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Tumor Necrosis Factor α Negatively Regulates the Expression of the Carcinoma-Associated Antigen Epithelial Cell Adhesion Molecule

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BACKGROUND. The epithelial cell adhesion molecule (EpCAM) is a homophilic and Ca2+ independent adhesion molecule that is expressed de novo in squamous cell carcinoma (SCC) but is absent in the majority of healthy squamous epithelia. EpCAM expression correlates with cell proliferation and dedifferentiation along with a progression in tumorigenicity. To date, nothing is known about the molecular mechanisms responsible for the regulation of the EpCAM gene.

METHODS. The authors analyzed the regulation of a fragment of the EpCAM promoter.

RESULTS. The analyzed fragment has significant activity in EpCAM positive cells, and it is regulated negatively by tumor necrosis factor α (TNFα). This negative regulation results in diminished mRNA expression and in the down-regulation of EpCAM protein at the cell surface in SCC cells. Both effects can be mimicked by the treatment of cells with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). TNFα-induced inhibition of the EpCAM expression is mediated by TNF receptor 1 through the TNF receptor-associated death domain protein (TRADD) and by the activation of nuclear factor κB (NF-κB), and it can be blocked by dominant-negative variants of TRADD and the NF-κB inhibitor, IκB. The authors provide further evidence that NF-κB represses EpCAM expression by competing for the transcriptional coactivator p300/CREB binding protein (p300/CBP).

CONCLUSIONS. The current results provide the first insights into the regulation of EpCAM expression, which is regulated negatively by TNFα and TPA through the activation of NF-κB. The repression may rely on the competition of NF-κB for p300/CBP histone acetyl transferase activity, because the overexpression of p300 reverts TNFα effects. ***Cancer* 2001;92:620–8.** *© 2001 American Cancer Society.*

KEYWORDS: epithelial cell adhesion molecule, promoter, tumor necrosis factor α, nuclear factor κB.

The epithelial cell adhesion molecule (EpCAM) is a homophilic, Ca2+ independent adhesion molecule. EpCAM is absent in the great majority of healthy squamous epithelia but, in contrast, is strongly expressed in squamous cell carcinoma (SCC).[1,2] Adenomatous epithelia as well as some types of differentiating cells, i.e., lung cells, hepatocytes, and germ line cells, express EpCAM. It is noteworthy that, when they are differentiated terminally, these cell types cease to express EpCAM.[1] EpCAM expression was shown to correlate with an increased proliferation rate of cells and with a decrease in cytokeratin 13 and involucrin expression levels, both markers of terminally differentiated epithelial cells.[2] These features are of great interest with respect to the strong expression of EpCAM in several types of carcinoma, especially in SCC. To date, nothing is known

about the molecular mechanism underlying the regulation of EpCAM expression. Part of the promoter of the EpCAM gene has been cloned from a genomic DNA bank,[3] but its regulation remains to be studied. The cloned 440-base pair (bp) fragment lacks a typical TATA box and a CAAT box but displays both AP1 and SP-1 consensus sequences. The current study shows that this fragment is active transcriptionally in EpCAM positive carcinoma cells but is inactive in EpCAM negative cells. Furthermore, we found that both tumor necrosis factor α (TNFα) and 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibit the activity of the EpCAM promoter fragment by approximately 50–75%. In line with these results, the treatment of EpCAM positive carcinoma cell lines with TNFα or TPA resulted in similar decreases of EpCAM cell surface expression and of total EpCAM levels. The down-regulation of EpCAM was not due to the induction of apoptosis but, rather, reflected a specific effect of TNFα and TPA on the promoter. Using dominant negative variants of the prime candidate molecules, we were able to delineate in part the signal transduction pathway responsible for the observed regulation. Thus, we provide first insights into the mechanism of EpCAM expression, showing that there is regulatory cross talk between signaling pathways, as has been shown for other proteins.

MATERIALS AND METHODS

Cell Lines

SCC-GHD-1 and SCC-ANT-1 are SCC cell lines generated from primary tumors (unpublished data), FaDu (American Type Culture Collection, Eugene, OR) is an SCC cell line, and HEK293 is an epitheloid cell line.[4] All cell lines were grown in standard Dulbecco modified Eagle medium essential culture medium containing 10% fetal calf serum and were propagated three times per week.

Plasmids, Transfections, and Luciferase Assays

The fragment of the EpCAM promoter, consisting of 440 bp, was amplified with polymerase chain reaction analysis and tagged with an Sac1 5′ sequence and a Xho1 3′ sequence (5′ primer, 5′-CCGAGCTCGATCCCTAACGCCGCCATGGAG-3′; 3′ primer, 5′-CCCTCGAGTGCCCCCCAGCCCAGCCC-3′). The amplified fragment was cut with Sac1 and Xho1 and ligated into the pGL3-basic luciferase vector. The nuclear factor κB (NF-κB) reporter plasmid, consisting of three κB sites in front of a minimal fos promoter, was a kind gift of Dr. B. Sugden.[5] Expression plasmids for TNF receptor-associated death domain protein (TRADD)-dominant negative (TRADD-DN), TNF receptor associated factor 2 (TRAF2-DN), and IκB-DN were kind gifts from Dr. A. Kieser.[6] The expression plasmid for p300/CBP was a kind gift from Dr. F. Kohlhuber. For transient transfection experiments, SCC-GHD-1, SCC-ANT-1, and HEK-293 cells (5×10^5 cells per well) were grown overnight in six well plates. Thereafter, cells were kept for 2 hours in serum free Optimem 1 medium and subsequently transfected with the CMV-LUC, EpCAM-prom-LUC, NF-κB-Luc, TRADD-DN, TRAF2-DN, DN-IκB, or p300/CBP plasmid, as indicated in the figure legends. Transient transfection was performed using Lipofectamin (Gibco Life Science, Gaithersburg, MD) following the manufacturer's protocol. The enzymatic activity of the luciferase was measured 20 hours after transfection. *P* values were calculated using the Instat software with a paired Student *t* test (*P* values ≤ 0.05 are considered significant) and untreated cells as reference. Where they are not indicated, *P* values were > 0.5 and were not considered significant (that is, reflecting variations within the standard deviation).

