Orthologs of the *Drosophila* Disabled that was identified as one of the proteins genetically interacting with Abi kinase in fly neuron development (1, 2). The three spliced forms (p96, p93, and p67) of murine Dab2 cDNA were first isolated as mitogen-responsive phosphoproteins functioning in the CSF-1 signal transduction pathway in macrophages (2). DAB2 is thought to be a tumor suppressor in ovarian cancer (3–6). Its expression is lost or greatly diminished in 85% of the breast and ovarian cancers analyzed (5), and forced re-expression of Dab2 suppresses cell growth and tumorigenicity (4, 6, 7). Gene deletions have been found to account for the loss of DAB2 expression in a small percent of tumors.2

In vertebrates, retinoic acid plays a role in inducing cell lineage in early embryonic development, and defects in retinoic acid metabolism or exposure may result in abnormal development (9, 10). The GATA transcription factors are believed to serve as mediators of retinoic acid in the induction of the heart, gut, and hematopoietic systems during development (9–13). Retinoic acid induces gene expression and differentiation in many cell types in culture and exhibits growth suppressive activity in a wide spectrum of tumor cells. Furthermore, retinoic acid has been used successfully to treat leukemia and has been explored for use in treating other malignancies (14–16). In *in vitro* studies of cultured tumor cells, retinoic acid suppresses cyclin D induction and saturation cell density but does not affect log phase cell growth (17, 18). One of the several possible mechanisms postulated for the effect of retinoic acid on cell growth inhibition is the suppression of AP-1 activity (19, 20), which is the target of activation of the Ras/MEK pathway, by many mitogens. Retinoic acid also induces the transforming growth factor-β pathway, another route for tumor/growth suppression in some systems (21). The action of retinoic acid is mediated through nuclear receptors that in turn modulate gene expression (9, 22). Although some of the direct transcriptional targets of retinoic acid are known, such as the GATA factors (11) and laminin (23), the principal retinoic acid-controlled growth regulator(s) has yet to be identified, and the mechanisms for retinoic acid regulation and resistance are as yet not fully understood. One of the remarkable changes in cell properties identified recently is that retinoic acid-induced differentiation of F9 cells accompanies the uncoupling of MAPK activation and c-Fos expression (24), although the mediators of this retinoic acid-induced alteration have not been identified.

In addition, some tumor cells develop resistance to growth suppression by retinoic acid (25). Loss of retinoic acid receptors accounts for some cases, but other unidentified mechanisms must exist (19, 25, 26). In this study using F9 (retinoic acid-

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**Disabled-2 Mediates c-Fos Suppression and the Cell Growth Regulatory Activity of Retinoic Acid in Embryonic Carcinoma Cells**

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Dab2 Suppresses c-Fos Expression of Teratocarcinomas

sensitive) and PA-1 (retinoic acid-resistant) teratocarcinoma cell lines, we identified the candidate tumor suppressor Dab2 as a retinoic acid-inducible gene in F9 cells but not in PA-1 cells. Dab2 was found to mediate the retinoic acid effect on cell growth inhibition by suppressing c-Fos induction without altering MAPK activation. Transfection/expression of Dab2 is sufficient for cell growth suppression, suggesting that Dab2 is the major mediator of retinoic acid in cell growth suppression. Moreover, the failure or inability to induce Dab2 may be a mechanism for the resistance of tumor cells to retinoic acid in growth suppression.

**EXPERIMENTAL PROCEDURES**

**Materials**—Retinoic acid (all-trans-, 9-cis-retinoic acid) and β-casein were purchased from Sigma. Tissue culture supplies were obtained from Fisher. DMEM medium was purchased from Meditech (Hermndon, VA); fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Atlanta, GA); TRITol reagent, 100× antibiotic-antimycotic solution, LipofectAMINE, and serum-free Opti-MEM I medium were purchased from Life Technologies, Inc.; the ECL Super-Signal West Dura extended detection substrate immunodetection reagents were purchased from Pierce; Hybrisol I hybridization solution came from Intergen (Purchase, NY); TRIzol reagent, 100× antibiotic-antimycotic solution, LipofectAMINE, and serum-free Opti-MEM I medium were purchased from Life Technologies, Inc.; the ECL Super-Signal West Dura extended detection substrate immunodetection reagents were purchased from Pierce; Hybridrol I hybridization solution came from Intergen (Purchase, NY), positively charged nylon membranes were from Roche Molecular Biochemicals; [m-32P]dCTP was from PerkinElmer Life Sciences. All other general chemicals and supplies including MeSO4, ethanol, isopropanol, and agarose were from Sigma or Fisher and were reagent grade or higher.

