The Transcription Factor EGR-1 Suppresses Transformation of Human Fibrosarcoma HT1080 Cells by Coordinated Induction of Transforming Growth Factor-β1, Fibronectin, and Plasminogen Activator Inhibitor-1*

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Re-expression of EGR-1 in fibrosarcoma HT1080 suppresses transformation including tumorigenicity (Huang, R.-P., Liu, C., Fan, Y., Mercola, D., and Adamson, E. (1995) Cancer Res. 55, 5054–5062) owing in part to up-regulation of the transforming growth factor (TGF)-β1 promoter by EGR-1 which suppresses growth by an autocrine mechanism (Liu, C., Adamson, E., and Mercola, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11831–11836). Here we show that enhanced cell attachment contributes to the suppression via increased secretion of fibronectin (FN) and also of plasminogen activator inhibitor-1 (PAI-1). The secretion of FN and PAI-1 is strongly correlated with EGR-1 expression (R<sub>PEARSON</sub> = 0.971 and 0.985, respectively). Addition of authentic TGF-β1 to parental cells greatly stimulated secretion of PAI-1 but not FN, whereas addition of TGF-β antibody or lipofection with specific antisense TGF-β1 oligonucleotides to EGR-1-regulated cells completely inhibits the secretion of PAI-1 but not FN. However, in gel mobility shift assays pure EGR-1 or nuclear extracts of EGR-1-regulated cells specifically bind to two GC-rich elements of the human FN promoter at positions −75 to −52 and −4 to +18, indicating that the increased secretion of FN is likely due to direct up-regulation by EGR-1. Moreover, adhesion was greatly enhanced in EGR-1-regulated cells and was reversed by treatment with Arg-Gly-Asp (RGD) or PAI-1 antibody indicating that the secreted proteins are functional. We conclude that EGR-1 regulates the coordinated expression of gene products important for cell attachment ("oikis" factor) and normal growth control.

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beit to variable extents (14). The most striking effects were observed in human fibrosarcoma HT1080 cells where proliferation was reduced by 50%, and tumorigenicity was reduced by 40.3% (14). The inhibition of proliferation was highly correlated with the level of EGR-1 expression (14).

An approach to understanding the underlying molecular basis for these EGR-1 functions has been to study signal transduction events associated with the expression of EGR-1. Previous studies of the monkey kidney epithelial cell line, CV-1, showed that WT-1 bound to two GCEs of the human TGF-β1 promoter, leading to strong suppression of transcription and that expression of EGR-1 reversed this effect (7). In HT1080 cells, transient expression of egr-1 strongly activated a TGF-β1 minimal promoter reporter containing the GCE consensus sequences, whereas coexpression of WT-1 inhibited this effect (18). Similarly, TGF-β1 reporter construct is strongly activated in HT1080 cells that stably expressed EGR-1 (18). Addition of a specific TGF-β1 antibody completely reversed the transactivation suppressive effect associated with stable expression of EGR-1, whereas addition of rhTGF-β1 to parental cells suppressed growth. These results indicated that EGR-1 binds to two potential GCE sites of the human TGF-β1 promoter, enhances the expression and secretion of functional TGF-β1 by EGR-1-expressing cells, inhibits cell proliferation, and restores anchorage-dependent growth (18–19).

TGF-β1 belongs to the TGF superfamily of cytokines that have been implicated in the regulation of growth, differentiation, development, and apoptosis (20–21). TGF-β1 is a potent growth-inhibitory protein in many cell types, including epithelial cells, endothelial cells, lymphocytes, and hematopoietic progenitor cells. Signal transduction by TGF-β1 has been studied intensively. TGF-β1 stimulates the synthesis and accumulation of several extracellular matrix (ECM) proteins, such as FN and several types of collagen and their receptors, respectively (22–24). In addition, TGF-β1 influences the function of pericellular proteases, such as by induction of plasminogen activator inhibitor-1 (PAI-1), which acts on urokinase and inhibits the fibrinolytic pathway thereby stabilizing the ECM (25–26). PAI-1 also promotes cell adhesion and spreading and acts as a molecular bridge between the cell surface and the ECM (27). The function of FN has been studied broadly. FN plays an important role in anchoring cells to the extracellular matrix. Inhibition of FN expression leads to a loss of FN from the cell surface and causes oncogenic transformation in vitro (28–30) and tumorigenic and metastatic phenotypes in vivo (31–32). Conversely, the addition of plasma FN to cultures of transformed fibroblasts restores a normal phenotype to cells (31). Indeed, overexpression of an intact form of recombinant FN in HT1080 human fibrosarcoma cells suppresses the transformed phenotype, reduces cell migration on the substratum, and suppresses tumor growth in vivo (33).

These observations suggest that TGF-β1 may suppress transformation of HT1080 or other tumor cells bearing functional TGF-β1 receptors by induction of one or more of the ECM proteins thereby enhancing the regulatory role of the ECM. We have investigated the production of ECM, specifically FN and PAI-1. Our results show that EGR-1 not only stimulates the expression of TGF-β1, but also greatly stimulates the accumulation of the ECM proteins including FN and PAI-1. We provide evidence that EGR-1 directly transactivates the TGF-β1 gene and binds and regulates the FN gene. In contrast, induction of the PAI-1 gene is a secondary effect of EGR-1 and is regulated by the TGF-β1 (40) transduction pathway. Addition of recombinant FN or PAI-1 proteins to parental HT1080 cells or addition of specific inhibitors of FN or PAI-1 to EGR-1-expressing HT1080 cells shows that all proteins are functional in mediating growth control with enhancing cell adhesion. These results indicate that EGR-1 function to initiate a coordinated program of gene expression leading to increased extracellular matrix formation with enhanced cellular regulations.

MATERIALS AND METHODS

Cells and Cell Culture—Fibrosarcoma HT1080 subclone H4 cells, EGR-1-expressing transfectants (H4E2, H4E3, H4E9), neomycin-resistant control cells (H4N), and EGR-1-null cells (H4E4, H4E6) were prepared by transfection of H4 cells with expression vectors for mouse wild-type egr-1 (pCMV-egr-1) as described (14) and were maintained in DMEM supplemented with 2% fetal bovine serum and grown in the presence of penicillin and streptomycin, and 200 μg/ml G-418 for all transfectants. Cell numbers were determined by direct cell counting (Coulter Electronics Inc., Hialeah, FL) similar to previous studies (14).

Protein Preparation and Western Blot—Cells were plated at the density of 4 × 105 cells/cm2, incubated overnight, washed twice with ice-cold phosphate-buffered saline (PBS), and lysed by scraping from the plates with RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 mM sodium orthovanadate). The lysates were passed through a 21-gauge needle to shear the DNA, incubated for 60 min on ice, and centrifuged at 12,000 × g for 20 min. The protein concentration was determined using Bio-Rad protein assay reagent (Bio-Rad). 100 μg of protein were resolved by 7% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electrophoretically transferred onto polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA), and incubated with rabbit polyclonal anti-Egr-1 (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). The intensity of EGR-1-containing bands was determined by image analysis using a Kodak Digital ScienceTM 1D image analysis system (Eastman Kodak Co.).

