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Ovarian Adenocarcinomas Express *fms*-Complementary Transcripts and *fms* Antigen, Often with Coexpression of CSF-1

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In earlier studies of oncogene expression in ovarian and endometrial neoplasms, the authors reported that high tumor levels of fms-***complementary transcripts correlate with high histologic grade and advanced clinical stage presentations. In this communication, they pursue these initial clinicopathologic investigations to demonstrate by*** in situ ***hybridization and immunohistochemistry that malignant epithelial cells of 14 of 14 invasive adenocarcinomas of the ovary express*** fms-***complementary transcripts. By Northern blotting and by reverse transcription, followed by polymerase chain reaction amplification, the authors also were able to demonstrate*** fms ***transcript expression in several ovarian and endometrial carcinoma-derived cell lines. Because about half (6/14) of the invasive adenocarcinoma specimens were shown to coexpress*** fms ***and colony-stimulating factor 1, the authors propose that the expression of this lymphohematopoietic cytokine and its receptor by ovarian adenocarcinomas could contribute to their proliferative and invasive characteristics*** in vivo. ***(Am J Pathol 1990, 137:135–147)***

Epithelial neoplasms of the ovary encompass a broad spectrum of lesions, ranging from localized benign hyperproliferative cystadenomas and neoplasms of borderline malignant potential to invasive adenocarcinomas and mixed-Müllerian tumors capable of intra-abdominal and distant spread.[1-5] For adenocarcinomas, degree of differentiation or histologic grade correlates strongly with likelihood of extraovarian spread, and the extent of disease at presentation or clinical stage is an excellent predictor of outcome after standard therapies.[4,5] How incremental stepwise changes in histology, extent of disease at presentation, and clinical behavior relate to parallel changes in the expression and/or activation of cellular proto-oncogenes, growth factors, or their receptors[6-11] remains unclear for ovarian epithelial neoplasms. In two preliminary studies of oncogene expression[1,2] in ovarian and endometrial epithelial neoplasms, we reported that tumor levels of *fms* colony-stimulating factor (CSF-1 receptor)-complementary transcripts correlated strongly with high-grade, high-stage presentations prognostic of poor outcome. In this report, we carry these investigations to their logical conclusion and demonstrate that neoplastic epithelial cells of ovarian adenocarcinomas indeed express *fms* transcripts and antigen with (or without) the *fms*-activating ligand (CSF-1) in tissue specimens and in adenocarcinoma-derived cultured cell lines.

Materials and Methods

Tissue Specimen Accrual, Preparation, and In Situ Hybridization

All tissue specimens were obtained from patients of the Hunter Radiation Therapy and Gynecologic Oncology Clinics of the Yale University School of Medicine, in accordance with Yale HIC protocol #3303. Lesions included two benign, three borderline neoplasms, and one grade 1 (mucinous), six grade 2 (serous), and seven grade 3 (serous and poorly differentiated) adenocarcinomas.[12] In-

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1 (mucinous), six grade 2 (serous), and seven grade 3 (serous and poorly differentiated) adenocarcinomas.[12] International Federation of Gynecology and Obstetrics Staging Classification (FIGO) clinical stage was determined from the clinical and operative findings by the involved gynecologic oncologists.[1,13]

Small samples (maximum 3-mm thick) of ovarian neoplastic or benign tissues were obtained during therapeutic or diagnostic procedures and placed into freshly prepared fixative (4% paraformaldehyde, 0.5% glutaraldehyde, 0.1 mol/l (molar) Na-phosphate (pH 7.5)) within 1 to 2 minutes of harvest; and aldehyde fixation continued for 4 to 6 hours. Specimens were processed for embedding in paraffin, serial 6-μ sections cut, floated onto polylysine-coated slides (three to four sections per slide), prepared for, and carried through ISH (50% formamide [37°C] followed by 4×, 2× [25°C, 5′ each] and 0.1× [37°C, 30′] saline sodium citrate [SSC] washes) and nuclear track emulsion autoradiography as previously described by ourselves and others.[2,3,14-22]

In situ hybridization probes were derived from the coding sequences of enterobacterial beta-lactamase[23] (EcoRI, PstI [0.7 kb]; a negative control) and v-*fms*[24] (PstI [0.4 kb]) and were labeled by random primer extension with alpha-^{35}S-labeled deoxycytidine triphosphate (dCTP) and thymidine triphosphate (dTTP) to specific activities of $\sim 5 \times 10^8$ dpm/μg DNA.[25] Sections of confluent monolayers of BeWo choriocarcinoma cells[26,27] (which show consistently elevated levels of expression of *fms*-complementary transcripts[2,27]) were grown in Weymouth's medium + 10% fetal calf serum (FCS) (37°C, 5% CO_2) and fixed, embedded, and processed with each experiment as positive control specimens, while samples of benign tissues (eg, cases 1 and 2) were employed as negative controls.

Computer-assisted Grain Count Acquisition and Data Analysis

The hematoxylin and eosin (H&E)-stained ISH autoradiograms were analyzed by light microscopy (Figure 1) and grain counts quantified with the aid of the Olympus Corporation Cue 2 VISION computer-assisted image analysis system (Tokyo, Japan). Randomly chosen fields of epithelium or stroma were identified by the microscopist, and the grain count density in each field quantified with the Cue 2 image analysis system, which is able to resolve black silver grains from other background cellular features and to count the total number of silver grains in each (100×) oil immersion field. Grain count density in grains per micron^2 is converted to hybrids detected (per micron^2 or high-power field) (Figure 2) by multiplicative factors that take into account field size, molecular weight, and specific activity of individual probes, and exposure duration, and assume an ideal emulsion efficiency of 1.0*.

*Immunohistochemical Staining with Anti-(feline)-*fms *and Anti-human-CSF-1 Antibodies*

Six-micron sections were prepared on polylysine-coated slides and heated at 65°C for approximately 1 hour. Tissue sections were deparaffinized in xylene and rehydrated in solutions of decreasing ethanol concentration. Endogenous peroxidase was quenched by a 30-minute incubation with 0.5% hydrogen peroxide in methanol. Sections were washed in phosphate-buffered saline (PBS, pH 7.4), and preincubated for 30 minutes in 10% normal goat or horse serum to block nonspecific antibody binding.

After PBS wash, sections were incubated for at least 1 hour with a 1:800 dilution of 2E8 mouse anti-feline *fms* monoclonal (ascites) prepared by from LR & VR[29] (Figure 3), or a 1:500 dilution of an anti-human CSF-1 rabbit polyclonal antibody obtained from E. R. Stanley (Figure 4) and coworkers.[30] Negative controls were run in parallel using the same dilutions of normal mouse ascites (Sigma Chemical company, St. Louis, MO) or normal rabbit serum (Vector Corp., Burlingame, CA) in place of the primary antibody. After PBS wash, sections were incubated for 30 minutes with biotinylated horse anti-mouse or goat anti-rabbit antibody. Tissue sections were rinsed in PBS and incubated 30 minutes in an avidin-peroxidase complex solution (Vector). After PBS wash, sections were incubated in 0.25% diaminobenzidine (DAB) and 0.5% hydrogen peroxide in phosphate buffer (pH 7.4), washed in tap water, stained with H&E, dehydrated in baths of increasing ethanol concentration, cleared in xylene, and coverslipped.