Immunoblot, Northern Blot, and Fluorescent-Activated Cell Sorter Analyses

FaDu cells (1×10^6 per sample) were grown in the presence or absence of TNFα (20 ng/mL) overnight and thereafter lysed in Tris-buffered saline containing 1% Triton and protease inhibitors. Equal protein amounts were separated on a 15% sodium dodecyl sulfate-polyacrylamide electrophoresis gel, and the expression of EpCAM was monitored using the specific antibody C215[7] in combination with a horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (Amersham/Pharmacia). The EpCAM mRNA expression was assessed by standard Northern blot analysis. Briefly, FaDu cells (3×10^6 cells per sample) were incubated with TNFα (20 ng/mL; 16 hours). Thereafter, cells were harvested, and mRNA was extracted and separated on a formaldehyde agarose gel (1.2%). EpCAM was detected using a radiolabeled, 1.4-kb, full-length cDNA fragment. Fluorescent-activated cell sorter (FACS) analysis of EpCAM, epidermal growth factor (EGF) receptor (EGF-R), and major histocompatibility complex (MHC) class I was performed with FaDu cells (5×10^5 cells per sample) after incubation in the specific antibodies (for 15 minutes at 4 °C), washing, and incubation in fluorescein isothiocyanateC-labeled secondary antibody (for 15 minutes at 4 °C). The analysis was performed using a FACScalibur (Becton Dickinson, San Jose, CA).

RESULTS

Transcriptional Activity of the EpCAM Promoter Fragment

To date, nothing is known about the regulation of EpCAM expression other than the fact that it is up-

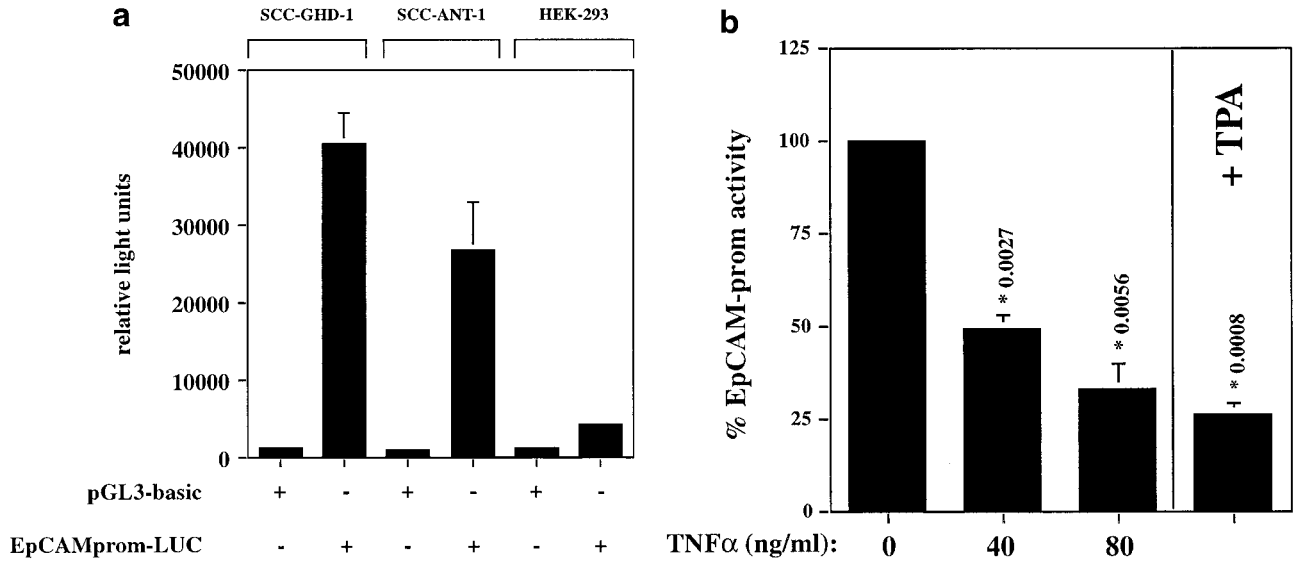

FIGURE 1. Activity of the epithelial cell adhesion molecule (EpCAM) promoter fragment. (a) Cells from the SCC-GHD-1, SCC-ANT-1, and HEK-293 cell lines (5×10^5 cells per well) were transfected transiently with the EpCAM promoter fragment plasmid that expresses the luciferase gene (EpCAMprom-LUC) (0.5 μg), and luciferase activity was monitored after 20 hours. Shown are the mean results given in relative lights units with standard deviations of three individual experiments performed in duplicate. (b) The EpCAMprom-LUC reporter plasmid was transfected transiently with SCC-GHD-1 cells (5×10^5 cells per well). Thereafter, the cells were incubated with the indicated amounts of tumor necrosis factor α (TNFα) or with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (400 ng/mL) for 16 hours. Cells were then harvested, and the luciferase activity was determined. To compare the levels of repression, the promoter activity in untreated cells was set to 100%. The mean results $\pm$ standard deviation are shown along with *P* values from five individual experiments performed in duplicate.