Cell Labeling, Extracellular Matrix Preparation, and Immunoprecipitation—For the plasminogen activator inhibitor (PAI-1) assay, 2 × 105 cells were plated in 6-well tissue culture plates in DMEM supplemented with 5% fetal bovine serum and incubated overnight. Then cells were subjected to cysteine/methionine-free DMEM in the presence or absence of various doses of recombinant human TGF-β1 (rhTGF-β1, R&D Systems Inc., Minneapolis, MN) in the range 0.011 to 100 ng/ml or 30 μg/ml monoclonal mouse anti-TGF-β1.2,3 (Genzyme Corp., Cambridge, MA) for 2 h at which time [35S]cysteine/methionine was added to 50 μCi/ml (1180 Ci/mmol, Trans-3S-label; ICN Biochemicals Inc., Costa Mesa, CA) for an additional 2 h. Extracellular matrix was prepared as described (31). Briefly, labeled cell monolayers were rinsed with PBS, and the cytosolic and nuclear proteins were extracted by subsequent washes with hypotonic buffer and sodium deoxycholate. The remaining labeled extracellular matrix proteins were recovered by addition of electrophoresis buffer to the washed wells following by scraping. The samples were subjected to 10% SDS-PAGE, and the gels were treated with Fluoro-Hancer™ autodigestion enhancer (Research Products International Corp., Mt. Prospect, IL) for 30 min followed by drying and autoradiography.

For the FN assay, 2 × 105 cells were plated in 6-well tissue culture plates. The cells were treated overnight with or without 10 ng/ml TGF-β1 or 30 μg/ml monoclonal mouse anti-TGF-β1.2,3 in cysteine/methionine-free media. The next day, [35S]cysteine/methionine was added to 50 μCi/ml for 2 h. The media were collected and subjected to adsorption on gelatin-Sepharose beads (Amersham Pharmacia Biotech) in the presence of 0.5% Triton X-100 as described (34). The samples were resolved by 7% SDS-PAGE, and the gels were treated with Fluoro-Hancer™ autodigestion enhancer (Research Products International Corp., Mt. Prospect, IL) for 30 min followed by drying and autoradiography.

Antisense TGF-β1 Oligodeoxynucleotides and Cell Transfection—Antisense 14-base phosphorothioate oligodeoxynucleotides corresponding to the human TGF-β1 mRNA and the corresponding scrambled sequence control were synthesized by Trilink Biotec. Inc. (San Diego, CA) and consisted of the antisense sequence of 5′-CGA TAG TCT TGT AG-3′ and scrambled control sequence of 5′-GTC CCT ATA CGA AC-3′ previously shown to completely and specifically eliminate TGF-β1 expression (64). To introduce the oligonucleotides into fibrosarcoma HT1080 subclone H4 cells or the egr-1-transfected clones, a cationic liposome-mediated transfection method was used (65). Briefly, oligonucleotides dissolved in 1 volume of antibiotic-free medium were mixed with Lipofect™ reagent (Life Technologies, Inc.) dissolved in same volume of antibiotic-free medium and incubated for 15 min at room temperature. Thereafter, the oligonucleotides-liposome complexes were diluted with 4 volumes of antibiotic-free medium and then added to cells that had...
been grown to 60% confluence and washed twice with antibiotic-free medium. The concentration of oligonucleotides and Lipofectin in the transfection medium was 1 μl and 1%, respectively. After 4 h, fresh normal growth medium containing 5% fetal bovine serum was added. Forty-eight hours later the cells were analyzed for the expression of PAI-1 or FN.

Oligonucleotides and Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared from the clone of maximum EGR-1 expression (H4E9) and from non-expressing cells (H4, H4N) as described (35). The protein concentrations in the nuclear extracts were determined by protein assay reagent (Bio-Rad). Synthetic double-stranded oligonucleotides bearing sequences corresponding to either −75 to −52 base pairs or −4 to +18 base pairs of the human FN promoter, termed sites A and B, respectively (36), were selected based on an analysis of the sequence of the human FN promoter region for the presence of GCEs (Transcription Element Search Software). The DNA sequences for the two oligonucleotides are for site A, 5′-GATCTCTCTCTCCCCCGCGCCGGCGCCGGCCGGCTCTGTG-3′; and for site B, 5′-GATCTCTCTCTCCCCCGCGCCGGCGCCGGCCGGCTCTGTG-3′. The prototypic EGR-1-binding sites are underlined. The oligonucleotides of sites A and B were end-radiolabeled with [γ-32P]ATP by use of T4 polynucleotide kinase according to supplier specification (Amersham Pharmacia Biotech) and used as “probes” A and B. Gel shift assays were performed as follows: nuclear extracts (20 μg) were incubated with radiolabeled DNA probe (1 × 106 cpm) for 20 min at 4 °C in a 20-μl reaction containing 25 mM HEPES, pH 7.9, 60 mM KCl, 2 mM MgCl2, 0.1 mM EDTA, 0.5 mM dithiothreitol, 100 μg/ml BSA, 10% glycerol, and 100 μg/ml bovine serum albumin. Protein-DNA complexes were separated from free DNA probe by electrophoresis through 6% non-denaturing acrylamide gels in 0.5X Tris borate/EDTA. The gels were dried and exposed to x-ray film (Kodak X-OMAT, Kodak) for autoradiography. For the competition experiments, excess unlabeled oligonucleotides for sites A and B, or oligonucleotides containing two consensus EGR-1-binding sequences (GCE) and mutated EGR-1-binding sequences (mGCE), were incubated with the reaction mixture for 15 min at 4 °C before the addition of the radiolabeled probes A and B. Similarly, in the antibody supershift experiments, the specific antibodies against Sp1 (Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit polyclonal EGR-1 antisera (Scios, Inc., New York, NY) were added to the binding reactions and incubated before the appropriate radiolabeled probe was added. Recombinant GST-EGR-1 fusion protein or wild-type FLAG-tagged-EGR-1 and FLAG-tagged-EGR-1-S348A/S350A mutant fusion proteins were used as controls. Mutations were introduced at the DNA-binding sites by the Quick Change method using polymerase chain reaction primers containing the S348A/S350A mutations (Strategene, La Jolla, CA).

