Oncogene Expression in Vivo by Ovarian Adenocarcinomas and Mixed-Mullerian Tumors

BARRY M. KACINSKI, M.D., Ph.D., a, b DARRYL CARTER, M.D., c ERNEST I. KOHORN, M.D., b KUSHBAKHAT MITTAL, M.B., B.S., c R. S. SHAEFFER BLOODGOOD, a JOHN DONAHUE, B.S., a CAROL A. KRAMER, M.D., a DIANA FISCHER, Ph.D., d ROB EDWARDS, B.S., e SETSUKO K. CHAMBERS, M.D., b JOSEPH T. CHAMBERS, Ph.D., M.D., b AND PETER E. SCHWARTZ, M.D. b

Departments of a Therapeutic Radiology, b Obstetrics and Gynecology, c Pathology, and d Biostatistics, Yale University School of Medicine, New Haven, Connecticut

Received July 3, 1989

Six-micron paraffin sections of paraformaldehyde-fixed specimens of 24 ovarian benign and neoplastic specimens were assayed for tumor cell-specific oncogene expression by a sensitive, quantitative in situ hybridization technique with probes for 17 oncogenes, beta-actin, and E. coli beta-lactamase. In the benign, borderline, and invasive adenocarcinomas, multiple oncogenes, including neu, fes, fms, Ha-ras, trk, c-myc, fos, and PDGF-A chains, were expressed at significant levels relative to a housekeeping gene (beta-actin). In the mixed-Mullerian tumors, a rather different pattern of oncogene expression was observed, characterized primarily by expression of sis (PDGF-B chain).

For the adenocarcinomas, statistical analysis demonstrated that expression of several genes (fms, neu, PDGF-A) was closely linked to others (c-fos, c-myc) known to have important roles in the control of cell proliferation, but only one gene, fms, correlated very strongly with clinicopathologic features (high FIGO histologic grade and high FIGO clinical stage) predictive of aggressive clinical behavior and poor outcome. The authors discuss the role that tumor epithelial cell expression of the fms gene product might play in the auto- and paracrine control of growth and dissemination of ovarian adenocarcinomas.

In some cell culture systems, serial transfection of morphologically benign primary cell lines with viral or cellular oncogenes can confer, stepwise, phenotypic traits characteristic of malignant cells [1,2]. Such observations have inspired hypotheses that a similar incremental progression of cellular oncogene activation (mutation, aberrant over- or underexpression, and so on) occurs during the development of spontaneous neoplasms [3–7]. Ovarian epithelial neoplasms are a system well-suited for testing such hypotheses, since they encompass a broad spectrum of lesions, ranging from benign hyperproliferative serous and mucinous adenomatous cysts, serous and mucinous adenomas of borderline malignant potential to invasively malignant well-differentiated, moderately differentiated, and poorly differentiated serous, mucinous, adenosarcomas.

Abbreviations: ISH: in situ hybridization M-CSF, CSF-1: macrophage colony stimulating factor MMT: mixed-Mullerian tumor

This research was supported by ACS grant CD-262, a Swebilius Foundation Cancer Research Award, NIH research grant CA-47292, a Leukemia Society Special Fellowship, and a Bristol-Myers Cancer Research Award to BMK. Tissue retrieval and processing was funded through the NIH Core Facility Grant to the Yale Comprehensive Cancer Center.

Address reprint requests to: Barry M. Kacinski, M.D., Dept. of Therapeutic Radiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

Copyright © 1989 by The Yale Journal of Biology and Medicine, Inc.

All rights of reproduction in any form are reserved.
and endometroid adenocarcinomas, and include less common histologies such as mixed-Mullerian (mixed mesodermal) tumors, and others [8–10]. Clinical presentations also vary and range from small lesions confined within the ovarian capsule to aggressively malignant neoplasms which have disseminated throughout the peritoneal cavity and metastasized to distant visceral sites. At least for the adenocarcinomas, clinical extent of disease and histologic grade at presentation correlate very strongly with outcome [8–10]. We anticipated, therefore, that studies of cellular changes of oncogene expression in ovarian neoplasia would provide us with valuable information on those relationships which exist between prognostically important clinical and pathologic characteristics and qualitative and quantitative changes in tumor cell-specific oncogene expression.

In 1989, over 30 different genes have been identified as potential oncogenes [6,7,11–13]. Most previously published reports have focused on the importance of the expression of a single oncogene or oncogene class in tumor tissue or tumor-derived cell lines [14–17]. In this paper, we report the use of a sensitive and quantitative in situ hybridization technique [18–22] to assay levels of expression of 17 different oncogenes in 24 ovarian benign and neoplastic specimens. This quantitative data was subjected to statistical analyses, revealing many interesting relationships, some of which link high-grade, high-stage presentations to genes not otherwise implicated in the biology of epithelial neoplasms.

