Matrix metalloproteinase (MMP) inhibitory proteins may negatively regulate MMP activity to suppress tumor metastasis. In this study, we demonstrate that the HER-2/neu oncogene inhibits the expression of the MMP inhibitor RECK to promote cell invasion. RECK was inhibited via transcriptional repression in B104-1-1 cells, which express constitutively active HER-2/neu. Overexpression of HER-2/neu in NIH/3T3 or HaCaT cells also suppressed RECK expression. Deletion and mutation assays showed that HER-2/neu repressed RECK via the Sp1-binding site localized in the −82/−71 region from the translational start site. DNA affinity precipitation and chromatin immunoprecipitation assays indicated that binding of Sp1 and Sp3 to this consensus site was increased in B104-1-1 cells. We also found that HER-2/neu inhibited RECK via the ERK signaling pathway. Sp1 proteins phosphorylated at Thr53 and Thr37 by ERK bound preferentially to the RECK promoter, and this binding was reversed by HER-2/neu and ERK inhibitors. Furthermore, our data indicate that HER-2/neu obviously increased HDAC1 binding to the Sp1-binding site localized in the −82/−71 region of the RECK promoter. The histone deactylase inhibitor trichostatin A reversed HER-2/neu-induced inhibition of RECK. HER-2/neu activation was associated with increased MMP-9 secretion and activation. Re-expression of RECK in HER-2/neu-overexpressing cells inhibited MMP-9 secretion and cell invasion. Taken together, our results suggest that HER-2/neu induces the binding of Sp proteins and HDAC1 to the RECK promoter to inhibit RECK expression and to promote cell invasion. Restoration of RECK provides a novel strategy for the inhibition of HER-2/neu-induced metastasis.

The HER-2/neu oncogene (also known as erbB2) encodes a transmembrane glycoprotein that belongs to the human epidermal growth factor receptor family (1, 2). Structural analysis of HER-2/neu and the epidermal growth factor receptor revealed significant sequence homology and identical gross structural organization between these two proteins (3–5). Amplification and overexpression of HER-2/neu have been found in breast, ovarian, lung, gastric, and cervical carcinomas (6–10). Up-regulation of this oncogene is frequently linked with increased metastasis and poor prognosis. The mechanism underlying HER-2/neu-induced metastasis is a field of intensive study, and several candidates involved in this process have been identified recently. The first candidate is the vascular endothelial growth factor. HER-2/neu may stimulate vascular endothelial growth factor expression, and neutralizing antibody against HER-2/neu suppresses vascular endothelial growth factor production and metastasis of cancer cells (11, 12). The second is the urokinase-type plasminogen activator. Induction of the urokinase-type plasminogen activator by HER-2/neu has been shown to be correlated with increased metastasis (13). The third is cyclooxygenase-2. Cyclooxygenase-2 plays a critical role in tumor metastasis and may be a potential target for chemoprevention (14). HER-2/neu up-regulates cyclooxygenase-2 through transcriptional activation, and the selective cyclooxygenase-2 inhibitor celecoxib has been shown to protect HER-2/neu-induced breast cancer (15, 16). Another important mediator for tumor metastasis triggered by HER-2/neu is the chemokine receptor CXCR4. A recent study has shown that HER-2/neu enhances the expression of CXCR4, which is required for HER-2/neu-mediated invasion in vitro and lung metastasis in vivo (17). Finally, recent studies have shown that matrix metalloproteinases (MMPs)2 are key players in the induction of tumor metastasis by HER-2/neu. HER-2/neu directly up-regulates MMP expression via ETS transcription factor-binding sites (18, 19).

The RECK gene was isolated as a novel suppressor gene using an expression cloning strategy designed to identify human cDNA inducing flat reversion in a v-Ki-ras-transformed NIH/3T3 cell line (20). This gene encodes a membrane glycoprotein that may inhibit tumor metastasis and angiogenesis by negatively regulating MMP activity (21, 22). Although RECK mRNA is expressed in most normal human tissues and untransformed cells, it is undetectable in many tumor cell lines (23). Additionally, clinical studies have also indicated that patients with high RECK expression in tumor tissues show better survival and that such tumors are less invasive (24, 25). These results strongly suggest that RECK is a novel suppressor gene for metastasis and angiogenesis. In this study, we investigate whether HER-2/neu may suppress RECK expression to promote cell invasion.