Cell Adhesion Assay—ELISA plates (Nunc Ltd.). were used for adhesion assays. For the cell adhesion assay in Petri dishes, the cells were added to polystyrene Petri dishes at 3 × 104 cells/cm2. After incubation for 4 or 7 h, non-attached cells were removed with gentle washing with warm PBS. Adherent cells were stained with 1% crystal violet in 20% methanol for 15 min and then washed with distilled water and solubilized with 2% SDS. The absorbance of the solution at 590 nm was quantified using a microtiter plate reader. For PAI-1 neutralization, monoclonal antibody against human PAI-1 (American Diagnostica Inc., Greenwich, CT) was added to a subclone of HT1080. Protein was extracted from parental H4 cells, empty vector transfectant (H4N), and from stable EGR-1 transfectants (H4E2, H4E3, H4E4, H4E6, and H4E9). 100 μg of protein of each extract was analyzed by 7% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and detected by using a polyclonal EGR-1 antibody as described under “Materials and Methods.” The band intensity for EGR-1 in H4E9 clone was considered as 100%, and the relative band intensities of the other samples were represented relative to that of H4E9. A, assay for the production of PAI-1. Cells were incubated with cysteine/methionine-free DMEM in the presence or absence of 10 ng/ml rhTGF-β1 for 2 h and labeled with [35S]methionine/cysteine for additional 2 h. After the cells were lysed by hypotonic buffer and sodium deoxycholate, ECM proteins were harvested by adding SDS-sample buffer and scraping the culture wells. PAI-1 is observed as 48-kDa bands after 10% SDS-PAGE and autoradiography. C, densitometric analyses of PAI-1 expression in the absence (white bars) or presence (black bars) of TGF-β1. The values plotted represent the average of five independent experiments. Inset, the expression of PAI-1 as a function of EGR-1 is shown.

RESULTS

EGR-1 Increases the Expression of PAI-1 in Fibrosarcoma HT1080 Cells—Fibrosarcoma HT1080 subclone H4 cells have been stably transfected with an expression vector for wild-type EGR-1 (14). A series of transfectants that express graded amounts of EGR-1 were used to determine whether PAI-1 or FN was synthesized and secreted in proportion to the amount of EGR-1 expressed by these cells. The relative expression of
PAI-1, whereas H4E2 also expressed more PAI-1 than parental cells or negative control clones (Fig. 1B). Quantitative analysis of the average of five independent experiments showed >5-fold elevation of PAI-1 in H4E9 clone (Fig. 1C). In contrast, The pCMV empty vector-transfected clones or EGR-1-negative clones, H4E6 and H4E4 (G418-resistant clones), expressed low levels of PAI-1 similar to the parental H4 cells (Fig. 1, B and C). Indeed, the expression of PAI-1 and EGR-1 is highly correlated with the expression of EGR-1 (R_{PEARSON} = 0.971, p < 0.0003) (Fig. 1C, inset).

We previously showed that expression and secretion of TGF-β1 was in direct proportion to the levels of expression of EGR-1 in these graded clonal series (R_{PEARSON} = 0.96) and functions in an autocrine loop to regulate growth of these cells (18). Moreover, TGF-β1 can stimulate the expression of PAI-1 (37–38), suggesting that EGR-1-induced expression and secretion of TGF-β1 may account for the increased expression of PAI-1 by the EGR-1-expressing cells. To test whether TGF-β1 could in fact stimulate the expression of PAI-1 in our clones, the cells were labeled with [35S]cysteine/methionine in the presence or absence of 10 ng/ml of recombinant human TGF-β1 (rhTGF-β1) for 4 h. The expression of PAI-1 was monitored by electrophoresis and autoradiography (Fig. 1B). All clones exhibited a significant (p < 0.05) increase in the secretion of PAI-1 following treatment with rhTGF-β1 (Fig. 1C). Thus, the secretion of PAI-1 in the clones of our series all responded to rhTGF-β1 treatment. Moreover, the total PAI-1 observed for each clone correlated with basal PAI-1 levels observed in the absence of stimulation (Fig. 1C) with exogenous rhTGF-β1, suggesting that the effect of addition of rhTGF-β1 was additive with stimulating effects of endogenous EGR-1/TGF-β1 system. These data demonstrated that TGF-β1 can regulate the induction of PAI-1 in H4-transfected clones, indicating that the correlation between EGR-1 and PAI-1 may be mediated by TGF-β1 induction.

**EGR-1 Increases the Expression of Fibronectin in Fibrosarcoma HT1080 Cells**—To determine the levels of FN secreted by the EGR-1-expressing or non-expressing clones, the cells were metabolically labeled for 2 h, and the labeled FN was adsorbed from the conditioned medium with gelatin-Sepharose beads that are known to specifically bind FN (42). The characteristic band of FN at 220 kDa was observed at maximum intensity in the medium from H4E9 cells, whereas a similar protein occurred in the medium from H4E2 and H4E3 clones with a band intensity intermediate between H4E9 cells, parental cells, and EGR-1-non-expressing clones (Fig. 2A). The quantitative analysis of the average of three independent experiments showed a 3.5-fold average induction of FN in the H4E2 clone and 38-fold average increase of FN in the H4E9 clone compared with the negative control cell clones (Fig. 2B). Similar to the analysis for PAI-1, we determined the correlation between EGR-1 expression and FN expression and found they are very highly correlated (R_{PEARSON} = 0.985, p < 0.00005) (Fig. 2B, inset). These results suggest that at least one factor that is important for FN secretion by the graded clonal series is EGR-1.

**TGF-β1 Regulates the Expression of PAI-1 but not FN in EGR-1-expressing Cells**—Previous studies indicated that the expression of FN was stimulated by TGF-β1 in prostate carcinoma cells (26), colon cancer cell Moser (24), and lung mink Mv1Lu (CCL64; American Type Culture Collection) (38). In order to determine whether EGR-1-induced TGF-β1 might regulate the expression of FN in HT1080 fibrosarcoma cells, rhTGF-β1 was added to all clones at the same concentration that stimulates a high level of expression of PAI-1. In contrast to the effects on PAI-1, in parallel experiments we observed only a weak induction of FN (<1.5-fold) for all clones (Fig. 2, A and B), suggesting the regulation of FN is distinct from PAI-1 regulation.

To determine further the effect of TGF-β1 on the secretion of PAI-1 and FN in fibrosarcoma HT1080 cells, the expressions of PAI-1 and FN were examined as a function of the concentration of rhTGF-β1 (0.001 to 100 ng/ml) on parental H4 cells (Fig. 3A). The induction of PAI-1 expression by rhTGF-β1 is dose-dependent in H4 cells. When the concentration of rhTGF-β1 is increased, the expression of PAI-1 dramatically increased (Fig. 3, A and B), up to 9-fold compared with basal level of PAI-1 in H4 cells. The half-maximal stimulation was achieved at a rhTGF-β1 concentration ~0.05 ng/ml (EC_{50} = 2 × 10^{-7} M), with the near-maximal effect being observed at ~100 ng/ml (4 × 10^{-5} M) (Fig. 3B), indicating that H4 cells are very sensitive to TGF-β1. In contrast, the induction of FN by rhTGF-β1 did not exhibit a detectable response at 0.05 ng/ml (Fig. 3A). A weak response of 2.4-fold was seen at a 200-fold higher concentration of 10 ng/ml (4 × 10^{-4} M) (Fig. 3B).