**Cell Culture**—F9 mouse teratocarcinoma and PA-1 human teratocarcinoma cells were purchased from American Type Culture Collection (ATCC). The PA-1 cells were cultured in DMEM supplemented with 10% FBS and 1× antibiotic-antimycotic solution. F9 cells were cultured on gelatin-coated tissue culture plates in DMEM containing 10% heat-inactivated FBS and 1× antibiotic-antimycotic solution. The plates were coated with an autoclaved 0.1% gelatin solution overnight at 4 °C, then washed three times with phosphate-buffered saline before use. Retinoids were added to cells from a 1× stock solution in MeSO4. If it is not specifically stated, all-trans-retinoic acid was used. Control cultures contained an equal volume of MeSO4 alone. Usually, retinoic acid was added 24 h after plating of cells. Cell growth was determined by either triplicate counting with a hemacytometer or measured using the MTT assay (Promega). The results of MTT assay agreed well with those from cell counting.

**Results**—Anti-Dab2 antibodies were characterized as previously described (2, 5, 6, 27). Anti-Dab2 (p96) monoclonal antibodies were purchased from Transduction Laboratories (Lexington, KY); anti-c-Fos came from Santa Cruz Technology; anti-Erk1/2 and anti-phospho-Erk1/2 came from Cell Signaling Technology, Inc. (Beverly, MA). Immunoblotting was performed according to standard procedures, as described previously (5, 6, 27). After confirmation of antibody specificity, in some cases two or more antibodies were used simultaneously in an incubation to detect various molecular weight proteins.

**Northern Blot Analysis**—Total RNA was isolated from cell monolayers according to the TRITol method (Life Technologies, Inc., RNA was separated on 1% agarose gel containing 7% formaldehyde and 20 mM MOPS buffer, transferred to positive-charged nylon membranes using 2× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) buffer, and fixed by baking. DNA probes were labeled with [α-32P]dCTP using a random prime labeling kit (Amersham Pharmacia Biotech). The hybridization and Northern blotting followed standard procedures as described previously (2, 5).

**Cell Transfection**—The full-length human DAB2 (28) or murine Dab2 (2) cDNA was inserted into the pcDNA3.1 (Invitrogen, La Jolla, CA) or pMT-CB6+ eukaryotic expression vectors. Plasmid DNA was purified using Qiagen Maxiprep columns. For transfection, 2 μg of Dab2 or vector plasmid DNA were mixed with 20 μl LipofectAMINE in 1 ml of Opti-MEM and added to PA-1 or F9 cells for 16 h. F9 cells were transfected with mouse Dab2 cDNA, and PA-1 cells were transfected with human Dab2 cDNA. The transfection medium was removed, and fresh DMEM containing 10% FBS was added. After 12 h, transfected cells were cultured in DMEM containing 10% FBS and 300 ng/ml zeomycin for selection of pcDNA3.1 vector or 400 μg/ml G418 for selection of pMT-CB6+ vector. This selection medium was changed every 2 days, and after 10–12 days cloning rings were used to isolate positive clones. Cultures were further expanded and examined for Dab2 expression by Western blotting.

F9 cells were also transfected with metallothionein promoter-regulated mouse Dab2 construct in pMT-CB6+ vector, and green fluorescent protein in pMT-CB6+ vector was used as a control. To induce expression, 0.1 mM ZnSO4 was added to the medium for 24–72 h.

