Association of the Cyclin-dependent Kinases and 14-3-3 Sigma Negatively Regulates Cell Cycle Progression*

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Christine Laronga‡, Heng-Yin Yang†, Christopher Neal, and Mong-Hong Lee§

From the Departments of Surgical Oncology/Molecular and Cellular Oncology and the Breast Cancer Research Program, the University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

14-3-3 sigma, implicated in cell cycle arrest by p53, was cloned by expression cloning through cyclin-dependent kinase 2 (CDK2) association. 14-3-3 sigma shares cyclin-CDK2 binding motifs with different cell cycle regulators, including p107, p130, p21CIP1, p27KIP1, and p57KIP2, and is associated with cyclin-CDK complexes in vitro and in vivo. Overexpression of 14-3-3 sigma obstructs cell cycle entry by inhibiting cyclin-CDK activity in many breast cancer cell lines. Overexpression of 14-3-3 sigma can also inhibit cell proliferation and prevent anchorage-independent growth of these cell lines. These findings define 14-3-3 sigma as a negative regulator of the cell cycle progression and suggest that it has an important function in preventing breast tumor cell growth.

Cyclin-dependent kinases (CDKs)1 are responsible for the transitions of the eukaryotic cell cycle and are tightly regulated by both extra- and intracellular signals. They act in concert with their regulatory subunits, the cyclins, to facilitate the cell cycle progression. The cell cycle regulatory machinery is controlled by both positive and negative regulators. Cyclin-dependent kinases (CDKs) and their cyclin partners are positive regulators or accelerators that help cell cycle progression. The recently characterized cyclin-dependent kinase inhibitors (CKIs) are important negative regulators that act as brakes to stop cell cycle progression in response to regulatory signals (1). Two families of CKIs have been characterized based on the specificity of interaction with CDKs and sequence homology. The CIP/KIP family, which shares homology at the N-terminal CDK inhibitory domain, includes p21CIP1/WAP1 (2–5), p27KIP1 (6, 7), and p57KIP2 (8, 9). They interact with the cyclin-CDK complexes and inhibit the kinase activity of cyclin A-CDK2, cyclin D-CDK4, and cyclin E-CDK2. Overexpression of CIP/KIP inhibitors in cells can cause G1 arrest, suggesting that they preferentially target G1 cyclin-CDK complexes.

The INK4 family includes p15 (INK4b) (10), p16 (INK4a) (11), p18 (INK4c) (12), and p19 (INK4d) (13). The INK4 family recognizes CDK4 and CDK6 but not CDK2 and may cause G1 arrest of the cell cycle by competing with cyclin D for binding with CDK4. Because the cell cycle regulatory machinery is such a complex system, it seems possible that more cell cycle regulators have yet to be discovered. Insights into the detailed regulation of the cell cycle machinery will help us understand the signals that render cells oncogenic. In order to understand the basic cell cycle regulatory machinery, we used an expression cloning method to search for CDK2-associated proteins and isolated a 14-3-3 protein.

