Journal of Cellular Biochemistry 44:189–198 (1990)

Oncogene Amplification Correlates With Dense Lymphocyte Infiltration in Human Breast Cancers: A Role for Hematopoietic Growth Factor Release by Tumor Cells?

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One hundred six primary breast cancer samples were analysed for c-erbB2, int-2, and c-myc gene amplification. Surgically confirmed nodal involvement was observed in 42%. Level of gene amplification was studied by Southern and/or slot blot techniques. Amplified c-erbB2 gene sequences were present in 21.5% of all samples. Int-2 was amplified in 13.1% and c-myc was amplified in 10.3%. In a non-parametric test (Kruskal-Wallis) a strong negative association was found between high levels of c-erbB2 amplification and absence of estrogen receptor (ER) ($P = .0009$) or progesterone receptor (PR) ($P = .011$) expression. No correlations were found between all or high levels of amplification of each oncogene separately or combined with T, N, grade, multifocality of tumor, or associated carcinoma in situ. There was a trend approaching statistical significance for patients with c-erbB2 amplifications to have positive lymph nodes at surgery ($P = 0.09$). A somewhat surprising finding however was a very strong association between oncogene amplification and dense lymphocyte infiltration of the tumor ($P = .05$). This correlation is even stronger when only high levels of amplification are considered, either for each oncogene separately ($P = .0048$) or in combination ($P = .0007$). We propose that malignant cell cytokine production may help explain this observation.

Key words: breast cancer, dense immune cell infiltrate, c-erbB2, int-2, c-myc, CSF-1

The gain and/or loss of genetic material are hallmarks of malignant tumors. The amplification of DNA sequences encoding growth factor receptors (EGFr, neu) and nuclear oncogenes (c-myc) or rearrangements which place them under an abnormal control of active promoters can lead to elevated levels of gene expression with the functional consequence of both an abnormal para- and/or autocrine growth stimula-

Received April 9, 1990; accepted July 10, 1990.

TABLE I. Clinical Parameters (T, N) + Pathological Stage (S)

T0	T1	T2	T3	T4
1	24 (23%)	69 (65%)	11 (10%)	1
N0	N1a	N1b		
81 (76%)	16 (15%)	9 (8%)		
S1	S2	S3	S4	
17%	72%	10%	1%	

tion as well as an abnormal phenotypic differentiation leading to invasion and metastasis. Furthermore if gene amplification occurs early in the course of a tumor and confers a selective growth advantage, the outgrowth of daughter cells deriving from this most rapidly growing cell clone will encompass the bulk of the tumor. Deletion or loss of expression of genes which normally inhibit cellular proliferation or maintain normal differentiation may also lead to abnormalities in neoplastic cell proliferation and differentiation. In this communication we evaluate the significance of gene amplifications of the c-myc, c-erbB2, and int-2 oncogenes and their correlations with known clinical, pathological, and biological prognostic factors in breast cancer.

MATERIALS AND METHODS

Patients

One hundred six tumor samples from previously untreated breast cancer patients were collected between June 1986 and July 1988. The distribution according to clinical parameters is indicated in Table I. Surgically confirmed nodal involvement was present in 42%. Most patients (71%) were postmenopausal, 27% were premenopausal, and 2% were unknown. Estrogen and progesterone receptor (ER, PR) status was assessed in 104 patients by the dextran-coated charcoal assay; 75.7% were ER positive and 57.5% were PR positive. Almost all tumors (94%) were histologically classified as infiltrating ductal; 3 tumors were infiltrating lobular, 2 were colloid, and one was medullary. Histological differentiation according to Scarff, Bloom, and Richardson (SBR) showed 20% to be of grade I, 68% of grade II, and 9% of grade III; 3 tumors could not be graded. All tumors had a repeat double-blind histological assessment for degree of tumor necrosis, associated carcinoma in situ, and marked versus slight lymphocytic infiltrates. The histological tumor dimensions and the number of positive nodes and total number of nodes dissected were also reported. Thirty-eight patients had been treated by lumpectomy and sixty-eight by mastectomy. Lumpectomy specimens had a microscopic assessment for tumor-free margins, followed by more surgery if needed. Only three patients did not have an axillary dissection; two of these had fine needle aspirates of clinically positive nodes.