**EXPERIMENTAL PROCEDURES**

Cell Culture and Experimental Reagents—NIH/3T3 and B104-1-1 (NIH/3T3 cells expressing constitutively active HER-2/neu) cells (26) were kindly provided by Dr. M. D. Lai (National Cheng Kung University). Human HaCaT keratinocytes were from Dr. R. H. Chen (National Taiwan University). Cells were cultured in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 containing 10% fetal calf serum and 2 The abbreviations used are: MMPs, matrix metalloproteinases; RT, reverse transcription; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DAPA, DNA affinity precipitation assay; ChIP, chromatin immunoprecipitation; MEK1, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1; TSA, trichostatin A.
antibiotics. A luciferase assay system was purchased from Promega Corp. (Madison, WI). The expression vector for constitutively active HER-2/neu was kindly provided by Dr. M. C. Hung (M. D. Anderson Cancer Center). The mouse Reck promoter-luciferase plasmid and human Reck cDNA were kindly provided by Dr. M. Noda (Kyoto University, Kyoto, Japan). The p21(WAF1) promoter-luciferase construct was kindly provided by Dr. B. Vogelstein. The OneStep reverse transcription (RT)-PCR kit was from Qiagen Inc. The kinase inhibitor AG825, P98059, and wortmannin were from Tocris (Northpoint, United Kingdom). Anti-phospho-AKT and anti-phospho-ERK antibodies were purchased from New England Biolabs. Anti-phospho-Sp1 antibodies were kindly provided by Dr. G. Pages.

**Construction of the Reck Expression Vector**—Human Reck cDNA in the pBluescript II vector was digested with Sall and NotI restriction enzymes and subcloned into the pBk-CMV vector (Stratagene).

**RNA Extraction and RT-PCR**—Total RNA was isolated from cells, and Reck expression was investigated using the OneStep RT-PCR kit according to the manufacturer’s protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to check the efficiency of cDNA synthesis and PCR amplification. cDNA synthesis was carried out at 50 °C for 30 min, and the PCR conditions were as follows: 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min and one cycle of final extension at 72 °C for 10 min. The predicted sizes of the PCR products for Reck and GAPDH were 477 and 512 bp, respectively. The primers used were as follows: Reck-forward, 5′-CCTCAATGACCAAGCTTAGG-3′; Reck-reverse, 5′-GACGACACACACTGCTGTA-3′; GAPDH-forward, 5′-GAGTGAACGGAGTGGTGGTGTG-3′; and GAPDH-reverse, 5′-TGTGCTCATGAGTCTTCCA-3′. After the reaction, PCR products were separated on a 0.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

**Western Blot Analysis**—Cells were harvested in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 50 mM NaF, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml aprotinin, and 1 mg/ml pepstatin A), scraped, and pelleted by centrifugation at 4 °C. Cells were resuspended in lysis buffer, incubated for 10 min on ice, and sonicated to shear DNA. After sonication, the lysate was centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was diluted in ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl, and protease inhibitors). Anti-Sp1, anti-Sp3, and anti-HDAC1 antibodies were added to the supernatant and incubated overnight at 4 °C with rotation. The immunocomplex was collected on Protein A/G-agarose and washed sequentially with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 200 mM Tris-HCl, pH 8.1, and 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 200 mM Tris-HCl, pH 8.1, and 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.1), and finally 1X Tris/EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The immunocomplex was eluted with elution buffer (1% SDS, 0.1 M NaHCO3, and 200 mM NaCl), and the cross-links were reversed by heating at 65 °C for 4 h. After the reaction, the samples were adjusted to 10 mM EDTA, 20 mM Tris-HCl, pH 6.5, and 40 μg/ml proteinase K and incubated at 45 °C for 1 h. DNA was recovered and subjected to PCR amplification using the primers specific for the detection of the −107/+52 region, which contains the Sp1(B) site of the Reck promoter. The primers used were as follows: sense, 5′-GAACTGAGACACTACATTCCAG-3′; and antisense, 5′-GACGATGAAGACGACCGGC-3′. The predicted size of the PCR product was 159 bp.