**Cell Cycle Analysis**—Cell monolayers were released from plates with 0.25% trypsin, 0.1% EDTA and collected by centrifugation. The cells were then fixed in 70% ethanol at 4 °C, pelleted, and re-suspended in 50 μg/ml propidium iodide in phosphate-buffered saline for 30 min at 4 °C. The stained cells were analyzed by flow cytometry performed on a FACSscan equipped with argon-ion laser and analyzed by Cell Quest software (Becton Dickinson).

**Transient Transfection of Dab2 Using Adenoviral Approach**—Replication-deficient adenovirus expressing Dab2 p96 or p67, allowed forms or β-galactosidase were produced, purified, and titrated as described previously (6). For transfection of F9 or PA-1 cells, 100 multiplicity plaque-forming units of adenovirus were added to the cells in medium with low serum (1% FBS) for 4 h. The cells were then used for further experimental manipulation. Under these conditions, more than 90% of the F9 cells expressed the transfected cDNA, as estimated using adenovirus-expressing β-galactosidase.

**RESULTS**

**Induction of Disabled-2 Expression by Retinoic Acid—**Dab2, a candidate tumor suppressor, is lost in a wide spectrum of tumor tissues and cultured carcinoma cells (5). To evaluate mechanisms for its loss, we examined potential factors that might affect Dab2 expression. Dab1, the human ortholog that is mainly expressed in brain, can be induced by retinoic acid in the embryonic P19 carcinoma cell line (29) and by thyroid hormone (T3 and T4) (30). Thus we investigated and found that Dab2 can also be induced by retinoic acid in the mouse embryonic teratocarcinoma F9 cell line, which is widely used as a model for studying effects of retinoic acid in gene transcription and cell differentiation. Another recent report also confirmed the ability of retinoic acid to induce Dab2 expression (31).

We found that retinoic acid induces expression of both of the two variably spliced forms of Dab2, p96 and p67 (2), in F9 cells. The effect is time (Fig. 1A) and dose-dependent (Fig. 1B). High levels of Dab2 protein were induced after treatment with retinoic acid for 4 days, and as little as 10−10 μM retinoic acid stimulated Dab2 protein expression. Retinoic acid treatment caused greater induction of the p67 form of Dab2, which differs from the expression pattern of Dab2 isoforms found in other cells in which p96 is generally the major or only isoform (2, 5). The induction of Dab2 by retinoic acid occurs at the transcriptional level, because the Dab2 message RNA is induced in a similar magnitude as the protein (Fig. 1C). Withdrawal of retinoic acid 4 days after induction did not reverse or decrease Dab2 protein levels (Fig. 1D), and even a month after retinoic acid removal F9 cells continued to express Dab2 (not shown).

These results correlate Dab2 expression with the irreversible endoderm differentiation of F9 cells by retinoic acid treatment. Among the retinoids tested, all-trans-retinoid acid is the most potent in the induction of Dab2 (Fig. 1F). 9-cis-Retinoic acid can induce Dab2 expression in F9 cells, but the required dosage is about 100 times more than that of all-trans-retinoic acid, and N-(4-hydroxyphenyl)retinamide (fenretinide or 4-HPR) and β-carotene (vitamin A) have no detectable activity (Fig. 1E).

In the PA-1 teratocarcinoma cell line, however, retinoic acid treatment for 4 days did not induce Dab2 expression (Fig. 2A). PA-1 cells were derived from a human ovarian germ cell tumor (32) and are resistant to growth suppression by retinoic acid (19, 33, 34), in contrast to F9 cells. Longer duration of treatment with 1 μM retinoic acid for 2 weeks still failed to induce Dab2 expression in PA-1 cells (data not shown). The lack of Dab2 induction occurs at the transcriptional level, because no changes in DAB2 mRNA were observed (Fig. 2B). RNA from ES2 cells, a Dab2-positive ovarian cancer cell line (5), was used as a positive control.
Changes in Both Cell Lines