Methods

All tissues were flash frozen and stored at −70°C until analysed. Small pieces of tissue were powdered in liquid nitrogen using a tissue pulverizer (Touzart Matignon, Vitry s/Seine, France). High molecular weight DNA was extracted and 10 μg was digested with the restriction endonuclease EcoRI. The digests were electrophoresed

on 0.8% agarose gels and the fractionated DNA's were denatured and transferred to nylon membranes (Genofit, Geneva Switzerland) by capillary blotting. Later samples were transferred to the Hybond N+ nylon membranes (Amersham) by alkaline capillary blotting as described by the manufacturer. In parallel experiments, 1 μg, 0.5 μg, and 0.1 μg samples of DNA were deposited in slot blot wells, dried, and all filters were in addition baked at 80°C for 2 hours. The membranes were hybridized to 10^6 cpm ml^{-1} radiolabeled probe in 10× Denhardt's, 6× SSC, 0.2% SDS, dextran sulfate 0.1 kg/liter at 65°C overnight. The membranes were washed at high stringency (0.1 × SSC at 65°C), dried, and autoradiographed for 1–7 days at −70°C using Kodak Lanex Fast film. After suitable autoradiographic exposure the membranes were stripped of the probe either in 0.4 M NaOH at room temperature for 30 min, neutralised in 0.2 M Tris-HCl (pH 7.5) (Genofit) or dipped in boiling water + 0.1% SDS and left to cool (Hybond N+ Amersham). Filters were exposed prior to rehybridization to assure complete removal of the probe and than prehybridized and re-hybridized using a second labeled probe as before. As a control for the amount of DNA immobilized, each filter was hybridized to control genes (fms and FcRII) for whom we found no quantitative variations in the samples studied. Fms is a single copy gene per haploid genome, Fc RII is present at 2 or 3 copies per haploid genome. The following probes were used:

c-myc: 1,029 bp PstI restriction fragment corresponding to the 3′ end of exon II and exon III [1].

c-erbB2: 0.44 kb KpnI-XbaI restriction fragment coding for the carboxyl terminal proximal sequence of the kinase domain [2].

int-2: 0.9 kb SacI restriction fragment [3].

Fc RII: 1.3 kb cDNA cloned into EcoRI site "HFc 3.0" [4].

fms: 1.4 kb PstI viral fms fragment [5].

All the above probes had been previously shown to hybridize to unique fragments in Southern blot analysis of total human genomic DNA. At the stringencies used for hybridization and washing, there was no hybridization of any probe to other relates sequences (e.g., the three related genes of the myc family).

Immunohistochemistry

Archival paraffin blocks (formalin and Bouin fixed) were labeled by standard methods using biotinylated secondary antibodies, streptavidin peroxidase, and DAB-H202 [6]. Primary antibodies were as follows:

To CSF-1 receptor (*fms:* monoclonal antibody 2E8) [7]
To CSF-1 (polyclonal 52P4) [8]
To human B cells (L 26 Dakopatts, Denmark)
To human macrophages, CD68 (KP1 Dakopatts, Denmark)
To human T cells (CD3, Dakopatts, Denmark)

Statistical Analysis

Association between qualitative criteria was tested by the CHI-2 test using the Yates correction for small numbers. Average ranks were compared by the non-parametric rank test of Kruskal-Wallis [9].

TABLE II. Oncogene Amplifications in Primary Breast Cancer*

		%
A c-erbB2	23/106	21,7
B int-2	13/106	12,3
C c-myc	11/106	10,3
A + B + C	40/106	37,7
No amplification	66/106	62.3
D c-erbB2 > 5	10/106	9,4
E int-2 > 5	3/106	2,8
F c-myc > 5	1/106	0,95
D + E + F	14/106	13,2
No amplification > 5	92/106	86,8

*The total No. of all positive patients is less than the sum of each oncogene amplification taken separately since 7 patients had two amplifications (4 × A + C, 2 × A + B, 1 × B + C).