Cell Lines

The endometrial carcinoma cells line AN3CA and the ovarian carcinoma cell line SKOV3 were obtained from the American-Type Culture Collection, while YAOVBIX, YAOVWEI, and YAOVDK were newly isolated (<20th passage) human papillary serous ovarian adenocarcinoma-derived cell lines (see below). HEY[31] ovarian carcinoma cells were obtained from the original investigator. All carcinoma cell lines were cultured in DME/F12 Ham's media supplemented with 10 μg/ml each insulin and transferrin

* Our estimates of hybrids per micron^2 and hybrids/100× field are presented in terms of an 'ideal' emulsion, as the absolute efficiency of NTB-2 emulsion for S beta particles is not precisely defined, although (by basic physical dosimetric considerations[25]) it is at most 10%.[28]

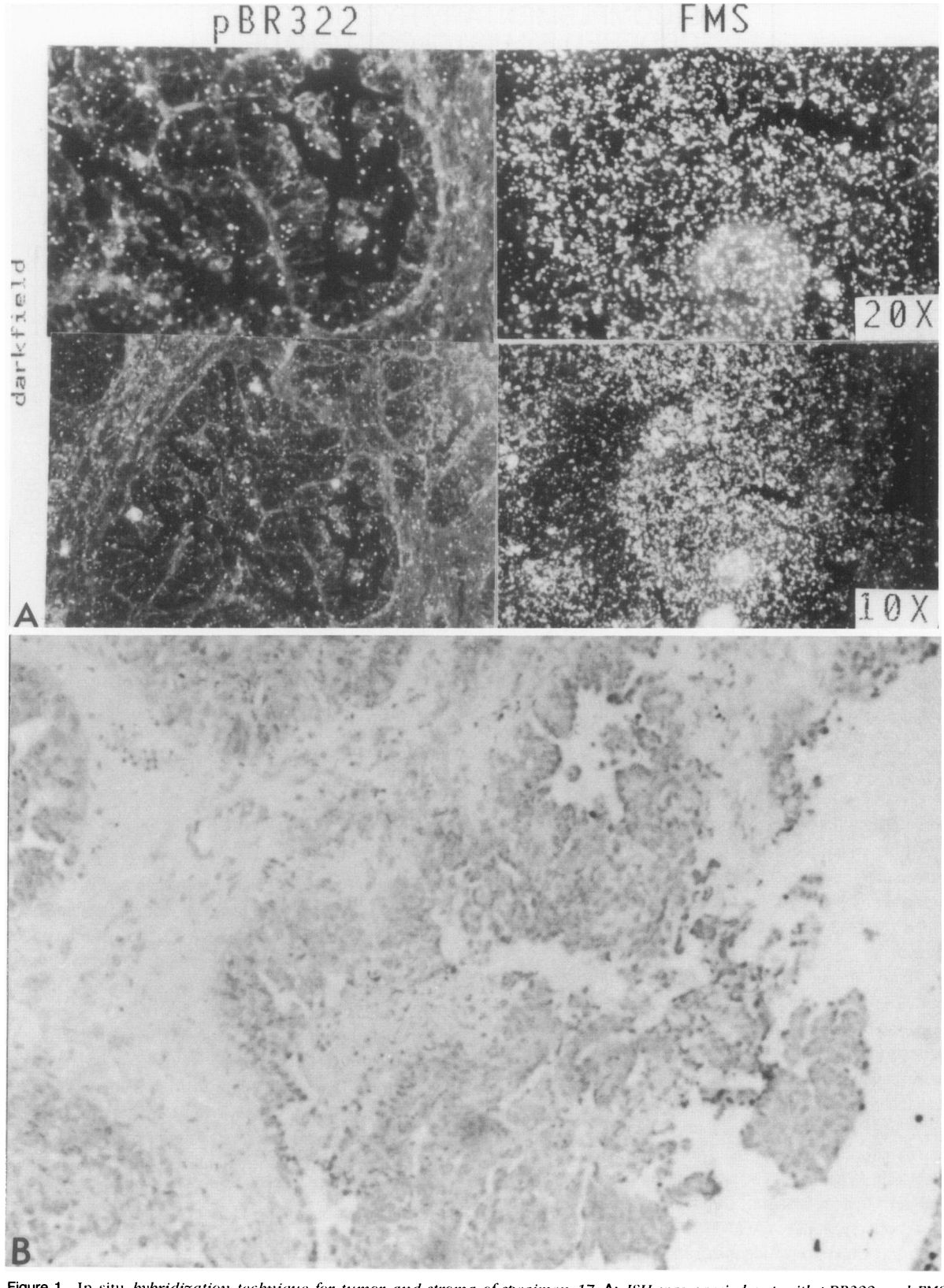

Figure 1. In situ *hybridization technique for tumor and stroma of specimen 17.* A: *ISH was carried out with pBR322 and FMS probes as described in Methods and photographed at ×10 and ×20 and darkfield illumination to clearly demonstrate grain count localization over the tumor epithelium as opposed to stromal or tissue-free areas of the section for the FMS but not the pBR322 probe.* B: *Same field as panel B for the FMS probe (×10) but photographed with bright field illumination to demonstrate (H&E stained) histologic detail.*