**In Vitro Invasion Assay and MMP-9 Secretion**—The in vitro invasion assay was performed using 24-well Transwell units with polycarbonate filters (8-μm pore size) coated on the upper side with Matrigel (BD Biosciences). Cells were transfected with the control or Reck expression vector for 48 h. Cells were collected, and 5 × 105 cells in 100 μl of medium were placed in the upper part of the Transwell unit and allowed to invade for 24 h. The lower part of the Transwell unit was filled with 10% fetal calf serum medium. After incubation, non-invaded cells on the upper part of the membrane were removed with a cotton swab. Invaded cells on the bottom surface of the membrane were fixed in formaldehyde, stained with Giemsa solution, and counted under a microscope. The medium from the same number of control or Reck vector-trans-
fected cells was collected and subjected to Western blot analysis to detect pro-MMP-9 and MMP-9.

RESULTS

HER-2/neu Down-regulates RECK via Transcriptional Repression—

We first compared the RECK protein level in NIH/3T3 and B104-1-1 cells. We found that the amount of RECK protein was dramatically reduced in B104-1-1 cells (Fig. 1A). RT-PCR analysis showed a 70–80% reduction of the RECK mRNA level in B104-1-1 cells (Fig. 1B). These results suggested that HER-2/neu suppresses RECK expression via transcriptional repression. We next performed a promoter activity assay to verify this issue. The mouse Reck promoter-luciferase construct was transfected into both cell lines, and we found that RECK promoter activity was significantly inhibited in B104-1-1 cells (Fig. 1C). Repression of RECK by HER-2/neu is specific because p21WAF1 promoter activity was not affected under the same experimental conditions (Fig. 1C). To rule out that this reduction may be due to the difference in transfection efficiency between these two cell lines, the Reck promoter-luciferase construct was cotransfected with the constitutively active HER-2/neu expression vector into NIH/3T3 cells, and RECK promoter activity was examined 48 h after transfection.

HER-2/neu Inhibits RECK via the Sp1-binding Site—

We next investigated the critical elements that mediate the inhibitory effect of HER-2/neu on RECK promoter activity using deletion mutants. Fig. 3A demonstrates that the element responsive to HER-2/neu is located within FIGURE 1. Down-regulation of RECK by HER-2/neu. A, total proteins of NIH/3T3 (N) and B104-1-1 (B) cells were extracted, and RECK protein levels were examined by Western blot analysis. B, RT-PCR experiments were performed to compare the mRNA levels of RECK in NIH/3T3 and B104-1-1 cells. C, the mouse Reck (R) or p21WAF1 (p21) promoter-luciferase construct was transfected into cells, and luciferase activity was determined 48 h after transfection. The promoter activities of NIH/3T3 cells were defined as 100%. D, NIH/3T3 cells were cotransfected with the mouse Reck or p21WAF1 promoter-luciferase construct and the control (C) or HER-2/neu (Neu) expression vector. Promoter activity was examined 48 h after transfection. The promoter activities of cells cotransfected with control vector were defined as 100%.

FIGURE 2. HER-2/neu suppression of RECK in human HaCaT cells. A, HaCaT cells were transfected with the control (C) or HER-2/neu (Neu) expression vector. After 48 h, cells were harvested, and RECK mRNA levels were examined by RT-PCR. The HER-2/neu protein level of transfected cells was investigated by Western blot analysis. B, the RECK promoter-luciferase construct was cotransfected with the control or HER-2/neu expression vector into HaCaT cells, and RECK promoter activity was examined at 48 h after transfection.
Repression of RECK by HER-2/neu