RESULTS AND DISCUSSION

Survival

The median follow-up is 24 months and too short to yield valid survival statistics. The patients included in this study had no treatment prior to surgery, which consisted in either a mastectomy (64%) or a lumpectomy (36%). An axillary node dissection and steroid receptor analysis was performed in 97% of all patients. We would expect, by comparison with historical survival curves, to see a 30–40% recurrence rate in 3 to 5 years. Due to the relatively limited number of patients and the short follow-up period, survival data are not available yet, but since less than 5% of patients did receive adjuvant chemotherapy, the spontaneous outcome for patients with and without oncogene amplification can be followed.

Early studies by Slamon et al. [10] document a highly significant correlation between high levels of c-erbB2 amplification in breast tumors and patient survival. These results are not observed by all investigators [11] but correlations with node invasion as well as overall survival in node positive patients have been reported [12,13]. A worsened prognosis has equally been advocated for tumors with either int-2 or c-myc gene amplifications [14].

Clinical Prognostic Factors

The overall incidence of total and high level amplification of the three oncogenes studied are indicated in Table II. All correlations with clinical and pathological parameters are calculated for each oncogene separately and for the sum of the three oncogenes. This procedure is repeated for high levels of amplifications. Southern blots for a group of patients hybridised with 2 probes in sequence are shown in Figure 1. The incidence of oncogene amplifications in our study is comparable to that published by others [10,14–16]. A higher incidence of c-myc amplification was reported by Varley et al. [14], which may reflect a selection bias in favour of node-positive patients in their clinical cohort.

No correlation with either the tumor size or the clinical nodal status could be elucidated. Table III shows the lack of any statistically significant difference for pre- or postmenopausal patients. There appear to exist equal numbers of patients with (amp-pos) or without (amp-neg) amplifications in the age classes under 50 years, with only ⅓ amp-pos patients in the age categories over 50 years, but these differences are

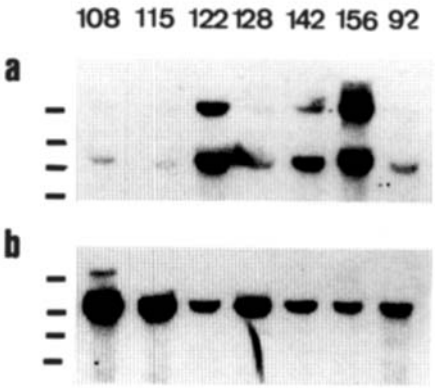

Fig. 1. Southern blot analysis of tumor DNAs hybridized with the 0.44 kb KpnI-XbaI restriction fragment coding for the carboxyl terminal proximal sequence of the kinase domain of c-erbB2 (**a**). The same filter, hybridised with the 0.9 kb SacI restriction fragment of int-2 (**b**). Size markers are 14, 10.6, 9, and 7.3 kb, respectively.

TABLE III. Correlation of c-erbB2, c-myc, and Int-2 Amplification With Several Factors

Age	N.S. ($P > .48$)
Menopause	N.S. ($P > .18$)
Tumor grade	N.S. ($P > .34$)
Stage 1 + 2/3 + 4)	N.S. ($P > .41$)

not statistically significant (Table IV). Of interest is the finding that the only inflammatory (and poor prognostic) tumor included in this study had a strong amplification of int-2, a member of the fibroblast growth factor family with known angiogenic potential.