**FIG. 1. Induction of Dab2 expression in F9 cells.** A, time course of Dab2 protein induction. F9 cells were seeded on 35-mm plates (5 × 10^5 cells/dish) on day 0. After incubation for 3 h, all-trans-retinoic acid (RA, 10 nM) was added. Cell lysates were prepared on days 1, 2, 3, and 4 and used to measure Dab2 protein level by Western blot. β-Actin was determined as a protein loading control, and a lysate of ES2 cells was used as a Dab2-positive control. B, dose dependence of retinoic acid induction. Dab2 protein expression was determined by Western blot in F9 cells incubated with increasing concentrations of retinoic acid for 4 days. M, molarity. C, retinoic acid induction of Dab2 mRNA. Approximately 1 × 10^6 F9 cells were plated on 100-mm plates and incubated with retinoic acid for the indicated days. Total RNA was isolated and analyzed for Dab2 mRNA levels by Northern blotting. D, irreversible retinoic acid-induced Dab2 expression. F9 cells were first stimulated with 1 μM retinoic acid for 4 days. On day 4, retinoic acid was removed, and the cells were washed, replated on new culture dishes, and cultured for additional days without retinoic acid. At the indicated times, cells were lysed and analyzed for Dab2 protein by Western blotting. β-Actin protein level was determined as a loading control. E, activity of retinoids in the induction of Dab2. F9 cells were treated with MeSO (DMSO) solvent alone or with 0.1, 1, or 10 μM all-trans-retinoic acid, 9-cis-retinoic acid, fenretinide (4-HPR), or β-carotene for 4 days. The cell lysate were used to determined Dab2 level by Western blotting.

**FIG. 2. Lack of effect of retinoic acid (RA) on Dab2 expression in PA-1 cells.** PA-1 cells were cultured and analyzed for Dab2 expression exactly as described for F9 cells in Fig. 1. A, retinoic acid effect on Dab2 protein expression. Dab2 protein expression was determined by Western blot in PA-1 cells incubated with increasing concentrations of retinoic acid for 4 days. B, effect of retinoic acid on Dab2 mRNA levels. Total RNA from ES2 cells was used as a positive control.

Retinoic Acid Induces Cell Growth Suppression in F9 but Not in PA-1 Teratocarcinoma Cells and Induces Morphological Changes in Both Cell Lines—In parallel experiments, retinoic acid inhibited the growth of F9 cells in a time (Fig. 3A)- and dose-dependent manner (Fig. 3C) and also caused morphological changes of the cells in culture (Fig. 3D). Suppression of cell growth correlated with the induction of Dab2 expression since both occurred at day 3 after treatment with retinoic acid. F9 cells treated with retinoic acid for 4 days were well separated and dispersed compared with non-treated cells, which appeared tightly packed and physically connected. In contrast, retinoic acid had no effect on PA-1 cell growth (Fig. 3, B and C), although a morphological change was seen, in agreement with previous reports (19, 33, 34). In PA-1 cells treated with 1 μM retinoic acid for 4 days (Fig. 3E), cells appear to be less elongated and the nuclei more pronounced. Thus, retinoic acid induces morphological changes and cell growth suppression in F9 cells and induces morphological changes but no growth suppression in PA-1 cells. Resistance to retinoic acid-induced growth suppression, therefore, correlates with a lack of Dab2 induction.

Transfection and Expression of Dab2 Mimics the Effect of Retinoic Acid on Cell Growth—To examine the effect of Dab2 on cell growth and morphology, a Dab2 expression construct was transfected into both F9 and PA-1 cells. In F9 cells transfected with Dab2, only three G418-resistant colonies were selected compared with 64 resistant colonies of vector controls in parallel transfection. After expansion of the three Dab2-transfected clones, none were found to express the Dab2 protein as detected by Western blotting. We then transfected F9 cells with mouse Dab2 p96 construct under the control of the metallothionein promoter (pMT-CB6+ vector). In 48 clones selected for analysis, at least 6 clones appear to express the Dab2 p96 protein (Fig. 4A). However, we have also observed that the ZnSO4-induced F9 cells undergo differentiation without retinoic acid; the cells also express the p67 form of Dab2 (although these were transfected with the p96 form of Dab2), and the cells also express GATA-4, GATA-6, collagen IV α2, and laminin, which are markers for differentiated endoderm cells. Thus, we
Fig. 3. Effect of retinoic acid (RA) on F9 and PA-1 cell growth and morphology. F9 (A) or PA-1 (B) cells were plated at 5 × 10^4 cells/well on day 0. After allowing the cells to attach for 3 h, retinoic acid (1 μM) was added. Cell number was determined by cell counting, and the MTT assay.
conclude that it is not possible to obtain stable Dab2-expressing F9 cells without also inducing spontaneous retinoic acid-independent differentiation. However, we are able to transiently express Dab2 by adeno-viral approach (Fig. 4B) without inducing differentiation of the F9 cells, as judged by the lack of expression of the p67 spliced form of Dab2, GATA-4, and GATA-6. Dab2 expression by the adeno-viral approach suppresses F9 cell growth (Fig. 4C), suggesting that retinoic acid-induced Dab2 expression is responsible for the cell growth inhibitory activity of retinoic acid in F9 cells.

