p53 Homologue p63 Represses Epidermal Growth Factor Receptor Expression

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The epidermal growth factor receptor (EGFR)† plays an important role in cell growth and development (1–3). Overexpression of the EGFR can lead to epidermal growth factor-dependent transformation (4, 5). High levels of the EGFR have been detected in several types of cancers such as glioblastomas due to gene amplification (6). Overexpression of EGFR transcripts in a variety of tumors such as ovarian, cervical, and kidney tumors results from transcriptional or post-transcriptional mechanisms (7). Also, a variety of agents have been shown to increase EGFR gene expression (8–10). Repression of EGFR gene transcription by different agents has been also reported (11, 12). Thus, transcriptional control plays a major role in regulation of EGFR gene expression.

The promoter of the EGFR gene lacks a TATA box and CAAT box but contains multiple GC boxes and multiple transcription initiation sites. A number of regions in the promoter have been identified that bind nuclear factors (13–15). Four repressor proteins, EGFR transcriptional repressor, GC-binding factor, GC-binding factor 2, and the Wilms’ tumor suppressor bind to sites within the EGFR promoter (16–19). Sp1, interferon-regulated factor-1, EGFR transcription factor, activator protein-1, and activator protein-2 have been shown to increase EGFR gene transcription (20–24). Two groups have shown that p53 can transactivate EGFR via binding to an upstream site of the EGFR promoter between −265 and −239 base pairs (25, 26).

p53 is the most frequently mutated tumor suppressor gene identified thus far in human cancers (27, 28). p53 appears to induce cell cycle arrest or apoptosis in response to cellular stresses such as DNA damage, mitotic spindle misassembly, and hypoxia. The actions are mediated through transcriptional regulation of p53 target genes (29, 30). Most functions of p53 involve its activity as a transcription factor. p53 binds to its consensus binding sequence (two copies of the 10-base pair motif 5’-PuPuPuPuC(A/T)(T/A)GPyPyPy-3’) separated by 0–13 base pairs) and transactivates expression of target genes (31).

Several biologically significant genes were found to contain this consensus sequence and to be subjected to p53 regulation. Among those commonly studied are p21Waf1/Cip1 (29), MDM2 (32), Gadd45 (33), BAX (34), proliferating cell nuclear antigen (35), and cyclin G (36). The p53 mutations found in human cancers are clustered in the DNA-binding domain of the p53 molecule (37). This leads to an inactivation of p53 function through abolition of p53-specific DNA binding and transactivation. By this means, p53 acts as a tumor suppressor; its loss of function appears to confer selective advantages to cells through deregulated growth and resistance to cell death (38, 39).

Two genes that are predicted to encode proteins with amino acid sequence homologous to p53 have been recently identified (40–47). Each of the p53 amino acid residues that are implicated in sequence-specific DNA binding is conserved in these proteins (40–47). One of these novel genes, termed p73, is mapped to chromosome 1p36.33 and encodes a protein with transactivation (transactivation) type and encode proteins with transactivational function (38, 40). Like p53, p73 activates the transcription of p21Waf1/Cip1 and also induces apoptosis in a p53-independent manner (40, 41). Another gene, termed p63/p51/p73L/p40/KET/CUSP, maps to the long arm of chromosome 3 (42–47). Although the amino acid sequences and the molecular weights were reported to be different, they have proven to be isotypes derived from a single gene (43). Thus far, there have been six reported isotypes of p63. Three of the variants are called TA (transactivation) type and encode proteins with transactivation, DNA binding, and oligomerization domains similar to p53. The three remaining variants, which lack the acidic N-terminal domain, are called dN (ΔN terminus) type. Both types, TAp63 and dNp63, have different C termini that are described as α, β,
and γ (43). Thus, the TA types are designated TAp63α, TAp63β, and TAp63γ and the dn types are designated dnP63α, dnP63β, and dnP63γ. TAp63γ has the shortest C terminus and the potential to induce apoptosis and growth suppression in a manner similar to p53. The mechanism is possibly through the p53 regulatory element and includes cellular responses similar to those induced by p53 (42, 43). TAp63γ transactivates several previously identified p53 target gene promoters such as p21WAF1/Cip1, BAX, and MDM2 (42, 48). Thus, TAp63 is considered to be a tumor suppressor gene and it may serve as an alternative tumor suppressor gene whose expression is induced by the loss of p53 function (42).

