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Actinomycin D as a novel SH2 domain ligand inhibits Shc/Grb2 interaction in B104-1-1 (*neu**-transformed NIH3T3) and SAA (hEGFR-overexpressed NIH3T3) cells

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Abstract Actinomycins, a family of bicyclic chromopeptide lactones with strong antineoplastic activity, were screened as inhibitors of Shc/Grb2 interaction in in vitro assay systems. To investigate the effects of actinomycin D on Shc/Grb2 interaction in cell-based experiments, we used SAA (normal hEGFR-overexpressed NIH3T3) cells and B104-1-1 (*neu-transformed NIH3T3) cells, because a large number of the Shc/Grb2 complexes were detected. Associated protein complexes containing Shc were immunoprecipitated from actinomycin D-treated cell lysates with polyclonal anti-Shc antibody. Then the association with Grb2 was assessed by immunoblotting with monoclonal anti-Grb2 antibody. The result of the immunoblotting experiment revealed that actinomycin D inhibited Shc/Grb2 interaction in a dose-dependent manner in both B104-1-1 and EGF-stimulated SAA cells. The inhibition of Shc/Grb2 interaction by actinomycin D in B104-1-1 cells also reduced tyrosine phosphorylation of MAP kinase (Erk1/Erk2), one of the major components in the Ras-MAP kinase signaling pathway. These results suggest that actinomycin D could be a non-phosphorylated natural and cellular membrane-permeable SH2 domain antagonist.**

Key words: Grb2; Shc; Actinomycin; Src homology 2 domain; Cyclopeptide antibiotic; Extracellular signal-regulated protein kinase

1. Introduction

Activation of Ras is an important convergence point in the mitogenic signaling pathway of receptor tyrosine kinases [1]. The most important components of the Ras signaling pathway are Shc, Grb2, and Sos [2–4]. The Shc proteins containing the src homology 2 (SH2) domain are cytoplasmic substrates of activated tyrosine kinases [5]. Shc is phosphorylated in response to a variety of receptor tyrosine kinases [6] and also by cytoplasmic tyrosine kinases such as Lck, Src, Fps or Sea [7].

Shc contains a SH2 domain that binds to either receptor tyrosine kinases upon ligand activation [5] or the phosphotyrosine-containing sequence [8]. Phosphorylated Shc then acts as a linker or adapter for other SH2 domain-containing proteins. One such protein is a 23–25 kDa Grb2 composed of two SH3 domains and one SH2 domain [9]. The SH2 domain binds to specific phosphotyrosine motifs on receptors or other adapter proteins such as Shc, whereas the SH3 domains associate with proline-rich motifs in the C-terminal part of SOS, a guanine nucleotide exchange factor for Ras proteins. Recruitment of the Grb2-SOS complexes results in relocalization of SOS to the membrane, in which the event is considered sufficient to induce Ras activation, then the activated Ras in turn leads to Raf and mitogen-activated protein (MAP) kinase activation [10].

Overexpression of the epidermal growth factor receptor (EGFR) and erbB-2 has been found in a number of human cancers [11]. The HER-2/*neu* (also known as *erbB-2*) protooncogene encodes a 185 kDa transmembrane glycoprotein (p185) with intrinsic tyrosine kinase activity homologous to the EGFR [12]. Unlike EGFR and other receptor tyrosine kinases, the mutated p185 tyrosine kinase is constitutively active in the absence of exogenously added ligands. The tyrosine phosphorylation of Shc and formation of the Shc/Grb2 complex occur in *neu** protooncogene-transformed NIH3T3 cells (B104-1-1) that express the mutation-activated p185 and in human breast cancer cells that overexpress p185 [13]. Interestingly, the transformed phenotypes of B104-1-1 are largely reversed to the normal phenotype by the amino-terminal SH3 deletion mutant of Grb2. These observations indicate that the Shc/Grb2/SOS pathway plays an important role in the oncogenic signaling pathway of the mutation-activated p185 tyrosine kinase [6]. Moreover, recent studies have indicated that communication from the EGFR to the mitogen-activated protein kinase can be inhibited by blocking protein-protein interaction at the Grb2 SH2 domain binding [14].