These data suggest that PAI-1 but not FN is preferentially regulated by TGF-β1. The high correlation between EGR-1 and FN (Fig. 2B, inset) without the role of TGF-β1 suggests, as one possibility, that EGR-1 directly regulates the FN gene. Therefore, we next examined whether the TGF-β1 is required for the
secretion of both PAI-1 and FN in egr-1-regulated HT1080 cells. Neutralizing TGF-β antibody was added to block the TGF-β1 effect in EGR-1-expressing clones. The results are shown in Fig. 4. The basal level of PAI-1 was reduced 1.4-fold in control cells (H4 and H4N), but there was 2.7-fold reduction of PAI-1 secretion in EGR-1-expressing cells (H4E2 and H4E9) in the presence of TGF-β antibodies, supporting the observation that the increased secretion of PAI-1 by EGR-1 may require the expression of TGF-β1. In contrast, the expression of FN was only slightly altered by the addition of TGF-β antibody (<1.4-fold) in the EGR-1–expressing cells (H4E2 and H4E9) (Fig. 4). Thus, consistent with the dose-response studies, the experiments using TGF-β neutralizing antibody suggest that the expression of PAI-1 by EGR-1 but not FN is mediated by TGF-β1.

Similar results were also obtained in experiments in which antisense TGF-β1 oligonucleotides were utilized to block TGF-β1 function. In previous studies, using a TGF-β1 antisense oligodeoxynucleotide complementary to the mRNA of human TGF-β1, both transcript levels and protein production were completely and specifically eliminated (55, 64). Therefore, we examined the expression of PAI-1 after lipofection with the same TGF-β1 antisense or the scrambled sequence control oligonucleotides (Fig. 5). The level of PAI-1 in EGR-1-expressing cells (H4E9) was reduced by over 75% in both EGR-1-expressing clonal cell lines to near basal levels similar to those of control cells (H4 and H4N), whereas the levels of PAI-1 were not influenced by transfection with the scrambled sequence oligonucleotide or by use of the lipofection reagent alone (Fig. 5, upper panel). In contrast, the expression of FN was not inhibited in EGR-1-expressing cells by transfection of either antisense TGF-β1 or scrambled sequence oligonucleotide (Fig. 5, lower panel). These experiments strongly support the results based on the TGF-β1 antibody blocking experiments, showing TGF-β1 is required for expression of PAI-1 but not FN by egr-1-transfected cells. In addition, the lack of effect of antisense TGF-β1 on FN secretion supports the observations on the dose-response studies (Fig. 3) that TGF-β1 is not involved in mediating expression of FN in the egr-1-transfected cells.

Nuclear and Recombinant EGR-1 Bind to the Proximal Region of the Human Fibronectin Promoter—The high correlation of FN secretion with EGR-1 expression independently of TGF-β1 suggests that EGR-1 may directly regulate FN gene. We examined the promoter region of the human FN gene (exon 1) consisting of 742 base pairs in 5′-flanking region (36), and we observed two potential EGR-1-binding sites by using Transcription Element Search Software. Two sequences containing potential EGR-1 consensus sites termed A and B were identified, and double strand probes containing these sequences were synthesized separately (Fig. 6A). In order to test whether EGR-1 binds to either of these motifs, electrophoretic mobility shift assays were carried out. A recombinant wild-type FLAG-tagged-EGR-1 fusion protein and control mutant FLAG-
tagged-EGR-1ΔS348A/S350A fusion protein were used for these assays. The FLAG-tagged-EGR-1ΔS348A/S350A fusion protein is a serine → alanine mutant at positions 348 and 350 in the EGR-1 zinc finger domain, thereby reducing DNA binding activity (79–80). Fig. 6B shows that a specific DNA-protein complex occurred when wild-type protein was combined with either of the DNA probes and that the complexes were absent when the EGR-1 mutant protein was used in the reaction in place of the wild-type protein (compare lanes 1 and 2 and lanes 6 and 7, arrow). When EGR-1 antibody was added to the reaction, this complex disappeared (lanes 5 and 8), consistent with previous studies showing that anti-EGR-1 did not promote a supershift but in fact caused a dissociation of the complex (67, 68). In contrast, addition of an anti-Sp1 antibody did not dissociate the complex or produce a supershift. In fact, the use of anti-Sp1 antibodies with the FLAG-tagged-EGR-1 fusion pro-
tein preparation is associated with a slight but reproducible increase in band intensity (Fig. 6B, lane 1 versus 3 and lane 2 versus 4). Moreover, experiments with FLAG-tagged-EGR-1 fusion protein preparation and probe B often lead to less intense bands than experiments with probe A. In order to clarify these observations, similar experiments were carried with a different EGR-1-containing preparation, GST-EGR-1 fusion protein (Fig. 6C). As before (Fig. 6B), specific DNA-protein complexes occurred when GST-EGR-1 protein was combined with either of the DNA probes (Fig. 6C, lanes 2 and 6). Furthermore, for both sites the addition of the unlabeled oligonucleotides with the consensus GCE sequence completely "competes-off" probe binding (Fig. 6C, lanes 3 and 7). Similarly, the addition of the unlabeled oligonucleotides with a mutated EGR-1-binding sequence (mGCE) has no effect (Fig. 6C, lanes 4 and 8). These results confirm the results of experiments with recombinant FLAG-tagged-EGR-1 fusion protein. Moreover, the experiments show that the complexes formed with the GST-EGR-1 preparation under the same conditions as with the FLAG-tagged-EGR-1 fusion protein are of similar intensity, suggesting similar binding affinities, whereas the intensity changes observed with the FLAG-tagged fusion protein are not representative of quantitative properties. In support of this, direct titration experiments with unlabeled probes demonstrated very similar binding for each site (Fig. 6C, lanes 10–13 and lanes 15–18). The sum of results indicates that both site A and B form specific complexes with recombinant protein. Similar results were obtained when nuclear extracts from the EGR-1-expressing clone (H4E9) and EGR-1-lacking cells (H4 and H4N) were used in place of the recombinant proteins. A prominent complex was observed when nuclear extracts from H4E9 cells were incubated with oligonucleotide probes A and B (Fig. 6D, lanes 3 and 13, arrow). This complex was not detected for the nuclear extracts from control cells H4 and H4N (Fig. 6D, lanes 1 and 2 and lanes 11 and 12). Specificity of binding was determined by titration experiments using unlabeled oligonucleotides. The complex formation by the H4E9 nuclear extracts were inhibited in dose-dependent manner by addition of the unlabeled oligonucleotides A or B (Fig. 6D, lanes 6–8 and lanes 16 and 17). Also, competition with oligonucleotides containing two consensus EGR-1-binding sequences (GCE), but not mutated EGR-1-binding sequences (mGCE), resulted in dissocia-