There are undoubtedly many differences in the genetic background and properties of mouse F9 and human PA-1 teratocarcinoma cells, although both cell lines have some properties of embryonic stem cells. Although both are undifferentiated and multipotent, PA-1 cells synthesize collagen IV and laminin (32–34), unlike F9 cells, which do not express collagen IV and laminin before retinoic acid-induced differentiation (26). After transfection of PA-1 cells with a Dab2 expression construct, 16 colonies were selected with 54 colonies from vector-transfected controls. All of the Dab2-transfected colonies developed much more slowly (estimated to be 20-fold less based on cell number) than colonies from vector-transfected controls, as shown in Fig. 5A for a typical example of a G418-selected colony. Under identical culture conditions, the Dab2-transfected cells appeared well separated from each other within a colony, whereas the vector-transfected control cells in a colony were aggregated and indistinguishable from parental cells. In an earlier passage with a cell number of about $1 \times 10^5$ cells/colony, Dab2 expression was detected. Only the p96 form of Dab2 and not the p67 form was expressed, suggesting that Dab2 expression was the result of cDNA transfection and not spontaneous differentiation. However, as cultures were expanded, the morphological difference diminished, and Dab2 expression was gradually lost in most of the clones. For three colonies, Dab2 expression remained after several passages, and the morphological changes, although not as obvious as for the cells in earlier passages, were still apparent compared with vector-transfected cells (Fig. 5B). Additionally, these Dab2-expressing cells exhibited a reduced growth rate compared with vector-transfected controls (Fig. 5C). The ability to form colonies on agar plates was suppressed upon Dab2 expression (Fig. 5D). Therefore, transfection experiments indicate that expression of Dab2 suppresses cell proliferation and anchorage-independent colony formation and alters cell-cell adhesion.

Moreover, these changes correlate well with alterations in the cell cycle. Under identical culture conditions as described above, the two transfected PA-1 clones with detectable Dab2 expression (clones 9 and 13) had an increase in the percentage of cells in G1 and a corresponding decrease of cells in S phase compared with vector-transfected or parental cells (Table I). Thus, Dab2 inhibits cell growth by suppressing G1 phase progression, which is similar to the effect of retinoic acid on the cell cycle (17, 18, 26).

**Dab2 Transfection and Expression Inhibits Serum-stimulated c-Fos Expression and Uncoupling from MAPK Activation**—We next examined the effect of Dab2 on end points of the mitogenic signaling pathway compared with the effect of retinoic acid on the signaling properties of F9 cells. Expression of Dab2 in breast cancer cells results in the disassociation of MAPK activation and c-Fos expression (35). It is thought that retinoic acid reduces cell growth by suppressing AP-1 activity of the Jun/Fos transcription complex (19, 20) as does Dab2 (36). In F9 cells, we have found that retinoic acid-induced differentiation results in a much weaker c-Fos induction by serum, although MAPK activation is not affected (Fig. 6A). By adenovirus transfection of Dab2, without induction of endo-derm differentiation, the Elk-1 phosphorylation and the c-Fos expression are inhibited (Fig. 6B). Thus, expression of Dab2 alone is sufficient to alter the signaling in F9 cells.