MATERIALS AND METHODS

Tissue Specimen Accrual, Preparation, and in Situ Hybridization (ISH)

All tissue specimens were obtained from patients of the Hunter Radiation Therapy and Ob/Gyn clinics of the Yale University School of Medicine, in accordance with Yale HIC protocol 3303. Small biopsies (maximum, ~3 mm thick) of ovarian neoplastic or benign tissues were obtained during therapeutic or diagnostic procedures (by PES, EIK, SKC, and JTC) and placed into freshly prepared PGP fixative (4 percent paraformaldehyde, 0.5 percent glutaraldehyde, 0.1 M Na-phosphate [pH 7.5]) within one to two minutes of harvest. Fixation was continued for four to six hours. Specimens were processed for embedding in paraffin, six-micron sections cut, prepared for, and carried through in situ hybridization and nuclear track emulsion autoradiography, as has been described elsewhere [18–24]. Probes for ISH were prepared by appropriate restriction digest of chimeric plasmids with cloned oncogenes, labeled with 35S-dCTP by random primer extension [25], using alpha-35S-labeled dCTP to give specific activities averaging 5 × 106 dpm/mcg DNA [26], and were complementary to coding sequences of beta-actin [27] (PstI), and enterobacterial beta lactamase [28] (EcoRI, PstI), c-myc [29] (third exon; ClaI, EcoRI), N-myc [30] (third exon; Accl, AvaI), L-myc [31] (second and third exon; SmaI, EcoRI), c-fos [32] (NcoI, XhoI), myb [33] (KpnI, XbaI), p53 [34] (EcoRI, BglII), Ha-ras [35] (SstI, PstI), Ki-ras [36] (EcoRI), N-ras [37] (SalI, NcoI), sis [38] (PstI, XbaI), PDGF-A chain [39] (SstI, HindIII), erbB [40] (BamHI), neu [41] (BamHI), fes [42] (PstI), fms [43] (PstI), ros [44] (EcoRI, PvuII), and trk [45] (NcoI, EcoRI). Sections of confluent monolayers of BeWo cells [46] (grown in Weymouth's + 10 percent fetal calf serum, 37°C, 5 percent CO2) were processed with each experimental run as positive controls. In our experiments, BeWo cells show consistently elevated levels of expression of c-myc, fms, and fos complementary mRNAs and were useful positive controls (see Fig. 1) [47,48]. Non-neoplastic ovarian tissues are included as negative controls (e.g., cases 1 and 2).
FIG. 1. In situ hybridization technique for tumor and stroma of specimen 17. A. In situ hybridization is carried out as described in the text with four oncogene, actin, and beta-lactamase (pBR322) probes. Sections are photographed at 100× (oil immersion) to demonstrate clearly the difference in grain counts for the negative control beta-lactamase probe and the other genes. B. Hybrids per micron^2 for beta-actin and seven oncogene probes are presented for BeWo choriocarcinoma cells (our positive control) and case 17 tumor and stroma. Tissue preparation, sectioning, processing of sections, in situ hybridization, autoradiography, and staining are carried out as described in Methods.
### TABLE 1

In Situ Hybridization Data (hybrids/cu micron × 1,000,000)

| No. | Grade | Stage | Actin | erb-B | fes | ros | trk | P53 | fos | myb | c-myc | L-myc | N-myc | Ha-ras | Ki-ras | N-ras | PDGF-A | sis |
|-----|-------|-------|-------|-------|-----|-----|-----|-----|-----|-----|-------|-------|-------|--------|--------|-------|--------|------|
| 1   | 0     | 0     | 290   | 0     | 0   | 630 | 5,097 | 0 | 0 | 508 | 0 | 124 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2   | 0     | 0     | 110   | 0     | 0   | 474 | 0    | 297 | 87 | 89 | 0 | 339 | 0 | 0 | 0 | 0 | 0 | 167 |
| 3   | 0.5   | II    | 359   | 1,083 | 1,490 | 0 | 0 | 146 | 349 | 238 | 512 | 305 | 148 | 1,279 | 112 | 356 | 437 | 296 |
| 4   | 0.5   | I     | 514   | 2,903 | 2,264 | 0 | 0 | 0 | 0 | 0 | 517 | 0 | 0 | 0 | 1,399 | 268 | 291 | 0 |
| 5   | 0.5   | III   | 773   | 3,719 | 2,946 | 0 | 0 | 295 | 0 | 517 | 0 | 2,995 | 170 | 0 | 0 | 4,246 | 0 | 0 | 0 |

**Benign and Borderline**

| Grade 1 and 2 Adenocarcinomas |
|--------------------------------|
| 6 | 1 | III | 87 | 3,670 | 815 | 217 | 212 | 95 | 453 | 558 | 347 | 141 | 355 | 1,814 | 0 | 0 | 327 | 0 |
| 7 | 2 | II | 717 | 787 | 2,214 | 0 | 0 | 286 | 516 | 0 | 2,489 | 0 | 260 | 2,643 | 0 | 119 | 2,468 | 0 |
| 8 | 2 | III | 85 | 1,786 | 724 | 0 | 98 | 229 | 363 | 163 | 123 | 0 | 164 | 262 | 0 | 297 | 634 | 132 |
| 9 | 2 | I | 249 | 0 | 0 | 0 | 1,153 | 626 | 0 | 1,392 | 0 | 773 | 1,083 | 2,482 | 0 | 1,350 | 159 |
| 10 | 2 | III | 148 | 2,285 | 22,285 | 1,097 | 328 | 0 | 548 | 2,657 | 1,961 | 0 | 170 | 1,835 | 0 | 0 | 1,040 | 165 |
| 11 | 2 | III | 193 | 2,545 | 3,023 | 4,162 | 12,513 | 138 | 1,139 | 0 | 4,527 | 190 | 0 | 3,037 | 0 | 0 | 1,195 | 0 |
| 12 | 2 | III | 412 | 0 | 2,517 | 0 | 1,167 | 181 | 1,800 | 0 | 1,357 | 89 | 352 | 4,281 | 401 | 1,160 | 1,097 | 0 |