regulated strongly during the process of carcinogenesis. In an attempt to clone the sequence of the EpCAM gene from a genomic DNA library, part of the EpCAM promoter has been isolated.[3] The cloned fragment (EpCAMprom) encompasses 440 base pairs 5′ of the ATG translation start of the EpCAM gene. It lacks a TATA box as well as a CAAT box but displays typical consensus binding sites for the transcription factors AP-1 and SP-1. To analyze the promoter regulation, we cloned the EpCAM promoter fragment into a reporter plasmid that expresses the luciferase gene. The resulting reporter plasmid, EpCAMprom-LUC, was transfected transiently into the EpCAM positive carcinoma cell lines SCC-GHD-1 and SCC-ANT-1 (unpublished data). Both cell lines derive from primary tumors and show a moderate EpCAM expression. In both cell lines, a robust luciferase enzyme activity was detectable after transient transfection of EpCAMprom-LUC. Control experiments with the empty vector resulted in very low background activity (Fig. 1a). In contrast, EpCAMprom-LUC was inactive in the EpCAM negative epitheloid cell line HEK293 (Fig. 1a). Thus, the cloned fragment displays minimal promoter activity and specificity.

TNFα-Mediated Repression of EpCAMprom

Next, we tested various agents for their effect on the activity of EpCAMprom. Transfected SCC-GHD-1 and SCC-ANT-1 cells were treated with TNFα, interferon γ, epidermal growth factor (EGF), or 12-O-tetradecanoylphorbol-13-acetate (TPA). Consistently, we could observe that TNFα and TPA had a repressing effect on the EpCAMprom-LUC activity (Fig. 1b). The reduction of EpCAMprom-LUC activity after TNFα or TPA treatment was significant ($P \leq 0.05$), in the range of 50–75%. To exclude the possibility that the effect observed was due to the induction of cell death, we first measured the induction of the transcription factor NF-κB, a known target of TNFα signaling. The treatment of SCC-GHD-1 and SCC-ANT-1 cells with TNFα resulted in a robust NF-κB induction of 130-fold compared with untreated cells (Fig. 2a and data not shown), suggesting that the treated cells were vital and were able to transduce signals into the nucleus. Then, we were able to show that TNFα does not have a detrimental effect on the activity of the luciferase itself. The activity of constitutively expressed luciferase (CMV-LUC) remained unchanged after TNFα treatment (Fig. 2b), and TNFα treatment did not result in a loss of vitality, as shown in Figure 2c. We could not detect any difference in the expression of cotransfected green fluorescence protein (GFP) in treated cells versus untreated cells, although the amount of TNFα used was twice as much compared with the promoter experiments. In addition, we performed