Whereas c-Fos induction and MAPK activation in PA-1 cells were not altered by retinoic acid treatment (Fig. 6C), we found that the expression of c-Fos was greatly reduced after serum stimulation in PA-1 cells expressing Dab2 (Fig. 6D). Remarkably, the MAPK activation in these cells was not affected by Dab2 expression. Thus, we conclude that Dab2 can suppress cell growth by inhibiting c-Fos expression as a result of uncou-
pling from MAPK activation, and expression of Dab2 mimics retinoic acid in inhibiting cell growth. Expression of Dab2 either by retinoic acid induction in F9 cells or transfection in PA-1 cells results in cell growth suppression and disassociation of c-Fos expression from MAPK activation.

**DISCUSSION**

Both F9 and PA-1 cells are well characterized teratocarcinoma lines derived from tumors of gonads (testes and ovary). F9 cells are undifferentiated, with characteristics resembling those of stem cells in early embryos and have been widely used to study early embryonic development and retinoic acid regulation (9, 26, 31, 37). The PA-1 line also shares similar properties to embryonic cells (19, 32, 33, 34). Although PA-1 cells can be differentiated or affected in morphology by retinoic acid (19, 33, 34), they are resistant to the growth suppressive activity of retinoic acid. Herein we have demonstrated that Dab2 is induced by retinoic acid in the F9 mouse teratocarcinoma cells.
but not in retinoic acid-resistant PA-1 cells. Moreover, Dab2 expression suppresses c-Fos expression by uncoupling it from MAPK activation and accounts for or contributes to the growth suppressive activity of retinoic acid in F9 cells. At least two additional proteins, KSR (kinase suppressor of Ras) (38) and Gab2 (39), have been reported to uncouple MAPK activation and c-Fos expression. This regulatory step adds additional complexity and flexibility in the Ras pathway.

Although the mechanism for growth suppression by retinoic acid is not yet certain, several possibilities have been investigated, including the induction of the transforming growth factor-β pathway (21) and inhibition of AP-1 activity by competition of the cofactor CBP (40). Here, we propose another pathway as follows. Retinoic acid inhibits AP-1 activity by reducing serum-stimulated c-Fos induction in a Dab2-dependent manner (Fig. 6). Dab2 binds the adapter protein Grb2 (growth factor receptor binding protein 2) and may affect Ras signaling (27) and suppresses AP-1 activity (37). We observe here that treatment of F9 cells with retinoic acid results in a much weaker induction of c-Fos by serum as a result of uncoupling it from MAPK activation, correlating with the expression of Dab2 (24). Remarkably, expression of Dab2 alone can inhibit serum-stimulated c-Fos expression by uncoupling from MAPK activation in transfected breast cancer cells (35), in F9 cells transfected with adenovirus carrying Dab2 cDNA (Fig. 6B), and in Dab2-transfected PA-1 teratocarcinoma cells (Fig. 6C). Thus, the suppression of F9 cell growth and c-Fos expression upon retinoic acid treatment is largely mediated by Dab2.

We were able to stably transfect and express Dab2 in PA-1 but not F9 cells. We reason that the presence of some additional partners such as collagen IV and laminin in PA-1 cells may have assisted for the tolerance of Dab2 expression in the cells. Additionally, during the transfection and selection of Dab2-expressing clones, the F9 cells often undergo retinoic acid-independent differentiation. Nevertheless, we were able to transiently express Dab2 without inducing endodermal differentiation of F9 cells using an adenoviral vector. Consistently, expression of Dab2 alone appears to be highly growth-suppressive (Fig. 4C). We have not observed spontaneous expression of Dab2 in PA-1 cells under various culture conditions and experimental procedures, making the PA-1 cell line a good model for analyzing the effect of Dab2 transfection/expression. PA-1 cells transfected with Dab2 appear less adhesive to each other, which leads us to speculate that Dab2 expression affects cell contact. Consistent with this idea, Dab2 has been shown to bind to the intracellular domain of Megalin (41). Megalin, a large glycoprotein that can act as a receptor for multiple extracellular ligands, is believed to function in cell-cell and cell-matrix interaction (42). Dab2 shows 30% sequence homology with Drosophila Dab and is 45% identical and 60% homologous to Dab1, the other mammalian ortholog (2, 29). Dab1 functions in controlling the positioning of brain cells (43–46) and serves as an adaptor protein in signaling from the extracellular matrix. This pathway consists of the matrix protein reelin binding to the glycoprotein cell surface receptor, which via Dab1 links to Src family kinases and subsequently further signal transduction (47–49). A similar function is proposed for Dab2 in epithelial