KACINSKI ET AL.
ONCOGENE EXPRESSION IN OVARIAN NEOPLASMS

| Grade 3 Adenocarcinomas | 13 3 III | 14 3 III | 15 3 III | 16 3 III | 17 3 II | 18 3 III | 19 3 III |
|-------------------------|---------|---------|---------|---------|---------|---------|---------|
|                         | 294 695 | 1,111 0 | 0 0 | 136 169 703 166 | 344 70 0 | 214 1,933 | 661 0 |
|                         | 186 0 | 3,473 459 | 0 | 170 580 1,378 1,329 | 0 306 297 1,272 | 0 | 2,721 745 |
|                         | 672 0 | 5,701 465 | 5,385 | 342 1,406 0 | 2,510 93 752 7,944 | 0 0 | 602 0 |
|                         | 766 13,216 | 0 | 306 6,172 | 476 2,369 3,909 | 0 0 0 3,895 | 0 949 1,223 |
|                         | 1,132 0 | 4,633 1,779 | 3,642 | 520 3,234 0 | 9,069 254 253 1,062 | 0 3,635 2,109 | 0 |
| Mixed-Mullerian Tumors | 676 43,586 | 3,824 2,320 | 414 | 400 4,879 0 | 6,918 103 527 5,990 | 0 787 3,185 218 |
|                         | 333 0 | 0 2,615 6,039 | 99 1,476 1,104 3,438 | 91 0 | 473 2,172 226 | 1,645 201 |

Specimen number and histology, grade, stage, cell volume, and hybrids per micron³ for actin and 14 oncogene probes.

In situ hybridization data expressed as hybrids per micron³ and cell volume in micron³ is presented for two benign (cases 1, 2), three borderline (cases 3–5), one grade 1 (case 6), six grade 2 (cases 7–12), seven grade 3 (cases 13–19) ovarian adenocarcinomas, and five anaplastic mixed-Mullerian tumors (MMT) (cases 20–24) with admixed carcinomatous and sarcomatous histologies. Histological designation and tumor grade and histology (grade 1: well-differentiated; grade 2: moderately well-differentiated; grade 3: poorly differentiated) were determined by our pathologists (DC and KM) by the conventions accepted by the Federation Internationale de Gynecologie et Obstetriques (FIGO) [65]. Likewise, clinical stage was determined by review of the operative findings by the involved clinicians (BMK, EIK, PES, SKC, and JTC) and quantified according to the FIGO designations [49] (stage I: confined to the ovary; stage II: spread to pelvic organs; stage III: intra-abdominal spread; stage IV: distant metastases). Data on individual cases for fms and neu are not included here since they will be submitted for publication elsewhere.
Computer-Assisted Grain Count Acquisition and Data Analysis

The hematoxylin- and eosin-stained ISH autoradiograms are analyzed by light microscopy and grain counts quantitated with the aid of the Olympus Corporation Cue 2 VISION Image Analysis System. Randomly chosen fields of epithelium or stroma are visualized and grain counts quantitated with the Cue 2 Image Analysis System, which automatically resolves black silver grains from cell features to size and count total silver grains for each ~7,750-micron\(^2\) (100\(\times\)) oil immersion field. Ideally, enough fields are viewed and silver grains counted for a specific histologic feature (e.g., tumor epithelium, tumor stroma, and adjacent normal tissue) to yield a total of 500–1,000 grains. Grain counts per field are converted to hybrids per micron\(^3\) by multiplicative factors\(^1\) which take into account section thickness, size and specific activity of probes, exposure duration, and microscope field size. Pearson's correlation test was applied to the tabulated data for each probe with the aid of the PRODAS Professional Database Analysis System, Version 3.2 (Conceptual Software, Houston, TX) to yield R-values and \(p\)-values of the pairwise comparisons of oncogene and actin mRNA hybrids per micron\(^3\) with each other, FIGO grade (0–3) and FIGO stage (0–IV). Borderline lesions were assigned a nominal grade of 0.5 to reflect their histologic status intermediate between benign (grade 0) and well-differentiated, invasive (grade 1) adenocarcinomas [8,49].

RESULTS

Quantitative in situ hybridization analyses were carried out with specific probes for beta-actin [27], pBR322 [28], and 17 oncogenes [29–45] on two benign, three borderline, one grade 1 (mucinous), six grade 2 (serous), seven grade 3 (serous and poorly differentiated) adenocarcinomas, and five anaplastic mixed-Mullerian tumors (MMT) of the ovary. Representative data sets of hybrids per micron\(^3\) are presented for the BeWo human choriocarcinoma cell line [46] and the tumor epithelium and stroma of a grade 3 papillary serous adenocarcinoma of the ovary (Fig. 1). For the adenocarcinoma, we demonstrate the localization of \(c-my\)c, \(f\)os, and \(f\)ms mRNA to tumor epithelium but not stroma.