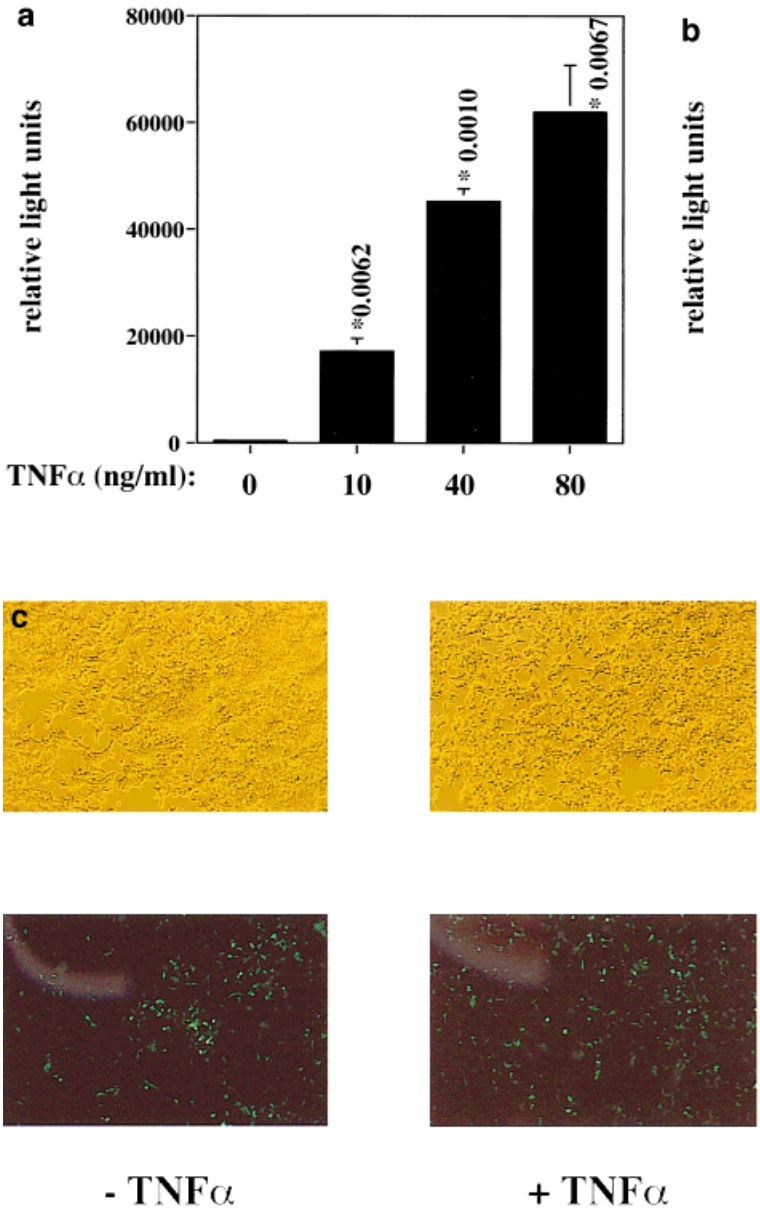

FIGURE 2. Effects of tumor necrosis factor α (TNFα) on transfection efficiency and luciferase activity. (a) The activation of nuclear factor κB (NF-κB) in SCC-GHD1 cells after TNFα treatment at the amounts indicated was determined using an NFκ-B reporter plasmid (100 ng). The mean results $\pm$ standard deviation and *P* values are shown from three individual experiments performed in duplicate. (b) SCC-GHD-1 cells were transfected transiently with the CMV-LUC plasmid (1 μg) and were kept untreated or were treated with TNFα overnight (80 ng/mL; 16 hours). Thereafter, the enzymatic activity of the luciferase was determined. The mean results $\pm$ standard deviation and *P* values are shown from three individual experiments performed in duplicate. (c) SCC-GHD1 cells (5×10^5 cells per well) were transfected transiently with the EpCAMprom-LUC plasmid (1 μg) and pEGFP-C1 (0.5 μg) and were treated overnight with TNFα (80 ng/mL). Thereafter, the expression of GFP was monitored after ultraviolet irradiation (bottom). The representative results of seven individual experiments are shown.

trypan blue exclusion experiments and could not see significant differences (data not shown). Thus, TNFα and TPA inhibit the activity of the EpCAM promoter fragment without significantly inducing apoptosis or loss of cell vitality by other means under these conditions.

TNFα Treatment Down-Regulates EpCAM in SCC Cell Lines

Because we observed a repression of the EpCAM promoter in transient reporter assays, next, we assessed the question whether TNFα and TPA treatment also results in a down-regulation of endogenous EpCAM at the cell surface SCC cells. To do so, the EpCAM positive carcinoma cell line FaDu was incubated with TNFα or TPA (TNFα, 10 ng/mL for 16–18 hours; TPA, 200 ng/mL for 16–18 hours). Thereafter, untreated and treated cells were harvested and analyzed for cell surface expression of EpCAM by FACS analysis. Figure 3a shows that treatment of FaDu cells with TNFα or TPA resulted in a substantial reduction of EpCAM expression at the cell surface. In contrast, EGF-R was induced weakly by TNFα, as reported previously,[8–10] but was unaffected by TPA. MHC class I was up-regulated by TNFα and, to a lesser extent, by TPA. Comparison of mean fluorescence values revealed a significant down-regulation (50%; $P \leq 0.05$) of EpCAM on the surface of TNFα-treated and TPA-treated, via-