### Table I

| Cell     | G1    | S     | G2 + M | % total cells |
|----------|-------|-------|--------|--------------|
| PA-1     | 48    | 27    | 25     | 90           |
| Vector   | 51    | 24    | 25     | 100          |
| Dab2 Clone 9 | 75    | 8     | 17     | 100          |
| Dab2 Clone 13 | 66    | 8     | 26     | 100          |
Dab2 Suppresses c-Fos Expression of Teratocarcinomas

Fig. 7. Dab2 mediates retinoic acid effects on signal transduction and cell growth in embryonic carcinoma cells. In embryonic carcinoma cells, retinoic acid (RA) induces laminin (Lam) expression directly (23) and induces expression of Dab2 and collagen IV (Col IV) through the GATA-6 transcription factor (50). Dab2 mediates the effect of retinoic acid on the uncoupling of MAPK activation by serum and growth factors (GP) from stimulation of c-Fos expression and cell growth (24). The induction of Dab2 is speculated to be defective in PA-1 cells.

cell-positioning control such that its loss may contribute to disorganized proliferation found in tumor growth (6). This is consistent with the ability of retinoic acid to induce the synthesis of basement membrane components and induce/maintain cell differentiation, hence organization, of epithelial cells.

Loss or mutations of retinoic acid receptors have been found as the means for tumor cells to acquire resistance to retinoic acid (25, 26), although other mechanisms also exist (9, 14). Because PA-1 cells still respond to retinoic acid with a change in morphology (19, 33, 34), the absence of an effect on growth is likely not because of a loss of functional retinoic acid receptors. Thus, the inability to induce Dab2 expression may be at least one reason for the resistance of PA-1 cells to the growth-suppressive activity of retinoic acid. Though the Dab2 gene is not disrupted in PA-1 cells (data not shown), the cause of Dab2 expression loss in PA-1 cells is not known. The Dab2 promoter lacks known retinoic acid-responsive elements (28), suggesting that retinoic acid indirectly induces Dab2 expression, perhaps through GATA transcription factors (Fig. 7). GATA factors can be induced by retinoic acid (11), and studies of GATA-deficient mouse embryos suggest that GATA-6 is required for Dab2 expression during embryonic development (50). Consistent with these findings, two GATA binding sites are present in the Dab2 promoter (28). Moreover, the transcription of other retinoic-acid-inducible genes such as the fibrolast growth factor (51) and the J6 gene (8, 52) also depends on GATA factors.

In conclusion, we found that Dab2 expression is absent or very low in teratocarcinomas such as PA-1 and F9 cell lines. Retinoic acid induces Dab2 expression in F9 but not PA-1 cells (Fig. 7). Furthermore, transfection of Dab2 in F9 and PA-1 cells mimics the retinoic acid-induced suppression on cell growth that occurs in F9 cells. PA-1 cells expressing the transfected Dab2 reach a much lower saturation density, and serum-stimulated c-Fos expression is greatly suppressed as a result of disassociation from MAPK activation. Similar events occur when Dab2 expression is induced in F9 cells, in which the cell growth is suppressed, and c-Fos expression and MAPK activation are uncoupled (24). Thus in F9 cells, Dab2 is one of the principal genes induced by retinoic acid involved in cell growth suppression, and expression of Dab2 alone is sufficient to suppress cell growth to a level that is achieved by retinoic acid treatment. Resistance to retinoic acid regulation in PA-1 cells is at least partially because of deficiency in retinoic acid up-regulation of Dab2 expression (Fig. 7).

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