Similar data for actin and 17 oncogene probes are presented for all 24 specimens in Tables 1 and 2. \(T\)-test comparison of hybrid values for the 14 adenocarcinomas to the five benign and borderline malignant neoplasms identify only \(f\)ms and PDGF-A hybrid levels as significantly higher in the invasive adenocarcinomas, although several other genes (\(n\)eu, \(f\)es, Hai-ras, \(t\)rk, \(c-my\)c, as well as \(f\)ms and PDGF-A) are expressed at higher levels in the neoplastic epithelial cells than a housekeeping gene, beta-actin. For the small collection of MMTs, \(f\)os and \(m\)yc were both expressed at higher levels than actin, while higher expression of \(f\)os transcripts differentiated the MMTs from the benign and borderline neoplasms.

\(^1\)For example, for a 1 kb probe labeled to \(5 \times 10^4\) dpm/\(\mu\)g specific activity, one hybrid emits \(~1.4\) \(^3\)S beta particles after a 48-hour (our standard) exposure to yield one silver grain in a photoemulsion whose detection efficiency was 100 percent. Our estimates of hybrids per micron\(^3\) and hybrids per 100 \(\times\) field are presented in terms of such an ideal emulsion, since the absolute efficiency of NTB-2 emulsion for isotopes such as \(^3\)S is not precisely known. Basic physical dosimetric constraints, however, limit photoemulsion detection efficiency for \(^3\)S-beta particles to \(~10\) percent (and conceivably much less), and, hence, one grain could represent the beta emissions of seven or more radiolabeled hybrids and the hybrids per cubic micron values which we derive actually underestimate the true number of radioactive hybrids present.
In Table 3, we present the statistically significant pairwise correlations observed for the data summarized in Tables 1 and 2 for the five benign and borderline and 14 ovarian adenocarcinoma specimens. Of particular interest are the strong correlations seen between FIGO histologic grade with levels of *fms*, PDGF-A, *fos*, and *c-myc* hybrids; the significant correlation of stage with levels of *fms* hybrids; and the strong correlations of *fms* and PDGF-A levels with each other and with *fos* and *c-myc* hybrid levels. Other less obvious but significant correlations between different oncogene probes are also revealed by this analysis and summarized in Table 3. When the five mixed-Mullerian tumors are compared as a group to the five benign and borderline neoplasms and the 14 ovarian adenocarcinomas, levels of *sis* (PDGF-B chain) expression were found to correlate significantly with the presence of MMT histologic features (R-value, 0.50801; p-value, 0.02638) while *fms* (R-value, −0.40290; p-value, 0.08721) and PDGF-A (R-value, −0.43548; p-value, 0.06328) expression correlated nearly significantly with their absence.

*Ras* oncogene mRNA expression was also noted in the tumor epithelium of most of the specimens but did not significantly correlate with either tumor grade or stage, nor

**TABLE 2**

* T-Test Comparison of Hybrids Per Micron³ (x 10⁶) Values of 14 Adenocarcinomas, Five Benign and Borderline, and Five Mixed-Mullerian Ovarian Tumors

| Probe | 14 Adenocarcinomas | | 5 Benign and Borderline | | 5 Mixed-Mullerian |
|-------|---------------------|---------|-------------------------|---------|------------------|
|       | Mean | SE Mean | Mean | SE Mean | Mean | SE Mean |
| Actin | 425  | 84     | 409  | 112     | 247  | 60      |
| p53   | 299  | 75     | 46   | 30      | 123  | 89      |
| *fms* | 2,662| 522    | 384  | 222     | 935  | 595     |
| *neu* | 2,955| 1,104  | 985  | 499     | 1,085| 465     |
| *erb-B* | 4,897| 3,117  | 1,540| 760     | 2,105| 1,231   |
| *fes* | 3,594| 1,519  | 1,434| 546     | 2,008| 822     |
| *ros* | 959  | 344    | 126  | 126     | 142  | 63      |
| *trk* | 2,568| 1,001  | 1,079| 1,006   | 828  | 412     |
| PDGF-A | 1,428| 234    | 145  | 92      | 550  | 323     |
| *sis* | 202  | 95     | 59   | 59      | 880  | 403     |
| *Ki-ras* | 744 | 331    | 302  | 275     | 3,780| 3,530   |
| *Ha-ras* | 2,193| 643    | 1,106| 824     | 1,959| 1,814   |
| *N-ras* | 582 | 280    | 158  | 71      | 289  | 129     |
| *fos* | 1,397| 355    | 146  | 74      | 820  | 190     |
| *myb* | 470  | 210    | 48   | 48      | 703  | 353     |
| *C-myc* | 2,824| 696    | 973  | 505     | 1,054| 289     |
| *L-myc* | 93  | 29     | 95   | 62      | 70   | 43      |
| *N-myc* | 284 | 68     | 54   | 34      | 118  | 49      |

* T-test comparisons were carried out on the means and standard errors (SE) of the mean of the hybrids per micron³ (x 10⁶) values for the 14 adenocarcinomas, five benign and borderline, and five mixed-Mullerian tumors relative to each other as well as to a housekeeping gene (actin) and a cell proliferation gene (p53). Statistically significant comparisons (p-value < .05) are indicated by symbols b,c,e, and a as defined in the key.