In an experiment of in vitro binding inhibitors screening for Shc/Grb2 interaction, we discovered actinomycin C2, VII and D as inhibitors from microbial origins [15,16]. Actinomycins anchor into a purine-pyrimidine (DNA) base pair by intercalation, and then inhibit the mRNA synthesis. For this reason, actinomycins have become a powerful tool for the study of molecular and cell biology. In a recent study, it was reported that cellular differentiation was induced in actinomycin D-treated rhabdomyosarcoma cells, whereas cell proliferation was inhibited [17]. However, the mechanism of inhibition is not well defined. In this report, we describe that actinomycin D inhibits Shc/Grb2 interaction in SAA and B104-1-1 cells with a large number of Shc/Grb2 complexes in the presence or absence of external stimulus, respectively. The association

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Abbreviations: SH2, src homology 2; MAP kinase, mitogen-activated protein kinase; Erk, extracellular signal-regulated protein kinase; hEGFR, human epidermal growth factor receptor; FBS, fetal bovine serum

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of Shc/Grb2 in B104-1-1 is constitutively formed without an extracellular growth signal, but in SAA cells it is inducible with EGF. We also elucidate that this inhibition of Shc/Grb2 interaction by actinomycin D results in a decrease of Erk1/Erk2 tyrosine phosphorylation in B104-1-1 cells.

2. Materials and methods

2.1. Antibodies

Peroxidase-conjugated secondary antibodies were obtained from Transduction Laboratory (Lexington, KY, USA). Polyclonal antibody against Shc was from Upstate Biotechnology Inc. (Lake Placid, NY, USA) and monoclonal antibodies against Grb2, Erk1, and phosphotyrosine (PY20) were from Transduction Laboratory. Polyclonal anti-phospho-Erk1 antibody was obtained from Promega (Madison, WI, USA).

2.2. Molecular modeling of the Grb2(SH2)-actinomycin D complex

The peptide extracted from the Grb2(SH2) domain co-crystal structure was used as a template to build the bound conformation of actinomycin D [18]. The three-dimensional conformation of actinomycin D was extracted from the X-ray crystal structure of the 2:1 complex between d(GAAGCTTC) and actinomycin D [19]. Calculations were performed on Silicon Graphics O_2 computers running the IRIX 5.x operating system. Energy minimizations were performed with the Insight II/DISCOVER program (release 97.2, 1997, Biosym/MSI) using the cff91 forcefield.

2.3. In vitro binding assay by ELISA

A 50 μl aliquot of actinomycin D (20 μg/ml) purified from *Streptomyces* sp. A1525 as previously described [15] was coated on polystyrene 96 well plates (Falcon, Franklin Lakes, NJ, USA) for in vitro binding assay by ELISA methods [20,24]. Non-specific binding to wells was inhibited by 5% bovine serum albumin (BSA) in TBS (10 mM Tris, pH 7.5, 0.15 M NaCl). Recombinant GST-Grb2 (with Grb2 whole protein) and GST-Grb2(SH2) (with the SH2 domain only of Grb2) were obtained from Santa Cruz Biotech (Santa Cruz, CA, USA). They were allowed to bind to the immobilized actinomycin D at the indicated concentrations for 2 h. For the competition assay, 40 μg/ml of Shc peptide (SpYVNVK, synthetic peptide with binding site to Grb2 from Amersham, Piscataway, NJ, USA) [15] was added to actinomycin D-coated wells with proteins. After extensive washing with TBS containing 0.1% BSA, the binding of proteins was detected using anti-GST antibody from Molecular Probes (Eugene, OR, USA) and anti-rabbit IgG (Santa Cruz Biotech) conjugated with horseradish peroxidase. Color reactions were performed with 3,3′,5,5′-tetramethylbenzidine (Boehringer Mannheim, Mannheim, Germany) as substrate.

2.4. Cell lines and culture

SAA cells (NIH3T3 cells transfected with wild-type human EGFR) were provided by Dr. Graham Carpenter (Vanderbilt University, Nashville, TN, USA). SAA cells were grown to 95% confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), serum-starved for 18 h, and then treated with actinomycin D in DMEM for 1 h [20]. Subsequently, cells were stimulated with or without EGF (Boehringer Mannheim) for 15 min and washed with cold phosphate buffered saline (PBS).