The 14-3-3 proteins comprise a family of highly conserved acidic proteins, and there are at least seven different mammalian isoforms. Several activities have been ascribed to these proteins, including signal transduction of Raf-1 (14, 15) and cell cycle regulation (16, 17). However, the molecular mechanism behind cell cycle regulation has remained elusive. First, 14-3-3 epsilon and 14-3-3 beta have been isolated in a yeast two-hybrid screen designed to identify proteins that interact with the human CDC25A and CDC25B phosphatases. They bind to CDC25 but do not affect the phosphatase activities of CDC25 (18). Second, in a yeast two-hybrid screen designed to identify Weel-associated proteins, the 14-3-3 zeta has been found to interact with the Weel kinase, which plays a key role in cell cycle progression by inactivating cyclin-dependent kinases (19). However, the binding of 14-3-3 zeta to Weel1 does not change the activity of Weel1 (19). Third, 14-3-3 has been shown to interact with polyoma middle T antigen (20) involved in cell proliferation, but it remains to be elucidated how regulation of 14-3-3 protein contributes to the development of neoplasia. Fourth, in yeast, two checkpoint genes, rad24 and rad25, encode 14-3-3 protein homologues that together provide a function that is essential for cell proliferation (16). The roles of RAD24 and RAD25 in regulating the activity of Chk1 or CDC25 in determining the progression of mitosis in response to DNA damage are not all clear (21, 22). Finally, 14-3-3 sigma (human mammary epithelial marker 1 or HME1) is expressed in epithelial cells, and its expression is dramatically low in human mammary carcinoma. However, its role in neoplastic formation is not understood (23). Furthermore, 14-3-3 sigma is induced by p53 in response to gamma irradiation and other DNA-damaging agents (24). 14-3-3 sigma induction results in a G2 arrest through an uncharacterized mechanism (24). Together, these observations imply that 14-3-3 proteins play important roles in cell cycle and that different 14-3-3 isoforms may bind to specific proteins for executing its biological function in cell cycle progression. Here, we describe the isolation, molecular cloning, and characterization of a CDK-associated protein 14-3-3 sigma. We show that 14-3-3 sigma could bind CDK2, CDC2, and CDK4 and emerges as a new class of CDK inhibitor.

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‡ Both authors contributed equally to this work.

† Recipient of the Fleming and Davenport research award. To whom correspondence should be addressed: Box 18, the University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Tel.: 713-792-8741; Fax: 713-796-6059; E-mail: mplee@notes.mdacc.tmc.edu.

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1 The abbreviations used are: CDKs, cyclin-dependent kinases; CKIs, cyclin-dependent kinase inhibitors; PCR, polymerase chain reaction; HA, hemagglutinin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4,6-diamidino-2-phenylindole; NES, nuclear exporting signal; m.o.i., multiplicity of infection; Ad, adenovirus; wt, wild type.
Expression Cloning of 14-3-3 Sigma cDNA—We prepared the FLAG-CDK2 following the procedure described before (8). Briefly, a PCR-generated NdeI-BamHI fragment of the human CDK2 cDNA containing the full-length coding region was subcloned into pET21a (Novagen), yielding a construct that encodes CDK2 with a FLAG tag sequence. Recombinant FLAG-CDK2 carrying the heart muscle kinase phosphorylation site was phosphorylated in vitro by protein kinase A with [γ-32P]ATP following the procedure previously described (25). The phosphorylated CDK2 were used to screen a mouse 16-day embryonic library that is a T7 RNA polymerase-driven λExIox mouse embryonic cDNA library (Novagen). At least 10x10^6 clones were screened. Several positive clones were sequenced, and one positive clone contained full-length cDNA of mouse 14-3-3 sigma.

In Vitro Association of 14-3-3 Sigma and CDK Proteins—A PCR-generated NdeI-BamHI fragment of the mouse 14-3-3 sigma cDNA containing the full-length coding region was subcloned into pET21a (Novagen) to yield a construct that encodes 14-3-3 sigma with a FLAG tag sequence. The protein was expressed in BL21(DE3), and FLAG-tagged proteins were prepared as described previously (8), except that proteins were immobilized on the beads without elution. Baculoviral proteins containing cyclins and CDKs were prepared by following the method described before (26). Cyclin D2 and CDK4 complex, cyclin B and CDC2 complex, or cyclin E and CDK2 complex was formed by preincubating in activating buffer (30 mM HEPES, 7.5 mM MgCl2, 0.2 mM phosphocreatine, 0.03 mM ATP). A T7 RNA polymerase-driven pET vector containing the coding region of the mouse cyclin E or CDK2 cDNA was transcribed in vitro and translated using a TNT kit (Promega). These products were labeled with [35S]methionine. These complexes, CDK, or the products translated by T7 RNA polymerase-driven pET vector containing the coding region of MCF-7, HER-18, and MDA-MB-361 cells were infected with Ad-HME1 in serum-free media for 1 h. Complete medium was added, and the cells were harvested 48 h later. The cells were lysed in RIPA buffer (100 mM NaCl, 20 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, 0.5% Triton X-100, 0.5% Nonidet P-40) and quantified with a Bio-Rad kit following the conditions suggested by the manufacturer. The proteins were then infected with the immobilized FLAG-tagged 14-3-3 sigma. After extensive washing in Nonidet P-40/RIPA buffer, the retained protein complexes were resuspended in SDS sample buffer. The complexes were resolved in SDS-polyacrylamide gel electrophoresis, followed by hybridization with CDK2 (PharMingen), CDK4 (PharMingen), and CDC2 antibody (Upstate Biotechnology Inc.) or by fluoroimmunoassay.