**Fig. 7.** Enhancement of cell adhesion in EGR-1-expressing cells to substratum. A, the parental H4 cells, empty vector control cells, and *egr-1* transfected clone H4E9 were plated in 35-mm plastic polystyrene Petri dishes or tissue culture dishes treated for the indicated times. The percentage of adhesion was calculated based on the numbers of starting cells. B, cells were plated in 96-well ELISA plates that were precoated with FN or PAI-1 as described under "Materials and Methods." After incubation for the indicated times, the cells were stained with crystal violet and the O.D. at 590 nm was read using microtiter plate reader. The percentage of adherent cells was calculated based on the number of starting cells. In this representative experiment the values plotted are means ± S.D. (n = four dishes). Similar results were obtained in three other independent experiments.
The induction of TGF-β1, Fibronectin, and PAI-1 by EGR-1

Enhanced Expression of Fibronectin and PAI-1 in EGR-1-Expressing Cells Increases Adherence to a Substratum—We have previously found that expression of EGR-1 in HT1080 slows proliferation, restores density-dependent growth arrest, and promotes a flattened and non-refractive phenotype (14, 15). Conversely, elimination of endogenous EGR-1 tends to slow proliferation, restore density-dependent growth arrest, and promote a more transformed phenotype (15). Both FN and PAI-1 have been shown to interact with FN receptors and to modulate cell adhesion and behavior in a variety of cell types including HT1080 cells (27, 74–78). To determine whether endogenous PAI-1 or FN influences the adhesion of HT1080 cells, we compared the attachment efficiency of the various cells to untreated polystyrene “Petri” dishes. Polystyrene plastic plates have high hydrophobic nature, inhibit cell adhesion, and are commonly used for growing cells in suspension culture. Many types of adherent cells fail to attach on this substratum (70), but EGR-1-expressing cells (H4E9 clone) can attach this kind of substratum significantly better than the parental cells (H4) or empty vector cells (H4N) (Fig. 7). For example, we observed that 88% of H4E9 cells adhere to polystyrene Petri dishes 4 h after plating, compared with 20% for the controls (H4 9% and H4N 7%) (< 0.0001) (Fig. 7). At 7 h after plating, 88% of H4E9 cells had attached to polystyrene Petri dishes, whereas only 58% of the control H4 or H4N cells adhered to Petri dishes (< 0.0001) (Fig. 7), consistent with functional roles for endogenous PAI-1 and/or FN.

To test whether authentic FN or PAI-1 in fact facilitates attachment, we carried out adhesion assays in the presence and absence of rhPAI-1 and human plasma FN (Fig. 7B). When the various cells were exposed to 96-well polystyrene “ELISA” plates precoated with 5 μg/ml rhPAI-1 for 4 h, they exhibited adhesion efficiencies similar to untreated plates (cf. Fig. 7, A and B). However, by 7 h about 70% of the cells attached to the PAI-1-treated plates although no consistent differences were observed between EGR-1-expressing cells and EGR-1-lacking control cells (Fig. 7B). Addition of neutralizing anti-PAI-1 antibodies (Fig. 8) had little effect on the adhesion of control cells but partially inhibited the attachment of the FN-secreting H4E9 cells by about 20%. The inhibition is reproducible and significant (p < 0.03). These results suggest, as one possibility, that any role of PAI-1 on the attachment of H4 cells and derivatives depends on other ECM components such as FN (see below).

To test whether authentic FN facilitates attachment, we tested the adhesion of cells on 96-well polystyrene plates precoated with 5 μg/ml FN for 4 h. We found that 88% of H4E9 cells attached to the plates and ~70% of H4 and H4N control cells attached (Fig. 7B). Thus, we found that FN promoted about 20 times greater adhesion than for uncoated or PAI-1-coated plates similar to the results reported by Planus et al. (27). Virtually all cells attached to FN–coated plates by 7 h (Fig. 7B). To determine whether the increased adhesion depended on specific FN interactions, we added Arg-Gly-Asp (RGD)-containing peptides which block FN binding to its receptors, such as α5β1 or αvβ3 integrin (61, 69). The addition of moderate amounts of GRGDSP peptide (10 μg/ml) reduced the adhesion of H4 or H4N to ~20% but had no effect on the adhesion of H4E9 cells (Fig. 9A). However, when the GRGDSP concentration was increased to 50 μg/ml, the adhesion of H4E9 cells was completely eliminated (Fig. 9A). The control peptide (GRGESP) had negligible effects even at 50 μg/ml (Fig. 9A). These results indicate that H4 cells and derivative cells interact with FN in an RGD-dependent manner. Moreover, the greater amount of GRGDSP required for the inhibition of attachment of the FN-secreting H4E9 cells supports a functional role of endogenous FN.

Since the attachment of FN-secreting H4E9 cells is partially inhibited by the addition of anti-PAI-1 antibodies (Fig. 9A), we asked whether anti-PAI-1 antibodies inhibit attachment to FN-coated plates. The addition of anti-PAI-1 antibodies to cells

FIG. 8. Cell adhesion on PAI-1-coated plates. 72196-well ELISA plate was precoated with recombinant active human PAI-1 in PBS for 1 h. Monoclonal antibodies against PAI-1 or GRGDSP and GRGESP peptides were added as indicated at the time of cell plating. Seven hours later, the adhesive cells were stained with crystal violet, and the O.D. at 590 nm was read using a microtiter plate reader. The percentage of adherent cells was calculated based on the number of starting cells. In this representative experiment the values plotted are mean ± S.D. (n = five wells). Similar results were obtained in three other independent experiments.
exposed to FN-coated plates (Fig. 9A) had the opposite results compared with the effect of the antibodies on cell adhesion to PAI-1-coated plates (cf. Figs. 8 and 9A). The antibody blocked the attachment of the control cells by 50–60% but had no effect on EGR-1-expressing H4E9 cells (Fig. 9A). Increased amounts of anti-PAI-1 had weak inhibitory effect on the adhesion of H4E9 cells (data not shown). However, addition of anti-PAI-1 antibodies to these cells when attached to plates coated with less FN (20 μg/ml) substantially and significantly reduced adhesion to levels indistinguishable from control cells (Fig. 9B). Consistent with this, the combination of small (10 μg/ml) amounts of GRGDSP peptide together with anti-PAI-1 treatment reduced attachment of all three cell types by approximately 50% compared with the effects of low dose GRGDSP alone (Fig. 9A). These results, together with the effects of anti-PAI-1 antibodies on cells adherent to PAI-1-coated surfaces (Fig. 8), show that PAI-1 facilitates attachment only in the context of FN-coated surfaces or FN-secreting cells.

