American Journal of Pathology, Vol. 148, No. 1, January 1996

Coexpression of Hepatocyte Growth Factor and Receptor (Met) in Human Breast Carcinoma

Alan B. Tuck,* Morag Park,†
Ernest E. Sterns,‡ Alexander Boag,* and
Bruce E. Elliott*

From the Departments of Pathology and Surgery,‡ Kingston General Hospital and Queen's University, Kingston, Ontario, and the Molecular Oncology Group,† Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada*

Expression of hepatocyte growth factor (HGF) and HGF receptor (HGFR, product of the* met *proto-oncogene) mRNA were examined by nonisotopic* in situ *hybridization in a spectrum of benign and malignant human breast tissues. mRNA for both HGFR and HGF was detected in benign ductal epithelium. Epithelial expression of HGF mRNA was particularly intense in regions of ductal epithelial hyperplasia. Positive expression of HGF (but not HGFR) mRNA was also found in adipocytes, endothelial cells, and to varying degrees in stromal fibroblasts. In 12 of 12 cases of ductal carcinoma* in situ *and infiltrating ductal carcinoma, carcinoma cells showed a heterogeneous pattern of expression for both HGFR and HGF mRNA. In infiltrating ductal carcinomas, intense expression of HGFR mRNA was not restricted to ductular structures but was also seen in non-duct-forming carcinoma cells. The same zones of the tumors (most commonly at the advancing margins) that expressed strongly HGFR mRNA often were also strongly positive for HGF mRNA, suggesting a possible autocrine effect. The expression pattern of HGFR protein in 25 cases including the same series of tissues used for* in situ *hybridization analysis was similar to that of HGFR mRNA, as determined by an immunoperoxidase technique. The finding that HGFR is expressed by both benign and malignant epithelium, and is not restricted to duct-forming structures, suggests that, although the potential for HGF/HGFR binding is maintained in malignancy, the response to ligand binding at the level of the receptor or the cellular response to receptor activation may change at some point during progression. (Am J Pathol 1996, 148:225–232)

Hepatocyte growth factor receptor (HGFR), also known as the product of the *met* proto-oncogene, is expressed by a variety of epithelial (and a few non-epithelial) cell types, including mammary epithelium.[1–4] The ligand, hepatocyte growth factor (HGF) was originally described as a mitogen for hepatocytes[5] and later found to be identical to scatter factor, a modulator of intercellular organization.[6] HGF has been found to be produced and secreted by cells of the mesenchymal/stromal compartment,[7–12] although recent evidence has shown epithelial expression of HGF in certain instances.[13–16] HGF has been shown to control a variety of cell functions including cell growth,[10,17,18] movement,[19–22] invasiveness,[21,22] and cell-cell adhesion,[23,24] as well as morphogenesis of epithelial (eg, ductular or tubular) structures.[2,25–27] Many of these functions have been shown to require activation of the Met receptor.[28,29]

There is presently controversy over the possible role of HGFR in human breast cancer. Both the HGF[6] and HGFR[30] genes have been mapped to human chromosome 7q. Loss of heterozygosity at 7q has been found to be a frequent event in human breast cancer (40% of informative cases), and loss of heterozygosity at 7q in human breast tumors has been found to correlate with shorter metastasis-free and overall survival times.[31] In addition, Tsarfaty et al[2] have reported that HGFR protein is expressed in benign ductal epithelium and is expressed at lower levels in the immediately adjacent malignant ductal epithelium. Although collectively these results are consistent with a possible tumor suppressor role for

Supported by grants from the National Cancer Institute of Canada (6233), the U.S. Army Breast Cancer Research Initiative (DAMD 17-94-J-4407), and the Clare Nelson Bequest Research Fund, Kingston General Hospital.

Accepted for publication September 12, 1995.

Address reprint requests to Dr. Bruce Elliott, Cancer Research Laboratories, Department of Pathology, Rm A307, Botterell Hall, Queen's University, Kingston, Ontario, Canada K7L 3N6.

HGF and/or HGFR in human breast carcinoma, this hypothesis has yet to be proven. Other studies have shown HGF to act as a motogen or morphogen in most breast carcinoma cell lines tested,[2,22] and there are a few reports of HGF acting as a mitogen for mammary epithelial cells.[10,18] Wang et al[16] reported that HGF mRNA is expressed in both benign and malignant mammary epithelium and that the most abundant expression in benign epithelium was in regions of proliferative activity. Although they suggested a possible autocrine role for HGF in inducing proliferation of benign and malignant mammary ductal epithelium, they had not examined expression of HGFR mRNA or protein in these same tissues to support this contention. Finally, the clinical import of HGF in human breast carcinoma was highlighted recently in the work of Yamashita et al,[32] who reported that a high level of expression of HGF protein is an even more significant factor in predicting poorer relapse-free and overall survival than is lymph node status. However, this study did not examine the stromal or epithelial source of the HGF in breast cancer tissues.

