Activating the ras-Mitogen-activated Protein Kinase Pathway and Phosphorylation of ets-2 at Position Threonine 72 in Human Ovarian Cancer Cell Lines

Suzanne E. Patton, Michele L. Martin, Lori L. Nelsen, Xianjun Fang, Gordon B. Mills, Robert C. Bast, Jr., and Michael C. Ostrowski

ABSTRACT

The activation status of the ras pathway was studied in eight ovarian tumor cell lines. Three biochemical parameters indicative of ras activation were tested: (a) the ratio of the ras-GTP:ras-GDP complex; (b) the activity of mitogen-activated protein kinases p42/p44; and (c) ets-2 phosphorylation at position threonine 72, a mitogen-activated protein kinase phosphorylation site in vivo. Four of the ovarian tumor cell lines had an activated ras pathway by these three parameters, whereas only one of these contained a mutated ras gene. In addition, ras/ets-2 responsive genes such as the urokinase plasminogen activator (uPA) were activated in these four cell lines. Transient transfection assays indicated that the compound ets-APl oncogene responsive enhancer present in the uPA gene was the target of ras signaling in ovarian tumor cells and that the combination of activated ras and ets-2 could superactivate the uPA enhancer element. Coexpression of the dominant-negative ras-Asn72 C DNA gene abrogated activity of this uPA element in ovarian tumor cells. These data indicate that ets-2 is a nuclear target of ras action in ovarian tumor cell lines and that ras signaling pathways may be activated in ovarian cancer by mechanisms independent of direct genetic damage to ras genes.

INTRODUCTION

The ras genes encode related Mr 21,000 plasma membrane-localized guanine nucleotide binding proteins that are capable of neoplastic transformation of cultured cells (1). ras genes that contain activating point mutations are often associated with human neoplasia; ~30% of all human solid tumors and leukemia have activating ras mutations (2). Several human cancers are associated with frequent ras gene mutations, including lesions of the pancreas (90%), colon (40%), and lung (30%; Ref. 2), whereas the rate in other cancers, including breast, colorectal, and bladder disease, is <10% (3). Furthermore, ras mutations are a negative prognostic factor (4, 5). Thus, activation of ras genes is likely to be a contributing factor in multistage carcinogenesis in humans.

The ras p21 protein acts as a molecular switch cycling between the GDP-bound inactive conformation and the GTP-bound active conformation, and activating point mutations lock the switch into the active GTP-bound conformation (2). The mechanism by which tyrosine kinase receptors switch ras to the active GTP-bound conformation has been determined. When activated by ligand, tyrosine kinase receptors recruit the proteins sem5/growth factor receptor binding protein 2 and SOS to the inner plasma membrane, the biologically relevant compartment for ras action (reviewed in Ref. 6). The Src homology domains Src homology 2 and Src homology 3 of growth factor receptor binding protein 2 and SOS are critical for the formation of this signal complex that promotes conversion of membrane-localized ras to the active GTP-bound conformation (6).

The ras-GTP complex is capable of directly associating with kinases and thereby initiating protein kinase cascades. For example, ras-GTP forms a complex with the c-raf protein kinase (7-9), resulting in translocation of this kinase to the cell surface and activation of kinase activity (10). The c-raf kinase then activates MAP/extracellular signal-regulated kinase-1, which in turn activates MAP kinases p42 and p44 (11, 12). A similar mechanism probably leads to the activation of the MAP kinase kinase 1/MAP kinase kinase-4/c-jun NH2-terminal kinase cascade in some cell types (13, 14), and the ras-GTP complex also associates with phosphatidylinositol 3'-kinase in some model systems (15). Therefore, one role of ras seems to be the recruitment of kinases to the correct cellular localization, and ras may activate at least three, and probably multiple, protein kinase cascades.

One target of ras action is the regulation of gene transcription in the nucleus. For example, MAP kinases phosphorylate the ets-family member elk-1 transcription factor and thus trigger expression of the immediate-early gene c-fos (16). In addition to regulating immediate early genes, ras can also stimulate the stable, sustained expression of genes (17). We have shown that the proto-oncogene products ets-1 and ets-2 may contribute to this sustained phase of gene expression (18). These transcription factors are activated by discrete phosphorylation events that occur at conserved threonine residues found at positions 38 and 72, respectively (18). Recent studies, using an anti-phospho-peptide antibody that specifically recognizes phospho-38. ets-2, demonstrated a strong correlation between activation of the ras/raf pathway, persistent phosphorylation of ets-2, and activation of oncogene-responsive genes, such as the heparin-binding EGF gene (19).