Immunofluorescence—NIH3T3 cells (2x10^5) were seeded onto a 0.7% agarose/complete media bottom layer. Three weeks later, 100 μl per well of 0.1% Trypsin, 0.02% EDTA in phosphate-buffered saline, and trypsinized. By using the cell counter, 3000 cells/well (96 well plate) were seeded for each cell line and type of infection. The number of cells was added and incubated for 16 h. Cells were lysed with 200 μl of 0.1 N KOH, and lysates were collected using the manufacturer’s kit (Amersham Pharmacia Biotech). Soft agar colony assays were performed on the same three cell lines and under the same infection conditions. The following day, 2000 cells per well mixed in a 0.35% agarose/complete media suspension were seeded onto 0.7% agarose/complete media bottom layer. Three weeks later, 100 μl per well of 0.1% Trypsin, 0.02% EDTA in phosphate-buffered saline, and trypsinized. By using the cell counter, 3000 cells/well (96 well plate) were seeded for each cell line and type of infection. The number of cells was added and incubated for 16 h. Cells were lysed with 200 μl of 0.1 N KOH, and lysates were collected using the manufacturer’s kit (Amersham Pharmacia Biotech).

A tritiated-thymidine incorporation assay was performed using the same three cell lines and infection method as mentioned previously. Ten thousand cells per well were seeded for each cell line and type of infection in a 96-well plate. Six hours later, 0.2 μC/well of tritiated-thymidine was added and incubated for 16 h. Cells were lysed with 200 μl of 0.1 N KOH, and lysates were collected using the manufacturer’s kit (Amersham Pharmacia Biotech).

Flow Cytometry—Indicated breast cancer cell lines (MCF-7, HER-18, MDA-MB-453, and MDA-MB-468) were infected with or without Ad-HME1 (m.o.i. = 10). Forty-eight hours after infection, cells were sorted, and their DNA content was analyzed by flow cytometry, all as described (7).