Steroid Receptor Levels

In a non-parametric test (Kruskal-Wallis), low levels of estrogen receptor ($P = .0009$) and progesterone receptor ($P = .011$) are associated with high levels of c-erbB2 amplification, while there is a trend for tumors with amplifications (all levels) of int-2 to show high values for estrogen ($P = .3$) and progesterone receptor ($P = .055$). The association of high levels of progesterone receptor with int-2 amplifications is interesting, in particular since both genes are located on the same arm of chromosome 11 and might share common regulatory elements. In a survey of 310 human breast tumors, Borg et al. detect a strong correlation between int-2 amplification and estrogen receptor positivity [15]. Our numbers are too small to confirm a statistically significant correlation with ER positivity, but our results, showing a correlation with PR levels (PR expression being under ER control) are not incompatible with these findings. Since int-2 amplification has been associated with a poor prognosis, a subset of ER-positive patients might be identifiable which could benefit from adjuvant therapy.

Pathological Prognostic Factors

The tumors we analysed were almost exclusively (94%) classified as infiltrating ductal, but one medullary carcinoma showed low level amplifications for both int-2 and c-myc and one tumor classified as infiltrating lobular appeared to have a low level amplification of int-2.

TABLE IV. Lack of Correlation Between Oncogene Amplification and Age*

	Age in years					
	<40	40–50	50–60	60–70	>70	*P* value
A c-erbB2		5	6	5	7	.59
B int-2	1	4	2	3	3	.87
C c-myc	1	4	2	1	3	.48
A + B + C >2	2	11	7	9	11	.80
No amplif.	2	13	15	19	17	
	4	24	22	28	28	
c-erbB2 >5		2	2	3	3	.91
All > 5		3	3	4	4	.87

*The total No. of patients presenting oncogene amplifications is different from the sum of A + B + C since 7 patients had two amplifications (4 × A + C, 2 × A + B, 1 × B + C).

Only a trend for tumors with c-erbB2 amplification to have spread into the axilla was apparent (N−: 15%; N+: 31%, $P = .08$); this correlation was not improved upon by summing all the amp-pos patients or selection of high levels of amplification. Similar results have been published by Slamon et al. ($P < .06$) [10] and were much more significantly correlated in another larger study of 310 patients ($P = .0018$) [13].

Tumor grade determined by the criteria of Scarff, Bloom and Richardson (SBR) did not correlate with any of the oncogenes separately or together, nor did pathological stage. High levels of amplification of c-erbB2 correlate with a high degree of tumor necrosis (60%+; 40%−; $P = .03$) a feature generally associated with extensive ductal carcinoma in situ (EDCIS). EDCIS in turn was shown to be significantly associated with local recurrence in a retrospective analysis on radiotherapeutic breast conservation [17]. Since Van de Vijver et al. reported a strong association [18] between comedocarcinoma and c-erbB2 antigen overexpression, we reassessed all pathological slides for the presence and amount (<10%; >10%) of carcinoma in situ (CIS) associated with the invasive tumor to determine whether there was any correlation of extent of intraductal carcinoma with c-erbB2 amplification. Our numbers are presently too small to demonstrate any difference in this patient population.

Dense lymphocytic infiltration has been proposed by some as a marker of either good or bad prognosis; a recent survey from the Memorial Sloan-Kettering Cancer Center on early breast cancer documents a significantly lower recurrence and better survival statistics for lesions with a slight lymphocytic infiltrate than for tumors with a marked infiltrate [19] ($P = .014$ for node-negative vs. $P = .03$ for node-positive patients). The slides of all our patients were evaluated for extent (marked/slight) of lymphocyte infiltrate in a double-blind analysis (Fig. 2). We were able to document a statistically significant trend for amp-pos tumors to have a marked "lymphoid cell" infiltrate. These results become highly significant if only high levels of amplifications are considered, and even more so if tumors presenting high-level amplification of c-erbB2 and int-2 are considered together (Table V).

Ongoing experiments to characterize these "lymphoid" cell infiltrates suggest a predominance of either macrophages or B cells for a given tumor (Fig. 2).* Moreover, whereas macrophages infiltrate the tumor diffusely, B cells are always arranged in a

*Mature T cells are frequently detected in large numbers as well (anti CD3 polyclonal rabbit antibodies; Dakopatts). Ongoing studies attempt to determine associations among different types of immune cells in conjunction with expression of CSF-1 and FMS by the tumour.