On the other hand, variants that are truncated with the acidic N terminus (dn type) or encode the longest C terminus (α type) are thought to possess oncogenic properties (43). These variants also act in a dominant-negative manner toward both p53 and transactivating versions of p63 through the reporter construct containing multiple copies of p53-binding sequence termed PG13 (43). In the present study, we examined potential TAp63-dependent transactivation of the EGFR promoter using transfection assays and also TAp63 binding to the EGFR promoter by electrophoretic mobility shift assays. Our results indicate a novel mechanism for regulation of EGFR gene expression through interaction of TAp63 with Sp1.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and DNA Plasmids**—The human non-small cell lung carcinoma cell line H1299, which is p53 deficient, was maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum and antibiotics. The human osteosarcoma cell line Saos-2, which is also p53 deficient, was maintained in McCoy’s 5A medium (Life Technologies) with 15% fetal bovine serum.

Luciferase reporter constructs containing the EGFR promoter, pER1-luc, pER9-luc, pER9A-luc, pER9C-luc, and pER10-luc, were prepared by ligation of the HindIII promoter fragments from EGRF-CAT constructs into pGL3-Basic (Promega, Madison, WI) (21). The 3′ end of each of the following EGFR-luciferase constructs is cut at 16 relative to the EGFR translational start site, while the 5′ end map to the following positions: pER1-luc (−1,109), pER9-luc (−388), pER9A-luc (−348), pER9C-luc (−292), and pER10-luc (−150) (Fig. 5A). PGL3-luc, which contains the full-length mouse β-galactosidase (β-gal) reporter gene (pGL3-BasicHindIII) was prepared by subcloning of the EcoRI/HindIII thymidine kinase (TK) promoter fragments from pRL-TK construct (Promega) into pGL3-Basic. TK-TATA-luc was prepared by removal of the EcoRI/HindIII fragments from TK-luc and then blunt and ligated. SV40-luc, pGL3-promoter, was purchased from Promega. nP63 7A-luc and pCRE-luc were purchased from CLONTech (San Francisco, CA). The pER348-luc was constructed by subcloning of four p53-binding site sequences from the EGFR promoter into the TK-TATA-luc construct using the XhoI site (Fig. 3). This site is located upstream of the TK promoter TATA region. The pER348−293-luc, pER348−293mt1-luc, pER348−293mt2-luc, or pER348−293mt1,2-luc has the EGFR promoter region between 348 and 293 with or without the Sp1 site mutation as an insert instead of p53 consensus sequences (Fig. 6). The pER1P53mt1luc, pER1SP1mt1,2-luc, and pER1P53mt-Sp1mt1,2-luc constructs contain the same mutations as the pER348−293mt constructs but are in the full EGFR promoter context. The pcMV-p53 expression vector was purchased from CLONTech and the p53 cDNA was subcloned into pcDNA3 (Invitrogen, Carlsbad, CA) with the EcoRI site to obtain pcDNA3-p53. TAp63γ cDNA was cloned from murine testis using PCR methods and subcloned into pcDNA3 vector.

**Transfections and Luciferase Assays**—H1299 and Saos-2 cells were seeded at 2.5 × 10⁵ cells/150-mm dish and incubated overnight at 37°C and then transfected with 15 μg of either pCDNA3 empty vector or constructs expressing TAp63γ tagged at its N terminus with influenza hemagglutinin (HA) by the LipofectAMINE method as described above. After 24 h, the media was changed to selective media containing 700 μg/ml Genetin (Life Technologies) to reduce the number of untransfected cells. Cells were harvested 4 days post-transfection and lysed on ice for 30 min in lysis buffer (10 mM Tris-HCl at pH 8.0, 1 mM EDTA, 400 mM NaCl, 0.5% Nonidet P-40, 5 mM sodium fluoride, 0.1 mM phenylmethylsulfonl fluoride, 1 mM dithiothreitol), containing complete protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, IN), the lysate was centrifuged at 14,000 rpm for 15 min and the soluble fraction was collected. Protein concentrations were measured with a Bio-Rad protein assay kit (Bio-Rad). Equal amounts of protein extract (40 μg) were loaded on a 4–12% SDS-polyacrylamide gel and subjected to electrophoresis at 200 V for 50 min. The proteins were transferred onto a polyvinylidene difluoride membrane and probed with anti-EGFR antibodies (1005) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-HA antibody (F-7) (Santa Cruz Biotechnology), and anti-actin antibody (CA) (Roche Molecular Biochemicals). The blot was probed with each antibody after stripping the membrane of the previous probe. EGFR was detected by horseradish peroxidase-conjugated secondary antibody coupled with enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham Pharmacia Biotech). The intensity of each EGF band was normalized based on the intensity of the actin band.