To reassess the extent of activation of ras signaling pathways in human ovarian tumor cell lines, we measured the activation of the ras pathway in eight established human ovarian cancer cell lines. We found that in four of eight cell lines tested, 40-60% of ras was found in the active GTP complex, MAP kinases p42 and p44 were activated, and ets-2 was phosphorylated at position threonine 72, and ets-target genes such as the uPA were transcribed. Only one of these cell lines was determined to contain an activating point mutation in one of the three ras genes, suggesting that the ras pathway is activated by other mechanisms.

MATERIALS AND METHODS

Cell Lines. OVCA420, OVCA429, OVCA432, OVCA433, OVCAR3, and OVCAR5 were grown in DMEM with 10% FCS, 1 mm sodium pyruvate, minimum essential amino acids, and 10 mm L-glutamine. DOVI3 cells were grown in the above medium modified by the addition of 1 μg of insulin

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3 The abbreviations used are: MAP, mitogen-activated protein; AP1, activating protein-1; uPA, urokinase plasminogen activator; EGF, epidermal growth factor; PMSF, phenylmethylsulfonyl fluoride; mAb, monoclonal antibody; SSCP, single-strand conformational polymorphism; RT-PCR, reverse transcription-PCR.
PERSISTENT ACTIVATION OF ets-2 BY ras SIGNALING

(ura/100 ml. SKOV3 cells were grown in McCoy’s medium with 15% FCS and 10 mM l-glutamine. In some experiments as indicated in the text, these cells were grown in serum-free media for 24 h before harvest.

**ras-GTP Assay.** Cells were grown to confluence on 60-mm tissue culture plates and were placed in serum-free, phosphate-free media for 45 min. After this initial incubation, 1 ml of fresh serum-free media containing 0.3 mM CaCl$_2$ [72P]PiP (ICN Biochemicals) was added, and incubation continued for 3 h. In experiments in which genistein was used, this drug was added 20 min before the labeling period. Cells were lysed in 0.5 M lysis buffer [50 mM Tris (pH 7.4), 20 mM MgCl$_2$, 150 mM NaCl, 1% NP40, 0.5 mM PMSF, 0.05 mg/ml leupeptin, 0.05 mg/ml aprotinin, 1 mM l-glutamine] and heated to 100°C for 5 min. After centrifugation, the protein content was determined by measuring the absorbance at 280 nm. The supernatant was then incubated at 30°C for 15 min with 100 ng of purified His-ras protein corresponding to amino acid residues 60–167.

**Western Blotting.** The protein samples were boiled in SDS sample buffer containing 2% SDS, 10% glycerol, 1% bromophenol blue, and 62.5 mM Tris (pH 6.8). The samples were resolved on 10% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in TBS-T (10 mM Tris, pH 7.4, 0.75 M NaCl, 0.05% Tween 20) and incubated with the primary antibody, then washed and incubated with the secondary antibody. The proteins were visualized by chemiluminescence using an enhanced chemiluminescence detection system.

**Antibodies.** The specific antibodies used for Western blotting were rabbit polyclonal antibodies against human ets-2 (Santa Cruz Biotechnology), human Ras (Cell Signaling Technology), and mouse monoclonal antibodies against human Ras (Cell Signaling Technology). The antibodies were detected using horseradish peroxidase-conjugated secondary antibodies and chemiluminescence detection system.

**Results**

**Activation of ras in Ovarian Carcinoma Cells.** Mutation or amplification of ras genes has only been observed in 2–12% of ovarian cancers (22–24). However, these tumor cells can produce extracellular matrix-degrading proteases, such as uPA or gelatinase A (25, 26), that are encoded by ras-responsive genes (18, 27). Because the ras pathway can be activated by mechanisms that do not depend on direct mutations in ras genes (e.g., constitutive activation of tyrosine kinases (28)), we reexamined whether ras signaling pathways were activated in ovarian tumor cell lines using criteria independent of whether ras gene point mutations were present.