We thus regarded it to be important to establish more clearly the cellular expression pattern of both HGF and HGFR in benign and malignant human breast tissues. We examined expression of both genes at the mRNA level in serial sections of the same tissues, using *in situ* hybridization (ISH).

Materials and Methods

Tissues

Mastectomy and segmentectomy/lumpectomy specimens were obtained fresh immediately after excision. Sections of both benign and malignant breast taken for ISH were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 4 hours, followed by alcohol cycle processing and embedding in paraffin. Sections of normal skin were taken for use as controls for HGFR expression from associated (usually nipple region) skin. Placental tissues as controls for HGF expression were obtained fresh from normal term deliveries and also fixed immediately in 4% paraformaldehyde for 4 hours. For ISH, tumor specimens included 2 cases of ductal carcinoma *in situ* alone and 2 cases of well differentiated, 5 cases of moderately differentiated, and 3 cases of poorly differentiated infiltrating ductal carcinoma (IDC). For immunohistochemistry, an additional 13 specimens, including 1 well differentiated, 7 moderately differentiated, and 5 poorly differentiated IDCs were analyzed. Access to tissues satisfied the requirements of the Kingston General Hospital ethics committee.

Plasmids

Both the HGF and HGFR (*met*) plasmids used for generation of riboprobes consisted of a cDNA fragment of the gene of interest cloned into a Bluescript KSII$^+$ vector between the T3 and T7 promoters, each with the 5′ end of the cDNA downstream to the T7 promoter. The HGF cassette consisted of the 540-bp *Bam*HI-*Xho*I fragment of the human HGF cDNA.[33] The HGFR cassette consisted of the 800-bp *Eco*RI-*Eco*RV fragment of the human *met* cDNA.[34] The platelet-derived growth factor α-receptor plasmid, used for generation of control riboprobe, consisted of the 1.5-kb *Eco*RI-*Pst*I fragment (extracellular domain) of the cDNA cloned into pGEM-Blue between the T7 and SP6 promoters, such that the 5′ end lay downstream of the T7 promoter. The platelet-derived growth factor α-receptor plasmid was obtained as a kind gift from Dr. Lena Welsh and Dr. Keiko Funa.[35]

Riboprobes

Riboprobes were generated by *in vitro* transcription from linearized templates with the appropriate phage RNA polymerase (Promega Corp., Madison, WI) in the presence of digoxigenin-UTP (Boehringer Mannheim, Montreal, Canada). Antisense riboprobes for HGF and HGFR were generated by transcription from the T3 promoter, whereas platelet-derived growth factor α-receptor antisense (control) riboprobes were generated by transcription from the SP6 promoter. Riboprobes generated from vector-only templates were also used as negative controls.

In Situ *Hybridization*

ISH was performed by a modification of the procedure of Yang and Park.[36] Six-micron paraffin sections were cut onto baked slides coated with triethoxysilane (Sigma Chemical Co., St. Louis, MO), dewaxed in toluene, and rehydrated. Permeabilization was performed by treating at room temperature sequentially with 0.2 mmol/L HCl, 0.2% Triton X-100 in PBS, and 40 μg/ml proteinase K for 10 minutes each. Slides were then washed in 0.1X PBS, refixed for 30 minutes at room temperature in 4% paraformaldehyde, washed again in 0.1X PBS, and acetylated with 0.25% acetic anhydride in 0.1 mmol/L triethanolamine HCl. Slides were then dehydrated, delipidated in 100% chloroform for 15 minutes followed by

absolute ethanol for 5 minutes and 95% ethanol for 15 minutes, and then air dried.