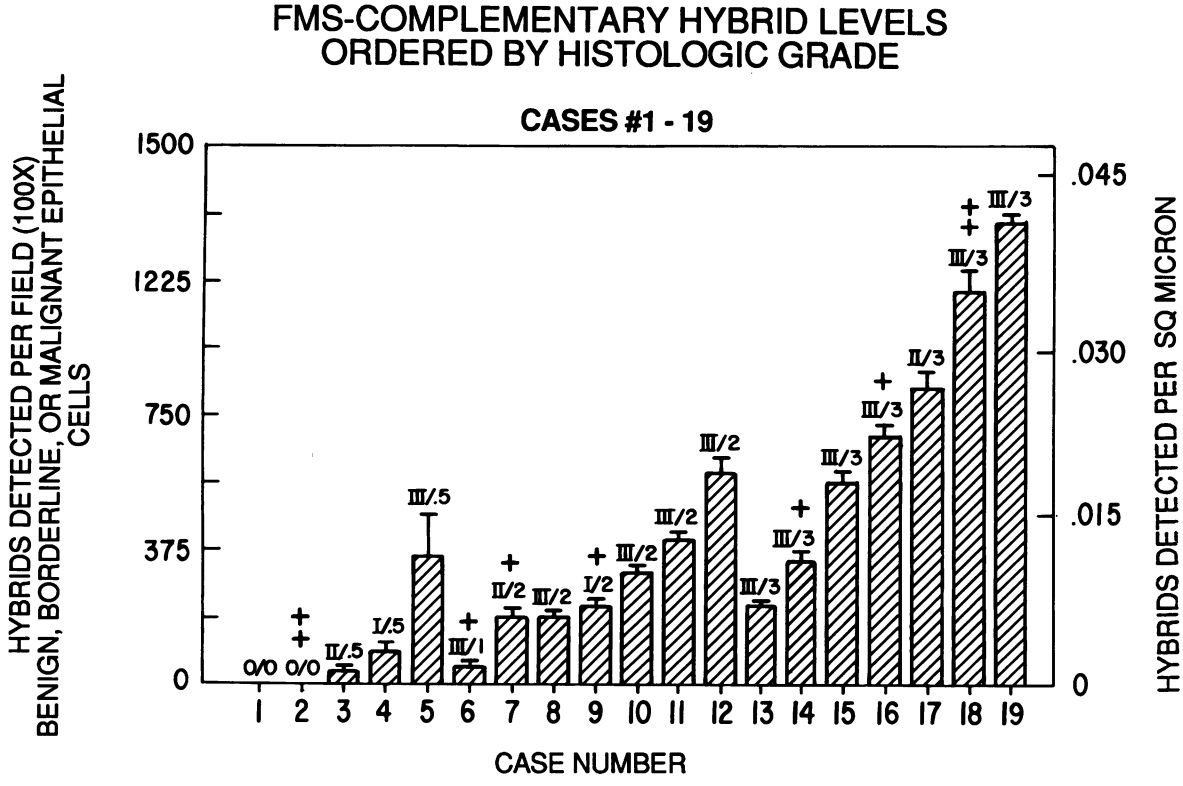

Figure 2. *Quantitated* in situ *hybridization (detected hybrids per micron² and per × 100 field) for 2 benign, 3 borderline, and 14 invasive ovarian adenocarcinomas with immunohistochemical staining score for coexpression of CSF-1 antigen.* In situ *hybridization and data analysis is carried out as described in Methods and cases are ordered by histologic grade and by* fms*-complementary hybrid levels. Clinical stage is indicated by the roman numeral and histologic grade by the arabic numeral above each number.* Fms*-complementary 'hybrids detected' per HPF and per micron² for benign, borderline, or invasively malignant epithelial cells is plotted* vs. *case number. IHC analysis for benign, borderline, or malignant epithelial cell coexpression of CSF-1 antigen was carried out as described in Methods and the semiquantitative score (no symbol = 0, + = 1+, ++ = 2+) of staining intensity is indicated above each data bar. Cases 2, 6, 7, 9, 14, 16, and 18 stained positively for CSF-1 antigen; the rest were negative.*

and 1% FCS until 48 hours before RNA or protein analyses, after which the same media with no FCS was employed until cell harvest. Such 'serum starvation' was necessary in most cases, for optimal *in vitro* expression of *fms*-complementary transcripts and protein (data not shown).

Three of our ovarian adenocarcinoma cell lines (YAOVBIX, YAOVDK, and YAOVWEI) were isolated from single epithelial clones isolated by culture (in the above-described medium) of (malignant cell-positive) ascites of patients with advanced grade 3 ovarian papillary serous adenocarcinomas. All three formed morphologically epithelial colonies and confluent monolayer layers in culture, and all stain strongly with anti-cytokeratin[32,33] (Boehringer-Manheim, Indianapolis, IN) and anti-epithelial membrane antigen[34] (Dako, Glostrup, Denmark) antibodies (unpublished observations). YAOVDK produced metastasizing tumors in four of four nude mice, and YAOVBIX produced tumors in two of three nude mice that disseminated intraperitoneally after the interperitoneal injection of approximately 10^6 cells (unpublished observations); data on the intraperitoneal tumorigenicity of YAOVWEI cells in nude mice is not yet available.

RNA Extraction and Northern Blot Hybridization

Total cellular RNA was isolated from flash-frozen tissue or cultured cells using the guanidium isothyocyanate/CsCl method. Absorbance of each RNA sample was measured at 260 nm and 5 to 50 μg of denatured RNA loaded per lane of a formaldehyde agarose gel (Figure 5A, B). The position of ribosomal 18S and 28S RNAs were determined by ethidium bromide staining. The agarose gels were blotted to nitrocellulose or Gene-Screen Plus filters (New England Nuclear, Boston, MA), baked, and hybridized with high specific activity labeled probes for (^{32}P: ~10^9 dpm/μg) human c-*fms* probes (3′-probe [terminal 1.1 kb; PstI[35]]; 5′-probe [proximal 1.3 kb; EcoRI[36]]), and beta-actin[37] probes (Figure 5A, B), washed to high stringency, and exposed to XAR-5 film for autoradiography.

PCR Amplification of c-fms *Complementary cDNA Sequences in Total RNA of Ovarian and Endometrial Carcinoma-derived Cell Lines*

21-mer oligonucleotides complementary to nucleotides 996-1016 (sense: ctttgatgtcttcctccaaca) and 1514-1494

Table 1. *Clinicopathologic Data on 2 Benign, 3 Borderline and 14 Malignant Ovarian Specimens*

Case	Age	Cell type	Grade	Stage
1	30	Cystadenofibroma	0	0
2	65	Mucinous cystadenoma	0	0
3	42	Serous, borderline	0.5	2
4	87	Serous, borderline	0.5	1
5	60	Serous, borderline	0.5	3
6	84	Mucinous carcinoma	1	3
7	79	Serous carcinoma	2	2
8	24	Serous carcinoma	2	3
9	45	Clear cell carcinoma	2	1
10	74	Serous carcinoma	2	3
11	73	Serous carcinoma	2	3
12	67	Serous carcinoma	2	3
13	77	Serous carcinoma	3	3
14	52	Serous carcinoma	3	3
15	57	Serous carcinoma	3	3
16	60	Serous carcinoma	3	3
17	67	Endometrioid carcinoma	3	2
18	43	Undifferentiated carcinoma	3	3
19	62	Endometrioid carcinoma	3	3

Patient #, age, diagnosis and histologic grade and clinical stage are presented for 24 specimens harvested as described in Methods.

Grade 0 = benign, grade 0.5 = borderline, and grades 1–3 and (FIGO) stage 0–3 are as described in Methods.

(anti-sense: cttacctctgggggtatcga) of the published c-*fms* transcript sequence[36] were synthesized and provided by the Genetics Institute of Cambridge, Massachusetts. Starting material was total RNA (100 ng) (Figure 6) isolated from BeWo choriocarcinoma cells (a c-*fms*–positive control), NIH3T3 fibroblasts (*fms*-negative), and human ovarian and endometrial carcinoma-derived cell lines. By the method of Kawasaki et al,[38] the antisense oligonucleotide was used first to prime the synthesis by reverse transcriptase of a cDNA copy and (along with the sense oligonucleotide) to amplify—by 25 cycles of the polymerase chain reaction (PCR) of Taq DNA polymerase—a 519 nucleotide fragment of the c-*fms*-transcript (cDNA) sequence-spanning nucleotides 996-1514 of the published sequence.