As a first step, an anti-ras mAb was used to compare the amount of ras present in the GTP complex with that in the GTP complex in situ in eight ovarian cell lines and four primary ovarian epithelial cell cultures. Fig. 1A shows data representative of these experiments for two fibroblast cell lines and three ovarian cancer cell lines; the data for all eight ovarian cell lines as well as for normal cultured primary human ovarian cells are presented in Table 1. The level of ras in the GTP complex in the normal ovarian cells was 12–25%, significantly higher than seen in the murine cell line NIH 3T3. Four of these cell lines (OVCA420, OVCA432, DOV13, and SKOV3) had levels of ras-GTP complex similar to those observed in the normal cell samples; however, four of eight of the cell lines had significantly more ras in the GTP complex than the controls (43–62%). The level of ras-GTP complex in the latter cell lines was similar to the levels of ras-GTP complex found in mouse NIH 3T3 fibroblasts that are transformed by a human Ki-ras L61 oncone. The experiments represented in Table 1 were performed twice. However, for three cell lines that represented normal ras-GTP levels (OVCA432, 14%), intermediate levels (SKOV3, 28%), or high levels (OVCA433, 62%), these experiments were repeated five times, indicating that the differences found in ras-GTP levels between these cell lines were reproducible and significant.

To further explore this phenomenon, we tested the effect of PCS on the levels of ras-GTP complexes in these ovarian cell lines. Representative data are presented in Fig. 1B and demonstrate that growing the cells in the presence or absence of the serum had no effect on the ratio of the ras protein found in the GTP complex in these cells.

The frequency of ras mutations in ovarian tumors is reported to be only 2–12% (22–24). To determine whether the results presented above reflected an unexpected high percentage of cell lines containing activating ras point mutations, we tested for mutations in ras genes in these cell lines using PCR-SSCP analysis (20). This technique was used to screen genomic DNA from the eight ovarian tumor cell lines.
with oligonucleotides capable of detecting codon 12–13 or codon 59–61 mutations in the Ha-, Ki-, and N-ras genes. This analysis showed that seven of eight of the cell lines lacked mutations in any of the ras genes (data not shown). Only the cell line OVCAR5 was revealed to contain an activating mutation in the Ki-ras gene at codon 12–13 (Fig. 1C). This cell line is one of those having a high level of the ras-GTP complex (Table 1) and was not used in the additional experiments described below.

Table 1 Ras activation in ovarian tumor cell lines

<table>
<thead>
<tr>
<th>Cell</th>
<th>% ras-GTP&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH 3T3</td>
<td>6</td>
</tr>
<tr>
<td>200L (normal ovary)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>201L (normal ovary)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>NOE4 (normal ovary)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.5</td>
</tr>
<tr>
<td>NOE7 (normal ovary)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td>OVCA420</td>
<td>8</td>
</tr>
<tr>
<td>OVCA432</td>
<td>14</td>
</tr>
<tr>
<td>D0V13</td>
<td>19</td>
</tr>
<tr>
<td>SKOV3</td>
<td>28</td>
</tr>
<tr>
<td>OVCA429</td>
<td>43</td>
</tr>
<tr>
<td>OVCA433</td>
<td>58</td>
</tr>
<tr>
<td>OVCAR3</td>
<td>45</td>
</tr>
<tr>
<td>OVCAR5</td>
<td>58</td>
</tr>
<tr>
<td>NIH 3T3rasL61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62</td>
</tr>
</tbody>
</table>

<sup>a</sup> ras-GTP = GTPdpm/(2GTPdpm + 3GDPdpm). See “Materials and Methods” for details.

<sup>b</sup> Primary cultures of normal human ovarian epithelium.

<sup>c</sup> NIH 3T3 transformed by c-Ha-ras leucine 61 gene.

A gene homologous to the three known ras proto-oncogenes, TC21/R-ras2, has been reported to contain an activating mutation in the human ovarian tumor cell line A2780 (21). This mutation renders the gene capable of transforming NIH 3T3 cells (21, 29). The ras mAb used for the GTP/GDP assays does weakly cross-react with TC21/R-ras2. Although the weak cross-reactivity of the antibody made it unlikely that the results shown in Fig. 1 and Table 1 were due to activation of TC21/R-ras2, we performed RT-PCR on mRNA prepared from the eight ovarian cell lines and directly sequenced the regions corresponding to the mutational hot spots in TC21/R-ras2 (codon 22 or 71). These experiments showed that TC21/R-ras2 was expressed in all of these cell lines, but no activating point mutations were uncovered by this analysis (data not shown).