Probes were diluted in prehybridization mix at 15 ng/μl, and 200 μl of hybridization mix was applied to each section. Slides were incubated in a humid chamber at 42°C overnight, washed in 1X standard saline citrate (SSC) at 55°C for 30 minutes, rinsed in RNAse buffer (0.5 mol/L NaCl, 10 mmol/L PIPES (pH 7.2), 0.1% Tween 20) at room temperature for 10 minutes, and incubated in 20 μg/ml RNAse A (Sigma) for 30 minutes at 37°C to remove unbound single-stranded RNA. Slides were washed in buffer 1 (100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.5) at room temperature for 10 minutes and blocked with 3% normal sheep serum in buffer 1 at room temperature for 30 minutes. To detect specific hybrids, slides were incubated with anti-digoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim; 1 to 1000 dilution in buffer 1 with 3% normal sheep serum) for 30 minutes, and then washed twice (10 minutes each) with buffer 1 and twice (5 minutes each) in buffer 2 (100 mmol/L Tris-HCl (pH 9.5), 100 mmol/L NaCl, 50 mmol/L $MgCl_2$). Hybrids bound to anti-digoxigenin antibody were then visualized by a color reaction containing nitroblue tetrazolium salt, 5-bromo-4-chloro-3-indolylphosphate, and levamisole (0.24 mg/ml) in buffer 2. An alkaline phosphatase substrate kit IV (Vector Laboratories, Burlingame, CA) was used. Color was allowed to develop for 12 hours in the dark. Slides were then dehydrated, washed in xylene, mounted with permount, and viewed and photographed by a light microscope. Positive areas showed brown-purple cytoplasmic staining (Figures 1 and 2).

Testing for RNA specificity of probe binding was performed by (1) treating sets of control slides with 20 μg/ml RNAse A in RNAse buffer for 30 minutes at 37°C before hybridization and (2) hybridizing with vector-only riboprobes generated from the parent vector without the HGF or HGFR cassette, transcribed in the same direction as the antisense probes. Controls for cell specificity of binding included testing of the pattern of binding of antisense HGFR and HGF probes to known positive tissue targets (eg, placenta (ie, trophoblast) for HGFR mRNA and skin (ie, epidermis and adnexal epithelium) for HGF mRNA).

Immunohistochemistry

Paraffin-embedded sections of formalin-fixed breast tissues from 25 cases of ductal carcinoma *in situ* and IDC including the same set of surgical pathological specimens used for ISH analysis were assessed for HGFR expression by an immunoperoxidase technique. Samples were immunostained with a polyclonal anti-Met antibody raised against a COOH-terminal peptide (1:200),[37] by a modification of the avidin-biotin peroxidase complex method previously described.[38] The immunoreaction was visualized with diaminobenzidine and H_2O_2. The antibody used has been shown previously to detect human Met (HGFR) specifically by both immunoprecipitation and Western blotting.[15,37]

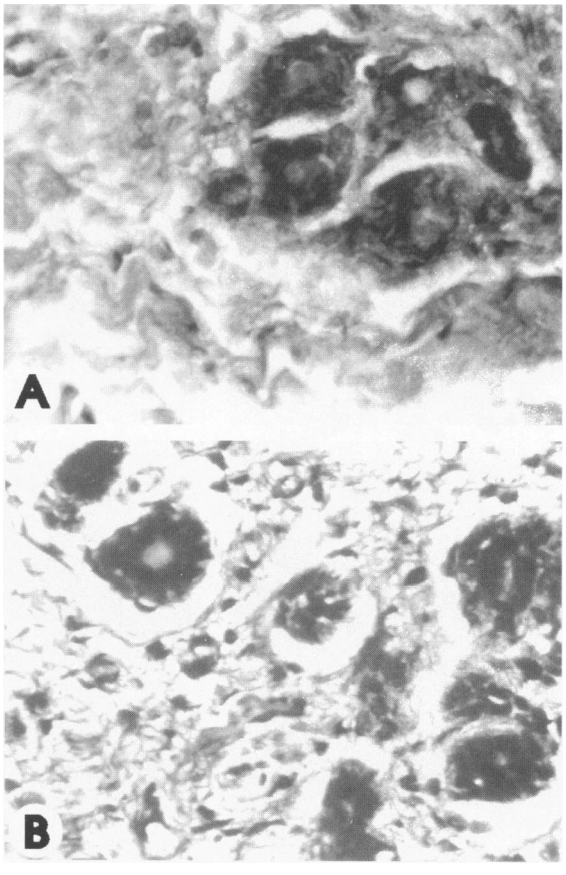

Figure 1. *Nonmalignant breast tissue.* **A:** *ISH for HGFR mRNA showing a strong cytoplasmic staining in ductal epithelial cells but negligible cytoplasmic staining of stromal cells (original magnification, ×630).* **B:** *ISH for HGF mRNA showing variable cytoplasmic staining of benign epithelium, with focal areas of strong positivity. Some cytoplasmic staining is also apparent in stromal fibroblasts and endothelial cells (original magnification, ×630).*