Significantly greater than actin
Significantly greater than p53
Significantly greater than same gene in benign and borderline tumors
Significantly greater than in adenocarcinomas

| Probes | 14 Adenocarcinomas |
|--------|---------------------|
| Actin  | 425                 |
| p53    | 299                 |
| *fms*  | 2,662               |
| *neu*  | 2,955               |
| *erb-B* | 4,897              |
| *fes*  | 3,594               |
| *ros*  | 959                 |
| *trk*  | 2,568               |
| PDGF-A | 1,428               |
| *sis*  | 202                 |
| *Ki-ras* | 744                |
| *Ha-ras* | 2,193              |
| *N-ras* | 582                |
| *fos*  | 1,397               |
| *myb*  | 470                 |
| *C-myc* | 2,824              |
| *L-myc* | 93                 |
| *N-myc* | 284                |
were any significant pairwise correlations noted between hybrid levels complementary to the three ras gene probes.

**DISCUSSION**

By the careful analysis of a collection of 24 human ovarian specimens specially fixed and processed for in situ hybridization, we have obtained quantitative data on tumor cell-specific expression of actin and 17 oncogene transcripts. Overall, ovarian neoplasm-
tic epithelial cells appear to express significant level (relative to actin) of many different oncogene transcripts, including neu, fes, fms, trk, c-myc, and PDGF-A. Of the 17 oncogenes studied, however, only fms hybrid levels correlate strongly with both high FIGO clinical stage and/or high histologic grade, which are the two clinicopathologic features of ovarian adenocarcinomas most strongly predictive of aggressive behavior and poor outcome [8–10]. Sis expression correlated with the presence of mixed-Mullerian as opposed to adenocarcinoma histologic features, an observation which is not wholly unexpected, since the sis gene product (PDGF-B chain) has been implicated by others as an autocrine mitogen in sarcomatous neoplasms [50]. Levels of fos transcripts (a gene expressed at higher levels in many types of rapidly proliferating cells) distinguished these aggressive, but rare, neoplasms from benign or borderline specimens.

Many statistically significant correlations were observed between levels of expression of different mRNAs (Table 3), and many are not surprising in the context of what is now known about the physiology of the genes involved. Thus, strong correlations should be and were observed between levels of fos and c-myc hybrids even though the probes themselves have no homology, since both genes are known to be expressed together in rapidly proliferating cells. Likewise, the correlations between the src family oncogenes erbB, neu, ros, fms with fos and c-myc are reasonable if the erbB, neu, ros, and fms protein kinases and their ligands play some role in the control of ovarian epithelial cell proliferation [51,52]. Indeed, the presence of such biologically reasonable correlations helps to provide valuable internal confirmation of the consistency and validity of our in situ hybridization data and its statistical analyses.

Other correlations, such as those found for different src family oncogenes with each other, may be a consequence of low-level homology and cross-hybridization between the probes for one oncogene and the mRNA of another; however, no evidence of significant correlation or cross-hybridization was even observed for the related (Ha-, Ki-, and N-ras and c-, L-, and N-myc) gene probes to suggest that the hybridization conditions used in our experiments were not adequately stringent. Hence, the strong correlations observed for pairs of different src family oncogene probes may indicate coordinate expression of multiple growth factor receptors (erbB, neu, fms, ros, trk) by the tumor epithelial cells of our specimens, an interpretation consistent with the strong correlations which we observed between expression of some of these src family genes and expression of c-myc and fos (refer to Table 3).

The interpretation of some of the correlations (such as that of fes with myb, sis with Ki-ras, c-myc with N-ras, n-myc with Ha-ras, n-myc with p53 and Ha-ras, and N-ras with neu) is not, however, apparent. They suggest possible coordinate expression of otherwise unrelated genes and may help to identify possible pathways of signal transduction in ovarian carcinoma cells involving co-expressed growth factor receptors, ras-encoded GTP-binding proteins, and nuclear protein oncogenes.

The observed correlation between fms with both grade and stage for ovarian adenocarcinomas warrants further discussion. The fms oncogene, first characterized in a feline retrovirus, is now known to code for the receptor for macrophage colony stimulating factor M-CSF or CSF-1, a mitogen, chemoattractant, and phenotypic activator of tissue macrophages [53,54] and trophoblast (which expresses high levels of the c-fms gene) with important roles in wound healing, immune response, and the implantation and development of the human placenta. Two other fms-related genes have also been identified, c-kit and PDGF-receptor, both of which are homologous to
fms primarily in the 3' protein domain with much less homology in 5' extracellular sequences. If the fms gene expressed in ovarian neoplasms is mutant or rearranged with a constitutively active protein kinase (as is the v-fms protein) [55,56], other physiologic changes may not be needed to produce uncontrolled cell growth and a malignant cell phenotype. Southern blot hybridization with probes derived from the 3' and 5' halves of the human c-fms gene for three benign and 23 malignant ovarian specimens failed to disclose any significant rearrangements of c-fms genomic structure (data not shown), while Northern blot, cDNA PCR, immunohistochemical studies, and immunological studies (to be submitted elsewhere) suggest the expression of a normal or near-normal c-fms mRNA and protein by ovarian adenocarcinoma cells both in vivo and in vitro.