the region −159 bp from the translational start site. Two potential Sp1-binding sites are found in this region; we used mutation mutants to address the importance of these Sp1 sites in the repression of RECK by HER-2/neu. As shown in Fig. 3B, mutation of the Sp1(B) site (localized in the −82/−71 region from the translational start site) up-regulated RECK basal promoter activity, so this Sp1-binding site functions as a transcriptional repressor. Additionally, HER-2/neu-induced inhibition of RECK was obviously attenuated after mutation of the Sp1(B) site. In contrast, mutation of the Sp1(A) site had little effect. We next performed DAPA to study the interaction between nuclear proteins and this consensus site. Fig. 4A shows minor increases in the levels of the nuclear Sp1 and Sp3 proteins (but not CDK4) in B104-1-1 cells. Western blot analysis indicated that the Sp1 and Sp3 protein levels were not significantly altered in B104-1-1 cells (Fig. 4B). In addition, RT-PCR data also showed that Sp1 and Sp3 mRNA levels were similar in NIH/3T3 and B104-1-1 cells (Fig. 4C). These results suggest that HER-2/neu increases Sp1 and Sp3 nuclear accumulation. DAPA demonstrated that binding of Sp1 and Sp3 to the DNA probe was increased in B104-1-1 cells (Fig. 4D), indicating that HER-2/neu activation potently stimulated the DNA binding affinity of Sp1 and Sp3. The binding between Sp1 and Sp3 to the DNA probe is specific because mutation of the Sp1-binding site obviously attenuated the interaction (Fig. 4D). This result is in agreement with the data of two previous studies demonstrating that HER-2/neu may increase the phosphorylation and DNA binding activity of Sp1 and Sp3 (30, 31). To verify that the Sp1 proteins bound to the Sp1-binding site of the DNA probe were in the phosphorylated form, we performed DAPA to precipitate the Sp1 proteins and investigated the phosphorylation status of Sp1 by immunoprecipitation/immunoblot assay after releasing the Sp1 proteins from the DNA probe. Previous studies have shown that ERKs, a major downstream mediator of the HER-2/neu signaling pathway, might phosphorylate Thr63 and Thr739 of the Sp1 protein to enhance its DNA binding and transcriptional activity (32, 33). Therefore, we probed the immunoprecipitated Sp1 protein with anti-phospho-Sp1 antibodies against these two phosphothreonine residues. As demonstrated in Fig. 4E, the Sp1 proteins bound to the DNA probe were mainly in the phosphorylated form, and phosphorylation at Thr63 and Thr739 was obviously increased in B104-1-1 cells. We next studied whether Sp1 and Sp3 bind to the RECK promoter in vivo using the ChIP assay. Fig. 4F shows that Sp1 and Sp3 indeed bound to the RECK promoter in vivo. Conversely, precipitation with the unrelated anti-p57Kip2 antibody (negative control) did not yield any PCR products under the same experimental conditions.

The ERK Signaling Pathway Is Involved in the Inhibition of RECK by HER-2/neu—We next tested the signaling pathways by which HER-2/neu suppresses RECK. ERK and phosphatidylinositol 3-kinase are the major mediators of HER-2/neu, so we tested the involvement of these two kinases. B104-1-1 cells were transfected with the Reck promoter-luciferase plasmid and incubated with different kinase inhibitors, AG825 (50 μM) for HER-2/neu, PD98059 (10 μM) for MEK1, and wortmannin (200 nM) for phosphatidylinositol 3-kinase, for 48 h. As shown...
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in Fig. 5A, the HER-2/neu kinase inhibitor AG825 completely suppressed ERK activity and reversed HER-2/neu-induced repression of RECK. The MEK1 inhibitor PD98059 also effectively inhibited ERK activity and counteracted the inhibition of RECK by HER-2/neu. Conversely, the phosphatidylinositol 3-kinase inhibitor had little effect. Because our results suggested that HER-2/neu increases Sp1 and Sp3 binding to the RECK promoter to inhibit gene expression, we investigated whether AG825 and PD98059 could reverse the binding of Sp1 and Sp3 to the RECK promoter using ChIP assays. Fig. 5B clearly demonstrates that these two kinase inhibitors attenuated the binding of Sp1 and Sp3 to the RECK promoter in vivo. These data suggest that HER-2/neu induces ERK activation and subsequently increases Sp protein phosphorylation and binding to the RECK promoter to repress gene expression. We also used SL2 cells (Drosophila cells that lack endogenous Sp proteins) to verify the importance of Sp proteins in the repression of RECK by HER-2/neu. As shown in Fig. 6, the expression of HER-2/neu in SL2 cells could not repress RECK promoter activity.