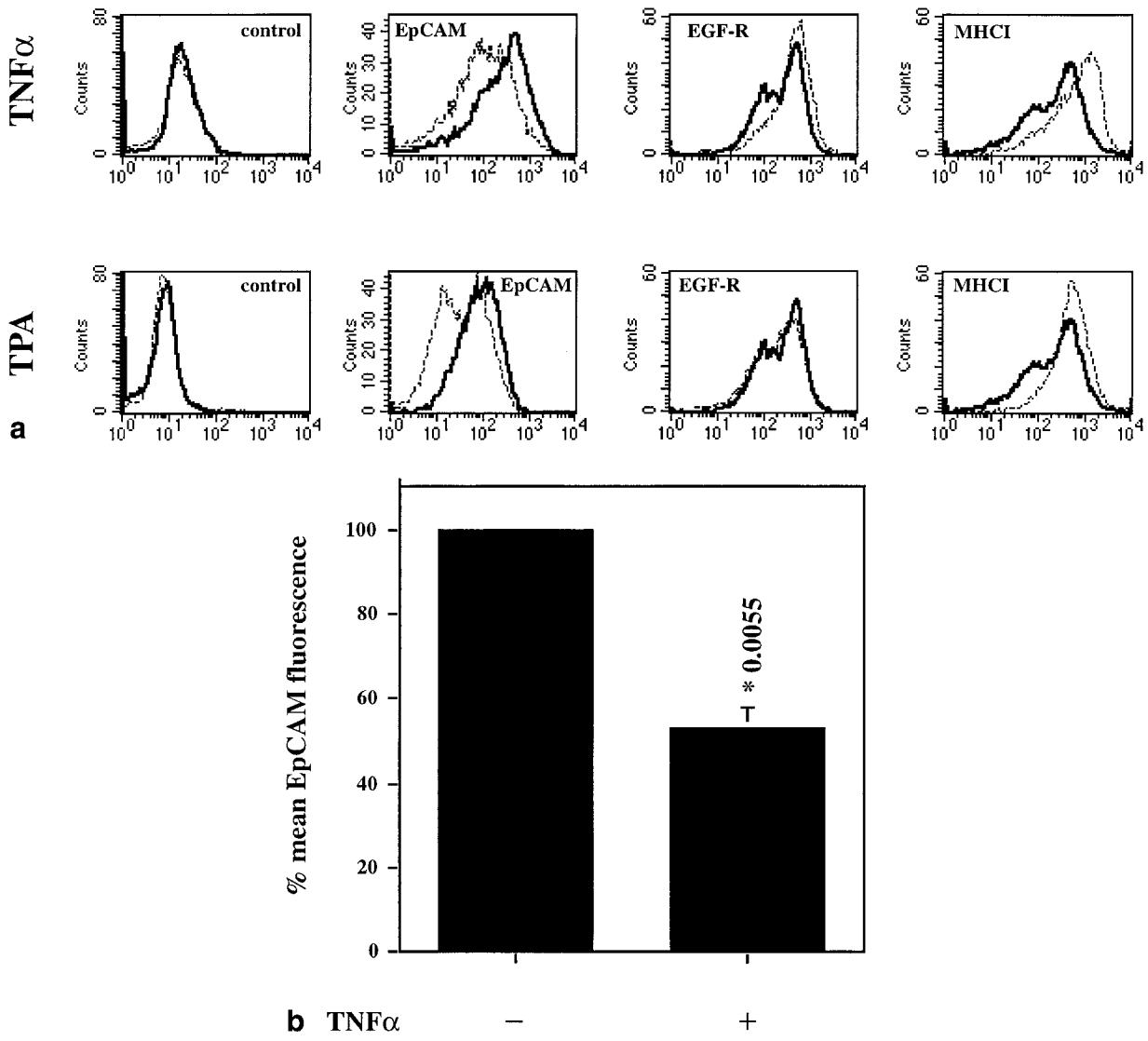

FIGURE 3. The effect of tumor necrosis factor α (TNFα) on epithelial cell adhesion molecule (EpCAM) cell surface expression on carcinoma cells. (a) FaDu cells (5×10^5 cells per sample) were either kept untreated (solid curves), incubated with TNFα (20 ng/mL; top graphs, dashed curves), or incubated with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) (200 ng/mL; bottom graphs, dashed curves) for 20 hours. Thereafter, the cell surface expression of EpCAM, epidermal growth factor receptor (EGF-R), and major histocompatibility complex (MHC) class I were monitored by fluorescent-activated cell sorter analysis using specific antibodies. The representative results of three individual experiments are shown. (b) The mean fluorescence of untreated cells was set to 100% and was compared with TNFα-treated cells. The down-regulation of EpCAM expression at the surface of FaDu cells was 50% on average. The mean results $\pm$ standard deviations and *P* values are shown from four individual experiments.

ble FaDu cells (Fig. 4b and data not shown). This closely correlated with the effects seen in transient reporter assays with EpCAMprom-LUC. Thus, TNFα down-regulates EpCAM at the cell surface, an effect that can be mimicked by TPA. Again, this effect was not due to a decreased vitality of the treated cells, because 3-(4,5-dimethylthiazol-2-yl)-2,5-dipenhenyltetrazolium bromide (MTT) assays did not detect any difference in the metabolic activity of treated cells versus untreated cells at the time of FACS analysis. A TNFα-induced decrease in vitality was not observed before 3 days after treatment (data not shown). Similar results were obtained with the EpCAM positive carcinoma cell line PCI-1. We were able to confirm the results of the FACS analysis in an immunoblot that detected EpCAM in whole cell lysates. The treatment of FaDu cells with TNFα, as anticipated, resulted in a reduction of EpCAM protein in whole cell lysates (Fig. 4a). In untreated cells, EpCAM was detected as a double band due to N-glycosylation, as reported else-

