Involvement of Co-activator p300 in the Transcriptional Regulation of the HER-2/neu Gene*

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Hua Chen and Mien-Chie Hung‡
From the Department of Tumor Biology and Breast Cancer Basic Research Program, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Overexpression of HER-2/neu is frequently found in human cancer and has been shown to enhance the metastatic potential of tumors and to induce the chemoresistance of cancer cells. The molecular mechanism(s) by which HER-2/neu expression is deregulated in cancer is not clear. We reported previously that adenovirus 5 E1A is capable of transcriptionally repressing the HER-2/neu promoter. We report here that the E1A-associated p300 protein can derepress the E1A-mediated repression of HER-2/neu in a dose-dependent manner. A p300 mutant, which lost its ability to bind to E1A, also effectively rescued the repressed HER-2/neu promoter in the presence of excess E1A inside the cells. A protein complex can bind to the p300 consensus sequences in HER-2/neu promoter. The intensity of the retarded band of the protein complex decreased significantly after preincubation of the nuclear extracts with beads that has been conjugated with anti-p300 antibody. The binding of E1A to p300 and the p300 consensus sequence in HER-2/neu promoter were crucial for the ability of E1A to repress HER-2/neu promoter, demonstrating that p300 is involved in the transcriptional regulation of HER-2/neu and serves as a target for E1A repression.

Overexpression of HER-2/neu oncogene, encoding a tyrosine kinase receptor protein (185 kDa) belonging to the epidermal growth factor receptor family, is frequently found in human cancer and is known to be involved in promoting the metastasis of tumor and enhancing the chemoresistance of the cancer cells (for a review, see Ref. 1). HER-2/neu overexpression in cancer cells has been shown to involve the transactivation of the HER-2/neu promoter (for a review, see Ref. 2), but the detailed molecular mechanisms are not clear. Studying the transactivation mechanism(s) of HER-2/neu may provide insight into the mechanisms by which HER-2/neu causes cancer and may also be useful for designing therapeutic agents for the treatment of HER-2/neu-overexpressing cancer. We previously found that adenovirus 5 E1A is capable of repressing the HER-2/neu gene at the transcriptional level, and thus it may serve as a tool to investigate the transcriptional regulation of HER-2/neu (3).

Because E1A is not a DNA-binding protein, the transcriptional repression of HER-2/neu by it is presumably mediated through the targeting of transcription factors. E1A is capable of targeting many transcription factors (for a review, see Ref. 4), among which the p300 protein is responsible for E1A-mediated transcriptional repression of several enhancers (5), p300 has been cloned recently (6), and it belongs to the family of co-activators, including the cAMP-responsive transcriptional enhancer-binding protein (CREB)1- binding protein or CBP (for a review, see Ref. 7). p300/CBP is capable of binding to either the enhancer-binding proteins (8, 9) or the proteins involved in basal transcriptional machinery including the TATA-binding protein or TBP (10) and the RNA polymerase II (11). p300/CBP serves as an adaptor that bridges the enhancer-specific factors to the basal transcriptional apparatus consequently transactivating gene. It is thought that binding of E1A to the p300/CBP inactivates the p300/CBP complex and represses the p300/CBP-responsive gene(s). By mapping the E1A domains responsible for repression of HER-2/neu, we demonstrated that domains containing the N-terminal nonconserved domain, and the conserved domain 1 (CR1) known to harbor the p300 binding site (12), are required for the repressive activities.2 Interestingly, there are two motifs (GGAGAAGGAGATT), which are very similar to the p300 consensus DNA binding sequence GGGAGTG (5), in an element of the HER-2/neu promoter shown previously to contribute to overexpression of this gene (13, 14). Among the HER-2/neu promoters of human, rat, and mouse (20), the first sequence is 100% homologous, and the second sequence conserved in the core sequence GGGAG, implying the importance of these sequences in the transcriptional regulation of HER-2/neu. These lines of evidence led us to investigate the possible role of p300/CBP in the transcriptional regulation of HER-2/neu and in the events of E1A-mediated transcriptionally repression of this gene. In this study, we report that p300 is involved in the transcriptional regulation of HER-2/neu and serves as a target for the E1A-mediated repression of HER-2/neu.