Immunofluorescence—NIH3T3 cells (2x10^5) were seeded onto chamber slides (Nunc) and infected with or without Ad-HME1 (m.o.i. = 10). R1b/L17 cells were transfected with pCMV5 plasmid containing FLAG-tagged HME1, FLAG-tagged HME1 (NES), T7-tagged-CDK2, or with empty vector. FLAG-tagged-HME1 (NES) is the NES mutant with point mutations (205A and 208A) at the NES sequence (STLIMQLRLN). This mutant was constructed by PCR mutagenesis. T7-tagged CDK2 was constructed by PCR cloning to contain the T7 gene 10 leader peptide tag sequence (MASTGGQMQG). Twenty-four hours after infection or infection, 2x10^5 cells were seeded onto tissue culture chamber slides (Nunc). Two days later, cells were fixed with methanol:aceton (1:1 v/v) and stained for 1 h with rabbit anti-CDK2, rabbit anti-CD28 antibodies (Santa Cruz), mouse anti-FLAG monoclonal antibody (Sigma), or mouse anti-T7 monoclonal antibody (Novagen), followed by 30-min exposures to anti-rabbit Cy3-conjugated or anti-mouse fluorescein isothiocyanate-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). Cells were then incubated with 0.1 μg/ml of 4,6-diamidino-2-phenylindole (DAPI) (Sigma) to stain...
Expression Cloning of 14-3-3 Sigma—The mouse 14-3-3 sigma gene was identified during a search for CDK2-associated proteins using an expression cloning method to screen a mouse 16-day embryonic cDNA library (27). Several clones were identified and sequenced. A GenBank™ search revealed that, at the amino acid level, one clone showed significantly high homology to human 14-3-3 sigma or HME1 (Human mammary epithelium marker 1) (23) and is the mouse ortholog of 14-3-3 sigma. The mouse 14-3-3 sigma (MME1) cDNA had an open reading frame of 744 base pairs, and it encodes a predicted protein of 248 amino acids. The human and mouse 14-3-3 sigma amino acid sequences are highly related, showing 96% identity (Fig. 1A). Sequence analysis revealed an important feature of a leucine-rich nuclear exporting signal (NES) located at amino acids 202–216 (STLIMQLLRDNLTLW) that plays an important role in cell cycle regulation (22).

14-3-3 Sigma Contains the Cyclin-CDK Binding Motif—Since we identified mouse 14-3-3 sigma using CDK2 as a bait through expression cloning, we compared the cyclin-CDK binding domain of all the important cell cycle regulatory proteins, including p27KIP1, p57KIP2, p21CIP1, p107, p130, and found that there is a consensus cyclin-CDK binding sequence (ZRXL, where Z and X are basic amino acids) that can be deduced in these proteins as shown in Fig. 1B. Structure determination of p27KIP1 has indicated that peptides around the ZRXL region are very critical for CDK binding (28). Therefore, 14-3-3 sigma is likely to use this CDK binding motif to interact with various CDKs.

14-3-3 Sigma Binds Cyclin-dependent Kinases—To confirm further the binding of 14-3-3 sigma to cyclin-CDK2, recombinant FLAG-tagged 14-3-3 sigma protein was purified by M2 monoclonal antibody-Sepharose and used for in vitro biochem-
plexes and prevents Thr 160 phosphorylation and activation of different from KIP1. KIP1 binds preactive cyclin E that 14-3-3 sigma does not block CDK activation, which is inactive forms of CDK (Fig. 2).

Cyclin E. The data show that 14-3-3 sigma binds both active and activating process. Affinity purified 14-3-3 sigma was used to test of CDK2 or CDC2 in the anti-HA immunoprecipitation complex (Fig. 3). Since CDKs are activated by their regulatory cyclins, we tested the role of 14-3-3 sigma in this dynamic activating process. Affinity purified 14-3-3 sigma was used to test the binding character during the activating process of CDK2 by cyclin E. The data show that 14-3-3 sigma binds both active and inactive forms of CDK (Fig. 2E). These results provide evidence that 14-3-3 sigma does not block CDK activation, which is different from KIP1. KIP1 binds preactive cyclin E/CDK2 complexes and prevents Thr160 phosphorylation and activation of CDK2 (7).