B104-1-1 cells obtained from ATCC were plated into T185 flask (Nunc, Naperville, IL, USA) in DMEM supplemented with 10% FBS and 50 μM 2-mercaptoethanol at 37°C in a humidified 5% CO_2 atmosphere. After 24 h, cells were replenished with fresh complete medium containing actinomycin D or 0.1% DMSO.

2.5. Preparation of non-denatured cell lysates

After EGF stimulation of SAA cells and in the case of B104-1-1 cells without any external stimulation, the cells were washed with PBS, and then lysed in ice-cold lysis buffer (20 mM Tris-HCl, pH 7.5, 1% Triton X-100, 50 mM NaCl, 5 mM EGTA, 1 mM sodium vanadate, 50 mM NaF, 30 mM $Na_4P_2O_7$, 10% glycerol, 1 mM PMSF, 5 μg/ml aprotinin, 10 μg/ml leupeptin) for 1 h [21]. The cell lysates were clarified by centrifugation at 12 000 rpm for 20 min at 4°C. Protein concentrations of the cell lysate were determined against BSA as standardized controls using the Bio-Rad^{DC} protein assay kit (Bio-Rad, Hercules, CA, USA).

2.6. Immunoprecipitation and immunoblotting

Equivalent amounts of protein (500 μg) of cell lysates were immunoprecipitated with anti-Shc antibody at 4°C for 1 h [21]. After incubation, pre-washed Pansorbin (Calbiochem, La Jolla, CA, USA) was added for 1 h at 4°C. The precipitation complexes were recovered by centrifugation, washed three times with lysis buffer, and then eluted with boiling in Laemmli SDS sample buffer. Samples were subjected to electrophoresis under reducing conditions, and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 0.1% gelatin in TTBS (10 mM Tris-HCl, pH 7.6, 150 mM NaCl and 0.05% Tween 20), incubated with primary antibodies for 1 h, washed three times, incubated with secondary antibodies for 1 h, and washed three more times. Specific antibody signals were detected with a chemiluminescence detection kit (Amersham) [21].

2.7. Determination of MAP kinase tyrosine phosphorylation

B104-1-1 cells were plated at a density of 1×10^6 cells/10 cm dish (Nunc) in DMEM supplemented with 10% FBS. One day after plating, the cells were starved of serum for 36 h, and either actinomycin D (10, 25 or 50 nM) or 0.1% DMSO (control) was present during the last 24 h [22]. Following removal of the medium, the cells were stimulated for 8 h by the addition of fresh complete medium containing