EXPERIMENTAL PROCEDURES

Cell Lines and Plasmids—The NIH 3T3 cells and MDA-MB-453 breast cancer cells were grown in Dulbecco’s modified Eagle’s medium/F-12 medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. The p300 plasmid CMVβ p300 and p300 mutant plasmid CMVβ p300dl30 were generously provided by Dr. David M. Livingston (Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA). The pE1a plasmid (3) was digested with EcoRI and SacI restriction enzymes, which generated an EcoRI and SacI DNA fragment containing the genomic E1a. This fragment was gel-purified and subcloned between EcoRI and SacI site of the vector pUK21 (15) and designated as pUK/E1A containing a kanamycin selection marker. The deletion mutant dl1101 was kindly provided by Dr. Stanley T. Bayley (Department of Biology, McMaster University, Hamilton, Ontario, Canada). The following plasmids, described previously, were used in this study.

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‡To whom correspondence and reprint requests should be addressed: Dept. of Tumor Biology, Box 79, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Tel: 713-792-3668; Fax: 713-794-4784.

1 The abbreviations used are: CREB, cAMP-responsive transcriptional enhancer-binding protein; EMSA, electrophoresis mobility shift assays; CAT, chloramphenicol acetyltransferase.
2 Chen, H. (1997) Oncogene 14, in press.
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RESULTS AND DISCUSSION

To investigate whether p300 can restore the activity of the HER-2/neu promoter repressed by E1A, increasing amounts of p300 plasmids were cotransfected along with E1A plasmid and pNeu-Stu1-CAT plasmid into NIH 3T3 cells. As shown in Fig. 1. A and B, 4 micrograms of E1A repressed the HER-2/neu promoter by 74% without the p300 plasmid. By increasing the amounts of the p300 plasmid from 2 to 12 μg, the repressed promoter activity by E1A was gradually restored from 26% to a complete recovery of the basal promoter activity (Fig. 1, A and B). The dose-dependent removal of E1A-mediated repression by p300 indicated that the effect observed is a p300-specific process. To confirm this, we cotransfected increasing amounts of E1A plasmid with or without p300 plasmids into NIH 3T3 cells. As shown in Fig. 1, C and D, the ability of p300 to restore HER-2/neu promoter activity was titrated with increasing amounts of E1A plasmid, demonstrating that p300 can indeed relieve the E1A-mediated HER-2/neu repression.

The results described above clearly demonstrated that p300 antagonizes the E1A-mediated repression of HER-2/neu. However, they did not address whether this effect was due to (i) p300 might regulate HER-2/neu expression or (ii) the transfected p300 sequestered the E1A. To distinguish between these two possibilities, we used a p300 mutant, which cannot bind to E1A yet maintains its transactivation function (6). Interestingly, transfection of the p300 mutant, which is unable to bind to E1A, significantly recovered the repressed HER-2/neu promoter activity even in the presence of excess E1A (Fig. 2A, lane 4). Under the identical condition (E1A to p300 plasmids ratio is 10 to 4), the wild type p300 did not rescue the HER-2/neu promoter from being repressed by E1A (Fig. 2A, lanes 1–3). The p300 and E1A protein levels in p300 mutant plasmid-transfected cells were comparable with that in the wild type p300 plasmid-transfected cells, as shown in Fig. 2B, lanes 3 and 4 (top and middle panels), demonstrating that the restoration of E1A-repressed HER-2/neu promoter activity by p300 mutant is not due to the differential expressions of p300 or E1A protein in different transfections. To further confirm that the transfections resulted in an excess amount of E1A, the cell extracts were depleted of p300 and E1A-p300 complex by immunoprecipitation using an anti-p300 antibody. Convincingly, after the depletion, there was still much E1A protein product left in the extracts from either the wild type p300 plasmid-transfected cells or the p300 mutant plasmid-transfected cells (Fig. 2B, lanes 3 and 4, bottom panel), showing that the amounts of E1A expressed were indeed greater than that of the p300 in all the transfections performed. Because the mutant p300 protein was incapable of sequestering E1A, due to failure to bind to E1A (6), the capability of this mutant to rescue the E1A-repressed HER-2/neu promoter strongly supported the notion that p300 is involved in the transcriptional regulation of HER-2/neu.