In summary, the observations support the conclusions that FN and, to a lesser extent, PAI-1 facilitate attachment of H4 and derivative cells and that endogenous FN is also functional in promoting increased and RGD-dependent cell adhesion interactions. Endogenous FN further functions to facilitate the attachment role of endogenous PAI-1.

**DISCUSSION**

**Regulation of Fibronectin and PAI-1 by EGR-1 Are Distinct—**

HT1080 and related clonal lines studied here express little or no EGR-1, a feature that is similar to a variety of human tumor cell lines including breast carcinoma and glioblastoma cells (13). Stable expression of EGR-1 in several tumor cell lines, such as U251 glioblastoma cell line, ZR-75 breast carcinoma, Saos2 osteogenic sarcoma cells that lack EGR-1 expression, leads to more normal cell morphology especially in HT1080 fibrosarcoma cells. The expression of EGR-1 in HT1080 stimulates the expression and secretion of TGF-β1 in direct proportion to the amount of EGR-1 expressed in a series of five clonal lines and inhibits cell growth. The addition of neutralizing anti-TGF-β1 antibody to the EGR-1 expression cells actually causes a near doubling of growth thereby completely reversing the growth inhibitory effect in EGR-1 in HT1080 cells (18). Moreover, EGR-1-expressing HT1080 cells but not control cells
strongly activate reporter constructs containing the EGR-1-binding sequences of the TGF-β1 promoter, and this effect is blocked by the TGF-β1 transcription suppressor, WT-1 (18). Thus, EGR-1 suppresses the growth and transformation of HT1080 cells by induction of a TGF-β1-dependent autocrine loop.

In this study, we provide evidence that the suppression of transformation by TGF-β1 involves the coordinated effects for the secretion of TGF-β1, FN, and PAI-1. Both the FN (23, 24, 26) and PAI-1 (25, 26, 72) genes have been reported to be regulated by the TGF-β1 signal transduction mechanism in several cell types. We reasoned that the secretion of FN and PAI-1 may be a consequence of the TGF-β1 autocrine loop known to be functional in the EGR-1-expressing cells (18). Indeed, addition of rhTGF-β1 to control HT1080 cells mimicked the effect of EGR-1 in that considerable increased secretion of PAI-1 was observed with an EC₅₀ ~10⁻¹⁵ m consistent with the presence of high affinity TGF-β1 receptors (41) that functionally regulate PAI-1 transcription and secretion. Conversely, the addition of neutralizing anti-TGF-β antibody to EGR-1-expressing cells but not control cells blocks the secretion of PAI-1. Moreover, the use of previously characterized antisense TGF-β1 oligonucleotides confirms this result thereby strongly supporting the presence of a functional TGF-β1 autocrine loop as responsible for the regulation of PAI-1 secretion. Thus, in HT1080 cells, the expression of EGR-1 appears to mediate the expression of PAI-1 by direct induction of TGF-β1, which in turn regulates the secretion of PAI-1 via a highly effective TGF-β1-autocrine loop. These relationships are summarized in Fig. 10.

However, similar regulation of the secretion of FN was not observed. Thus, the addition of rhTGF-β1 did not stimulate the secretion of FN to an appreciable extent, and the secretion that was observed occurred at over 2 orders of magnitude higher concentration of rhTGF-β1 than that known to mediate TGF-β1-dependent PAI-1 secretion. Conversely, specific anti-TGF-β antibodies that effectively blocked PAI-1 secretion had no effect on FN secretion. These results were obtained in parallel with the results for PAI-1 that, therefore, provided a convenient positive control. The sum of observations strongly argues against regulation of FN secretion by TGF-β1 in HT1080 cells.

The human FN gene contains at least four GC-rich sequences at least two of which consistent with the consensus sequence for EGR-1-binding site (36). Interestingly, the site termed A here has been observed to positively regulate transcription of FN by Sp-1 in embryonal carcinoma cells (66). Sp-1 binds GC-rich sequences and commonly interacts with EGR-1 in the regulation of a variety of genes (19). In direct binding studies of either recombinant EGR-1 or nuclear extracts of EGR-1-expressing cells, we observed complex formation with both sites. These complexes were disrupted by anti-EGR-1 antibodies, a known characteristic of EGR-1 antibodies on specific EGR-1-DNA complexes (67–68). Anti-Sp-1 sera, on the other hand, had no effect on the complex indicating that Sp-1 was not likely associated with either GCE in HT1080 cells. These observations indicate that EGR-1 interacts with known positive transcription activation sites of the FN promoter and strongly supports the view that EGR-1 directly induces the transcription of FN. Since we have not examined the transcription properties of these sites, it remains an hypothesis that EGR-1 directs the expression of FN via sites A and/or B. However, this hypothesis provides an explanation for the strong correlation of FN secretion and EGR-1 expression observed here. These relationships are summarized in the model of Fig. 10.

**Phenotypic Consequences of Coordinated Gene Expression by EGR-1.—**Several studies have shown that one of the most important growth regulatory mechanisms of TGF-β1 is its stimulatory effects on the accumulation of ECM proteins such as FN (23, 24, 26). The functional effects of FN on oncogenic transformation have been studied in detail. The expression level of FN in fibrosarcoma cell lines is commonly very low. For instance, in JEG-3 cells it is 0.01% of total protein, in TE671 is 0.002%, in HeLa Bu25 is 0.008%, and in HT1080 is 0.004% (43). In contrast, normal fibroblasts exhibit high basal levels of FN (0.3% of total protein) (43). FN expression is decreased also in a variety of other transformed cells (44–45). Both FN and its receptor are down-modulated during the chemical transformation of murine fibroblasts (46–47). Ha-ras transformation of fibroblasts significantly reduced the expression of FN and its receptor (48). Absence or reduced expression of FN and its receptor is thought to play a major role in determining the malignant phenotype (49–50). Down-modulation of FN in melanocytes promotes malignant behavior (51). Conversely, the addition of FN peptides has been shown to inhibit experimental metastasis of melanoma cells (52). Similarly, the level of expression of FN is significantly correlated with low metastatic potential of breast carcinoma (53). Overexpression of recombinant FN suppresses the transformed phenotype in fibrosarcoma HT1080 (33). The HT1080 cells that re-express FN adopt a more flattened morphology, have reduced proliferation and growth in soft agar, suppressed tumorigenicity in vivo, and reduced cell migration (33).

It is likely that the EGR-1-induced expression of TGF-β1, PAI-1, and FN have functional roles in the suppression of transformation of HT1080 cells. Here we used adhesion assays to provide an indication of the functional role of the secreted products. Anti-PAI-1 antibodies preferentially inhibited the...
attachment of control cells but not EGR-1-expressing cells. Moreover, RGD-containing peptides that are known to specifically disrupt the association of FN with FN receptors completely inhibited the adhesion of EGR-1-expressing cells in a dose-related manner. Strong inhibition also could be achieved by using RGD-containing peptides at lower doses in association with anti-PAI-1 antibodies. Thus, it appears that the actual products of the regulation proposed here (Fig. 10) are both secreted and participate in the formation of specific ECM-cell association to effect enhanced cell-substratum attachment.