Results

To further investigate and localize the *fms* gene expression in ovarian carcinomas, ISH and immunohistochemical staining (Figures 1 to 3) were carried out with *fms*-complementary DNA probes and the 2E8 MAb[29] prepared against the feline *fms* protein. *In situ* hybridization localized *fms*-complementary transcripts to tumor epithelium (Figures 1, 2) in 14 of 14 adenocarcinomas and one of the borderline neoplasms, with higher levels of *fms*-complementary transcripts observed in the high-grade, advanced-stage neoplasms (Figure 2). However, the level of resolution of autoradiographic ISH with ^{35}S-labeled probes could not exclude a 'tumor-infiltrating' macrophage origin for these *fms*-complementary transcripts, and a more precise cellular localization of *fms* expression was needed. Immunohistochemical staining carried out with the anti-*fms* 2E8 antibody clearly localized *fms* antigen expression to malignant epithelial cells (and histiocytes—a valuable positive control for *fms* protein: Panels C′ and D′) of all 14 ovarian tumor specimens in which significant levels of *fms*-complementary hybrids had been observed by ISH (Figure 2—Cases 6 through 19; Figure 3A—Panels C and D) and to BeWo choriocarcinoma cells (Figure 3A—Panel E), a positive control cell line already known to express moderately high levels of *fms* mRNA and protein.[27,29] These same *fms*-positive tumor cell structures were keratin (A1+A3)-positive and *Leu*-M1 negative (Figure 3: Panels F and G), and hence are true epithelial cells and not contaminating histiocytes. The anti-*fms* antibody did not stain the tumor epithelial cells of benign or borderline neoplasms (eg, Figure 3, Panels A and B), where little or no *fms*-complementary hybrids were detected by ISH (Figure 2).

Confirmatory Northern blot analyses (Figure 5A) were carried out on total cellular RNA isolated from those cases where sufficient, flash-frozen tumor tissue was available. Despite technical problems of uneven sample load and degradation, *fms*-complementary transcripts were observed that hybridized well to human c-*fms* probes derived from both 5′ and 3′ regions of the coding sequence in RNA isolated from both the tumor and the (positive control) normal placental specimens. The "smears" of low (<4 kb) molecular weight species observed both in the tumors (Lanes C, D, E) and the positive control (Lane F) RNA are most likely the consequence of RNA degradation in both the tumor and placental specimens, rather than an indication of true heterogeneity of transcripts *in vivo*.

In no case, however, did the tumor RNA contain *fms*-complementary transcripts larger than the ~4 kb transcript of the placental control. Hence, such results could still reflect tumor infiltration by *fms*-positive macrophages; and confirmatory studies *in vitro* with ovarian adenocarcinoma-derived cultured cell lines were mandatory.

Of the ovarian carcinoma cell lines studied, SKOV3 (ovarian), HEY (ovarian), and YAOVBIX (ovarian) cells expressed detectable levels of a normal (or near normal) ~4-kb *fms*-complementary transcript by Northern blot analysis (Figure 5B), as did a BeWo choriocarcinoma positive control. YAOVDK (ovarian) and AN3CA (endometrial) expressed lesser amounts, whereas YAOVWEI (ovarian) and NIH3T3 cells express little or no *fms*-complementary message. Reverse transcription followed by PCR (Figure 6) of cDNA sequences revealed the specific amplification of the same 0.52-kb cDNA sequence fragment in BeWo choriocarcinoma cell RNA and in RNA isolated from three ovarian and one endometrial adenocarcinoma-derived cell lines, but not from NIH-3T3 cell RNA. No amplified *fms*-complementary sequences were observed when ei-