HER-2/neu Inhibits RECK via Recruitment of HDAC1—We (36) and others (34, 35) have shown previously that histone deacetylases may interact with Sp1 to repress gene expression, so we investigated whether binding of Sp1 and Sp3 proteins to the Sp1(B) site might subsequently recruit HDAC1 to the RECK promoter. Western blot analysis showed a minor increase in nuclear HDAC1 in B104-1-1 cells (Fig. 7A). However, total cellular HDAC1 protein and mRNA levels were not significantly changed (Fig. 7, B and C). DAPA showed that binding of HDAC1 to the DNA probe was obviously enhanced in B104-1-1 cells (Fig. 7D). This binding is specific because mutation of the Sp1-binding site abolished the interaction between HDAC1 and the DNA probe (Fig. 7D). Because the aforementioned results suggest that the ERK signaling pathway is involved in the inhibition of RECK by HER-2/neu, we also tested the effect of AG825 and PD98059. Fig. 7E clearly shows that these two inhibitors potently attenuated the binding of HDAC1 to the DNA probe. The ChIP assay confirmed significant increases in HDAC1 binding to the RECK promoter in B104-1-1 cells in vivo (Fig. 8A). The addition of AG825 and PD98059 also obviously reduced the binding of HDAC1 to the RECK promoter in vivo (Fig. 8B). We then tested the effect of the histone deacetylase inhibitor trichostatin A (TSA) on HER-2/neu-induced inhibition of RECK. The promoter activity assay clearly indicated that TSA only mildly (~1.8-fold increase) stimulated RECK promoter activity in NIH/3T3 cells. In contrast, TSA potently activated RECK promoter activity and effectively reversed HER-2/neu-induced inhibition of RECK (Fig. 8C). These results indicate that histone deacetylases are involved in the down-regulation of RECK by HER-2/neu. Collectively, our data suggest that activation of HER-2/neu induces the recruitment of HDAC1 to Sp proteins that bind to the RECK promoter to suppress RECK expression.

Re-expression of the RECK Gene Inhibits HER-2/neu-induced Cell Invasion—To investigate whether inhibition of RECK by HER-2/neu increases cell invasiveness, we first analyzed the invasive ability of NIH/3T3 and B104-1-1 cells. Fig. 9A shows that the invasive ability of B104-1-1 cells was much higher compared with the parental NIH/3T3 cells. We also found that HER-2/neu-induced invasiveness was closely linked with the secretion and activation of MMP-9 because the amounts of
pro-MMP-9 and active MMP-9 in the culture medium of B104-1-1 cells were obviously increased compared with that of NIH/3T3 cells (Fig. 9B). Re-expression of the RECK gene in B104-1-1 cells significantly reduced the secretion of pro-MMP-9 and active MMP-9 in the culture medium and potently inhibited HER-2/neu-induced cell invasion (Fig. 9, A and B). However, it should be noted that RECK has been shown to suppress several MMPs, including MMP-2, MMP-9, MMP-14, and membrane-type MMP-1 (21), so MMP-9 may not be the only one activated by HER-2/neu and inhibited by RECK. More experiments are needed to clarify the functional role of other MMPs in HER-2/neu-induced cell invasion.