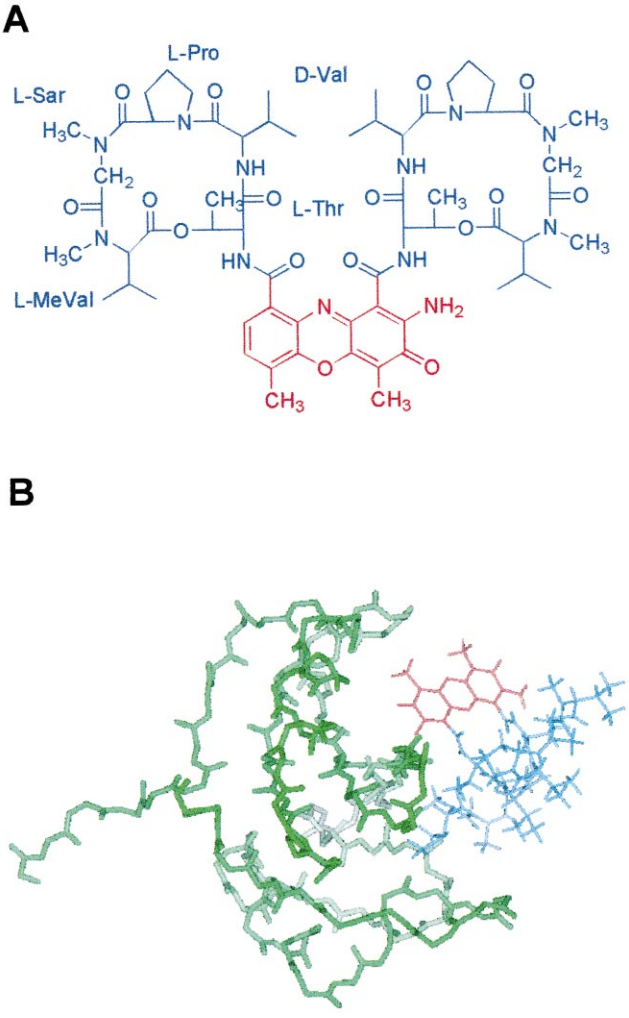

Fig. 1. The structure of actinomycin D (A) and molecular modeling of the Grb2(SH2)-actinomycin D complex (B). Actinomycin D consists of two cyclopeptides (blue) and a chromophore moiety (red) (A and B). The SH2 domain of Grb2 is in green (B).

10% FBS in the absence or presence of actinomycin D. To determine Erk tyrosine phosphorylation, cell lysates were resolved by SDS-PAGE and then immunoblotting was performed with anti-phospho-Erk or monoclonal anti-Erk antibody.

3. Results

3.1. Affinity of actinomycin D for Grb2

Molecular modeling studies were performed to see the binding mode of Grb2 and actinomycin D. Key elements for recognition are the phosphotyrosine and asparagine residues of the phosphopeptide Lys-Pro-Phe-pTyr-Val-Asn-Val-NH_2 whose side chains are seen to make multiple electrostatic and hydrogen bond interactions with the protein in the X-ray structure [18]. The SH2 domain-actinomycin D complex was obtained by replacing the phosphotyrosine residue of the X-ray structure with the chromophore moiety of actinomycin D and then minimized. Fig. 1B shows that within the binding cavity, the complex has similar interactions between the SH2 domain of Grb2 (green color) and the bound ligand (blue color, peptide ring of actinomycin D).

To verify the Grb2 binding activity of actinomycin D, we used the ELISA method. In the assay, an actinomycin D-coated 96 well plate was incubated with recombinant proteins, GST-Grb2 or GST-Grb2(SH2). Fig. 2 shows that Grb2 and Grb2(SH2) could bind to actinomycin D in a dose-dependent pattern. In this assay, GST protein did not affect binding between Grb2/Grb2(SH2) and actinomycin D (data not shown). This binding was also inhibited when Shc peptide with binding site to Grb2(SH2) was added with proteins in a reaction mixture.

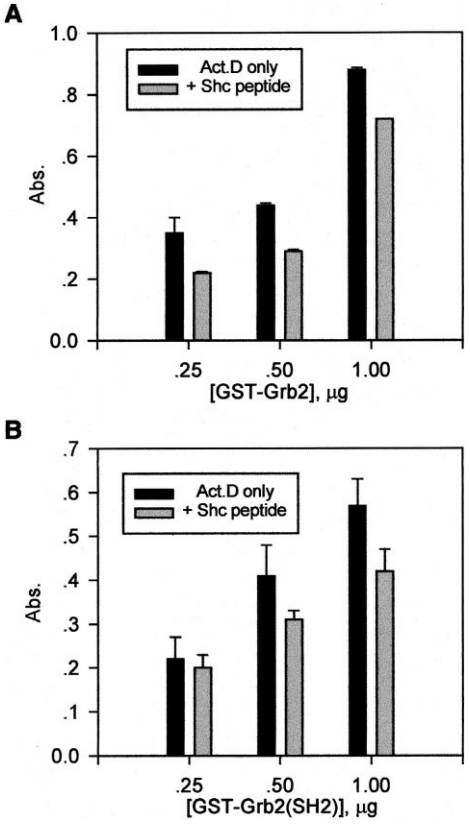

Fig. 2. In vitro binding assay of actinomycin D to Grb2 and Grb2(SH2). An actinomycin D-coated polystyrene 96 well plate (Falcon) was incubated with GST-Grb2 (A) or GST-Grb2(SH2) (B) at the indicated concentration. For the competition assay, Shc peptide was added to actinomycin D-coated wells with proteins. These results were obtained from at least three independent experiments. The means are shown with standard deviation.