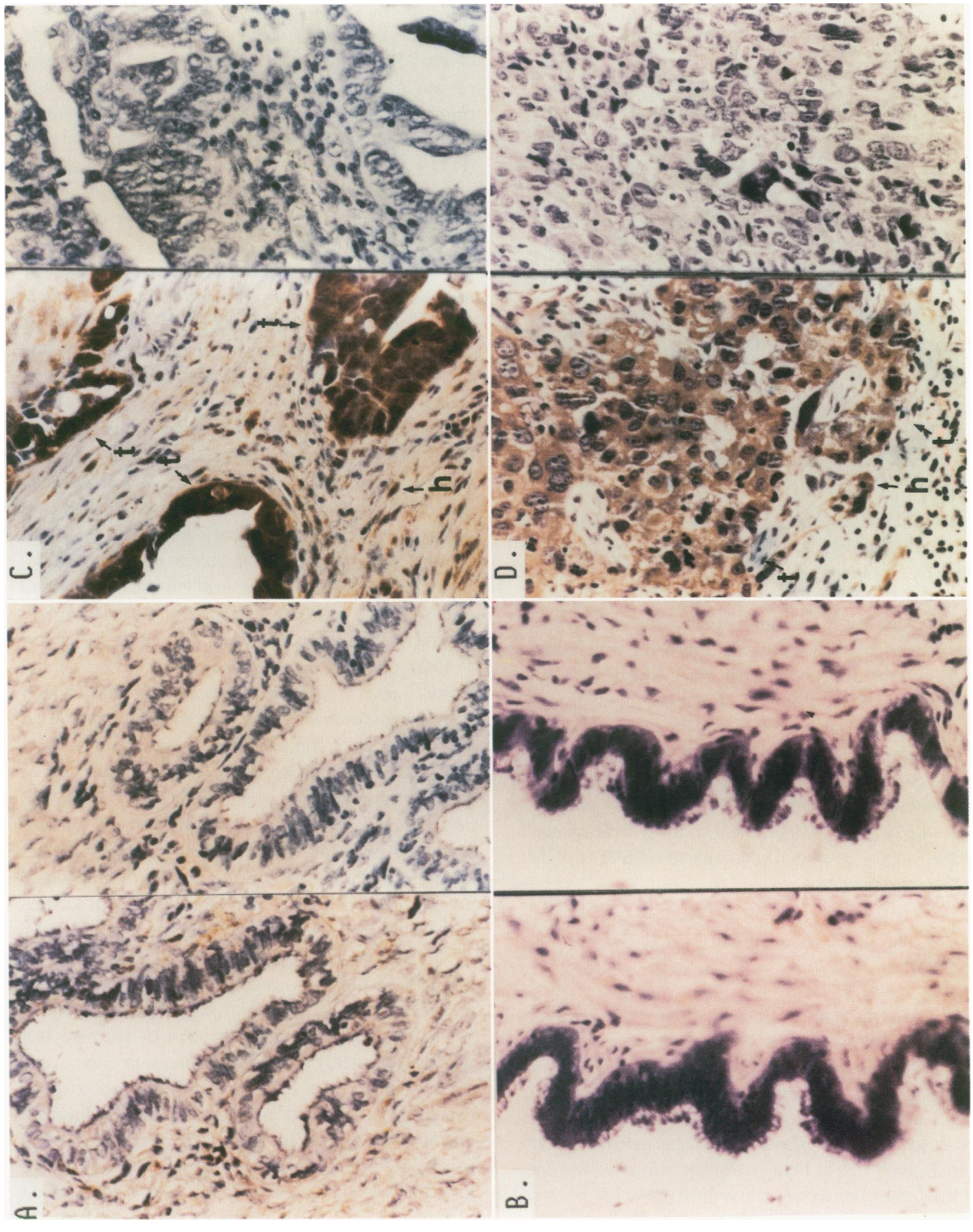

Figure 3

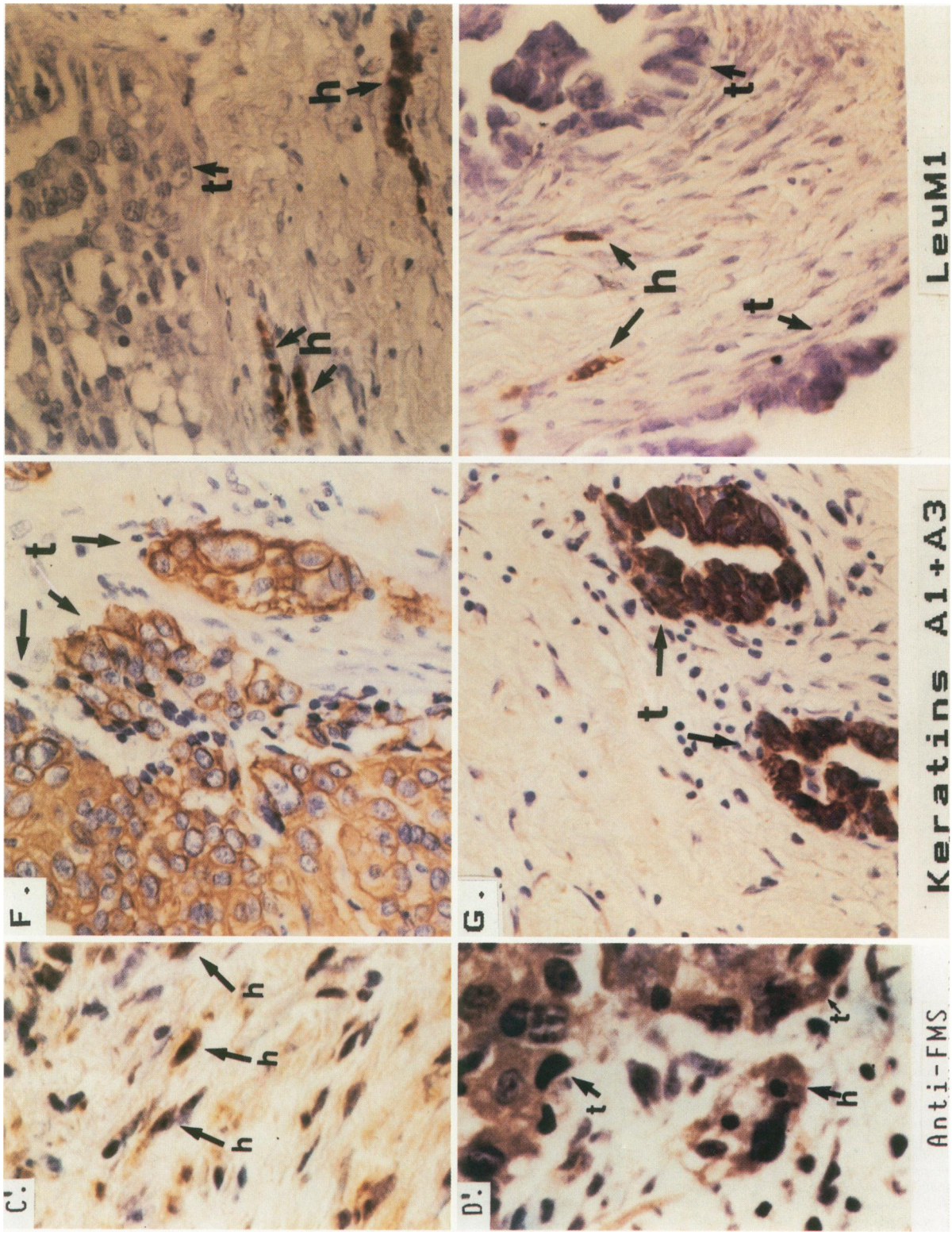

Figure 3 (*continued*)

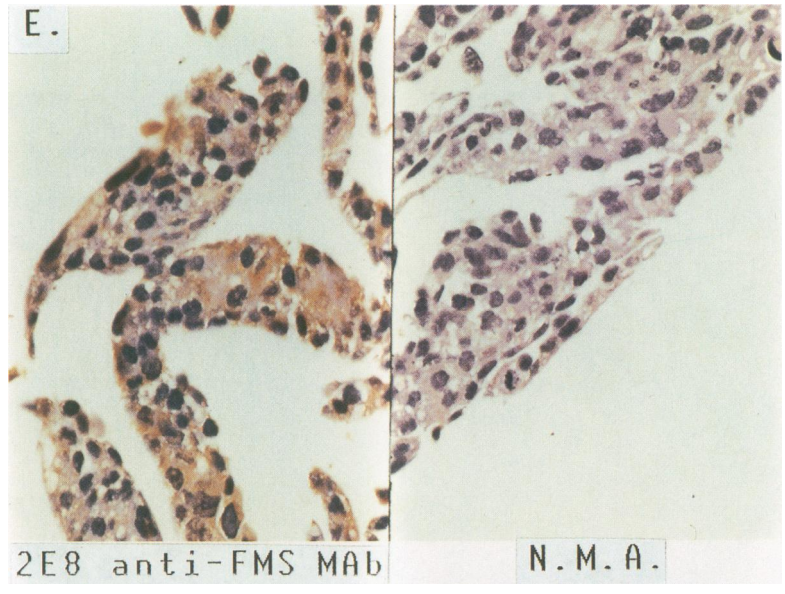

Figure 3. *Immunohistochemical staining with anti-*FMS *monoclonal antibody for a benign, borderline and two invasive ovarian adenocarcinoma specimens and (positive control) BeWo choriocarcinoma cells: IHC staining with the 2E8 anti-*fms *monoclonal antibody and a normal mouse ascites (N.M.A.) negative control as described in Methods is carried out for a benign cystadenoma [case 1; ×40] in* **A**, *a borderline ovarian adenocarcinoma [case 3; ×40] in* **B** *and two grade invasive adenocarcinomas in* **C** *[case 13: ×20] and* **D** *[case 18; ×20] with BeWo choriocarcinoma cells in* **E** *[×40] as a positive control. Arrows indicate putative tissue histiocytes ('h') and tumor cell ('t') structures.* **C'** *and* **D'** *present 'blow-up' photos of some of these regions from* **C** *and* **D**, *respectively.* **F** *and* **G** *present sections from cases 13 and 18 IHC-stained with monoclonal antibodies prepared against either cytokeratins A1 + A3[32,33] (a marker of carcinoma cells that is not expressed by histiocytes)—or against* LeuM1 *(Dako), an IHC marker for histiocytes and leukocytes[56] in paraffin sections. Both monoclonals were used at final concentrations of 1 μg/ml.*