Inhibition of ras-GTP Complex Formation by Genistein. To begin to address the mechanism of ras activation in the ovarian tumor cell lines, the inhibitor of tyrosine kinase activity genistein (30) was used to determine whether tyrosine kinase activity was required. The cell line OVCA433, which exhibited the highest ras-GTP complex levels, was used for this analysis (see Table 1). A dose-response titration indicated that 50 µg/ml of genistein was sufficient to inhibit ~90% of the ras-GTP complex in these cells, in good agreement with the concentration of this inhibitor used in previous studies (Fig. 2A; Ref. 31). Four additional experiments were performed with 50 µg/ml of genistein with the result that the amount of the ras-GTP complex was consistently lowered by 7-fold (86% decrease in the ras-GTP complex) by this treatment (Fig. 2B).

Activation of MAP Kinases p42/p44 in Ovarian Tumor Cell Lines. One hallmark of activation of the ras signaling pathway is activation of the MAP/extracellular signal-regulated kinase kinases p42 and p44 (32). Immunoprecipitation-kinase assays using antibody that recognized both MAP kinase p42 and p44 were performed using as substrate a portion of ets-2 that contains threonine 72 (Fig. 3A). We have demonstrated previously that this recombinant ets-2 protein is a specific substrate for these MAP kinases (19).

Consistent with the results of the ras-GTP assays, these experiments revealed that three cells lines, OVCA3, OVCA429, and OVCA433 (Fig. 3A, Lanes 5–7), had detectable MAP kinase activity, whereas SKOV3, DOV13, OVCA420, and OVCA432 did not (Fig. 3A, Lanes 1–4). However, all seven cell lines expressed similar amounts of the MAP kinases, as determined by Western blotting of the immunoprecipitates (Fig. 3B).

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4 M. C. Ostrowski, unpublished observations.
Expression and Phosphorylation of ets-2 in Ovarian Tumor Cell Lines. Our work has demonstrated that ets-2 is a nuclear target for ras/raf/MAP kinase signaling pathways in fibroblasts and that this protein is activated by phosphorylation at threonine residue 72 (18, 19). A nondiscriminating ets-2 polyclonal antibody and an anti-pT72 ets-2 affinity purified antibody that only recognizes the phosphorylated form of ets-2 (19) were used to analyze extracts prepared from the ovarian tumor cell lines by Western blotting (Fig. 4). These experiments revealed that ets-2 could be detected with the former antibody only in the cells OVCAR3, OVCA 429, and OVCA433, which exhibited higher ratios of the ras-GTP complex (Fig. 4, compare Lanes 1, 4, and 5 with Lanes 2, 3, and 6). Using the anti-phospho-T72 antibody, we determined that ets-2 was phosphorylated at threonine 72 in all three cell lines that expressed this protein (Fig. 4B, Lanes 1, 4, and 5). The phosphorylated ets-2 protein that was detected was found exclusively in the nuclear fraction, and cytosolic ets-2 was not detected in any of the cell lines examined (data not shown).

Expression of uPA and the Activity of the uPA Promoter/Enhancer in Ovarian Tumor Cells Correlates with ras-GTP Levels. The uPA gene is a ras- responsive gene (17, 18, 33). Activation of this gene by ras signaling pathways occurs via an enhancer element termed the ras-responsive element (17), which consists of two DNA binding sites, one specific for the ets-family transcription factors and one specific for the AP1 family transcription factors (18, 33). This enhancer element is conserved in mouse and human genes (33).

Because ets-2 is phosphorylated at position threonine 72 in the three ovarian cell lines, we predicted that uPA mRNA expression should be high in these cells. Analysis of mRNA isolated from seven ovarian tumor cells by Northern blotting indicates that uPA mRNA expression correlates with the level of ras-GTP complexes and ets-2 phosphorylation (Fig. 5). Expression of uPA mRNA was detected in OVCA433, OVCA429, and OVCAR3 cells (>40% ras-GTP complex), but low expression of the 2-kb uPA mRNA was detected in the other four cell lines.