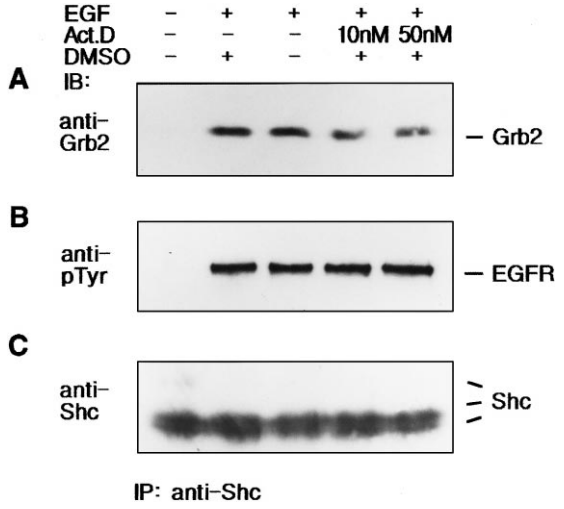

Fig. 3. Inhibition of inducible Shc/Grb2 protein-protein interaction in SAA cells. SAA cells (A) were grown as described in Section 2, and then treated with indicated concentrations of actinomycin D in DMEM for 1 h. Subsequently, cells were stimulated with or without EGF for 15 min, washed with cold PBS, and lysed in lysis buffer. The Shc/Grb2 complexes were immunoprecipitated with rabbit antiserum against Shc. The immunocomplexes were analyzed by immunoblotting using anti-Grb2 antibody (A). The blot used in A was stripped and then probed with anti-phosphotyrosine (PY20) antibody (B) or with anti-Shc antibody to ensure equal loading of immunoprecipitated protein (C).

3.2. Inhibition of Shc/Grb2 interaction in both SAA and B104-1-1 cells

To learn the inhibitory activity of Shc/Grb2 interaction by actinomycin D in cells, we used EGF-stimulated (SAA) cells and endogenously activated (B104-1-1) cells due to expression of activated growth factor receptor, erbB-2. To examine the effect of actinomycin D on EGF-induced Shc/Grb2 interaction in cells, we delivered actinomycin D in SAA cells, and then cells were stimulated by EGF. Co-immunoprecipitation of Grb2 from cell lysates with anti-Shc antibody showed that Grb2 proteins associated with the Shc protein when cells were stimulated with EGF (Fig. 3A, lane 2). The inducible Shc/Grb2 interaction was inhibited by treatment of actinomycin D (10 nM, extracellular concentration) (Fig. 3). This result indicates that actinomycin D inhibits Shc/Grb2 interaction induced by EGF.

To learn the inhibitory effect in B104-1-1 cells with endogenously activated Shc/Grb2 complex, we first examined the effects of incubation time from 1 to 48 h. After continuous exposure to actinomycin D for 48 h, Shc/Grb2 interaction was sufficiently inhibited in B104-1-1 cells (data not shown). Thus cells were incubated with actinomycin D for 48 h in dose-dependence experiments. To examine the concentration-dependent inhibition of Shc/Grb2 interactions, we treated actinomycin D at concentrations of 10, 25, or 50 nM for 48 h. The protein complexes were immunoprecipitated from cell lysates with anti-Shc antibody and the presence of Grb2 was

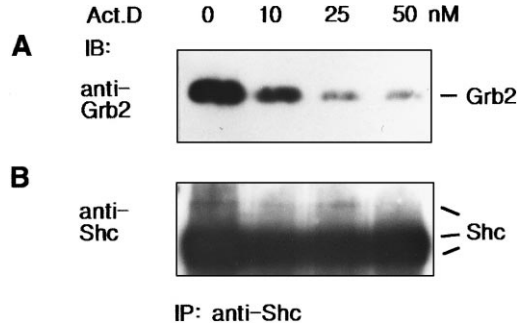

Fig. 4. Immunoprecipitation of Shc/Grb2 complexes in B104-1-1 cells. B104-1-1 cells were maintained in growth medium with increasing concentrations of actinomycin D for 48 h. Cells were lysed in lysis buffer, and then the Shc/Grb2 complexes were immunoprecipitated with rabbit antiserum against Shc. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted using anti-Grb2 antibody (A). The blot used in A was stripped and then probed with anti-Shc (B).

assessed by immunoblotting with anti-Grb2 monoclonal antibody. We found that the formation of Shc/Grb2 complexes was inhibited about 50% by treatment with 10 nM actinomycin D for 48 h (Fig. 4A). When the cells were treated with 50 nM of the SH2 domain antagonist, the Shc/Grb2 complexes were almost completely dissociated. The results demonstrated that actinomycin D inhibited Shc/Grb2 interaction in a dose-dependent manner. The blots used in Fig. 4A were stripped, re-blocked and re-probed with anti-Shc antibody to ensure equal loading of immunoprecipitated protein (Fig. 4B).