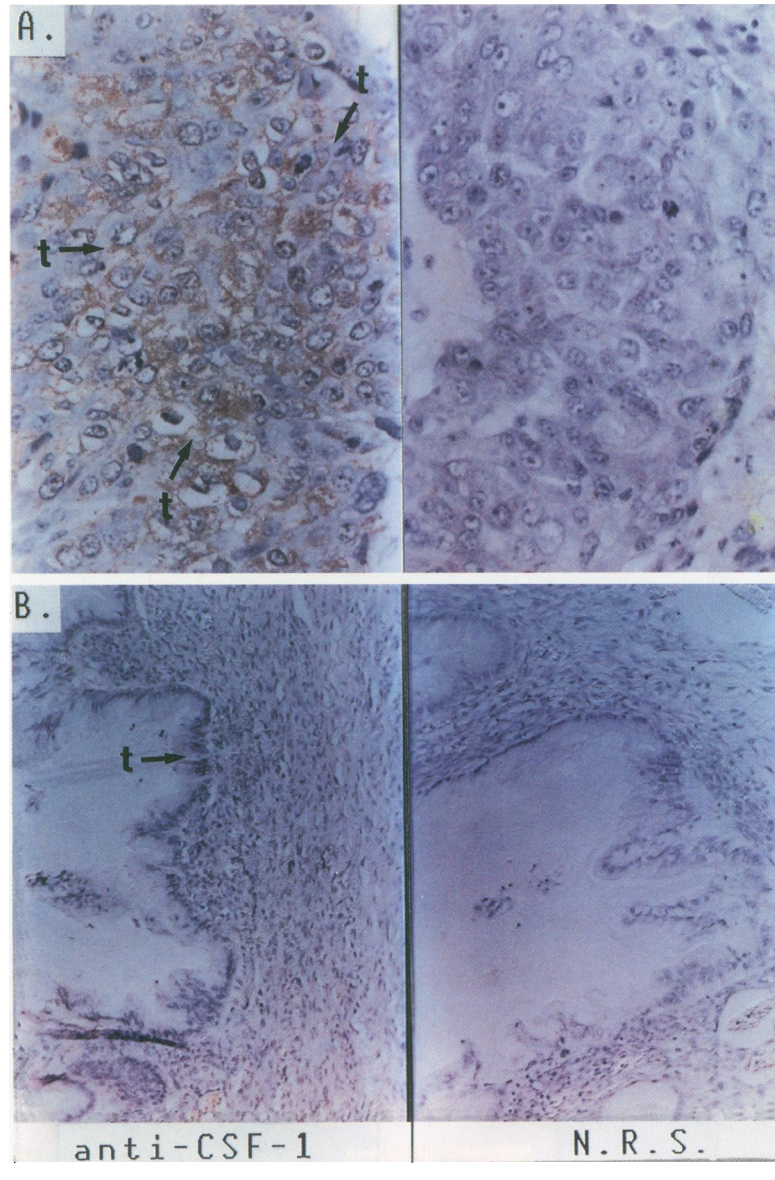

Figure 4. *Immunohistochemical staining with anti-human-CSF polyclonal antiserum for a benign (case 1) and an invasive grade 3 adenocarcinoma specimens (case 18): IHC staining with the rabbit polyclonal anti-human-CSF-1 antiserum and a normal rabbit serum (N.R.S.) negative control as described in Methods is carried out for case 18 (***A***; ×20) and case 1 (***B***; ×20). IHC staining of malignant cells is heterogeneous with some cells stained much more strongly than others; arrows indicate a cluster of positively staining tumor cells (t). Staining of stromal elements cannot, however, be excluded and is indeed expected, as CSF-1 is a known product of proliferating fibroblasts and other benign mesenchymal cells.[40,52]*