CDK Inhibitory Activity—Most CDK-associated proteins can either negatively or positively regulate the activity of the CDKs. To investigate if 14-3-3 sigma can regulate the activity of the CDKs through protein association, we overexpressed 14-3-3 sigma in various cell lines by virus gene transfer (24), and we examined whether 14-3-3 sigma regulates the activity of CDKs. Recombinant 14-3-3 sigma adenovirus (Ad-HME1) was used to infect breast cancer cells under conditions in which the majority of the cell population was infected (m.o.i. = 10). The activity of both CDK2 and CDC2 was inhibited by the expression of 14-3-3 sigma as demonstrated in the CDK2/CDC2-associated histone H1 kinase assays (Fig. 4). Also, immunoblotting bands demonstrate that when these cells were infected with Ad-HME1, HME1 associated with more than 95% of endogenous CDK2 or CDC2 as quantitated by PhosphorImager Imagequant program (data not shown), suggesting that 14-3-3 sigma can bind to most of the CDK2/CDC2 to inhibit their activities. All breast cancer cell lines tested were sensitive to the inhibitory activity of 14-3-3 sigma regardless of p53, Rb (retinoblastoma protein), or HER-2/neu status. These results provided evidence that 14-3-3 sigma can physically associate with CDKs and inhibit their activities. The inhibitory activity is also observed in a cell line with non-epithelial origin (NIH3T3) that does not express 14-3-3 sigma (data not shown).

14-3-3 Sigma Overexpression Inhibits Cell Proliferation and Anchorage-independent Growth of Breast Cancer Cells—The fact that 14-3-3 sigma can inhibit CDK activity suggests that 14-3-3 sigma may inhibit cell proliferation by cell cycle arrest. To determine if 14-3-3 sigma could inhibit cell proliferation, we assessed its inhibitory effect by MTT assay (29) and flow cytometry. Cell cycle analysis of several breast cancer cell lines infected with recombinant 14-3-3 sigma adenovirus by fluorescence-activated cell sorter demonstrated that 14-3-3 sigma has an effect on G2/M progression (Table I). Three breast cancer cell lines (MDA-MB-361, MCF7, and HER-18) were infected with recombinant 14-3-3 sigma adenovirus and assayed for live cells every 24 h. Overexpression of 14-3-3 sigma markedly decreased the viable cell number during a 7-day growth period as indicated by the decrease of A570 reading (Fig. 5A). In a parallel virus infection assay, the cells infected with adenovirus expressing β-galactosidase (Ad-β-galactosidase) grew normally as the cells that did not receive 14-3-3 sigma. Also, the rate of [3H]thymidine incorporation into DNA was reduced by half in cells infected with 14-3-3 sigma as compared with cells receiving no 14-3-3 sigma. Also, the rate of

Table I

| % of cell cycle | MCF7 | MCF7 + Hme1 | HER-18 | HER-18 + Hme1 | G1 | G2 |
|----------------|-----|------------|-------|--------------|----|----|
| MCF7           | 74  | 72         | 79    | 75           | 61 | 51 |
| HER-18         | 17  | 12         | 14    | 7            | 21 | 23 |
| G1             | 8   | 16         | 7.5   | 18           | 18 | 25 |
| G2             | 74  | 72         | 79    | 75           | 61 | 51 |
| MCF7 + Hme1    | 17  | 12         | 14    | 7            | 21 | 23 |
| HER-18 + Hme1  | 8   | 16         | 7.5   | 18           | 18 | 25 |

a MDA-MB-453 cells.
b MDA-MB-468 cells.
CDC2—To support the hypothesis that interaction of 14-3-3 sigma and CDK proteins could have a physiological role, subcellular localization of these proteins was analyzed. As shown in Fig. 7, 14-3-3 sigma caused a decrease in the nuclear staining for both CDK2 and CDC2 compared with the controls. These results indicate that 14-3-3 sigma can sequester CDK2 and CDC2 from the nucleus, thereby preventing the activity of CDK2 and CDC2. Because 14-3-3 sigma contains a leucine-rich nuclear exporting signal at amino acid residues 202–216 (Fig. 1), we determined whether the nuclear accumulation of CDK2/CDC2 is mediated by the nuclear exporting activity of 14-3-3 sigma. An NES mutant of 14-3-3 sigma was constructed by mutating the leucine-rich NES sequence with alanine (I205A and L208A). Immunolocalization studies showed that wt 14-3-3 sigma was detected in the cytoplasm, whereas the NES mutant of 14-3-3 sigma was mainly detected in the nucleus (Fig. 8, A and C). We also found that CDK2 is distributed in the cytoplasm in the presence of wt 14-3-3 sigma (Fig. 8G), but CDK2 remains in the nucleus when coexpressed with the NES mutant of 14-3-3 sigma (Fig. 8I). CDK2 transfection was used as a control and was demonstrated as a nuclear protein (Fig. 8K). DAPI is used to stain the nuclei. These results demonstrated that the binding of 14-3-3 sigma to CDK can cause the sequestration of CDK2 and CDC2 into the cytoplasm, and the NES of 14-3-3 sigma is required for this biological function.