3.3. *Actinomycin D does not inhibit tyrosine phosphorylation of Shc and expression levels of Shc/Grb2*

We examined whether expression levels of Shc and Grb2 protein were affected by actinomycin D, since actinomycin D is a well-known DNA intercalating agent. We did not observe any differences between actinomycin D-treated cells and untreated ones in the expression levels of Shc and Grb2 up to 50 nM (Fig. 5B,C). At that concentration, actinomycin D completely blocked the formation of Shc/Grb2 complexes. Also, actinomycin D did not inhibit tyrosine phosphorylation of Shc, as shown in Fig. 5A. Therefore, these results show that the number of Shc/Grb2 complexes in B104-1-1 was decreased by the inhibition of actinomycin D of the protein-protein interaction itself.

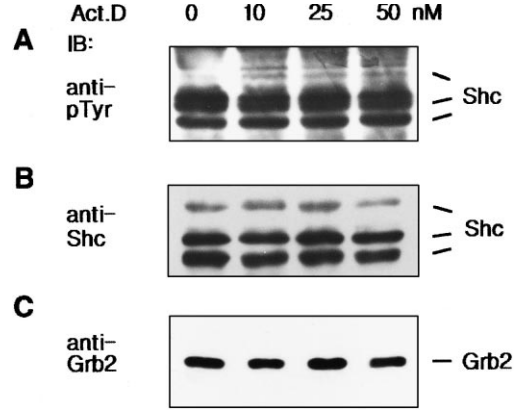

Fig. 5. Effects of actinomycin D on tyrosine phosphorylation of Shc and protein expression levels of Shc and Grb2. B104-1-1 cells were maintained in growth medium with increasing concentrations of actinomycin D for 48 h. Total cell lysates were separated by SDS-PAGE and analyzed by immunoblotting with monoclonal anti-phosphotyrosine PY20 (A), polyclonal anti-Shc (B) and monoclonal anti-Grb2 antibody (C).

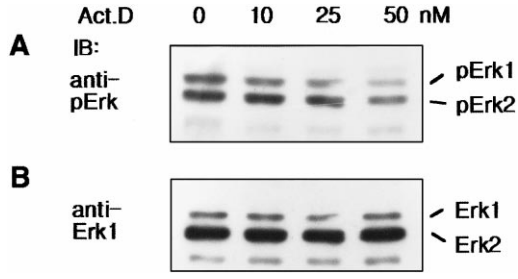

Fig. 6. Tyrosine phosphorylation of Erk1/Erk2 in B104-1-1 cells treated with actinomycin D. Total cell lysates to examine tyrosine phosphorylation of Erk1/Erk2 were prepared as described in Section 2. Cell lysates were resolved by SDS-PAGE and then immunoblotting was performed with polyclonal anti-phospho-Erk1 (A) or monoclonal anti-Erk1 antibody (B).

3.4. *Inhibition of MAP kinase activity in actinomycin D-treated cells*

MAP kinases (Erk1/Erk2) are a member of the Ras signaling pathway and play a crucial role in the transmission of signals initiated at the plasma membrane by a variety of signals, including growth factors, oncogenes and differentiating agents. The inhibition of Erk1/Erk2 activity in actinomycin D-treated cells was studied using monoclonal anti-Erk1 and antiserum to phosphorylated Erk1, which can detect the tyrosine phosphorylation level of the Erk1/Erk2 proteins. As shown in Fig. 6A, actinomycin D decreased the tyrosine phosphorylation of the Erk1/Erk2 in a dose-dependent manner and the antagonist of Grb2-SH2 domain inhibited the phosphorylation of Erk1 and Erk2 with an IC_{50} of 50 and 40 nM, respectively. However, the compound did not inhibit the expression levels of MAP kinases in lysates prepared from actinomycin D-treated B104-1-1 cells (Fig. 6B).