These results suggest that E1A-mediated HER-2/neu repression is through binding of E1A to p300, which is involved in HER-2/neu transcriptional regulation. To further confirm that the binding between E1A and p300 is required for E1A-mediated repression of HER-2/neu, a set of E1A mutants was used to examine their abilities to repress the HER-2/neu promoter (Fig. 2. C and D). Consistent with the above notion, the E1A mutants (dl343, pE1AN80), which failed to bind to p300 (Fig. 2D, lane 4), lost the ability to repress HER-2/neu, whereas the E1A mutant (pE1AN80), capable of binding to the p300 protein (Fig. 2D, lane 3), repressed the HER-2/neu promoter as effectively as the wild type E1A (Fig. 2C, lanes 1–3), indicating that the p300 is the target of E1A through which E1A represses the HER-2/neu gene.
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With the idea that p300 is involved in the transcriptional regulation of HER-2/neu, we examined whether there is any protein or protein complex that can bind to the p300 consensus sequences in HER-2/neu promoter. We performed EMSA using the oligonucleotide probes with the sequence derived from HER-2/neu promoter containing the p300 consensus sequences. By incubating nuclear extracts from the MDA-MB-453 breast cancer cell line with the probe, we reproducibly detected a retarded band, which can be competed by a 50-fold wild type probe (Fig. 3A, lane 3) but not by a 50-fold mutant competitor (Fig. 3A, lane 2), which contained two base mutations within the p300 consensus sequences. This suggests that this protein complex is p300 binding site-specific. To confirm this, the mutant probe was then incubated with the nuclear extracts. The results showed a dramatically reduced retarded band at the same position compared with the wild type probe (Fig. 3B).

To further examine whether this protein complex is related to the p300 protein, the nuclear extracts were first incubated with the protein A-Sepharose beads conjugated with affinity-purified rabbit anti-p300 antibody for 1 h at 4°C. After centrifugation at 12,000 rpm for 5 min, 4 μg of the supernatants depleted of the p300 protein complex were then incubated with the 4 μg of the supernatants depleted with anti-p300 antibody, or 4 μg of the supernatants incubated with the anti-IgG antibody were incubated with the same probe as that used in A and analyzed by EMSA. The arrow indicates the retarded complex at the position of the specific retarded complex shown in A.
Interestingly, there is a MyoD consensus sequence (CANNTG) within a previously identified E1A-responsive element (5'-TCTTGCTGGAATGCAATTTG-3') in HER-2/neu promoter (3). Furthermore, p300 has been shown to be a co-activator of MyoD (8). As both the p300 consensus sequence and the MyoD consensus sequence are crucial for E1A-mediated repression of HER-2/neu, it would be interesting to investigate whether the p300-MyoD complex is involved in the transcriptional regulation of HER-2/neu through binding to these consensus sequences and whether the E1A-p300-MyoD complex would interfere with the transactivation of the HER-2/neu promoter.

In addition to E1A, other proteins of various origins have been shown to be capable of down-regulating the HER-2/neu promoter, including c-Myc, the SV40 large T antigen, the estrogen receptor, the HER-2/neu protein product p185, and RB (for a review, see Ref. 2). Among these proteins, the SV40 large T antigen (21) and the estrogen receptor (22) were shown to be capable of binding to the p300 protein and repressing the p300-responsive gene. With the finding that p300, a co-activator in gene regulation, is involved in the HER-2/neu regulation, it would be very interesting to see whether these proteins may also repress HER-2/neu through targeting the common co-activators.

In summary, we demonstrated that the p300 is involved in the transcriptional regulation of HER-2/neu and serves as a target of E1A to repress the HER-2/neu. These findings will facilitate the investigation of the mechanism(s) by which HER-2/neu expression is deregulated in cancer.

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