To more directly link these correlations between ets-2 phosphorylation and uPA expression, the activity of the uPA ets-AP1 ras-responsive enhancer was measured by transient transfection assays in the OVCA432 (low ras-GTP) and OVCA433 cells (high ras-GTP; Fig. 6A). This analysis revealed that the murine uPA promoter-enhancer was approximately eight times more active in OVCA433 cells than in OVCA432 cells when compared with an internal control Rous Sarcoma Virus-Secreted Alkaline Phosphatase reporter (see "Materials and Methods"). In contrast, a uPA reporter that contained a mutation at the ets-binding site in the compound enhancer element was equally active in both cell types (Fig. 6A).

The effect of the dominant-negative ras A57 (34) mutation on uPA reporter activity in these ovarian tumor cell lines was determined (Fig. 6B). In these experiments, the dominant-negative ras allele had little effect on the reporter activity in OVCA433 cells, but it decreased
the uPA reporter activity 8-fold in OVCA433 cells. Thus, either a cis
mutation in the ets-binding site of the enhancer or a coexpression of
the dominant-negative ras Asn17 allele could abolish the increased
uPA reporter gene activity in OVCA433 cells relative to OVCA432
cells.

In fibroblasts, ras and ets-2 can collaborate to superactivate the
uPA reporter (18). Similarly, in OVCA432 cells, the combination of
ras and ets-2 resulted in a 20-fold activation of the uPA reporter,
5-fold or 15-fold higher than the effect of ras or ets-2 alone, respecti-
vely (Fig. 6C). When an ets-2 protein that contained the alanine 72
substitution was used, superactivation of the reporter was not ob-
served (Fig. 6C). We have demonstrated previously that the alanine
substitution at position 72 does not grossly affect protein stability,
DNA binding activity, or nuclear localization ets-2 (18) but seems to
alter the association of ets-2 with co-activators necessary for transac-
tivation of the uPA reporter.4 These results are consistent with direct
activation of the compound ets-APl element by ras signaling path-
ways in the ovarian tumor cells tested.

DISCUSSION

In murine fibroblast models, increases in the ras-GTP complex
mediated by growth factor receptors (e.g., the c-fms proto-oncogene
product) results in the stable expression of genes that have enhancers
containing binding sites for the ets and API families of transcription
factors (33, 35, 36). The ets-2 gene product is one likely direct nucle-
tar target for this ras signaling pathway (18), and phosphorylation of this
factor at position threonine 72 correlates closely with the kinetics of

MAP kinase p42/p44 activation and ras/raf-responsive gene activa-
tion (19). To extend these observations beyond the fibroblast model
systems for cell transformation, studies using human ovarian tumor
cell lines were initiated.

In four normal ovarian epithelium primary cell culture samples as
well as in eight ovarian carcinoma cell lines, we found ras-GTP levels
to be substantially elevated above levels present in the fibroblast
control cell line. Only one of these cell lines, OVCAR5, was found to
contain a mutation in one of the three ras-family proto-oncogenes or
in the related gene TC21/R-ras2. When MAP kinase activation and
ets-2 phosphorylation status were tested in the group of ovarian cell
lines, a distinction could be drawn between cells exhibiting levels of
ras-GTP similar to the normal ovarian epithelial cells and those with
ras-GTP levels >40%, similar to the levels observed in fibroblasts
transformed by activated ras genes. Only in the latter group could
MAP kinase activation and ets-2 expression and phosphorylation at
residue threonine 72 be detected. In addition, expression of tras-
responsive genes, such as uPA, were increased in the latter group of
cells.

Analysis of the uPA promoter-enhancer in ovarian cell lines pro-
vided additional evidence, supporting the functional significance of
the correlations observed between ras/MAP kinase activation, ets-2
phosphorylation at threonine 72, and activation of uPA expression.
These experiments demonstrated significantly higher uPA reporter
activity in the OVCA433 cells (the cell line in which the ras pathway
is activated) compared with the activity in the OVCA432 cells.