**RNA Isolation and Northern Analysis**—H1299 cells were seeded at 2.5 × 10⁵ cells/150-mm dish and incubated overnight at 37°C and then transfected with 15 μg of either pCDNA3 empty vector or constructs expressing TAp63γ by the LipofectAMINE method as described above. After 24 h, the media was changed to selective media containing 700 μg/ml Genetin (Life Technologies) to reduce the number of untransfected cells. Cells were harvested 4 days post-transfection, and total cellular RNA was isolated using TRIzol reagent (Life Technologies) and quantified by A260/A280 measurement using an Ultraspec 3000 (Amersham Pharmacia Biotech). Total RNA samples (20 μg) were subjected to Northern blot analysis. After electrophoresis in MOPS electrophoresis buffer, the RNA was transferred to a nylon membrane in 10 × SSC buffer by capillary action. The RNA was fixed to the nylon membrane by ultraviolet light exposure with a UV Stratalinker (Stratagene, La Jolla, CA). The membrane was hybridized with random-primed 32P-labeled DNA probe in ExpressHyb hybridization solution (CLONTECH) according to the manufacturer’s recommendation. After hybridization at 68°C, the membrane was washed twice in 2 × SSC containing 0.5% SDS at room temperature and then washed twice at 50°C using 0.1 × SSC containing 0.1% SDS. The filters were autoradiographed with Kodak X-AR film for 24–72 h at −80°C. The signal obtained from the Northern blots was normalized to the signal for β-actin.

**Electrophoretic Mobility Shift Assays**—The p53 and TAp63 cDNAs were subcloned into pcDNA3 and tagged at their N termini with HA peptide. To generate C-terminally truncated proteins, polymyrase chain reaction was used to amplify the regions encoding HA and amino acids 1–363 for p53. This region was also subcloned into pcDNA3 and checked for fidelity of DNA sequence. Protein was synthesized in vitro in the presence of unlabeled amino acids with the coupled transcription/translation system (TNT) from Promega. Translated products were analyzed by Western blotting using anti-HA antibody (F-7) (Santa Cruz Biotechnology). For immunoprecipitation, 35S-labeled p53 and TAp63γ were synthesized in the presence of 40 μCi of 35S-labeled methionine and other unlabeled amino acids with TNT system.

**Electrophoretic Mobility Shift Assays**—Electrophoretic mobility shift assays were performed as described previously (26). Briefly, a double-stranded oligonucleotide containing the p53 consensus DNA-binding site (PG) was prepared by annealing two complementary oligonucleotides, 5’-AGCTTAGACATGCCTACAGCTACGCTA-3’ and 5’-TAGGCAT-GTCTAGGCTATGCTAGCT-3’, in a buffer containing 10 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 1 mM EDTA. Equimolar amounts of the complementary oligonucleotides were mixed in a 1.5-mL microcentrifuge tube and placed in a heat block at 95°C. The tube was returned to cool to room temperature, and the sample was desalted on a G-25 micro spin column (Amersham Pharmacia Biotech). The double-stranded oligonucleotide was end-labeled with 32P using T4 polynucleotide kinase and γ-32P]ATP. For electrophoretic mobility shift assay, the end-labeled double-stranded oligonucleotide 5000 cpm was incubated with 2 μl of p53 and TAp63γ at room temperature (22°C) for 30
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RESULTS

TAp63γ Transrepresses EGFR Expression—In this study, we sought to determine whether p63 gene products, which are p53-related molecules, had a similar activating function on EGFR expression. To examine potential p53 and TAp63γ-dependent transactivation of the EGFR promoter, we co-transfected the EGFR luciferase reporters, pER1-luc, with wild-type p53, TAp63γ, or empty vector into p53-deficient H1299 and Saos-2 cells. Similar to the effect on PG13 (43), p53 activated pER1-luc reporter activity (Fig. 1, A and B). While TAp63γ also transactivated PG13-luc, TAp63γ surprisingly repressed pER1-luc reporter activity (Fig. 1, A and C). A 83 and 75% decrease in luciferase activity was observed when compared with the empty vector in Saos-2 and H1299 cells, respectively. Additional co-transfection analysis showed that TAp63γ, but not dNp63, repressed pER1-luc reporter activity in a dose-dependent manner (Fig. 1C).