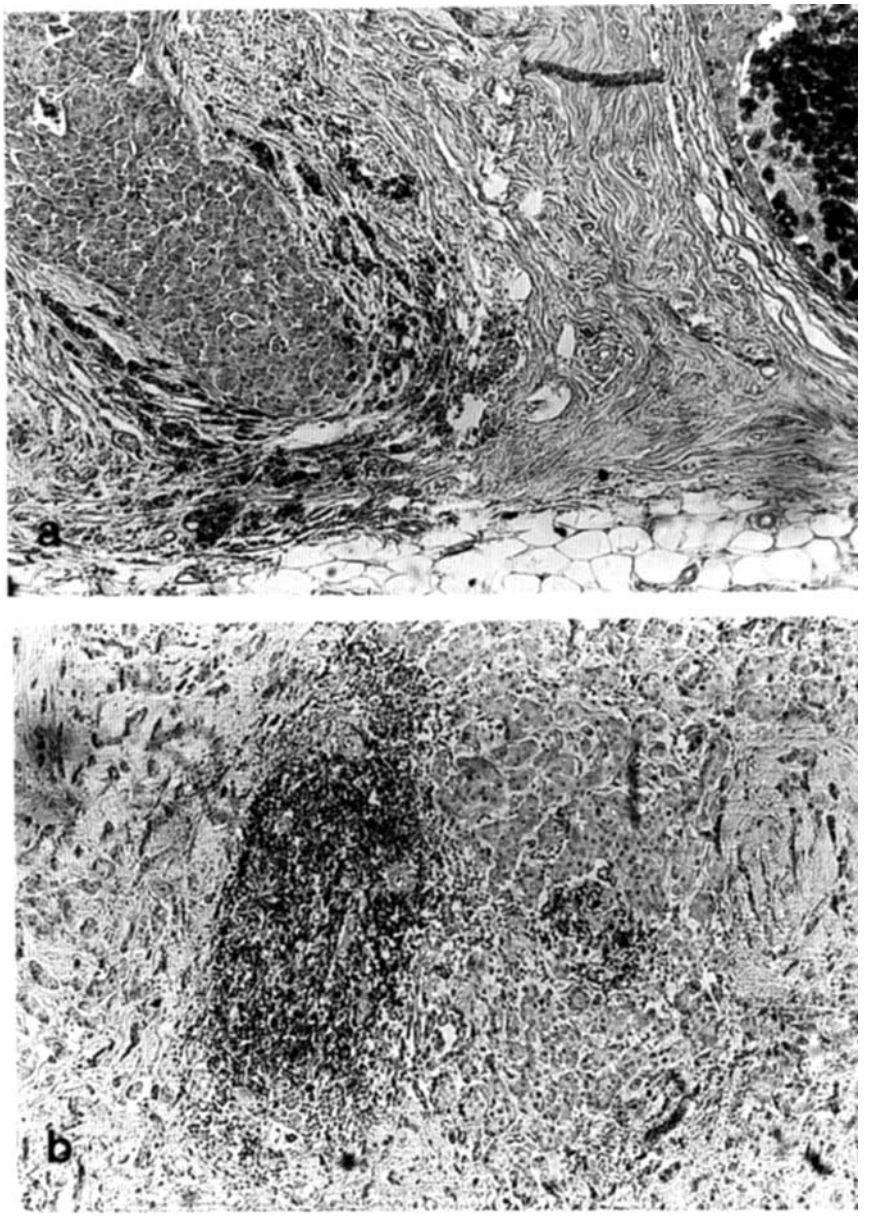

Fig. 2. **a:** Immunohistochemical staining with a specific anti-macrophage antibody (KP1 Dakopatts) showing macrophages in and around a cluster of tumor cells. Necrotic tumor (upper right corner) equally shows positive staining with this marker. **b:** Immunohistochemical staining with a specific anti-B cell marker (L26 Dakopatts) showing a typical lobular arrangement of B cells. This pattern is most frequently seen around blood vessels and in situ carcinoma, rarely interspersed with tumor cells.

