and a high-affinity IFNα-MCF-7 fusion molecule IMBP was constructed. Our aim was to investigate whether this reconstructed cytokine had enhanced cell growth inhibition activity compared to the wild-type IFNα.

Materials and methods

Cell culture. Human breast cancer cell line MCF-7, lung cancer cell line A549 and prostate cancer cell line PC-3 were kept in our lab. Human embryonic kidney 293T cell line was used for lentiviral packaging. All cell lines were routinely cultivated.
in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen) at 37°C in a humidified atmosphere containing 5% CO₂.

Phage display library screening of breast cancer binding peptides. Phage display library (Ph.D.-12 library, #E81105, New England BioLabs, Ipswich, MA, USA) was employed to screen a short 12-peptide that specifically binds to MCF-7 breast cancer cells (12, 13). Briefly, 1x10⁹ phage in 1 ml DMEM medium was incubated with 1x10⁶ MCF-7 cells at room temperature (RT) for 1 h to develop phage-cell complexes. After binding, cells were washed 5 times with 5 ml TBST [TBS (10 mmol/l Tris pH 7.5, 150 mmol/l NaCl) containing 0.1% Tween-20] to remove the unbound phages. Cells were collected by centrifuging at 3200 x g. The surface-bound phages were eluted in 1 ml elution buffer [0.2 N Glycine-HCl (pH 2.2), 1 mg/ml BSA] for 20 min and was neutralized by 150 µl 1 N Tris-HCl, pH 9.0. The eluted phages were reproduced by infecting E. coli ER2738 and purified using polyethylene glycol (PEG)-8000/NaCl solution (20% PEG-8000 and 2.5 N NaCl).

After 4 rounds of surface panning, the DNA sequences of MCF-7-binding peptide (MBP) were amplified by PCR (Fig. 1A). Primers used for PCR amplification were as follows: 5'-CTTTGATGTTACCTTTACCTCAGTCT-3' (forward primer with Xho I) and 5'-CTTTCAACAGTTTCG TCTAGA primer with Xba I 5'-CTTTAGTGGTACCTTTCTATTCTCGAGTCT-3' (reverse primer with Xba I). The PCR production was purified from 3% agarose gel and cloned into plso vector using CloneJET PCR Cloning kit (Thermo Fisher Scientific, Waltham, MA, USA) for sequencing and constructing the engineered IFNα molecules.

Phage-ELISA assay. Phage-ELISA was carried out to determine the binding affinity of the isolated MBP. Approximately 2x10⁵ MCF-7 cells were amplified by PCR (Fig. 1A). Primers used for PCR amplification were as follows: 5'-CTTTAGTGGTACCTTTCTATTCTCGAGTCT-3' (forward primer with Xho I) and 5'-CTTTCAACAGTTTCG TCTAGA primer with Xba I 5'-CTTTAGTGGTACCTTTCTATTCTCGAGTCT-3' (reverse primer with Xho I). The PCR production was purified from 3% agarose gel and cloned into plso vector using CloneJET PCR Cloning kit (Thermo Fisher Scientific, Waltham, MA, USA) for sequencing and constructing the engineered IFNα molecules.

Detection of the secreted IMBP fusion protein. On day 5 post-transduction, 100 µl supernatant of MCF-7 cells was collected and the secreted IFNα and IMBP fusion protein were quantitated with IFNα assay kit according to the manufacturer's instruction (Cusabio, Hubei, China).

Western blotting. Protein was extracted with RIPA buffer (KeyGEN Biotech, Jiangsu, China) supplemented with cocktail protease inhibitor (Roche, Shanghai, China) and quantified with BCA protein assay kit (Beyotime Biotechnology, Jiangsu, China). Whole cell lysates were resolved on 5-10% or 5-12% SDS polyacrylamide gel electrophoresis (SDS-PAGE). To detect the secreted IFNα and IMBP proteins in cell supernatants, on day 5 post-transduction, 100 µl MCF-7 cell supernatant was collected and condensed to 20 µl in a vacuum-freeze dryer (Boyikang, Beijing, China). Solutions were resolved on Mini-PROTEIN TGX gradient gel (Bio-Rad, Beijing, China). Proteins were transferred to 0.45 µm PVDF membranes (Roche) and immunoblotted at 4°C overnight or at RT for 1 h with the following antibodies: IFNα (1:1,000, Abcam, Shanghai, China), STAT1 (phospho Y701, 1:1,000, Abcam), Cleaved CASP-9 (1:1,000, Cell Signaling Technology, Beijing, China), p33 (1:1,500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p21 (1:500, Santa Cruz Biotechnology), CDK2 (1:1500, Cell Signaling Technology), Cyclin A (1:1,500, Abcam) and β-actin (1:3,000, Santa Cruz Biotechnology). Membranes were incubated with horseradish peroxidase-conjugated secondary antibody...
(1:3,000, ZSGB-BIO, Beijing, China) at 37°C for 1 h before chemiluminescence reading. Protein expression levels were determined semi-quantitatively by densitometric analysis with the Quantity One software (Bio-Rad). Western blotting was performed in triplicate, and data showed a representative finding of these triplicate analyses.