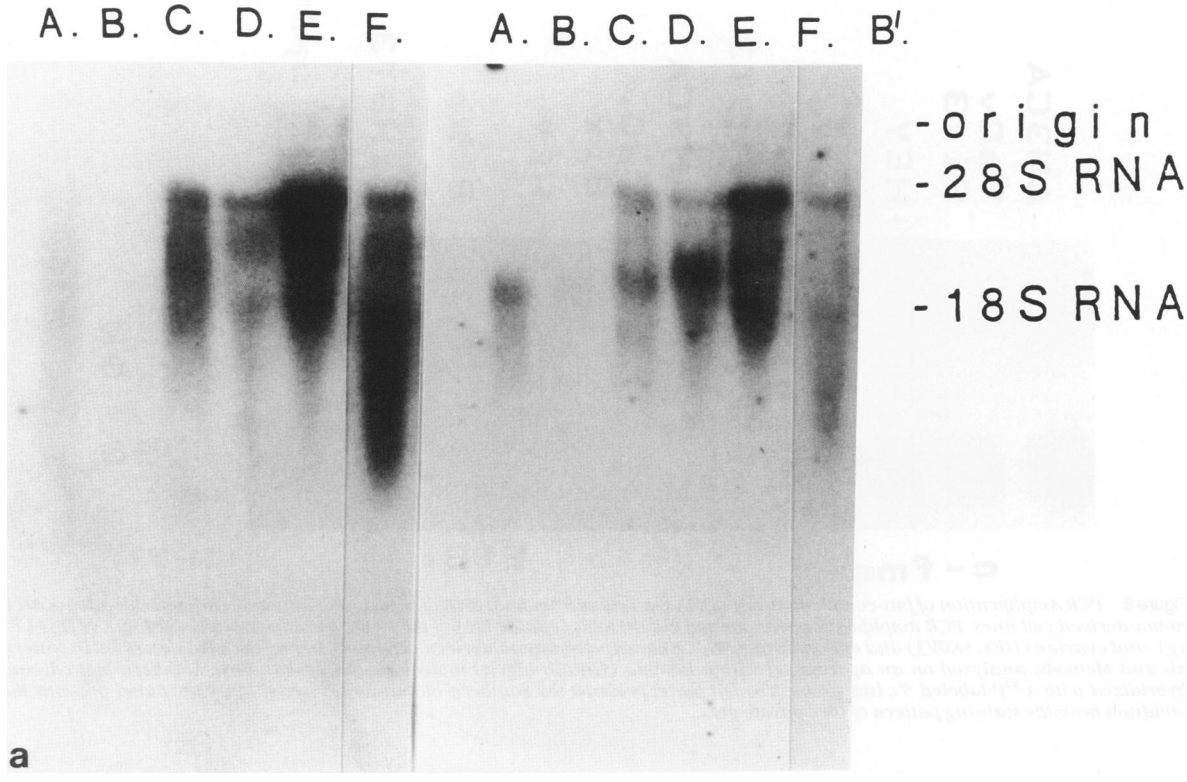

Figure 5. *Northern blot hybridization to total cellular RNA isolated from ovarian adenocarcinoma and ovarian and endometrial adenocarcinoma-derived cell lines. Total cellular RNA was isolated and analyzed by formaldehyde/agarose gel electrophoresis as described in Methods.*[18] *The gel was blotted to nitrocellulose, baked, and hybridized with labeled (^{32}P: ~ 10^9 dpm/μg) c-*fms *probe or c-*fms *probe + probe for beta-actin.*[37] *The 3' protein kinase domain human c-*fms *probe (PstI) is that of Browning, et al,*[35] *and the 5' domain probe (EcoR1) that of Coussens et al.*[36] *Panel A presents the autoradiogram of the filter hybridized to the 3' domain probe (left half of photograph), which was then stripped and rehybridized with probes to the 5' domain of c-*fms *and to beta-actin. Specimens are: Lane A: Case 6 Grade 1 Stage I ovarian mucinous adenocarcinoma (10 μg); Lane B: Cultured human fetal foreskin fibroblasts (5 μg); Lane C: Stage III, Grade 3 ovarian adenocarcinoma (20 μg); Lane D: Grade 3, Stage III adenocarcinoma (50 μg); Lane E: Case #8 Grade 2, Stage III adenocarcinoma (50 μg); Lane F: Culture villous trophoblast (20 μg); Lane B': Same as Lane B but printed with high contrast. Cultured villous trophoblast tissue was included as a positive control for* fms *and actin, cultured human fetal foreskin fibroblasts were included as a positive control for actin and a negative control for* fms.

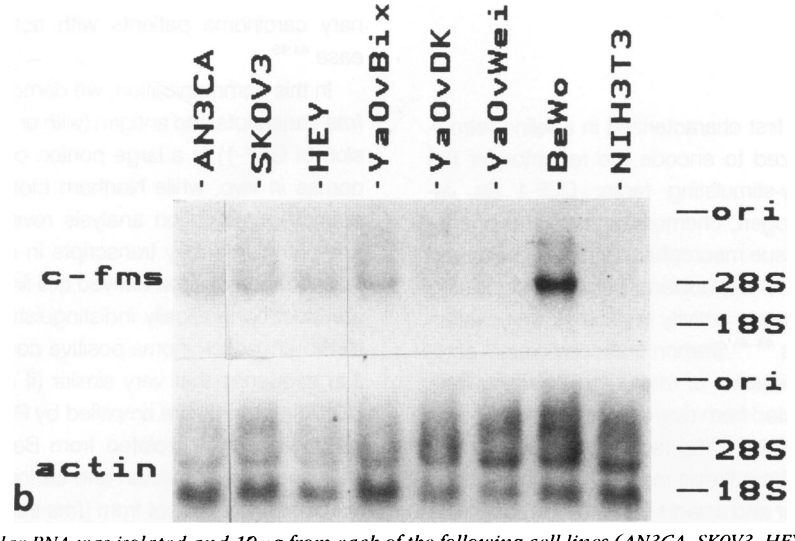

Figure 5B. *Total cellular RNA was isolated and 10 μg from each of the following cell lines (AN3CA, SK0V3, HEY, YAOVBIX, YAOVDK, YAOVWEI, BeWo, and NIH 3T3) was electrophoresed, and Northern blotted as described in Methods and hybridized first to a 5' c-*fms *probe (upper half of figure), washed to high stringency, and then stripped and rehybridized to a probe for beta actin and washed to high stringency (lower half of figure). A monodisperse ~ 4kb* fms*-complementary RNA is observed in the AN3CA, SK0V3, HEY, and YAOVBIX, and to a lesser extent to YAOVDK cells, but not in the YAOVWEI or NIH3T3 cell lines.*

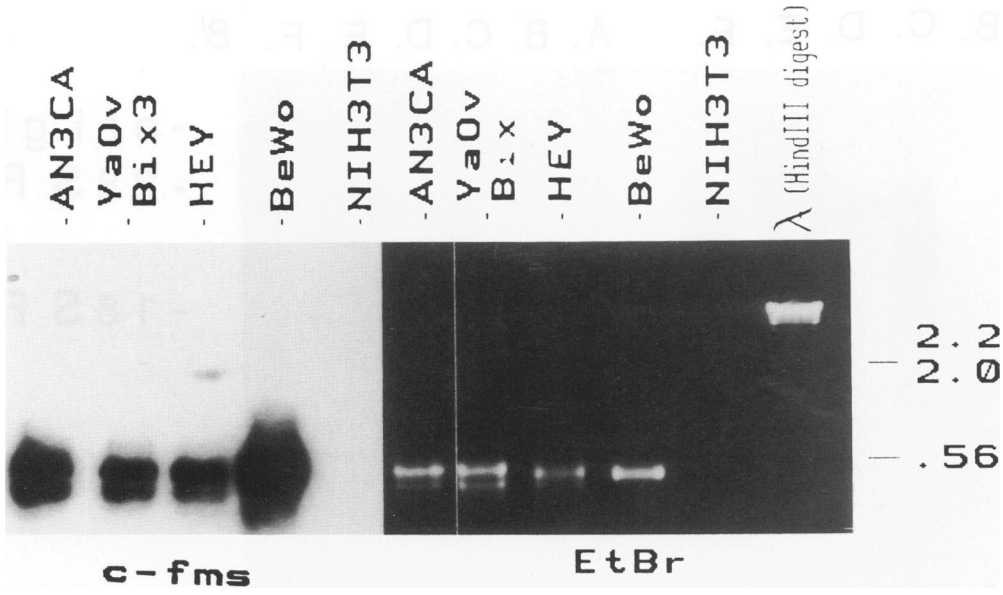

Figure 6. *PCR Amplification of fms-complementary cDNA sequences from total RNA of BeWo, ovarian, and endometrial adenocarcinoma-derived cell lines. PCR amplification was carried out on total cellular RNA of the BeWo (positive control—10 ng), NIH3T3 (1 μg), and ovarian (HEY, SKOV3) and endometrial (AN3CA) adenocarcinoma-derived cell lines (100 ng each) as described in Materials and Methods, analyzed on an agarose gel with a lambda (HindIII digest) molecular weight standard, Southern blotted, and hybridized with a ^{32}P-labeled 5'c-fms probe. The left panel presents the Southern blot autoradiogram; the right panel presents the ethidium bromide staining pattern of the agarose gel.*

ther the reverse transcriptase or either of the primer oligonucleotides was omitted (not shown).