TABLE V. Correlations Between Oncogene Amplifications and Marked Lymphocyte Infiltrate

		Intense lymphocytic infiltrate, %	*P* value (Yates cor)
A	c-erbB2	10/23	43	.46
B	int-2	7/13	54	.22
C	c-myc	5/11	45,5	.65
A + B + C		19/40	47,5	.05
0 amplif.		18/66	27	
D	c-erbB2 > 5	8/10	80	.0048
E	int-2 > 5	3/3	100	.07
F	c-myc > 5	0/1	—	—
D + E + F		11/14	78,5	.0007
0 amplif.		26/92	28	

follicular fashion and most frequently found surrounding vessels or areas of in situ carcinoma.

Of particular interest to us is the finding that oncogene amplifications correlate strongly with a pathological feature which in a large retrospective study [19] has been shown to be of pejorative value in early breast cancer patients. These results suggest that tumor cell-dependant cytokine production may be associated with paracrine immunological phenomena which themselves are associated with a poor prognosis. There is evidence from in vitro and animal studies implying a cooperation between tumor cells and resulting in an enhancement of the invasive and metastatic potentials of carcinoma cells. Lymphocytes [20,21], mast cells [22,23], macrophages [24], and polymorphonuclear cells [25] have all been shown to enhance metastatic potential in various animal tumors. More recently, receptors for the colony-stimulating-factor (CSF-1) of the macrophage/monocyte lineage (fms) have been discovered on solid tumors including tumors of the endometrium and ovary [6, 26–28], and a positive correlation between degree of fms and CSF-1 expression by the tumor and high grade and stage of the tumors has been documented. In preliminary experiments, using immunohistochemical techniques, phenotypic markers for the macrophage/monocyte lineage (fms and CSF-1) have been found to be expressed on infiltrating macrophages as well as on invasive epithelial tumor cells. This cytokine, if expressed, is regularly associated with the more infiltrating part of the tumor and weak or absent in in situ carcinoma (Fig. 3). Fifteen out of 20 tumors (75%) with a marked immune cell infiltrate show an equally strong expression of CSF-1 on both the tumor cells and the immune cell infiltrate. In the absence of this lymphoid cell infiltrate, CSF-1 expression is weak (<30% of cells stained, weak intensity of staining); but 9 out of 12 tumors (75%) with neu and/or int2 amplification (and no marked infiltrate) showed strong positive staining. Only one out of eight tumors without oncogene amplification and withoout a marked immune cell infiltrate had more than 30% positive staining. In this last group, small foci of intensive staining are visible, however, at sites of maximal tumor invasion. Serum levels of CSF-1 in patients with metastatic disease are consistently more elevated than in patients with primary tumors (data not shown) and ongoing studies test its value as a marker protein for early metastatic relapse.

CSF-1 and its receptor have previously been implicated in the control of cellular proliferation and invasive differentiation of an invasive immune cell (macrophage) and a normally invasive epithelium (syncytiotrophoblast of the placenta) [29], an observa-