**Cell binding assay of IMBP.** The binding affinity of the engineered hybrid molecule to the MCF-7 cell membrane was measured by FACS. MCF-7 cells were collected and stained with Trypan blue to make sure that viable cells were more than ninety percent. Approximately 1x10⁶ normal MCF-7 cells were incubated with equal amount of the secreted IFNα or IMBP at 37°C for 1 h. After washed with PBS, cells were incubated with FITC-conjugated IFNα antibody (PBL, Piscataway, NJ, USA). The FITC-conjugated mouse IgG (Abcam) was used as the isotype control. Cells were analyzed using BD LSRII Fortessa flow cytometer (BD Biosciences) and FlowJo software (FlowJo, OR, Ashland, USA) to calculate the fluorescence intensity (14,15).

**Cell viability assay.** On day 7 post-transfection, approximately 1x10⁶ viable stable MCF-7 cells transduced by lentivirus carrying IMBP, IFNα, MBP and empty vector DNA were seeded into a 96-well plate 24 h before cell viability assay. Then, cell growth was analyzed by WST-1 Cell Proliferation Reagent (Roche). According to the manufacturer's instructions, 20 µl WST-1 reagent was added to 200 µl cell culture medium and incubated at 37°C in the dark for 2 h. The absorbances of 450 and 630 nm were measured with microplate reader (Biotek). Final OD was designated as OD₄₅₀-OD₆₃₀-ODblank.

**Cell cycle assay.** On day 7 post-transfection, 1x10⁶ stable MCF-7 cells were collected and washed by cold PBS. Cells were resuspended in 1 ml fixation solution (300 µl PBS and 700 µl ethanol). After incubation at 4°C for 4 h, cells were centrifuged and the fixation solution was removed. After washed twice with PBS, cells were pelleted, stained with 0.5 ml propidium iodide (PI, Sigma) staining solution (50 µg/ml PI, 20 µg/ml RNase A (Takara, Liaoning, China) and 0.2% Triton X-100 (Sigma)), and incubated in the dark at 37°C for 10 min. Cell suspensions were filtered with a 400-mesh sieve. Cell cycle was analyzed by BD LSRII Fortessa flow cytometer.

**Flow cytometry for cell apoptosis.** Annexin V-FITC and PI double staining flow cytometry analyses were used for cell apoptosis assay. Approximately 1x10⁶ stable MCF-7 cells were collected and washed three times with cold PBS and binding buffer. Then cells were stained with Annexin V-FITC and PI (Annexin V-FITC Apoptosis Detection kit, BD Bioscience) for apoptosis detection. Briefly, MCF-7 cells were first resuspended in 1 ml binding buffer. Then, 5 µl of Annexin V-FITC was added to the tubes, and cells were incubated for 10 min at RT followed by the addition of 5 µl PI. After 15 min incubation in PI buffer at RT, cells were immediately analyzed with a flow cytometer (BD Biosciences). The cells in the different portions represented the different cell states as follows: the late-apoptotic cells were present in the upper right portion, the viable cells were present in the lower left portion, and the early apoptotic cells were present in the lower right portion.

**Treatment with chemotherapeutic drug doxorubicin.** The anticancer drug doxorubicin (DOX, Sigma) was dissolved in water. We harvested 12 ml of cell supernatant containing the secreted interferons from the stable MCF-7 cells transduced by lentivirus carrying IMBP, IFNα, MBP and empty vector. The cell supernatant was centrifuged at 3,500 x g for 5 min to remove cell debris. Then the supernatant was successively passed through two centrifugal filter units with MW cut-off 30 and 10 kDa (Millipore, Temecula, CA, USA) to remove large and small molecules. The interceptions were dissolved in 6 ml DMEM complete medium and kept in 4°C. Normal MCF-7 cells were seeded into 6-well plates at a density of 3x10⁵ cells/well. After 24 h, cell medium was changed to the interception-DMEM supplemented with 0.1 µg/ml DOX. MCF-7 cells were treated with this therapeutic medium for 3 days, with the medium changed every day. After that, cells were harvested and applied for the next step of cell viability assay or molecule detection.