DISCUSSION

The possible involvement of MMPs in HER-2/neu-induced metastasis is suggested by several observations. First, the expression of HER-2/neu is associated with up-regulated gelatinases (18). Second, heregulin may stimulate the expression of MMP-9 (37). Third, increased MMP-2 levels are found in HER-2/neu-transfected mammary epithelial cells (38). Consistent with these data, we found that MMP-9 expression and activation were obviously induced in B104-1-1 cells, which express constitutively active HER-2/neu. Therefore, MMPs are important mediators of HER-2/neu-induced metastasis. However, few studies have addressed the effect of HER-2/neu on the expression of MMP inhibitory proteins. In this study, we have provided evidence that HER-2/neu inhibits RECK, an important MMP inhibitor and metastasis suppressor, to promote cell invasion. Because proenzyme processing is essential for MMP function, it is rational to speculate that, in addition to up-regulating MMP expression, HER-2/neu needs to inhibit the expression of MMP inhibitory proteins to guarantee full activation of MMP and to promote tumor metastasis. Our data indeed support this speculation and show that the enforced expression of RECK in B104-1-1 cells suppresses MMP-9 secretion and attenuates the invasiveness of HER-2/neu-transformed cells (Fig. 9). These results suggest that RECK is a critical target for HER-2/neu and may be a suitable target for the suppression of HER-2/neu-mediated metastasis.

Using microarray analysis, Wilson et al. (39) compared HER-2/neu-positive and -negative breast cancer cell lines and tissues and showed that 46 and 132 genes were down-regulated in HER-2/neu-positive cancer cell lines and tumor tissues, respectively. These data suggest that repression of gene expression is a critical step for HER-2/neu-induced tumorigenesis. However, the molecular mechanism by which HER-2/neu represses gene expression is largely unknown. We have provided the first evidence that HER-2/neu represses RECK expression via an Sp1- and deacetylation-dependent mechanism. Four members of the Sp transcription factor family have been identified. Within this gene family, Sp1 and Sp3 are ubiquitously expressed in mammalian cells. Although a number of reports have demonstrated that Sp1 is mainly a transcriptional activator, Sp1 has also been shown to repress gene expression (34–36). Therefore, both Sp1 and Sp3 may act as positive or negative regulators of gene expression. Two models can be used to explain the mechanism of RECK inhibition by HER-2/neu via the Sp1-binding site. First, HER-2/neu may change the relative level of Sp1 and Sp3 binding to the Sp1-binding site of the RECK gene. Several previous studies have

FIGURE 7. Involvement of HDAC1 in HER-2/neu-induced inhibition of RECK. A, the nuclear protein levels of HDAC1 in NIH/3T3 (N) and B104-1-1 (B) cells were analyzed by Western blot analysis. CDK4 was used as an internal control to verify equal loading of each sample. B, total cell lysates were prepared from NIH/3T3 and B104-1-1 cells, and the HDAC1 protein level was examined. C, total RNA was harvested, and the mRNA level of HDAC1 was investigated by RT-PCR. GAPDH was included as an internal control to check the efficiency of cDNA synthesis and PCR amplification. D, the biotinylated wild-type or mutant (Mut) DNA probe corresponding to the Sp1-binding site localized in the −82/−71 region from the translational start site of the RECK gene was used for interaction with nuclear proteins, and the DNA-protein complex was precipitated by streptavidin-coated beads. The amount of HDAC1 protein bound to the probe was analyzed by Western blot analysis. E, cells were incubated with vehicle (0.02% dimethyl sulfoxide (DMSO)), AG825 (50 μM), or PD98059 (PD; 10 μM) for 48 h. DAPAs were performed to investigate the interaction of HDAC1 and the DNA probe.