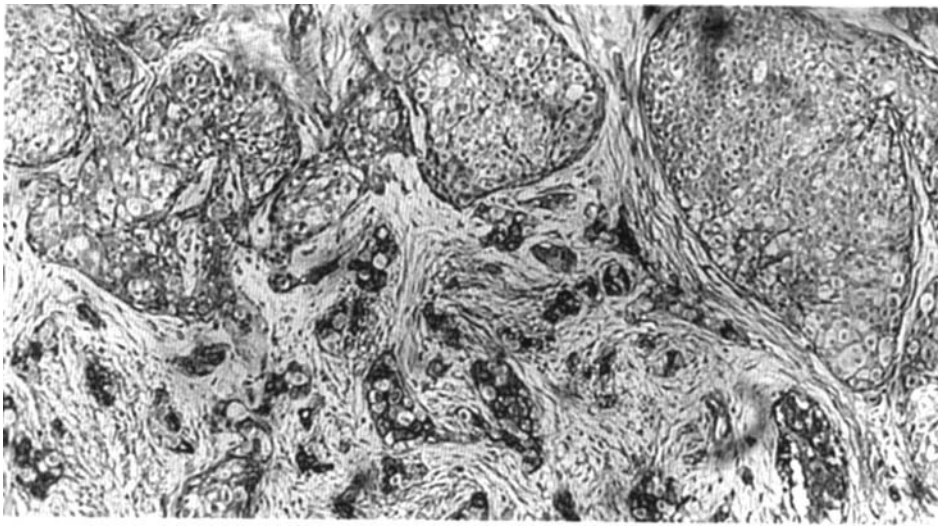

Fig. 3. Immunohistochemical staining with an anti CSF-1 antibody (polyclonal 52P4 R.Stanley [8]). The more invasive tumor cells are strongly positive with this macrophage marker whereas in situ carcinoma is almost completely negative.

tion which led us to propose that this macrophage-like differentiation might contribute to the invasive phenotype in epithelial cell tumors. CSF-1 is produced by a variety of cells including macrophages, activated T cells, and uterine gland epithelial cells [30]. CSF-1 production in the pregnant mouse uterus is under estrogen and progesterone control; it is constitutively expressed by many mesenchymal cells including fibroblasts and bone marrow stromal cells [30] and following stimulation by physiologic inflammatory mediators in monocytes and macrophages [31]. CSF-1 serum levels increase throughout pregnancy and have been shown to promote an increase in placental and fetal weight [31]. Circulating levels of this cytokine are equally markedly elevated in a majority of ovarian and endometrial carcinoma patients [26, 32].

Another macrophage-produced cytokine implicated in placental implantation is TGF-beta. This peptide has equally been shown to be secreted by many tumor cells, to induce fibrosis and angiogenesis, and in particular to be a direct chemotactic signal for monocytes at extremely low concentrations [33].

In summary, the meaning of the observed correlation between oncogene amplification and the pathological changes at this point remains speculative. Ongoing studies attempt to determine a potential role of cytokines (CSF-1 and TGF-beta) produced by tumor cells in the recruitment and proliferation of the tumor-infiltrating immune cells. Ten to 80% of tumor cells stain positively for CSF-1 (data not shown) in a mosaic-like pattern but the site of CSF-1 production (tumor, macrophages, or stromal cells) remains to be assessed. Tumor-associated macrophages in turn may secrete enzymes (Cathepsin D, heparanase, type IV collagenase) which may paradoxically facilitate tumor spread as well as synthesize a variety of cytokines, some of which may inhibit tumor growth (TNF), but others could stimulate stromal and even tumor cell proliferation and dissemination, closely reminiscent of the paracrine stimulatory (immunotrophic) effects which immune cells exert on placental proliferation and implantation.

ACKNOWLEDGMENTS

We thank Martine Coutant for her secretarial help and Mme. Bussières for her assistance in microphotography. We gratefully acknowledge Claudie Gallet's advice on immunohistochemical techniques.

This work was supported by grants from the Institut Curie, the Ligue Nationale Française contre le Cancer (Comité de Paris) the Association de la Recherche Contre le Cancer (ARC), the FEGEFLUC, NIH grant CA47292, and a Bristol-Myers award 100-R063.