DISCUSSION

New Class of CKI—In a search for CDK2-associated proteins, we have identified a 14-3-3 protein, 14-3-3 sigma, that specifically associates with CDK2. 14-3-3 proteins are important and highly related dimeric factors found in eukaryotic organisms, including yeast, mammals, Drosophila, and plants (17). Members of the 14-3-3 family are involved in regulating the activities of tyrosine and tryptophan hydroxylases and protein kinase C, exocytosis, transcriptional activities, and the cell cycle (17). Here we demonstrated the specific interaction between CDKs and 14-3-3 sigma, and we characterized the biological functional role of the interaction in cell cycle progression. Interestingly, one CDK-like protein PCTAIRE-1 was shown to interact with several isoforms of 14-3-3, including eta, tau, and zeta isoforms (30). However, the significance of these interactions in cell cycle regulation remains to be determined. Sequence analysis showed that the 14-3-3 sigma protein is highly conserved with 96% identity in human and mouse. Its distinctive feature is a cyclin-CDK binding motif that is shared by many cyclin-CDK-binding proteins (Fig. 1), including CIP/KIP.
family members. In vitro binding experiments confirm that 14-3-3 sigma binds to various cyclin-CDK complexes (Fig. 2). Interestingly, purified 14-3-3 sigma cannot inhibit cyclin-CDK activity (data not shown) in vitro although overexpression of 14-3-3 sigma in the cells can inhibit CDK2/CDC2-associated kinase activity (Fig. 4) and cell cycle progression (Fig. 5), suggesting that a biological environment is required to assay its CDK inhibitory activity. Because it binds and inhibits various cyclin-CDKs to regulate cell cycle progression and cell proliferation, 14-3-3 sigma emerges as a new class of CKI.

CDK Inactivation and Growth Inhibition—We showed that 14-3-3 sigma affects the subcellular localization of CDK2 and CDC2 to inhibit their accumulations in the nucleus, thus preventing the activation of CDK2/CDC2 in the nucleus. This mislocation of CDK2 and CDC2 in the cytoplasm can cause inhibition of their biological activities (31). Sequence analysis revealed a leucine-rich NES-like sequence (202STLIMQLLR-DNLTLW212) in the 14-3-3 sigma. Indeed, we showed that 14-3-3 sigma can bind CDK proteins and sequester them in the cytoplasm (Fig. 7) through the nuclear exporting activities. The nuclear exporting sequence is involved in the sequestration of CDK2 since the NES mutant of 14-3-3 sigma cannot sequester the CDK2 into the cytoplasm (Fig. 8). Other 14-3-3 proteins have similar characteristics to export their target proteins to the cytoplasm, thus preventing their activities at the nucleus. For example, CDC25 is phosphorylated by Chk1 (32) or Chk2 (33) to create a binding site in CDC25 for 14-3-3 proteins and is exported by Rad24/Rad25 (also 14-3-3 proteins) in response to DNA damage (22), thereby inhibiting the activity of CDC25 and causing G2/M arrest. But it also remains to be determined which kinase is responsible for the serine phosphorylation on CDK2/CDC2 since targeted proteins of 14-3-3 required serine phosphorylation before binding to 14-3-3 (34). We demonstrated that 14-3-3 sigma can affect cell cycle distribution at the G2/M (Table I), which highlights its role at the G2/M control. The significance of the role of 14-3-3 sigma in inhibiting CDK2 activity and CDK2 nuclear accumulation remains to be determined in terms of cell cycle distribution and checkpoint control. However, both inhibition of CDC2-associated kinase activity and sequestering of CDC2 into the cytoplasm can account for its role in G2/M control.