**Real-time PCR.** Total cellular RNA was isolated using an Eastep Super total RNA isolation kit (Promega, Beijing, China). First-strand cDNA was synthesized from 800 ng of total RNA using Transcriptor First Strand cDNA Synthesis kit (Roche). Real-time PCR was performed using an aliquot of first-strand cDNA as a template in a 20 µl reaction system containing 10 µl 2X SYBR premixed buffer (Roche), 2 µl forward and reverse primers. The primers were as follows: IFIT1 (interferon induced protein with tetratricopeptide repeats) sense 5'-TCTCAGAGGAGCCTGGCTAA-3', IFIT1 antisense 5'-CCAGACTATCTCTGGACATGTA-3'; MX1 (MX dynamin-like GTPase 1) sense 5'-CCTTTCCAGTCCAGCTCGGCA-3', MX1 antisense 5'-AGCTGCTGGCCCGTACGTCTG-3'); STAT1 sense 5'-GGACACAGAACGAAATGG-3', STAT1 antisense 5'-CCATCGTGACATGGTGAG-3'; IFITM1 antisense 5'-ATGTTAGACTGTCGACAGGAG-3'; β-actin sense 5'-TCACCCACACTGTGCCCATCTACGA-3', β-actin antisense 5'-CAGCGGAACCGCTCATTGCCAA-3' (19). The PCR amplification process was one cycle at 95°C 10 min, 40 cycles at 95°C for 10 sec and 60°C for 30 sec (ABI StepOnePlus, Beijing, China).

**Statistical analysis.** All data and results were calculated from at least three replicate measurements and presented as means ± SD. The significance was determined by SPSS 20.0 (IBM). Student's t-test was used to compare statistical differences for variables among treatment groups. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Construction of the IFNα/MCF-7-binding peptide fusion protein.** In order to enhance the antitumor activity of IFNα in breast cancer therapy, we used a NEB Ph.D.-12 peptide library to select a short peptide with 12 amino acids that specifically binds to the cell membrane of MCF-7 cells. Our aim is to add a short, high-affinity peptide to the C-terminus of IFNα and construct an IFNα-MCF-7 fusion molecule (IMBP), which may facilitate the binding of IFNα to the
According to the manual of NEB Ph.D.-12 peptide library, MCF-7 cells were incubated with 1x10^11 phage particles in DMEM tissue culture medium. The unbound phage particles were stripped off by stripping buffer. The bound phages on the cell membrane were eluted and recovered for the second round of screening. After four rounds of screening, the phages specifically binding to MCF-7 cells were enriched and identified (Fig. 1A). After cloning and sequencing, one sort of phage encodes short peptide with the sequence of ‘C E H I K D E L V C Q N’ was selected for further cell studies (Fig. 1B). Using phage-ELISA, we showed that this phage displayed short peptide was able to bind preferentially to MCF-7 cells compared to A549 lung cancer and PC-3 prostate cancer cells (Fig. 1C).

In order to examine the role of this short peptide and compare the antitumor activity of wild-type IFNα and IMBP, we linked the short peptide to the C-terminus of IFNα and the IMBP fusion molecule was synthesized (Fig. 1D). Then we used software on the website (http://zhanglab.ccmb.med.umich.edu) to predict the protein structure of the synthetic IMBP. The structure of wild-type IFNα and the putative structure of IMBP is illustrated in Fig. 1E. Arrow indicates MBP fused into wild-type IFNα.

Detection of the secreted IFNa and IMBP fusion protein.
We used 293T cells to produce lentivirus carring the DNA...
sequences of IMBP, wild-type IFNα, MBP and empty vector. Then, MCF-7 cells were transducted by lentiviruses and the stable cells were selected with puromycin. On day 5 post-transduction, 100 µl supernatant of MCF-7 cells was collected and the secreted IFNα and IMBP fusion protein were quantitated with IFNα assay kit. Based on the result of IFNα ELISA, the concentration of secreted wild-type IFNα in MCF-7 supernatant was 885 pg/ml and that of the IMBP fusion molecule was 763 pg/ml (Fig. 2A). We further used western blotting to confirm the secretion of wild-type IFNα and IMBP fusion molecule. The blots are shown in Fig. 2B.