Claims for importance for fms (or a closely related gene) in non-hematopoietic neoplasms are not totally without precedent. Walker et al. [57] have recently reported that overexpressed length fms-complementary transcripts were observed in tumorigenic cell lines derived from MNNG- or gamma ray-mutagenized primary tracheal epithelial cells. Similarly, Feldman and Eisenbach [58] have reported the association of the expression of a fms-complementary transcript with metastatic phenotype in several mouse carcinoma cell lines, while we have reported in vivo fms transcript expression in many endometrial and breast carcinomas. If tumor cells do indeed express a normal or near-normal c-fms gene product, then a source of CSF-1 would be necessary to allow this receptor to exert phenotypic consequences on tumor cells which express it. CSF-1 (M-CSF) [53,54,59] is present in many tissues where it is synthesized by proliferating fibroblasts, activated macrophages, and other mesenchymal cells, and it is possible that the ubiquitous low levels of this mitogen are adequate to stimulate the proliferation of cells with high levels of M-CSF receptor. Such stromal cell production of CSF-1 could be facilitated by tumor cell synthesis of stroma mitogens such as PDGF-A, whose expression is strongly linked to tumor grade and expression of fms in our ovarian adenocarcinoma specimens (Table 1); however, stromal CSF-1 production is not the only available source for this cytokine in ovarian carcinoma patients. We, and others, have reported ovarian tumor cell line expression of CSF-1 in vitro and have observed markedly elevated plasma CSF-1 levels in ovarian carcinoma patients with active disease [60]. Such high levels of circulating cytokine may facilitate tumor growth and spread to metastatic site, a possibility under active investigation. Less complete information in breast, lung, and endometrial adenocarcinoma supports the hypothesis that similar CSF-1/CSF-1 receptor para- and autocrine interactions may be important in the development and progression of aggressive epithelial malignancies at other sites. This mechanism, in and of itself, does not exclude or diminish potentially important roles for the neu, erbB, ros, or ras oncogenes in ovarian adenocarcinomas; it merely suggests that their expression is not closely linked to those high-grade, high-stage presentations prognostic of poor clinical outcome.

One particular gene, neu, has been the focus of much controversy concerning its role in determining the prognosis of breast and, perhaps, ovarian carcinoma patients [61,62] and is worthy of further discussion here. We have observed that levels of neu expression (like PDGF-A and fms) strongly correlate with c-fos and myc expression (Table 2) in ovarian (as well as breast [21]) carcinomas even though levels of neu expression in benign and borderline lesions are not significantly different from those observed in invasively malignant neoplasms (Table 2). In addition, we observed
statistically significant correlations between levels of neu and fms expression in both ovarian and breast neoplasms. Such observations have led us to predict that the neu gene product is in some way involved in the control of epithelial cell proliferation in the ovary and breast and that it is at least co-expressed (and may interact) with the fms gene product. We are, however, still wary of any attempts [61] to relate levels of neu gene expression with prognosis in ovarian cancer, since, in our analysis, neu expression levels did not correlate significantly with either tumor grade or stage—both of which are extremely strong prognosticators of short- and long-term outcome in ovarian carcinoma patients treated with either standard chemotherapy or radiotherapy [8–10].

Likewise, in many human tumors, expression, and often overexpression, of a mutated ras oncogene is recognized to be an important step in the development of neoplastic specimens [14–16]. In fact, nearly all of our borderline and invasively malignant specimens show significant levels of ras-complementary hybrids (refer to Table 1), and several of our anaplastic ovarian MMT specimens even show significant hybridization to more than one ras probe. Our in situ hybridization techniques are not able to discriminate mutant from wild-type ras gene expression in our specimens, but we hope that further refinements of in situ hybridization and in situ transcription and PCR techniques [63] and the recent development of antibodies able to discriminate mutant from wild-type ras proteins [64] will help us to elucidate the role these overexpressed ras oncogenes play in determining the malignant phenotypes of ovarian adenocarcinoma cells.

REFERENCES

1. Lee W, Schwab M, Westaway D, Varmaus HE: Augmented expression of normal c-myc is sufficient for cotransformation of rat embryo fibroblast with a mutant ras gene. Mol Cell Biol 5:3345–3346, 1985
2. Land H, Parada LF, Weinberg RA, Wolf D, Rotter V: Cooperation between the genes encoding p53 tumor antigen and ras in cellular transformation. Nature 312:649–651, 1984
3. Land H, Parada LF, Weinberg RA: Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304:596–602, 1983
4. Land H, Parada LF, Weinberg RA: Cellular oncogenes and multistep carcinogenesis. Science 222:771–778, 1983
5. Foulks L: The experimental study of tumor progression: A review. Cancer Res 14:327–339, 1954
6. Vogt PV: Spontaneous segregation of non-transforming viruses from cloned sarcoma viruses. Virology 46:939–946, 1971
7. Bishop JM: Cellular oncogenes and retroviruses. Ann Rev Biochem 52:301–354, 1983
8. Scully RE: Ovarian tumors. Am J Pathol 87:686–720, 1977
9. Ozols RF, Garvin AJ, Costa J: Advanced ovarian cancers: Correlation of histologic grade with response to therapy and survival. Cancer 45:572–581, 1980
10. Bargmann F: Carcinoma of the ovary, a clinicopathological study of 86 autopsied cases with special reference to mode of spread. Act Gyneol Scand 45:211–231, 1966
11. Seemayer TA, Cavenee WK: Molecular mechanisms of oncogenesis. Lab Invest 60:585–599, 1989
12. Kraus MH, Pierce JH, Fleming TP, Robbins KC, et al: Mechanisms by which genes encoding growth factors and growth factor receptors contribute to malignant transformation. Ann NY Acad Sci 551:320–335, 1988
13. Serunian LA, Cantley LC: Growth factor and oncogene influences on cell growth regulation. Ann NY Acad Sci 551:309–319, 1988
14. Thor A, Hand PH, Wunderlich D, Caruso A, Muraro R, Schlom J: Monoclonal antibodies defined differential ras gene expression in malignant and benign colon disease states. Nature 311:562, 1984
15. Viola MV, Fremwerk F, Oravez S, Deb S, Funkel G, Lunde J, Hand PH, Thor A, Schlom J: Expression of the ras oncogene p21 in prostate cancer. N Engl J Med 314:133–137, 1986
16. Ohuchi N, Hand PH, Merlo G, Fujita J, Constantini-Mariani R, Thor A, Nose M, Callahan R, Schlom J: Enhanced expression of c-Ha-ras p21 in stomach adenocarcinomas defined by immunoassays using monoclonal antibodies and in situ hybridization. Cancer Res 47:1413–1420, 1987

17. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182, 1986

18. Lawrence JE, Singer RH: Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. Nucleic Acids Res 13:1777–1799, 1985

19. Angerer LM, Angerer RC: Detection of poly-A RNA in sea urchin eggs and embryos by quantitative in situ hybridization. Nucleic Acids Res 9:2819–2840, 1981

20. Kacinski BM, Carter D, Mittal K, Kohorn EI, Bloodgood RS, Donahue J, Donofrio L, Edwards R, Schwartz PE, Chambers JT, Chambers SK: High level expression of fms proto-oncogene mRNA is observed in clinically aggressive human endometrial adenocarcinomas. Int J Rad Onc Biol Phys 15:823–829, 1988

21. Yee L, Kacinski BM, Carter DC: Oncogene structure, function and expression in breast cancer. Sem Diag Path 6:110–125, 1989

22. Kacinski BM, Yee LD, Carter D: Quantitation of tumor cell expression of the p-glycoprotein (MDR1) gene in human breast carcinoma clinical specimens. Cancer Bulletin 41:44–48, 1989

23. Hayashi S, Gillam JC, Delaney AD, Tener GM: Acetylation of chromosome squashes of Drosophila melanogaster decreases the background of 125I-labeled RNA. J Histochem Cytochem 26:677–679, 1978

24. Godard CM, Jones KW: Detection of AKR MuLV-specific RNA in AKR mouse cell by in situ hybridization. Nucleic Acids Res 6:2849–2861, 1979

25. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, Cold Spring Harbor Press, 1982, pp 75–96, 129–132

26. Bartocci A, Pollard JW, Stanley ER: Regulation of colony stimulating factor 1 during pregnancy. J Exp Med 164:956–961, 1986

27. Cleveland DW, Lopata MA, MacDonald RJ, Cowan NJ, Rutter WJ, Kirschner WJ: Number and evolutionary conservation of alpha and beta tubulin and cytoplasmic beta and gamma actin using specific cloned cDNA probes. Cell 20:95–105, 1984

28. Bolivar F, Rodriguez RL, Greene PJ, Heyeden HL, Boyer HL: Construction and characterization of new cloning vehicles II. A multipurpose cloning system. Gene 26:197–203, 1977

29. Battey J, Moulding C, Taub R, Murphy W, Stewart T, Potter H, Lenoir G, Leder P: The human c-myc oncogene: Structural consequences of translocation into the IgH locus in Burkitt’s lymphoma. Cell 34:779–782, 1983

30. Kohl NE, Legouy E, DePinho RA, Nisen PD, Smith RK, Gee CE, Alt FW: Human N-myc is closely related in organization and nucleotide sequence to c-myc. Nature 319:73–77, 1986

31. Nau MM, Brooks BJ, Battey J, Sauesville E, Gazdar A, Kirsch IR, McBride J, Bartness V, Hollis GF, Minna JD: L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. Nature 318:69–73, 1985

32. Curran T, MacCormell WP, vanStraaten F, Verma IM: Structure of the FBJ osteosarcoma virus genome. Molecular cloning of its helper virus and the cellular homologues of the fos gene from mouse and human cells. Mol Cell Biol 3:914–921, 1984

33. Klempnauer KH, Gonda TJ, Bishop JM: Nucleotide sequence of the retroviral leukemia gene v-myc and its cellular homologue c-myc; the architecture of a transduced oncogene. Cell 31:453–462, 1982

34. Jenkins JR, Rudge K, Redmond S, Wade-Evans A: Cloning and expression of a full length mouse cDNA sequence encoded in the transformation associated protein p53. Nucleic Acids Res 12:5609–5626, 1984

35. Ellis RW, DeFeo D, Furth MF, Scolnick EM: Mouse cells contain two distinct ras gene mRNA species that can be translated to a p21 protein. Mol Cell Biol 2:1339–1345, 1982

36. Shimizu K, Goldfarb DM, Suarez Y, Peruchio M, Li Y, Kamata T, Ferramisco J, Stavnezer E, Fogh J, Wigler M: Three transforming genes are related to the viral ras oncogenes. Proc Natl Acad Sci USA 80:2112–2116, 1983

37. Shimizu K, Goldfarb M, Peruchio M, Wigler M: Isolation and preliminary characterization of the transforming gene of a human neuroblastoma. Proc Natl Acad Sci USA 80:383–387, 1983

38. Robbins K, Devare SG, Aaronson SA: Molecular cloning of an integrated simian sarcoma virus: Genome integration and organization of infectious DNA clones. Proc Natl Acad Sci USA 78:2918–2922, 1981
39. Betsholtz C, Johnson A, Helder C-H, Westernmark B, Lind P, Urdea MS, Eddy R, Shows TB, Philpott K, Mellor AL, Knott TJ, Scott J: CDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumor cell lines. Nature 320:695–700, 1986