REFERENCES

1. Rushdi A, Nishikura K, Erikson K, Watt R, Rovera G, Croce CM: Science 222:390, 1983.
2. Yamamoto T, Ikawa S, Toyoshima K, et al.: Nature 319:230, 1986.
3. Casey G, Smith R, McGillivray D, Peters G, Dickson C: Mol Cell Biol 6:502, 1986.
4. Hibbs ML, Bonadonna L, Scott BM, McKenzie IEC, Hogarth PM: Proc Natl Acad Sci USA 85:2240, 1988.
5. Donner L, Fedele LA, Garon CF, Anderson SJ, Sherr CJ: J Virol 41:489, 1982.
6. Kacinski BM, Carter D, Yee LD, Mittal K, Scata K, Donofrio L, Chambers SK, et al.: Am J Pathol 137:135–147, 1990.
7. Woolford J, Rothwell V, Rohrschneider L: Mol Cell Biol 5:458, 1985.
8. Das SK, Stanley ER, Guilbert LJ, Forman LW: Blood 58:630, 1981.
9. Kruskal-Wallis: In Wonnacott TH, Wonnacott RJ (eds): "Statistics for Business and Economics." New York: John Wiley & Sons, 1984, p 508.
10. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Science 235:177, 1987.
11. Ali IU, Campbell G, Lidereau R, Callahan R: Science 240:1795, 1988.
12. Paik SM, Fisher ER, Fisher B, Sass RE, Lippman ME, King CR: 'Abstract' Proc Am Assoc Can Res 30:1752, 1989.
13. Borg A, Sigurdsson H, Ferno M, McGuire WL: 'Abstract' Biology and Therapy of Breast Cancer. Third IST Int. Symposium: 105, 1989.
14. Varley JM, Swallow JE, Brammar WJ, Whittaker JL, Walker RA: Oncogene 1:423, 1987.
15. Borg A, Sigurdsson H, Olsson H, Dickson C, McGuire WL: Third IST Symposium: 104, 1989.
16. Callahan R: Breast Cancer Res Treat 13:191, 1989.
17. Fourquet A, Campana F, Zafrani B, Vilcoq J, et al.: Int J Radiat Oncol Biol Phys 17:719, 1989.
18. Van de Vijver MJ, Peterse JL, Mooi WJ, Nusse R, et al.: N Engl J Med 319:1239, 1988.
19. Rosen PP, Groshen S, Saigo PE, Kinne DW, Hellman S: J Clin Oncol 7:1239, 1989.
20. Gorelik E, Wiltrout RH, Copeland D, Herberman RB: Cancer Immunol Immunother 19:35, 1985.
21. Fidler IJ, Gersten DJ, Budmen MB: Cancer Res 36:3160, 1976.
22. Dabbous MH, Walker R, Haney L, Carter LM, Nicolson GL: Br J Cancer 54:459, 1986.
23. Dabbous MH, Wooley DE, Haney L, Carter LM, Nicolson GL: Clin Exp Metastasis 4:141, 1986.
24. Dabbous MH, North SM, Haney L, Nicolson GL: Cancer Res 48:6832, 1988.
25. Welch D, Schisseler R, Howrey RP, Aeed PA: Proc Natl Acad Sci USA 86:5859, 1989.
26. Kacinski BM, Chambers SK, Carter D, Filderman AE, Stanley ER: In Dinarello CA, Kluger MJ, Powanda MC, Oppenheim JJ (eds): "The Physiological and Pathological Effects of Cytokines," New York: John Wiley & Sons, 1990, p 393.
27. Kacinski BM: Int J Radiat Oncol Biol Phys (in press).
28. Kacinski BM, Carter D, Kohorn EI, Mittal K, Bloodgood RS, et al.: Yale J Biol Med 62:379–382, 1989.
29. Pollard JW, Bartocci A, Arceci R, Orlovski A, Ladner MB, Stanley ER: Nature 330:484, 1987.
30. Rettenmeyer CW, Sherr CJ: "Hematology/Oncology Clinics of North America." 1989, vol 3, No 3, p 479.
31. Wegman TG: Am J Reprod Immunol Editorial (in press).
32. Kacinski BM, Bloodgood RS, Schwartz PE, Carter DC, Stanley ER: CSHSQB "Cancer Cells" 7:333, 1989.
33. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: Proc Natl Acad Sci USA 84:5788, 1987.