PAI-1 participates in the stabilization of the extracellular matrix and enhanced adhesion by at least two broad mechanisms (27, 54). First, it interacts with an organized structure consisting of the urokinase plasminogen activator (uPA)-urokinase plasminogen activator receptor (uPAR) and inhibits the serine protease activity of uPA, thereby blocking the conversion of serum plasminogen to plasmin, a broad specificity protease that activates collagenases and metalloproteases (54). These and related proteases destabilize the ECM thereby facilitating motility and the metastatic phenotype (54, 72–73). The organization of the PAI-1-uPA-uPAR complex on HT1080 cells has been examined in detail and is thought to preferentially associate with the \( \beta_1 \) integrin subunit for cells adherent to FN, laminin, or vitronectin-coated surfaces (74). PAI-1 directly influences the nature of FN and vitronectin cell surface associations (27, 75–78). For example, the addition of antibodies against PAI-1 to primary human muscle satellite cells, which secrete PAI-1, is reported to cause near complete inhibition of adhesion (27). Similarly, the role of PAI-1 in the adhesion of the cells studied here was only apparent in the presence of FN. On uncoated surfaces cells that secrete ample amounts of both FN and PAI-1, such as H4E9, are at least partially susceptible to the blocking of adhesion by anti-PAI-1, whereas the control cells, which express considerably less of each factor, establish attachments to ELISA plates that are not susceptible to inhibition by anti-PAI-1 (Fig. 8). Conversely, when FN is supplied as precoated surfaces, the interactions formed by the small amounts of PAI-1 secreted by control cells (H4 and H4N, Fig. 1) are readily inhibited by anti-PAI-1, whereas the increased amounts of PAI-1 and FN secreted by H4E9 cells appear too extensive to be neutralized readily (Fig. 8). Finally, the use of increased anti-PAI-1 antibody or decreased FN at the same antibody concentration (Fig. 9B) demonstrated the role of endogenous PAI-1 on the adhesion of EGR-1-regulated H4E9 cells. The combined use of RGD-containing peptides and anti-PAI-1 lead to complete inhibition of adhesion by the minimal PAI-1/FN-expressing control cells and over 60% inhibition of adhesion to FN-coated surfaces. These results argue that PAI-1 expressed by EGR-1-regulated cells is indeed a functional product that facilitates attachment and that this effect is at least partially dependent upon the presence of FN as described previously (27, 74–78). Similarly, the complete inhibition of attachment in a dose-dependent manner by RGD-containing peptides but not RGE control peptides strongly argues that the EGR-1-induced FN is a fully functional product. Of the two factors, FN has by far the major effect on adhesion of H4 cells.

Control of Transformation by EGR-1 (Fig. 10)—The enhanced PAI-1- and FN-mediated attachments observed here likely have important consequences in suppressing the transformation of HT1080 cells. There is considerable evidence that FN receptors, especially the \( \alpha_5\beta_1 \) integrin, play a role in modulating cellular transformation and metastasis (56–57) and that HT1080 cells express \( \alpha_5\beta_1 \) integrin (33, 74). Conversely, reduced expression of integrin \( \alpha_5\beta_1 \) in Chinese hamster ovary cell variants leads to increased tumorigenicity (58), whereas overexpression of integrin \( \alpha_5\beta_1 \) inhibits anchorage-independent growth and tumorigenicity (59–60). In our system, integrin \( \alpha_5\beta_1 \) might be regulated in two ways, by TGF-\( \beta_1 \) stimulation (24, 62, 71) and/or by FN itself (63) (Fig. 10). Consistent with this, our adhesion assays showed that GRGDSP peptides disrupted adhesion in a dose-related manner. Thus, the EGR-1-dependent secretion of TGF-\( \beta_1 \) and FN, in addition to directly facilitating adhesion, may control increased expression of FN receptors to enhance further attachment (Fig. 10).

These features argue that EGR-1 functions to promote enhanced cell-cell and cell-substratum interactions that are known to facilitate density-dependent growth control. An early observation of the effects of stable expression of EGR-1 on HT1080 cells was the 3-fold decrease in the saturation density in a manner strictly correlated with the level of EGR-1 expression (14). We conclude that the mechanism of the restored density arrest is due to the coordinated expression of TGF-\( \beta_1 \), FN, and secondarily regulated genes such as PAI-1. A diagrammatic model representing this relationship is summarized in Fig. 10. It is very likely that this mechanism is significant in a variety of cells including glioblastoma cells (data not shown).

**EGR-1 as “Oikis” Factor**—Loss of integrin-mediated cell-cell contact by normal cells has profound consequences such as loss of normal growth and induction of apoptosis, a phenomenon termed “homelessness” or anoikis (81). Transformed cells circumvent this process by developing means of anchorage-independent growth involving growth-promoting oncogenes, such as activated ras, and subvert apoptosis by deletion or mutation of p53 or overexpression of Bcl-2. It is likely that the coordinated expression of TGF-\( \beta_1 \), FN, and PAI-1 by Egr-1 plays an important role in maintaining the anchorage-dependent growth. Recent observation show that the focal adhesion kinase is activated in EGR-1-expressing HT1080 cells and that apoptosis is suppressed even in the presence of overexpressed wild-type p53 (82). Thus, we propose that EGR-1 functions as a true oikis factor, which works to establish normal contact and cell attachment growth control. Now, it is important to examine the generality of this mechanism in normal cells and tissues.