Since the phage particles bind to MCF-7 cell membrane specifically, we used FACS to further examine whether the IMBP fusion protein binds to MCF-7 cell surface easier than the wild-type IFNα. Therefore, FITC conjugated-IFNα antibody was used in FACS assay and the binding affinity of the two proteins was quantitatively evaluated according to the fluorescence intensity in flow cytometry (Fig. 2C). Quantitative analysis result showed that the binding affinity of the IMBP fusion protein was 1.5-fold increase compared to the wild-type IFNα (P<0.05), and the binding ability of both the wild-type IFNα and the IMBP fusion protein was significantly higher than the isotype control (Fig. 2C, P<0.05).

**Synthetic IMBP fusion protein inhibits cell growth of MCF-7 cells.** We compared the cell growth inhibition effect between the IMBP and the wild-type IFNα. MCF-7 breast cancer cells were transducted with lentiviruses carrying IMBP, IFNα, MBP and empty vector, respectively. Cell proliferation was determined by WST-1 assay. We found that although both the secreted IFNα and IMBP inhibited the growth of MCF-7 cells, IMBP was superior to IFNα (Fig. 3A, P<0.05).

Then we examined cell apoptosis of the stable transfected MCF-7 cells with flow cytometry. As was shown in Fig. 3B, we found that the transfection of both IFNα and IMBP induced an increased apoptosis ratio, but the IMBP group showed a significantly higher apoptosis ratio than the IFNα group (26 vs. 18%, P<0.05). The cell morphology also showed a similar result (Fig. 3C).

**IMBP potentiates the therapeutic efficacy of doxorubicin-based chemotherapy.** Since doxorubicin is one of widely used anthracyclines to treat breast cancer, we examined the synergistic effect on cell killing by the combined use of DOX and IMBP fusion molecule. First, using WST-1 cell proliferation assay, we tested a serial working concentration of DOX including 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 µg/ml for the cell growth inhibition of MCF-7 cells. We selected a relative low concentration of 0.1 µg/ml of DOX with a cell growth inhibition ratio approximately 25% for the chemotherapy (data not shown). Then, we investigated the cell killing ability of the co-administration of DOX/IFNα or DOX/IMBP. The WST-1 assay result showed that cell proliferation in both the DOX/IFNα and DOX/IMBP treatment groups was apparently inhibited compared to the PBS control group and the DOX group. Of note, cell killing ability of the co-administration of DOX and IMBP was superior to the co-administration of DOX and IFNα (Fig. 4A, 48 vs. 32%, P<0.05).

Then we examined the cell cycle distribution in the treated MCF-7 cells with flow cytometry. We found that the co-administration apparently induced an S phase cell cycle arrest, and the DOX/IMBP treatment group showed a more obvious arrest effect than the DOX/IFNα group (Fig. 4B, 43 vs. 37%, P<0.05).
To unveil the underlying molecular mechanism that explains the enhanced therapeutic efficacy of the combined use of DOX and IMBP in chemotherapy, we first examined the expression of the cell cycle pathway related genes by western blotting. We found that the expression of p53 and p21 in the DOX/IMBP chemotherapy group was dramatically up-regulated compared to the DOX/IFNα group; the expression of CDK2 and cyclin A was dramatically down regulated (Fig. 4C left panel and D, P<0.05). We then examined the expression of the cell apoptosis pathway-related genes and found that the caspase 9 and Bcl-2/Bax apoptosis pathways were activated (Fig. 4C right panel and D, P<0.05).

**IMBP fusion molecule activates the STAT1 pathway.** To further delineate the mechanism of the enhanced activity of the combination chemotherapy, we used real-time PCR to examine the expression of the interferon pathway genes in the treated MCF-7 cells. The quantitation result showed that both the DOX/IMBP and DOX/IFNα co-administration activate several inducible genes of the STAT1 pathway, including...
STAT1, IFIT1, IFITM1, and MX1. However, DOX/IMBP group showed a stronger activation effect than the DOX/IFNα group (Fig. 5A). The western blot analysis result also showed an activation of the phosphorylated STAT1 (Y701, Fig. 5B).

Discussion

Anthracyclines, one type of anticancer therapy, are commonly used to treat both early and metastatic breast cancer; however, their toxicity, especially cardiac toxicity, is high (20,21). Additionally, frequent use of single therapeutic agent may result in chemoresistance, which is a major obstacle to the successful treatment of breast cancer. To minimize the toxicity of DOX and maximize the therapeutic efficiency of DOX, a combination therapeutic strategy is important.

The functions of IFNs are represented by three major biological activities, antiviral activity, antitumor activity and immunoregulatory activity (22). Although IFNα is widely used in the treatment of various types of cancer, its antineoplastic potency for breast cancer is low. Therefore,
improvements in this cytokine are valuable and further investigation is still needed.