To determine whether TAp63γ represses endogenous EGFR expression, EGFR mRNA and protein levels were examined by introducing the exogenous TAp63γ and parental vector plasmids, respectively, into H1299 cells, followed by Northern and Western blot analysis. EGFR mRNA level was decreased by 67% in the TAp63γ-transfected cells as compared with the vector-transfected cells (Fig. 2A). KB cells which have rel-
amounts of cellular protein (40
41/H92362
41/ and TAp63
41/ (Fig. 3). To examine potential p53-dependent transactivation
41/ the thymidine kinase TATA region and named pERp53RE-luc
41/ated a luciferase construct which has four tandem repeat of the
41/ the p53-binding site (PG) (Fig. 4,
41/upper panel
41/) were loaded.
41/ EGFR m-seRNA in H1299 cells transfected with TAp63γ. H1299 cells
41/ were transfected with plasmid constructs expressing TAp63γ and
41/pcDNA3 by the LipofectAMINE method. Total RNAs isolated after
41/transfection were subjected to Northern blot as detailed under “Experimental Procedures.” Equal amounts of RNAs (20 µg) were loaded. Total RNA isolated from KB cells was used as a positive control. B, EGFR level in H1299 cells transfected with TAp63γ. H1299 cells were transfected with plasmid constructs expressing HA-tagged TAp63γ and pcDNA3 by the LipofectAMINE method. Cell lysates were subjected to Western analysis as detailed under “Experimental Procedures.” Equal amounts of cellular protein (40 µg) were loaded.

Fig. 2. TAp63γ represses endogenous EGFR expression. A, EGFR m-seRNA in H1299 cells transfected with TAp63γ. H1299 cells
41/ were transfected with plasmid constructs expressing TAp63γ and
41/pcDNA3 by the LipofectAMINE method. Total RNAs isolated after
41/transfection were subjected to Northern blot as detailed under “Experimental Procedures.” Equal amounts of RNAs (20 µg) were loaded. Total RNA isolated from KB cells was used as a positive control. B, EGFR level in H1299 cells transfected with TAp63γ. H1299 cells were transfected with plasmid constructs expressing HA-tagged TAp63γ and pcDNA3 by the LipofectAMINE method. Cell lysates were subjected to Western analysis as detailed under “Experimental Procedures.” Equal amounts of cellular protein (40 µg) were loaded.

Fig. 3. Effect of TAp63γ expression on pERp53RE-luc reporter
41/ gene activity. In the upper panel, the sequence of the EGFR p53
41/ response element and a schematic of pERp53RE-luc are shown. In the
41/ lower panel, H1299 cells were transfected with pERp53RE-luc (0.1 µg)
41/ and 1.0 µg of the p53, TAp63γ, or pcDNA3. Luciferase assays were
41/performed after 24 h.

To address this issue further, we asked whether TAp63γ could interact with p53 target DNA sites. We used HA-tagged
41/p53 and TAp63γ proteins prepared in vitro which were approximately equal in concentration as examined by Western blot
41/(data not shown). Ludes-Meyers et al. (25) showed that p53 could bind the oligonucleotide consisting of the p53-binding site
41/identified in the EGFR promoter (EGFRp53RE) (26). However,
41/ we could not detect binding of TAp63γ to the EGFRp53RE (data
41/not shown). The binding to p53 was only slightly competed by
41/30-fold excess of the p53 response element and a schematic of pERp53RE-luc are shown. In the
41/lower panel, H1299 cells were transfected with pERp53RE-luc (0.1 µg)
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41/ performed after 24 h.
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**Fig. 4. Analysis of TAp63γ interaction with the EGFR p53 response element.** The DNA sequence of PG, the p53-binding site from p21 promoter (p21p53RE), and the p53-binding site from EGFR promoter (EGFRp53RE) are shown in the top. The mismatches are depicted as lowercase letters. The p53 consensus DNA-binding site (PG) was end-labeled and used in binding reactions with p53 protein (3 μl) or TAp63γ protein (3 μl). The thick arrow indicates p53 complex and thin arrow indicates TAp63γ complex. Rabbit reticulocyte lysate devoid of in vitro expressed protein was used as a control (the first lane). For the competition assays, 30-fold molar excess of the p21p53RE and 100-fold molar excess of the EGFRp53RE were used, respectively. In the lower panel, the band intensities were quantified using BAS2000 image analyzer (Fuji film) and the results of three independent experiments are plotted. Error bars indicate standard deviation.