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**REFERENCES**

1. Herschman, H. R. (1991) *Annu. Rev. Biochem.* 60, 281–319
2. Christy, B., and Nathans, D. (1989) *Mol. Cell. Biol.* 9, 4889–4895
3. Gashler, A. L., and Sukhatme, V. P. (1991) *Proc. Natl. Acad. Sci. USA* 88, 151–154
4. Lebreton, B., Sukhatme, V. P., Roberts, A. B., Sporn, M. B., Rauscher, F. J., and Kim, S. J. (1994) *Mol. Endocrinol.* 8, 595–602
5. Liu, C., Calogero, A. R., Gagana, G., Adamson, E., and Mercola, D. (1996) *Critt. Rev. Oncog.* 7, 101–125
6. Nagaraj, L., Zavadil, J., Claxton, D., Lu, X., Fairman, J., Wasmuth, J. J., Chinault, A. C., Willman, C. L., and Deisseroth, A. B. (1994) *Blood* 83, 199–208
7. Fairman, J., Chumakov, I., Chinault, A. C., Nowell, P. C., and Deisseroth, A. B. (1994) *Cancer Genet. Cytogenet.* 76, 47–54
8. Levin, W. J., Press, M. F., Gaynor, R. B., Sukhatme, V. P., Boone, T. C., Reissmann, P. T., Figlin, R. A., Holmes, E. C., Souza, L. M., and Slamon, D. J. (1995) *Oncogene* 11, 1261–1269
9. Huang, R.-P., Liu, C., Fan, Y., and Deisseroth, A. B. (1994) *Cancer Res.* 55, 5054–5062
10. Huang, R.-P., Darland, T., Okumura, D., Mercola, D., and Adamson, E. (1995) *Int. J. Cancer* 59, 102–109
11. Huang, R.-P., Liu, C., Fan, Y., Mercola, D., and Adamson, E. (1995) *Cancer Res.* 55, 1367–1377
12. Kieser, A., Seitz, T., Adler, H. S., Cofer, P., Kremmer, E., Crespo, P., Gutkind, J. S., Hender, D. W., Mushinski, J. F., Kolch, W., and Mischak, H. (1996) *Genes Dev.* 10, 1455–1466
13. Powell, C. T., Guschewd, J. E., Fair, W. R., Britts, N. J., Stie, D., and Huryk...
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R. (1990) Cancer Res. 56, 4137–4141
18. Liu, C., Adamson, E., and Mercola, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5233–5237
19. Liu, C., Rangnekar, V. M., Adamson, E., and Mercola, D. (1998) Cancer Gene Ther. 5, 3–28
20. Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641
21. Roberts, A. B., and Sporn, M. B. (1990) in Peptide Growth Factors and Their Receptors (Stern, M. B., and Roberts, A. B., eds) pp. 419–472, Springer-Verlag, Heidelberg, Germany
22. Chakrabarty, S. (1992) Int. J. Cancer 50, 968–973
23. Ignetz, R. A., and Massague, J. (1986) J. Exp. Med. 163, 437–445
24. Huang, S., and Chakrabarty, S. (1994) Int. J. Cancer 57, 742–746
25. I I, M., Sakaeda, O., and Kekki-Oja, J. (1987) J. Biol. Chem. 262, 17467–17474
26. Franzen, P., Ichijo, H., and Miyazono, K. (1993) Cell 72, 1–7
27. Planus, E., Barovitz-Meimon, G., Rogers, R. A., Bonavaud, S., Ingber, D. E., and Wang, N. (1997) J. Cell Sci. 110, 1091–1098
28. Carmeloh, B., Balza, E., Sari, A., Zardi, L., Nicotta, M. R., Bigotti, A., and Natali, P. G. (1998) J. Cell Biol. 136, 303–313
29. Oyama, F., Hirohashi, S., Shimazato, Y., Titani, K., and Sekiguchi, K. (1989) J. Biol. Chem. 264, 10331–10334
30. Chandler, L. A., and Bourgeois, S. (1991) Cell Growth Differ. 2, 379–384
31. Der, C. J., Ash, J. F., and Stanbridge, E. J. (1981) J. Cell Biol. 85, 151–166
32. Zajchowski, D. A., Band, V., Trask, D. K., Kling, D., Connolly, J. L., and Sager, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2314–2318
33. Akamatsu, H., Ichihara-Tanaka, K., Osumo, O., Kameike, W. H., Matsuda, H., and Sekiguchi, K. (1986) Cancer Res. 46, 4541–4546
34. Càrcamo, J., Weis, F. M. B., Ventura, F. W., Rana, R., Wrana, L. J., Attisano, L., and Massague, J. (1994) Mol. Cell. Biol. 14, 3810–3821
35. Espinosa, D. A., Kondo, K. L., Wong, S. W., and Diamond, D. J. (1992) Eur. J. Immunol. 22, 2419–2422
36. Dean, D. C., Bowlus, C. L., and Bourgeois, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1876–1880
37. Laib, M., Baur, I., Huettner, C., Schmauber, B., Roggendorf, W., Schlingensiepen, K. H., and Brysch, W. (1995) J. Neuropathol. Exp. Neurol. 54, 286–244
38. Elgner, P. L., Gadek, T. R., Holm, M., Roman, R. Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M., and Danielson, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7413–7417
39. Suzuki, M., Oda, E., Nakajima, T., Sekiya, S., and Oda, K. (1998) Mol. Cell. Biol. 18, 3010–3020
40. Huang, R.-P., Fan, Y., Ni, Z., Mercola, D., and Adamson, E. (1993) J. Cell Biol. 126, 265–273
41. Huang, R.-P., Fan, Y., Ni, Z., Mercola, D., and Adamson, E. (1997) J. Cell. Biochem. 66, 489–499
42. Hynes, R. O. (1992) Cell 69, 11–25
43. Ramsey, W. S., Hertl, W., Nowlan, E. D., and Binkowski, N. J. (1984) In Vitro 20, 802–808
44. Roberts, C. J., Birkenmeier, T. M., McQuillan, J. J., Akiyama, S. K., Yamada, S. S., Chen, W.-T., Yamada, K. M., and McDonald, J. A. (1988) J. Biol. Chem. 263, 4377–4382
45. Dano, K., Andreasen, P. A., Grondahl-Hansen, G., Kristensen, P., Nielsen, L. S., and Skriver, L. (1995) Adv. Cancer Res. 69, 139–266
46. Liotta, L. (1992) Sci. Am. 266, 54–59
47. Xue, W., Mirukami, I., Todd, R. F., Ill, and Petty, H. R. (1997) Cancer Res. 57, 1662–1689
48. Nusrat, A. R., and Chapman, H. A. (1991) J. Clin. Invest. 87, 1091–1097
49. Qazaz, P. H. A., Fracchi, E., Pedersen, N., Benayoun, S., Thibert, P., Martelly, I., Verheijen, J., Blasi, F., and Barlovitz-Meimon, G. (1999) J. Exp. Med. 151, 161–175
50. Walters, D. A., Sailor, L. Z., and Chapman, H. A. (1993) J. Clin. Invest. 91, 1541–1552
51. Gyetko, M. R., Todd, R. F., III, Wilkinson, C. C., and Sitrin, R. G. (1994) J. Clin. Invest. 93, 1380–1387
52. Winsen, V. E., Day, M. L., Patek, T., Padgett, K. J., Johnston, M., and Milbrandt, J. (1992) J. Biol. Chem. 267, 3718–3724
53. Pavletich, N. P., and Pabo, C. O. (1991) Science 250, 809–817
54. Frisch, S. M., and Ruoslabhi, E. (1997) Curr. Opin. Cell Biol. 9, 701–706
55. de Belle, I., Huang, R.-P., Fan, Y., Liu, C., Mercola, D., and Adamson, E. (1999) Oncogene, in press
The Transcription Factor EGR-1 Suppresses Transformation of Human Fibrosarcoma HT1080 Cells by Coordinated Induction of Transforming Growth Factor-β1, Fibronectin, and Plasminogen Activator Inhibitor-1

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