40. Vennstrom B, Fanshier L, Moscovici C, Bishop JM: Molecular cloning of the avian cytotblast virus genome and recovery of oncogenes by transfection of chicken cells. J Virology 36:575–585, 1980

41. Bargmann C, Hung MC, Weinberg RA: The neu oncogene encodes a growth factor receptor related protein. Nature 319:230–233, 1986

42. Franchini G, Evans J, Sherr CJ, Wong-Staal F: Onc sequences (v-fes) of Snyder-Theilen feline sarcoma virus are derived from noncontiguous regions of a cat cellular gene c-fes. Nature 290:154, 1984

43. Hampe A, Gohet M, Sherr CJ, Galibert F: Nucleotide sequence of the feline retroviral oncogene v-fms shows unexpected homology with oncogenes controlling tyrosine-specific protein kinases. Proc Natl Acad Sci USA 81:85–89, 1984

44. Matsumura H, Wang LH, Shibuya M: Human c-ros-1 gene homologous to the v-ros sequence of the UR2 sarcoma virus encodes a transmembrane receptor molecule. Mol Cell Biol 7:3000–3004, 1986

45. Martin-Zanca D, Hughes SH, Barbacid M: A human oncogene formed by the fusion of truncated tropomysin and protein tyrosine kinase sequences. Nature 319:743–748, 1986

46. Pattilo RA, Gey AO: The establishment of a cell line of human hormone trophoblastic cells in vitro. Cancer Res 28:1231–1236, 1968

47. Rettenmier CW, Sacca R, Turmman WL, Roussel MF, Holt JT, Nienhuis AW, Stanley ER, Sherr CJ: Expression of the human c-fms proto-oncogene product (colony-stimulating factor-1 receptor) on peripheral blood mononuclear cells and choriocarcinoma cell lines. J Clin Invest 77:1740–1746, 1986

48. Mueller R, Tremblay JM, Adamson ED, Verma IM: Tissue and cell type-specific expression of two human oncogenes. Nature 304:454–456, 1983

49. FIGO: Annual Report on the Results of Treatment in Gynecological Cancer. Edited by F Pettersson. Stockholm, Panorama Press AB, 1986, p 111

50. Ross R, Raines E, Bowen-Pope DF: The biology of platelet-derived growth factor. Cell 46:155–169, 1986

51. Muller R, Bravo R, Bureckhardt J, Curran T: Induction of c-fos gene and protein by growth factors precedes activation of c-myc. Nature 312:716–720, 1984

52. Macara IG: Oncogenes, ions, and phospholipids. Am J Physiol 248:C3–C11, 1985

53. Stanley ER: The action of the colony stimulating factor CSF-1. In Biochemistry of Macrophages. Ciba Foundation Symposium 118. Edited by D Evered, J Nugent, M O’Connor. London, Pitman, 1986, pp 29–41

54. Clark SC, Kamen R: The human hematopoietic colony stimulating factors. Science 236:1229–1237, 1987

55. Coussens L, Van Beveren CV, Smith D, Chen E, Mitchell RL, Isacke CM, Verma IM, Ultrrich A: Structural alteration of viral homologue of receptor proto-oncogene fms at carboxyl terminus. Nature 320:277–280, 1986

56. Roussel MR, Dull TJ, Rettenmeier CW, Ralph PW, Ultrrich A, Sherr CJ: Transforming potential of the c-fms proto-oncogene (CSF-1 receptor). Nature 325:549–552, 1987

57. Walker C, Nettesheim P, Barrett JC, Gilmer TM: Expression of a fms-related oncogene in carcinogen-induced epithelial cells. Proc Natl Acad Sci USA 84:1804–1808, 1987

58. Feldman M, Eisenbach L: What makes a tumor cell metastatic? Scientific American (November):60–85, 1988

59. Rajavashisth TB, Eng R, Shadduck RK, Waheed A, Ben-Avram CM, Shively JE, Lusis AJ: Cloning and tissue-specific expression of mouse macrophage colony-stimulating factor mRNA. Proc Natl Acad Sci USA 84:1157–1161, 1987

60. Kacinski BM, Stanley ER, Carter D, Chambers JT, Chambers SK, Kohorn EI, Schwartz PE: Circulating levels of CSF-1 (M-CSF), a lymphohematopoietic cytokine, may be a useful marker of disease status in patients with malignant ovarian neoplasms. Int J Rad Onc Biol Phys 17:159–164, 1989

61. Van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, Nusse R: Neu-protein overexpression in breast cancer: Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med 319:1239–1246, 1988

62. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707–712, 1988
63. Longley J, Merchant MA, Kacinski BM: In situ transcription and detection of CD1a mRNA in epidermal cells: An alternative to standard in situ hybridization techniques. J Invest Dermatol, in press

64. Carney WP, Hamer P, Petit D, Wolfe H, Cooper G, Lefebvre M, Rabin H: A monoclonal antibody reactive with an activated ras protein expressing valine at position 12. J Cell Biochem 32:207–214, 1986

65. Scully RE: World Health Organization classification and nomenclature of ovarian cancer. Natl Cancer Inst Mono 42:5–7, 1975