Immunohistochemical staining with an anti–CSF-1 polyclonal antibody[30] (Figure 4) demonstrated the presence of CSF-1 antigen in tumor epithelial cells for 6 of 14 invasive adenocarcinomas (cases 6 through 19), which also express *fms*-complementary transcripts and *fms* antigen (Figure 2).

Discussion

The *fms* oncogene, first characterized in a feline retrovirus, is now recognized to encode the receptor for the macrophage colony-stimulating factor CSF-1 (ie, M-CSF)—a known mitogen, chemoattractant, and phenotypic 'activator' of tissue macrophages,[39,40] a growth and differentiation factor for trophoblast,[41,42] and a circulating 'tumor marker' of disease activity in patients with ovarian and other neoplasms.[43–45] Slamon and coworkers[11] actually did report the presence of *fms*-complementary transcripts in RNA extracted from ovarian carcinomas 6 years ago, but their RNA dot blotting technique could not discriminate tumor cell from tissue macrophage transcripts. More recently, Walker and coworkers[46] have reported the expression of *fms*-like transcripts in (rat) bronchial carcinoma derived cell lines, Feldman and Eisenbach[47] have implicated *fms* or a close-related gene in the lung metastasizing phenotypes of certain subclones of mouse tumor cell lines, and at least one breast carcinoma cell line has been reported to express a '*fms*-complementary' transcript.[48] In addition, we have reported that tumor epithelial expression of *fms*-complementary transcripts, often with coexpression of CSF-1, is commonly observed *in vivo* in many endometrial[2] and breast[20] adenocarcinomas. We also have observed CSF-1 (transcript and/or protein) expression by a variety of ovarian and endometrial cell lines[43,45] and have noted marked elevations in circulating CSF-1 levels in ovarian, endometrial, and some pulmonary carcinoma patients with active or recurrent disease.[44,45]

In this communication, we demonstrate expression of *fms* transcripts and antigen (with or without the coexpression of CSF-1) in a large portion of ovarian adenocarcinomas *in vivo*, while Northern blot and PCR cDNA sequence amplification analysis reveal the expression of *fms*-complementary transcripts in ovarian and endometrial adenocarcinoma-derived cell lines. These transcripts are electrophoretically indistinguishable from those of a BeWo choriocarcinoma-positive control and so similar to it in sequence that very similar (if not identical) 0.52-kb cDNA fragments are amplified by PCR after reverse transcription of RNA isolated from BeWo choriocarcinoma cells and several ovarian and endometrial carcinoma-derived cell lines, but not from (*fms* transcript) negative controls. Hence, this transcript is unlikely to be derived from either c-*kit*[49] or c-PDGF[50] receptor (two genes known to be related to c-*fms* but known to encode receptor proteins that recognize ligands other than CSF-1), and is most likely transcribed from either the c-*fms* locus or a

gene so closely related to the latter in sequence that oligonucleotides chosen from its cDNA sequence can be used to amplify electrophoretically indistinguishable (*fms*-complementary) cDNA sequence fragments from BeWo and ovarian adenocarcinoma cell total RNA (Figure 6).

Why or how c-*fms* or a very closely related gene come to be expressed in ovarian adenocarcinomas remains unclear. We have failed (data not shown) to detect genomic DNA c-*fms* rearrangements or amplification in any ovarian tumor (23 tumor, three benign specimens) or tumor-derived cell line by Southern blotting, while our Northern blot and PCR analyses do not reveal evidence for grossly abnormal *fms*-complementary transcripts in ovarian adenocarcinomas or tumor-derived cell lines. Complete sequence analysis (currently in progress by cDNA cloning and PCR) will be necessary to determine whether the transcripts we observe are actually those of c-*fms* and, if so, to further determine whether they contain point mutations or small insertions or deletions able to affect gene expression and/or function. It also is very possible that levels of the expression of this ovarian adenocarcinoma *fms*-complementary transcript are under the control of other genes (transcriptionally or post-transcriptionally) whose function or expression is abnormal in invasively malignant neoplasms (eg, a recessive 'oncogene,' etc). We are now investigating such possibilities in several ovarian, endometrial, and breast adenocarcinoma-derived cultured cell lines that, in preliminary studies, bind ^{125}I CSF-1 and display marked changes in tyrosine phosphoprotein content after CSF-1 treatment (unpublished observations).

Because macrophage colony stimulating factor (M-CSF or CSF-1), the activating ligand for the *fms* protein, is a ubiquitous mitogen synthesized by proliferating fibroblasts and other stromal elements,[39,40,51] CSF-1 should be readily available at primary or metastatic sites to act on tumor epithelial cells of invasively malignant ovarian adenocarcinomas that express its receptor. Low-grade or benign ovarian neoplasms may be much less susceptible to this omnipresent stimulus by virtue of lower or non-expression of the *fms*-complementary transcripts and antigen (Figure 2). The physiologic importance of such a CSF-1–'responsive' protein or 'receptor' in ovarian neoplastic epithelial cells, however, remains unclear. We are actively investigating the possibility that (tumor or stromally produced) CSF-1 alters the physiology and phenotypes of CSF-1 receptor-positive carcinoma cells, macrophages, and trophoblast similarly, and thus contributes to the genesis and dissemination of ovarian carcinomas *in vivo.*

Our demonstration by IHC (Figures 2, 3) that benign and malignant ovarian epithelial cells can express CSF-1 and *fms* antigens (in 6 of 14 adenocarcinomas) is also not surprising in the context of observations published by Pollard and coworkers,[42] who reported significant levels of CSF-1 gene expression in benign mouse endometrial glandular epithelium, and by ourselves and several others who have described CSF-1 transcription and synthesis by ovarian,[43–45] endometrial,[43,45] breast,[52] pancreatic,[53] and pulmonary[50] tumor cell lines. Relevant too are our own observations of markedly elevated plasma CSF-1 levels in patients with ovarian and other carcinomas with active or recurrent disease.[43–45] Such autocrine and/or circulating CSF-1 is obviously available to act on (and perhaps influence the growth and metastatic behavior of) tumor cells expressing a CSF-1 receptor.

More recently, several other investigators have reported that other lymphohematopoietic cytokines exert direct mitogenic effects on breast[54] (GM-CSF) and colonic[55] (GM-CSF, G-CSF, IL3) epithelial neoplasms. In light of these observations, our original proposal[1,2] for paracrine or autocrine functions of for CSF-1 and its receptor in invasive ovarian adenocarcinoma becomes much less surprising, as it now is apparent that the role of lymphohematopoietic cytokines in tumor biology is not strictly limited to their actions on the host immune and hematopoietic systems, but extends to their synthesis by and actions on neoplastic epithelial cells. Both this tumor cell receptor and its activating ligand, CSF-1, could then become logical targets for pharmacologic manipulation, as agents able to interrupt CSF-1 receptor/ligand paracrine and autocrine interactions might exert therapeutically beneficial effects on ovarian adenocarcinoma cell growth and dissemination.

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