4. Discussion

In previous studies, phosphotyrosine (pTyr) peptidomimetics and non-phosphorus-containing pTyr mimetics as SH2 domain antagonists were discovered or synthesized for blocking intracellular signaling pathways via the SH2 domain [23]. These compounds strongly bind to the SH2 domain and some of them are stronger antagonists than natural ligands. However, the inhibition activity of these antagonists, when applied to intact cells, is limited because of their highly charged moieties in physiological conditions [24,25]. In a recent report, a non-phosphorylated cyclic peptide, which strongly binds the SH2 domain of Grb2, does not show any inhibition activity in cells, probably because the compound is also not permeable to intact cellular membranes [26].

In contrast, actinomycin D could alleviate the limitations because of its membrane permeability. Actinomycins are DNA intercalating chemotherapeutic agents in the treatment of various tumors. They also prevents the estrogen-induced changes in glycolysis in human breast cancer cells [27]. Even

though actinomycins have complex functions, the mechanism of their biological functions, except a DNA intercalating function, is not well known yet. In our results, an interesting aspect is that actinomycin D directly inhibits the protein-protein interaction via the SH2 domain at 10 nM, a much lower concentration. Because the DNA binding constant of actinomycin D is around $2.3–3.3\times10^7$ M^{-1}, which is dependent on the DNA sequences [28], the inhibition of transcription expression by actinomycin D is usually observed at a concentration of 1–3 μM [29].

Human cancers develop as a result of the progressive accumulation of genetic alterations in genes whose protein products play critical roles in cell proliferation [30]. Overexpression of the EGFR and also erbB-2 has been found in a number of human cancers, including bladder, colon, and lung cancers. Previous studies on the EGFR signaling pathway indicated that the Grb2/Sos complex could be recruited to tyrosine-phosphorylated Shc or directly associated with the activated EGF receptor. Xie et al. [6] reported that the Shc/Grb2/Sos pathway was the most dominant one in coupling the activated ErbB-2 to Ras since interference of the interaction between Shc and Grb2 led to a dramatic inhibition of Ras activation [13]. They suggested that the controlling agents of the Shc/Grb2/Sos pathway might shed light on developing therapeutic agents to block the oncogenic signaling pathway of the ErbB-2 oncoprotein. Therefore our results suggest the possibility that actinomycin D could be used as a chemotherapeutic agent for EGFR- or ErbB-2-overexpressed tumors in addition to ras-dependent tumors.

In conclusion, we report here that actinomycin D blocks Shc/Grb2 interaction in EGFR-overexpressed NIH3T3 (SAA) and *neu**-transformed NIH3T3 (B104-1-1) cells. Therefore, this work provides a mechanistic explanation for growth inhibition of cells with activated Ras signaling pathways, including SAA and B104-1-1, by actinomycins and a new biological function of actinomycins. Our results also suggest that actinomycin D will be a useful agent for the treatment of specific tumors caused by EGFR or the ErbB-2 oncogene. In addition, such a structure will give an idea for the design and development of new SH2 domain antagonists.