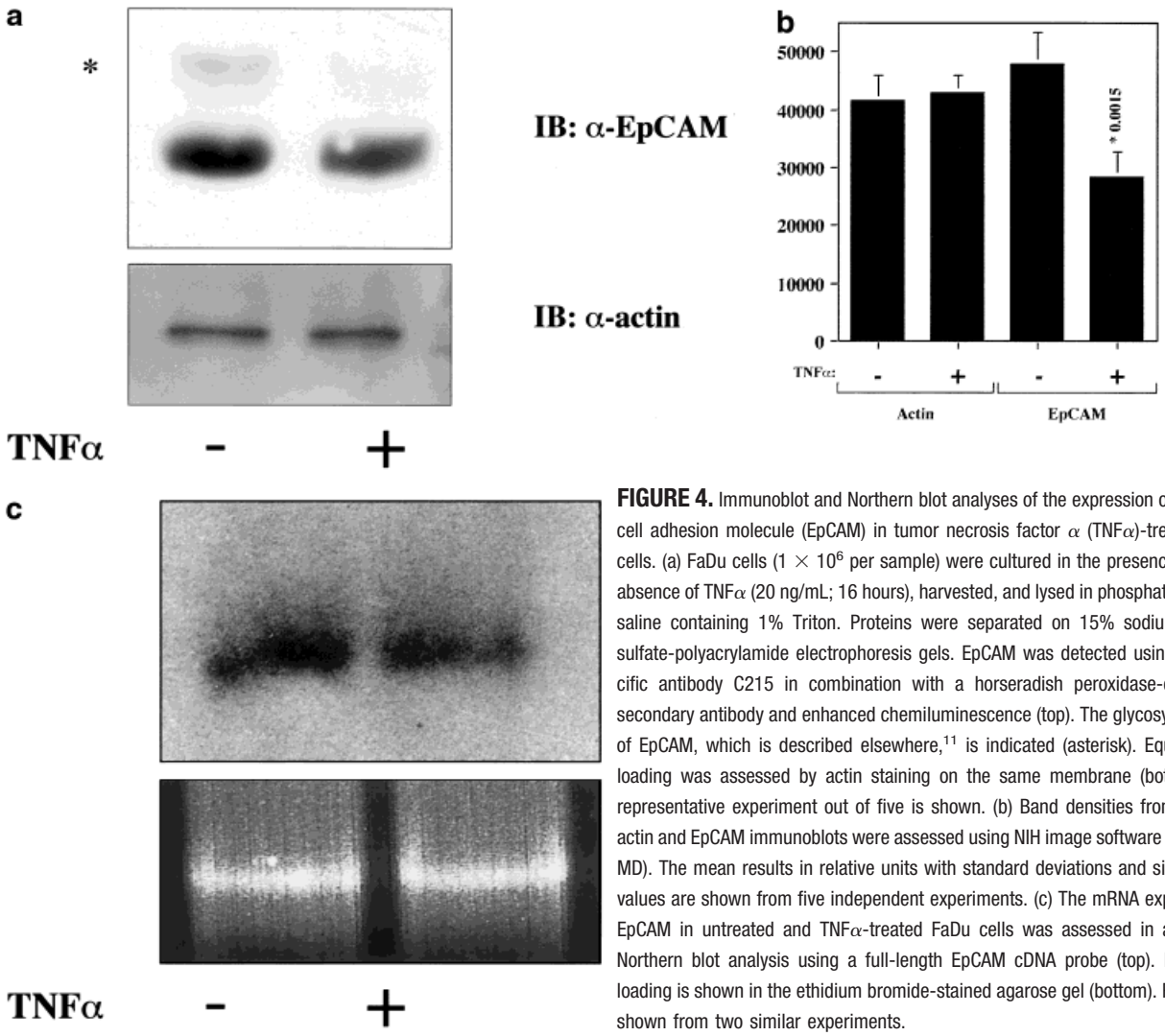

FIGURE 4. Immunoblot and Northern blot analyses of the expression of epithelial cell adhesion molecule (EpCAM) in tumor necrosis factor α (TNFα)-treated FaDu cells. (a) FaDu cells (1×10^6 per sample) were cultured in the presence or in the absence of TNFα (20 ng/mL; 16 hours), harvested, and lysed in phosphate-buffered saline containing 1% Triton. Proteins were separated on 15% sodium dodecyl sulfate-polyacrylamide electrophoresis gels. EpCAM was detected using the specific antibody C215 in combination with a horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (top). The glycosylated form of EpCAM, which is described elsewhere,[11] is indicated (asterisk). Equal protein loading was assessed by actin staining on the same membrane (bottom). One representative experiment out of five is shown. (b) Band densities from scanned actin and EpCAM immunoblots were assessed using NIH image software (Bethesda, MD). The mean results in relative units with standard deviations and significant *P* values are shown from five independent experiments. (c) The mRNA expression of EpCAM in untreated and TNFα-treated FaDu cells was assessed in a standard Northern blot analysis using a full-length EpCAM cDNA probe (top). Equal RNA loading is shown in the ethidium bromide-stained agarose gel (bottom). Results are shown from two similar experiments.

where.[11] For a control, actin levels were assessed in parallel and remained unchanged after treatment (Fig. 4a). A density scan analysis of the bands revealed a significant down-regulation of EpCAM (40% on average; Fig. 4b). Similar down-regulation of mRNA levels was detected by Northern blot analysis using radiolabeled, full-length cDNA of EpCAM as a probe (Fig. 4c).

Activation of NF-κB Is Necessary for the TNFα-Mediated Down-Regulation of EpCAM Expression

To identify the TNFα-induced signaling pathway(s) involved, we cotransfected dominant-negative variants of potential candidates. First, we cotransfected a dominant-negative mutant of TRADD (TRADD-DN), which is a direct cytoplasmic ligand of TNF receptor 1 (TNF-R1) and a key mediator of TNF-R1 signal transduction[12,13] Figure 5a shows that cotransfection of TRADD-DN repressed the TNFα-induced inhibition of EpCAMprom-LUC activity completely. Thus, the repression induced by TNFα is mediated by a signaling cascade involving TNF-R1 and TRADD. This assumption was supported further by the fact that all cell lines investigated expressed TNF-R1 but not TNF-R2 (data not shown). Next, we asked whether the activation of NF-κB by TNFα is necessary for the TNFα-mediated down-regulation of EpCAMprom-LUC. To inhibit the activation of NF-κB, we cotransfected SCC-GHD-1 cells with a dominant-negative variant of the NF-κB inhibitor IκB (DN-IκB). The induction of NF-κB after the treatment of SCC-GHD-1 cells with TNFα was repressed to basal levels in the presence of DN-IκB (Fig. 5b). It is interesting to note that the expression of DN-IκB also inhibited the effect of TNFα on the EpCAM promoter (Fig. 5c). Thus, these results point toward a role of NF-κB in the process of EpCAM down-regulation by TNFα. The involvement of NF-κB is