with TAp63γ, whereas pER9C-luc and pER10-luc that contain −292 to −16 and −150 to −16, respectively, were not repressed (Fig. 5B). A similar effect was also found in Saos-2 cells (data not shown). These results suggest that nucleotides between −348 and −293 within the EGFR promoter are critical for TAp63γ-mediated repression.

To confirm whether this EGFR promoter region was crucial for TAp63γ repression, we generated a luciferase construct, pER348 and p21p53RE, and EGFRp53RE. The p53 consensus DNA-binding site (PG) was end-labeled and used in binding reactions with p53 protein (3 μl) or TAp63γ protein (3 μl). The thick arrow indicates p53 complex and thin arrow indicates TAp63γ complex. Rabbit reticulocyte lysate devoid of in vitro expressed protein was used as a control (the first lane). For the competition assays, 30-fold molar excess of the p21p53RE and 100-fold molar excess of the EGFRp53RE were used, respectively. In the lower panel, the band intensities were quantified using BAS2000 image analyzer (Fuji film) and the results of three independent experiments are plotted. Error bars indicate standard deviation.

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In the present study, we investigated the roles of p53-related p63 gene products in the regulation of EGFR expression. We demonstrated reporter assays that showed TAp63\(_{\gamma}\)-mediated repression of EGFR promoter activity (Fig. 1C). The results from Northern blot and Western blot analyses revealed that TAp63\(_{\gamma}\) repressed endogenous EGFR expression (Fig. 2, A and B). This was surprising since TAp63\(_{\gamma}\) has been shown to possess p53-like transcriptional activating activity (43). To clarify such a discrepancy between p53 and TAp63\(_{\gamma}\) in the regulation of EGFR expression, we demonstrated another set of reporter assays using pERp53RE-luc, which harbors four copies of the p53-responsive element found in the EGFR promoter region (Fig. 3). The results showed that TAp63\(_{\gamma}\) did not transactivate pERp53RE-luc activity to the same level as p53 (Fig. 3). EMSAs using EGFRp53RE as a probe showed that TAp63\(_{\gamma}\), in contrast to p53, did not bind to the EGFRp53RE (Fig. 4 and data not shown). This is not surprising because p53 and TAp63\(_{\gamma}\) showed
different profiles of DNA binding affinity to the same p53-responsive elements such as p21p53RE, mdm2p53RE, cyclinGp53RE, and BAXp53RE. It is, therefore, possible to speculate that bindable elements such as p21p53RE, mdm2p53RE, cyclinGp53RE, and BAXp53RE are responsible for this region of TAp63-mediated repression was largely dependent on the intact Sp1-binding sites (Figs. 6 and 7). Sp1 is a well known basal transcription factor that is involved in the regulation of several important genes (53). Therefore, we next examined possible association between Sp1 and TAp63α by immunoprecipitation analysis. The results show that TAp63γ could physically associate with Sp1 as does p53 (Fig. 8). In this point of view, p53, as well as TAp63α, transactivates genes harboring their binding site(s) in the promoter region and that p53/p73/TAp63α repression by TAp63γ was decreased by deletion of Sp1 sites. The HSV-thymidine kinase promoter and minimal TK promoter were assayed for TAp63γ repression. Error bars indicate standard deviation in triplicate assays.

**Fig. 8.** Analysis of TAp63γ interaction with Sp1 in vitro. 35S-Labeled p53 or TAp63α was analyzed in immunoprecipitation assays as described under “Experimental Procedures.” No binding was observed using rabbit normal IgG instead of anti-Sp1 antibody. The input represents 10% of the amount of labeled protein used in each immunoprecipitation assay.