Sequence analysis of human CDK2 reveals that there are two possible 14-3-3-binding sites located at the C-terminal region of CDK2 (GVTS229MP and YKPS236FP), which share a homologous sequence to what has been predicted as the 14-3-3 sigma-binding sites (35). Therefore, it is possible that 14-3-3 sigma may bind to this region to regulate CDK activities and cell cycle progression. Our data show that overexpression of 14-3-3 sigma in vivo could bind and inhibit the activities of CDK2 and CDC2 and render cells unable to reach cell cycle transition. In this aspect, it is functionally similar to the CIP/KIP family members. The strong reduction in the growth rate and the decrease in [3H]thymidine incorporation caused by 14-3-3 sigma expression are also consistent with a role of 14-3-3 sigma as a negative regulator of cell cycle. Through IR, 14-3-3 sigma was shown to be a mediator of p53 growth-inhibitory signaling (24). As cells received ionizing radiation, p53 quickly induced 14-3-3 sigma expression within 2 h, and this induction led to G2/M arrest (24). The mechanism behind this 14-3-3 sigma-induced cell cycle arrest is not clear, although it was proposed that 14-3-3 sigma may sequester CDC25C to cause G2 arrest (24) based on the observations that other 14-3-3 isoforms can interact with CDC25 (18, 36, 37). It remains to be elucidated if 14-3-3 sigma can interact with CDC25 directly to block G2/M progression in response to DNA damage. Our data show that 14-3-3 sigma can directly bind and sequester CDC2 into the cytoplasm to inhibit its activity required for G2/M progression, which may contribute to IR-induced p53-mediated G2/M arrest.

Inhibition of Cell Transformation—After 14-3-3 sigma was cloned, it was determined that the expression level is significantly reduced both in v-Ha-Ras transformed mammary epithelial cells and mammary carcinoma cells (23). Furthermore, expression of 14-3-3 sigma was also reduced in SV40-transformed human keratinocytes compared with primary cells (38). Finally, the transcript of 14-3-3 sigma was down-regulated in head and neck squamous cell lines in comparison with keratinocytes (39). Together these observations support the idea that loss of its expression contributes to malignant transformation. Recent studies in breast carcinomas and colon cancer suggested that p27 expression is decreased when cells become tumorigenic (40–42). These observations lend further support to the concept that the CDK activity controls cell proliferation and is involved in the development of human cancer. The decreased expression of HME1 in neoplastic mammary cells is reminiscent of p27 down-regulation in breast cancer. We hypothesize that the expression of 14-3-3 sigma, like the function of p27kip1, is one of the mechanisms cells employ to ensure the maintenance of a nonproliferative state. The fact that 14-3-3 sigma suppressed the anchorage-independent growth of some breast cancer cell lines (Fig. 6) supports the idea that loss of 14-3-3 sigma function correlates with cell transformation. Taken together, these results suggest that a reduction of 14-3-3 sigma plays an important role in cell transformation. Most significantly, fluorescence in situ hybridization analysis of metaphase chromosomes with a 14-3-3 sigma probe showed that 14-3-3 sigma was localized to chromosome 1p35 (24) where high percentages of loss of heterozygosity (43) were seen in
Cloning of 14-3-3 Sigma as a CDK Interaction Protein

In summary, our characterization of 14-3-3 sigma as a CDK interaction protein suggests that 14-3-3 sigma could be the candidate for this tumor suppressor gene in the 1p35 region. Further investigation of the expression pattern and gene function of 14-3-3 sigma in tumors will shed light on its potential role as a tumor suppressor.

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