Mutation of the ets-binding site in the compound ets-APl ras-respon-
sive enhancer element or expression of the dominant-negative ras
Asn17 gene eliminated the difference in reporter activity between the
two cell types. Additionally, ras and ets-2 could superactivate the uPA
reporter in OVCA432 cells, a cooperation that depended on an intact
threonine 72 phosphorylation site. Taken together, the data indicate
that a ras/MAP kinase/ets-2 signaling pathway defined in model
systems is also functional in human tumor cells, in which it promotes
expression of metastasis-related genes such as uPA (31, 37, 38).

The activation of ras p21 signaling can have diverse effects, even in
the same cell. For example, in the PC12 cell culture system for
neuronal differentiation, activation of ras signaling by the EGF re-
ceptor leads to mitotic cell growth, whereas activation of ras signaling
by the nerve growth factor receptor leads to neuronal differentiation
(39). An emerging hypothesis to account for these diverse effects of
ras on cell growth and differentiation is that the dose and duration of ras signaling are the crucial factors for determining how ras will affect a particular cell (reviewed in Ref. 32). In the PC12 cell line, the nerve growth factor is capable of converting a higher percentage of ras to the GTP complex versus the GDP complex for a longer period of time than is EGF; thus, differentiation versus cell growth is promoted (39). In the present study, our results are consistent with the hypothesis that the length and strength of the ras signal in the ovarian tumor cell lines studied is critical in determining the final outcome on uPA gene activation and, ultimately, cellular phenotype (32). The functional significance of the elevated ras-GTP levels (15–25% ras-GTP complex) in the normal ovarian cells and four of the eight tumor cell lines is unclear. The results suggest that the threshold of ras-GTP complex necessary for activation of the MAP kinase cascade is higher in the ovarian epithelial cells than in PC12 cells or fibroblasts, in which considerably lower levels of the ras-GTP complex can trigger MAP kinase activity (39, 40). Whether this is true for additional ras effector pathways [e.g., the phosphatidylinositol 3'-kinase pathway (15)], remains to be determined.

The tyrosine kinase inhibitor genistein (30) blocked formation of the ras-GTP complex in OVC443 cells. This suggests the possibility that activation of a growth factor/tyrosine kinase receptor autocrine loop could account for ras activation in the ovarian tumor cell lines. Ovarian tumor cells or cell lines have been reported to express the growth factors insulin-like growth factor I, basic fibroblast growth factor, transforming growth factor α, and colony-stimulating factor-1, as well as the cognate tyrosine kinase receptors (41–44). Furthermore, ovarian cancer cell lines can produce lysophosphatidic acid, which can activate ras signaling. Thus, several candidates for this model are available. Additionally, ras signaling itself can trigger the expression of a large number of growth factors and cytokines (17), suggesting a manner by which such an autocrine loop could be stably maintained once initiated.

A second possibility is that cell adhesion characteristics and/or the cytoskeletal structure of the tumor cells might lead to constitutive activation of ras. Activation of integrins by the appropriate ligand can activate ras/MAP kinase signaling (45). In this second model, initiating events (for example, mutation of tumor suppressor genes such as APC) might alter cell adhesion properties and the cytoskeleton (46, 47), resulting in stable activation of ras signaling pathways.

The low level of ras mutations present in certain types of tumors, including ovarian and breast tumors, has been interpreted to indicate that ras signaling pathways may be less important in multistage neoplastic progression of these tumor types (22–24, 28). However, as suggested by the present studies, ras signaling may be activated at multiple levels independent of actual point mutations in the ras genes (reviewed in Ref. 28). This leads to the conclusion that ras signaling pathways may contribute to neoplastic progression, even in tumors that are not frequently associated with mutations in ras genes (28). This is supported by a recent report demonstrating that MAP kinase expression and activity are amplified in breast cancer cells (48). What might the role of ras signaling be in these types of tumors? Results presented here demonstrate that one consequence of ras signaling in these cells is activation of ras-responsive genes such as uPA. uPA has long been associated with the processes of tumor invasiveness and metastasis (31, 37, 38). For example, the inhibition of uPA by specific anti-human uPA antibodies blocked infiltration of chick chorioallantoic membrane mesenchyme by human HEP-3 carcinoma cells and inhibited metastasis in this model (31). Thus, by activating the expression of uPA as well as metalloproteases (17, 24, 25), ras signaling may contribute to the invasive and metastatic potential of ovarian carcinomas.

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