We determined by deletion mutants of pER1-luc that the responsible region of TAp63γ-mediated repression was between nucleotides −348 and −293 (Fig. 5B). Previous studies have shown that there are two Sp1-binding sites in this region (13). Mutation analysis in these two Sp1-binding sites showed that TAp63γ-mediated repression was largely dependent on the intact Sp1-binding sites (Figs. 6 and 7). Sp1 is a well known basal transcription factor that is involved in the regulation of several important genes (53). Therefore, we next examined possible association between Sp1 and TAp63α by immunoprecipitation analysis. The results show that TAp63γ could physically associate with Sp1 as does p53 (Fig. 8). In this point of view, p53, as well as TAp63α, transactivates genes harboring their binding site(s) in the promoter region and that p53/p73/TAp63α repression by TAp63α was decreased by deletion of Sp1 sites. The HSV-thymidine kinase promoter and minimal TK promoter were assayed for TAp63α repression. Error bars indicate standard deviation in triplicate assays.

**Fig. 9.** Effect of TAp63γ expression on promoter activity. A, H1299 cells were co-transfected with 1.0 μg of pcDNA3 or the TAp63γ expression plasmid and 0.1 μg of each promoter reporter plasmid. Luciferase assays were performed after transfection. B, transcriptional repression by TAp63γ was decreased by deletion of Sp1 sites. The HSV-thymidine kinase promoter and minimal TK promoter were assayed for TAp63γ repression. Error bars indicate standard deviation in triplicate assays.

**Fig. 10.** TAp63γ impairs the Sp1 binding to DNA. Electrophoretic mobility shift assays were performed with the end-labeled Sp1 consensus oligonucleotide. Sp1 from HeLa nuclear extract (NE) bound to Sp1 consensus oligonucleotide. For the supershift assay, anti-Sp1 antibody (0.2 μg) was added into binding reaction. For the competition assays, 100-fold molar excess of the unlabeled Sp1 consensus oligonucleotide was used. To examine the TAp63γ effect on Sp1 binding to DNA, different amounts of TAp63γ were added into each reaction. p53 was used as a positive control and rabbit reticulolysate (RL) devoid of in vitro expressed protein was used as a negative control.

showed that TAp63γ, as well as p53, inhibited Sp1 binding to the target DNA fragment in a dose-dependent manner (Fig. 10). Altogether, it seems possible to generalize that p53/p73/TAp63γ transactivates genes harboring their binding site(s) in the promoter region and that p53/p73/TAp63γ can act as a negative regulator when the promoter lacks their binding site(s) and is regulated by Sp1. The results in Fig. 9, a finding that repression by TAp63γ depended on the copy number of Sp1-binding site in the promoter region, also supports this speculation.

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* M. Senoo, unpublished observation.
We have clearly established that TAp63 represses EGFR promoter activity through a mechanism that involves the Sp1-binding sites. One initially could speculate that the interaction of Sp1 and TAp63 would result in a complex that inhibits the role of Sp1 in EGFR activation. However, p53, which interacts with Sp1 with a similar affinity, is able to activate EGFR promoter activity through direct binding to the promoter region. On the other hand, in the absence of a binding site, p53 leads to repression of promoter activity to a similar extent as TAp63 (Fig. 6). Thus, the mechanisms by which changes in EGFR promoter activity are mediated by TAp63 may involve its interaction with Sp1 and/or another as yet unidentified factor.

Overexpression of EGFR has been implicated in a number of malignant tumors (4–7). Despite the extensive studies on the regulation of EGFR gene expression, little is known so far concerning the negative regulator(s) of its expression. Such negative regulators are potential explanations for the elevated level of EGFR gene expression, when they are inactivated, in human cancer cells. Our present results suggest that TAp63 might be a good candidate as a negative regulator of EGFR expression. p63 is necessary for limb and craniofacial development and a mutation has been found in an autosomal dominant disorder (54–56). Up-regulation of the EGFR has been linked to defects in development (57). Although the p63 gene was originally isolated as a p53 relative, it is not frequently mutated in human cancers examined to date (58–61). However, we and Park et al. (61) have reported that expression of TAp63 was frequently lost in certain epithelial cancer cells, but was constantly expressed in normal epithelial cells (61–63).

In summary, we have shown that TAp63 represses EGFR gene expression through a mechanism involving factors associated with the Sp1-binding site. These findings, showing that TAp63 can act as a transcriptional repressor of the EGFR promoter, may suggest an anti-oncogenic role of TAp63 in human epithelial cells. The ability of TAp63 to prevent malignant transformation of epithelial cells by repressing EGFR gene expression, and the correlation between TAp63 and EGFR expressions in human tumors are under investigation.

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