FIGURE 8. Binding of HDAC1 to the RECK promoter in vivo and effect of TSA on RECK promoter activity. A, cells were fixed in 1% formaldehyde, washed twice with ice-cold phosphate-buffered saline containing protease inhibitors, and pelleted by centrifugation at 4 °C. Cells were resuspended in lysis buffer and sonicated to shear DNA. Anti-HDAC1 antibody was added to immunoprecipitate (IP) the DNA-protein complex. Anti-p57Kip2 antibody was used as a negative control. DNA was recovered and subjected to PCR amplification using the primers specific for the detection of the −107/+52 region, which contains the Sp1(B) site of the RECK promoter. B, cells were incubated with vehicle (0.02% dimethyl sulfoxide (DMSO)), AG825 (50 μM), or PD98059 (PD; 10 μM) for 48 h. The ChIP assay was performed to investigate the binding of HDAC1 to the RECK promoter in vivo. Anti-p57Kip2 antibody was used as a negative control. C, NIH/3T3 (N) and B104-1-1 (B) cells were transfected with the RECK promoter-luciferase construct and treated with vehicle (0.02% dimethyl sulfoxide) or TSA (100 nM) for 48 h. Luciferase activity was then determined.
demonstrated that a high ratio of Sp1 to Sp3 may activate gene expression, whereas a high ratio of Sp3 to Sp1 represses gene expression (40–43). This is not the case in our study because our previous study (36) already clearly showed that both Sp1 and Sp3 are transcriptional activators for the RECK gene. Similar results have also been reported by Sasahara et al. (23). Second, Sp1 may recruit histone deacetylases to repress gene expression. Our results support this model by showing that HDAC1 is recruited by Sp1 to the RECK promoter after HER-2/neu activation and that the histone deacetylase inhibitor TSA may effectively counteract HER-2/neu-induced down-regulation of RECK. These results are in agreement with a number of studies showing that the Sp1 protein may recruit histone deacetylases to inhibit gene expression (34–36). How HER-2/neu activation increases the interaction between HDAC1 and Sp1 is currently unknown. According to our data, we suggest that HER-2/neu may change the phosphorylation status of Sp1 and HDAC1 to promote their interaction. Previous studies have already demonstrated that HER-2/neu may increase Sp1 phosphorylation and DNA binding activity. Because ERKs are a critical mediator of HER-2/neu signaling, it is rational to speculate that HER-2/neu activates ERKs, which in turn phosphorylate Sp1 to enhance its DNA binding activity. The results of this study indeed support this hypothesis. First, the DNA-binding form of Sp1 is a predominantly phosphorylated form, and the major phosphorylation sites are Thr453 and Thr739, two consensus sites that have been reported to be phosphorylated by ERKs (33). Second, Sp1 may recruit histone deacetylases to inhibit gene expression (34–36). How HER-2/neu activation increases the interaction between HDAC1 and Sp1 is currently unknown. According to our data, we suggest that HER-2/neu may change the phosphorylation status of Sp1 and HDAC1 to promote their interaction. Previous studies have already demonstrated that HER-2/neu may increase Sp1 phosphorylation and DNA binding activity. Because ERKs are a critical mediator of HER-2/neu signaling, it is rational to speculate that HER-2/neu activates ERKs, which in turn phosphorylate Sp1 to enhance its DNA binding activity. The results of this study indeed support this hypothesis. First, the DNA-binding form of Sp1 is a predominantly phosphorylated form, and the major phosphorylation sites are Thr453 and Thr739, two consensus sites that have been reported to be phosphorylated by ERKs (33). Second, ChIP results demonstrated that the MEK1 inhibitor PD98059 reverses the HER-2/neu-stimulated binding of Sp1 and Sp3 to the RECK promoter in vivo. Thus, HER-2/neu indeed stimulates Sp1 phosphorylation and DNA binding activity. After phosphorylation, the interaction between Sp1 and HDAC1 may be modulated. A recent study demonstrated that the phosphorylation status of NF-kB determines its association with CBP (cAMP-responsive element-binding protein) p300 or HDAC1 (44). In addition, cyclin-dependent kinase-mediated phosphorylation of RB protein also regulates histone deacetylase binding (45). Similar conditions may be existed in Sp1 and HDAC1. However, it cannot be excluded that HER-2/neu may modulate HDAC1 phosphorylation to promote its binding to Sp1. A previous study has already shown that HDAC1 is a phosphoprotein and that phosphorylation of HDAC1 promotes the formation of a transcriptional repression complex and enhances HDAC1 enzymatic activity (46). More experiments are needed to clarify the molecular mechanism that regulates Sp1 and HDAC1 interaction. Collectively, our data provide the first evidence that HER-2/neu inhibits the metastasis suppressor RECK via an Sp1- and HDAC1-dependent mechanism to promote cell invasion.

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