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References

[1] Downward, J. (1992) BioEssays 14, 177–184.
[2] Basu, T., Warne, P.H. and Downward, J. (1994) Oncogene 9, 3483–3491.
[3] Pruett, W., Yuan, Y., Rose, E., Batzer, A.G., Harada, N. and Skolnik, E.Y. (1995) Mol. Cell Biol. 15, 1778–1785.
[4] Benjamin, C.W. and Jones, D.A. (1994) J. Biol. Chem. 269, 30911–30916.
[5] Songyang, Z., Shoelson, S.E., McGlade, J., Olivier, P., Pawson, T., Bustelo, X.R., Barbacid, M., Sabe, H., Hanafusa, H., Yi, T., Ren, R., Baltimore, D., Ratnofsky, S., Feldman, R.A. and Cantley, L.C. (1994) Mol. Cell Biol. 14, 2777–2785.
[6] Xie, Y., Pendergast, A.M. and Hung, M.-C. (1995) J. Biol. Chem. 270, 30717–30724.
[7] Migliaccio, E., Mele, S., Salcini, A.E., Pelicci, G., Lai, K.-M.V., Superti-Furga, G., Pawson, T., Fiore, P.P.D., Lanfrancone, L. and Pelicci, P.G. (1997) EMBO J. 16, 706–716.
[8] Pawson, T. and Gish, G. (1992) Cell 71, 359–362.
[9] Matuoka, K., Shibata, M., Yamakawa, A. and Takenawa, T. (1992) Proc. Natl. Acad. Sci. USA 89, 9015–9019.
[10] Aronheim, A., Engelberg, D., Li, N., Al-Alawi, N., Schlessinger, J. and Karin, M. (1994) Cell 78, 949–961.
[11] Verbeek, B.S., Adriaansen-Slot, S.S., Vroom, T.M., Becker, T. and Rijksen, G. (1998) FEBS Lett. 425, 145–150.
[12] Ben-Levy, R., Paterson, H.F., Marshall, C.J. and Yarden, Y. (1994) EMBO J. 13, 3302–3311.
[13] Xie, Y., Li, K. and Hung, M.-C. (1995) Oncogene 10, 2409–2413.
[14] Williams, E.J., Dunican, D.J., Green, P.J., Howell, F.V., Derossi, D., Walsh, F.S. and Doherty, P. (1997) J. Biol. Chem. 272, 22349–22354.
[15] Nam, J.-Y., Kim, H.-K., Son, K.-H., Kim, S.-U., Kwon, B.-M., Han, M.Y., Chung, Y.J. and Bok, S.H. (1998) Bioorg. Med. Chem. Lett. 8, 2001–2002.
[16] Koh, W.S., Yoon, S.Y., Lee, E.K., Kwon, B.-M., Kim, J.W. and Han, M.Y. (1997) Cancer Lett. 120, 1–7.
[17] Marchal, J.A., Prados, J., Melguizo, C., Fernandez, J.E., Velez, C., Alvarez, A. and Aranega, A. (1977) J. Lab. Clin. Med. 130, 42–50.
[18] Rahuel, J., Gay, B., Erdmann, D., Strauss, A., Garcia-Echeverria, C., Furet, P., Caravatti, G., Fretz, H., Shoepfer, J. and Grutter, M.G. (1996) Nature Struct. Biol. 3, 586–589.
[19] Kamitori, S. and Takusagawa, F. (1992) J. Mol. Biol. 225, 445–456.
[20] Rojas, M., Yao, S.Y., Donahue, J.P. and Lin, Y.-Z. (1997) Biochem. Biophys. Res. Commun. 234, 675–680.
[21] Wolfson, M., Yang, C.-P.H. and Horwitz, S.B. (1997) Int. J. Cancer 70, 248–252.
[22] Gana-Weisz, M., Haklai, R., Marciano, D., Egozi, Y., Ben-Baruch, G. and Kloog, Y. (1997) Biochem. Biophys. Res. Commun. 239, 900–904.
[23] Burke Jr., T.R., Smyth, M.S., Otaka, A., Nomizu, M., Roller, P.P., Wolf, G., Case, R. and Shoelson, S.E. (1994) Biochemistry 33, 6490–6494.
[24] Xiao, S., Rose, D.W., Sasaoka, T., Maegawa, H., Burke Jr., T.R., Roller, P.P., Shoelson, S.E. and Olefsky, J.M. (1994) J. Biol. Chem. 269, 21244–21248.
[25] Ye, B., Akamatsu, M., Shoelson, S.E., Wolf, G., Giorgetti-Peraldi, S., Yan, X., Roller, P.P. and Burke Jr., T.R. (1995) J. Med. Chem. 38, 4270–4275.
[26] Oligino, L., Lung, F.-D.T., Sastry, L., Bigelow, J., Cao, T., Curran, M., Burke Jr., T.R., Wang, S., Krag, D., Roller, P.P. and King, C.R. (1997) J. Biol. Chem. 272, 29046–29052.
[27] Neeman, M. and Degani, H. (1989) Proc. Natl. Acad. Sci. USA 86, 5585–5589.
[28] Chen, F.M. (1998) Biochemistry 37, 3955–3964.
[29] Duff, J.L., Monia, B.P. and Berk, B.C. (1995) J. Biol. Chem. 270, 7161–7166.
[30] Huang, P.S. and Heimbrook, D.C. (1997) Curr. Opin. Oncol. 9, 94–100.