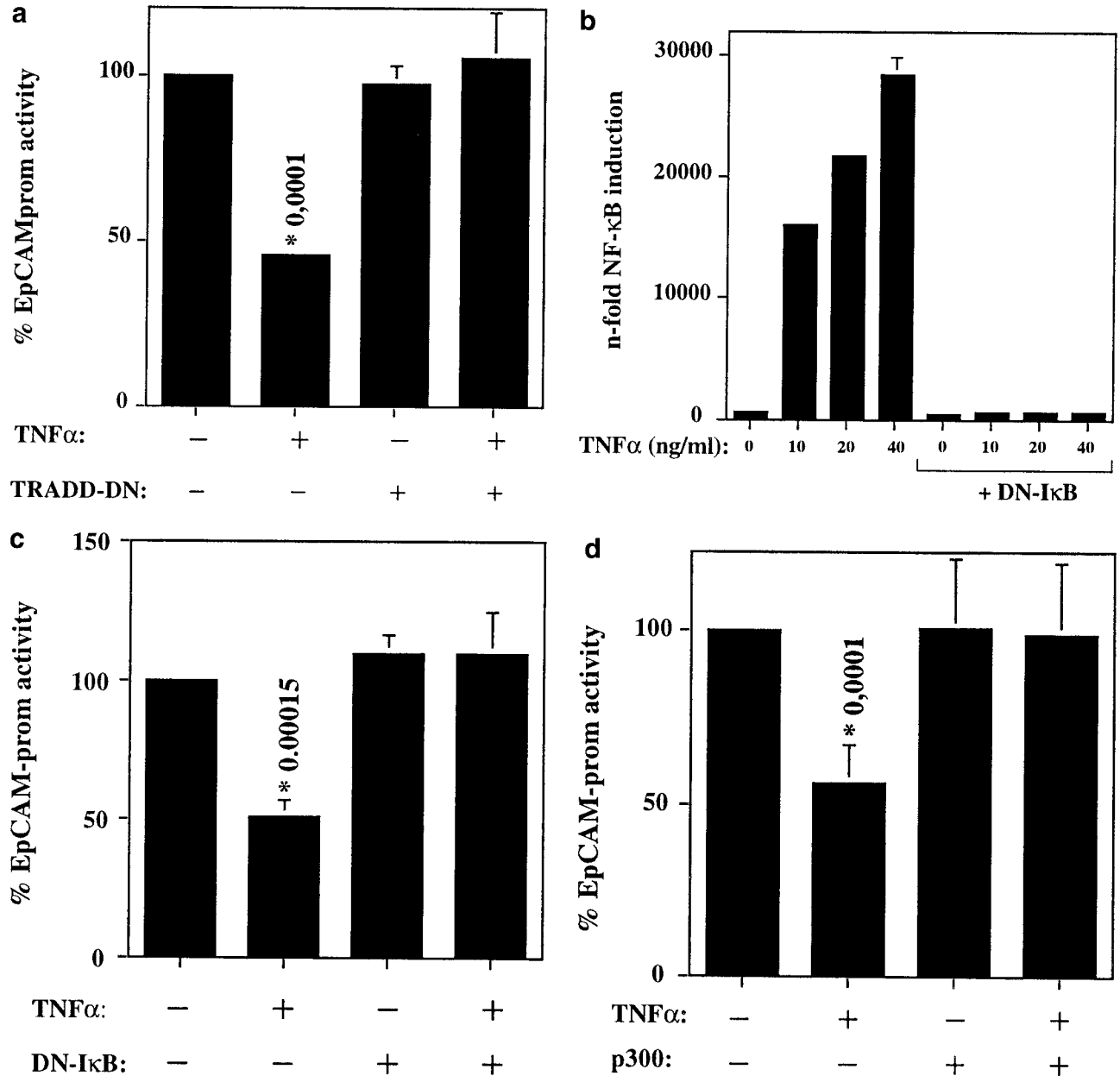

FIGURE 5. Tumor necrosis factor receptor-associated death domain protein-dominant negative (TRADD-DN), the dominant-negative variant of the NF-κB inhibitor (IκB-DN), and p300 block tumor necrosis factor α (TNFα)-mediated effects on the cloned epithelial cell adhesion molecule (EpCAM) promoter fragment plasmid that expresses the luciferase gene (EpCAMprom-LUC). (a) EpCAMprom-LUC (0.5 μg) and TRADD-DN (0.5 μg) were cotransfected in SCC-GHD-1 cells (5×10^5 per well), and the luciferase activity was measured after 20 hours. The observed repression of EpCAMprom-LUC induced by TNFα was inhibited by the cotransfection of TRADD-DN. The mean results $\pm$ standard deviations and significant *P* values are shown from three independent experiments performed in duplicates. (b) GHD-1 cells (5×10^5 per well) were transfected with a 3x-κB reporter plasmid (100 ng per well). Cells were treated with increasing amounts of TNFα as indicated (16 hours), and the luciferase enzyme activity was determined. Where indicated, a dominant-negative variant of IκB (DN-IκB) was cotransfected (1 μg per well). The mean results $\pm$ standard deviations are shown from four independent experiments performed in duplicate. (c) GHD-1 cells (5×10^5 per well) were transfected with the EpCAMprom-LUC plasmid (0.5 μg per well) then treated with TNFα (20 ng/mL; 16 hours) or left untreated. IκB-DN was cotransfected where indicated. The mean results $\pm$ standard deviations and significant *P* values are shown from five independent experiments performed in duplicate. (d) Transfected cells as shown in c, except that an expression plasmid for the histone acetyl transferase p300 (0.1 μg) was transfected where indicated. The mean results $\pm$ standard deviations and significant *P* values are shown from six independent experiments performed in duplicate.