In our previous study, using a cDNA in-frame library screening approach, we identified a short peptide derived from placental growth factor-2 (PLGF-2). We demonstrated that fusing this short peptide to IFNα and IFNγ induced greater activity than the wild-type counterparts in inhibiting tumor cell growth, invasion and colony formation (14,15). In this study, using a Ph.D.-12 peptide library, we selected a short peptide that specifically binds to the cell membrane of MCF-7 cells, and we synthesized a high-affinity IFNα-MCF-7 fusion molecule (IMBP). Using lentiviral DNA delivery system, we obtained a stable MCF-7 breast cancer cell line that secretes IMBP. Compared with wild-type IFNα, the IMBP fusion molecule demonstrated higher antitumor activity potency in the inhibition of cell growth, promotion of cell cycle arrest and induction of cell apoptosis. Recently, using the same approach, we also identified a short peptide that specifically binds to Jurkat T lymphocyte leukemia cells (JBP). We demonstrated that a JBP and IFNα fusion protein (IFNP) also had significantly better antitumor activity than wild-type IFNα (unpublished data). Through this series study, we evaluated the value of engineering an IFNα fusion molecule, which may serve as a novel antitumor agent in cancer treatment.

IFNs bind to receptors on the cell surface, activate specific signaling pathways and exert antitumor actions (23). Unfortunately, we still do not know the function of how this Ph.D.-12 peptide binds to the cell membrane. According to the result of ‘Blastp’ on the NCBI website, this 12-peptide is not homologous to any existing protein sequences. We speculate that it may bind to some unknown receptor(s) or biomacromolecule(s) on the surface of the cell membrane. Using a cell binding assay, we demonstrated that MBP promoted the adherence affinity of IFNα to its receptor (Fig. 2C), which is probably why we detected a lower level of free IMBP in the cell supernatant (Fig. 2A). We speculate that because more IMBP molecules adhere to the cell surface of MCF-7 cells than IFNα, the IMBP possesses a higher tumor cell killing ability than wild-type IFNα.

The STAT pathway plays a critical role in the anti-infection role of IFNs (24-27). When binding to its receptor on the cell surface, IFNα initiates the phosphorylation of TYK2 and JAK1 kinases, which is followed by the activation of STAT family transcription factors (28,29). Several IFN inducible
genes can be co-activated in the STAT pathway, including IFIT1, IFIT3, MX1, IFITM1, OAS1 (2'-5'-oligoadenylate synthetase 1), PAREP9 and PAREP12 (poly[ADP-ribose] polymerase family) (30). It was reported that IFIT3 promotes IFNα adjuvant therapeutic effects by strengthening IFNα effector signaling in hepatocellular carcinoma patients (31); IFNγ inhibits the growth of human liver cancer cells through activating IFITM1, which enhances the transcriptional activity of p53 and stabilizes the p53 protein by inhibiting p53 phosphorylation on Thr55 (19).

The JAK–STAT signaling pathway also plays an important role in doxorubicin-based breast cancer chemotherapy (32). It was demonstrated that the combined use of doxorubicin and IFNs may have a significant synergistic effect. Thomas et al showed that doxorubicin potentiates STAT1 activation in response to IFNγ; and the combination of doxorubicin and IFNγ enhances apoptosis in breast cancer cells (33). Hannesdóttir et al reported that STAT1 is crucial to the susceptibility of breast cancer cells to chemotherapeutic drugs (e.g., doxorubicin) by contributing to the induction of a productive antitumor immune response, which is based on IFNγ-producing T cells (34). In this study, we found that a combination of low dose DOX antitumor drug (0.1 μg/ml) and endogenous secretory IFNα had an advantage over the single use of either DOX or IFNα in inhibiting breast cancer cell growth. Of note, we found that low dose DOX combined with synthetic IMBP fusion protein had a significantly better therapeutic effect than the DOX/IFNα combination. When we examined the molecular mechanism of this enhanced antitumor activity, we observed that the DOX/IMBP combination activated cell cycle arrest and cell apoptosis pathway genes more efficiently than the DOX/IFNα combination (Fig. 4), which was also correlated with the activation of STAT1 pathway target genes (Fig. 5).

In summary, by using a phage library screening, we have identified a short peptide that specifically binds to MCF-7 breast cancer cells. Fusion of this short peptide to IFNα significantly enhanced the antitumor activity. The combined use of DOX and IMBP fusion protein potentiated the effectiveness of chemotherapy. Since using lentivirus to deliver molecules into cells is hard to apply in clinical gene therapy, targeting short peptides can be a genetically modifiable supramacromolecule in chemistry, materials and medicine. Acc Chem Res 49: 1111-1120, 2016.