supported further by the finding that TRAF2-DN also is counteracting in part the effect of TNFα (data not shown). The inhibition of promoters by TNFα through the induction of NF-κB has been reported for the transforming growth factor β (TGFβ) signaling molecule Smad7,[14] vitamin D receptor signaling,[15] and others. In some cases, it has been shown that the observed inhibition relies on the competition of NF-κB with other transcription factors for transcription coactivators, such as p300/CBP.[14,16] This effect can be overcome by the overexpression of p300/CBP. Therefore, we sought to determine whether the overexpression of p300/CBP has any effect on the TNFα-mediated down-regulation of the EpCAM promoter. p300/CBP was cotransfected with EpCAMprom-LUC in SCC-GHD1 cells, and the cells either were kept untreated or were treated with TNFα. Figure 5d shows that the overexpression of p300 has no significant effect on the promoter activity itself ($P > 0.5$) but clearly reverts the effect of TNFα on EpCAMprom-LUC. Thus, it seems conceivable that TNFα inhibits the EpCAM promoter by the induction of NF-κB, which, in turn, competes for the rate-limiting coactivator of transcription p300/CBP in SCC-GHD1 cells.

DISCUSSION

The expression of the pan carcinoma antigen EpCAM correlates with an increase in proliferation as well as a decrease in differentiation.[3] In SCC, EpCAM is synthesized de novo, raising questions about the regulation of its gene. In the current study, we used a 440-bp fragment of the EpCAM promoter to analyze its regulation. We showed that the fragment has a robust promoter activity in EpCAM positive cells. TNFα and TPA, both of which induce the transcription factor NF-κB, had a down-regulating effect on the EpCAM promoter fragment (Fig. 1). Using different experimental approaches, we showed that the effects observed after treatment with TNFα or TPA were not due to the induction of apoptosis or to decreased viability (Fig. 2 and data not shown). In an attempt to identify the molecules involved in the down-regulation of EpCAM by TNFα, we used dominant-negative variants of mediators of TNF-R1 signal transduction. We were able to delineate in part the signaling cascade responsible for the effects of TNFα on the EpCAM promoter. This cascade includes TRADD, TRAF2, and the transcription factor NF-κB (Fig. 5 and data not shown). The activation of NF-κB was revealed as a crucial point, because the repression of NF-κB activation using a dominant-negative variant of IκB abrogated the effect of TNFα on the EpCAM promoter. A comparable repression has been reported in the context of TGFβ signaling. The promoter of Smad7, which is a negative regulator of TGFβ signaling, is inhibited by TNFα.[14] TNFα induces the activity of NF-κB, which then recruits the transcriptional coactivator p300/CBP and thereby competes for a limited pool of cotransactivators. p300 and CBP are histone acetyl transferases that loosen the chromatin structure and, thus, are essential coactivators of transcription factors. Overexpression of p300/CBP in our assay system abrogated the effect of TNFα on EpCAMprom-LUC (Fig. 5d), strongly suggesting that NF-κB competes for the p300/CBP transcription coactivator. A regulatory cross talk of transcription factors that results in the inhibition of one of the factors involved has been shown for other receptors, such as the macrophage scavenger receptor, the expression of which is inhibited by the signal transducer and activator of transcription (STAT) family member 1 (STAT1).[17] It was concluded that STAT1 indirectly inhibits the transcription of the macrophage scavenger receptor gene by competition with AP-1 for CBP/p300 binding. Hottiger and colleagues[18] reported an analogous effect of STAT proteins competing with NF-κB-mediated transactivation in the context of human immunodeficiency virus gene transcription. STAT2 binds p300 and competes with RelA, thereby inhibiting it. Thus, cross talk between signal transduction pathways may result in the selective inhibition of promoters, which is one explanation for the effects of NF-κB on the EpCAM promoter. Whether additional mechanisms of repression, such as the inhibition of phosphorylation of transcription factors, as described for the β-casein gene,[19] also influence the transcription of EpCAM remains unknown. However, the cotransfection of p300/CBP fully restores the promoter activity in the presence of TNFα. Thus, although we cannot exclude the possibility, it is unlikely that further repression mechanisms are predominant. The effect of TNFα could be mimicked by TPA in all of our experimental settings, most likely due to its ability to induce NF-κB activity. Similar down-regulating effects of TNFα and TPA on the expression of genes was described for the granulocyte-colony stimulating factor (G-CSF) receptor.[20] TNFα and TPA treatment of carcinoma cell lines, as expected, translated into the down-regulation of EpCAM mRNA and protein (Figs. 3 and 4). Comparable effects were observed with the Smad7 protein,[14] the G-CSF receptor,[20,21] corticosteroid receptors,[22] and the constitutive form of nitric oxide synthase.[23]

It is conceivable that the induction of NF-κB in healthy squamous epithelia by extracellular signals triggered by surrounding cells represses the expression of EpCAM, although this clearly remains to be proven. A relief from such extracellular signals would then allow positive regulators, which are yet to be

identified, to transactivate the EpCAM promoter. The restricted length of the promoter fragment used in this study may have had an impact on the experimental outcome. However, the effects observed on this fragment clearly translated into endogenous mRNA and protein levels in carcinoma lines, thus suggesting a major role for the fragment studied. Nevertheless, further experiments should aim at the cloning of a larger fragment of the promoter to gain more insight into the regulation of the pan carcinoma antigen EpCAM.

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