Results

Both HGFR and HGF mRNA were expressed cytoplasmically in regions of nonmalignant ductal epithelium in all tissues examined (Figure 1). Expression of HGF mRNA in benign ductal epithelium was variable but appeared most intense in regions showing architectural evidence of ductal epithelial hyperplasia. Expression of HGF (but not HGFR) mRNA was also

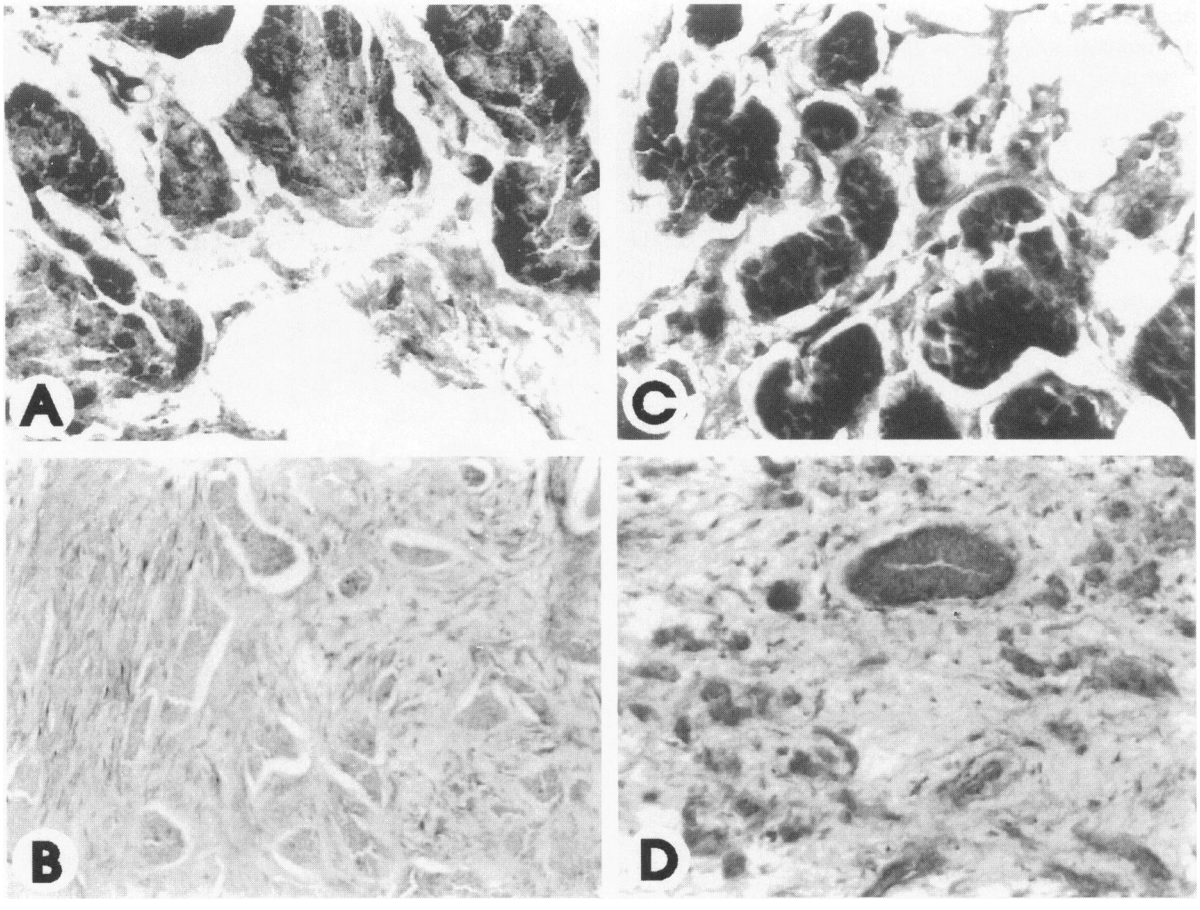

Figure 2. *Moderately* (A to C) *and well* (D) *differentiated ductal carcinomas.* A: *ISH for HGFR mRNA showing intense cytoplasmic positivity at the advancing margin of the tumor* (*original magnification,* ×*630*). B: *ISH for HGFR mRNA showed much weaker staining in the malignant epithelium in more central regions of the same tumor* (*original magnification,* ×*250*). C: *ISH for HGF mRNA showing strong cytoplasmic positivity of the malignant epithelium at the advancing margin of the same tumor, in the same region that stained intensely for HGFR mRNA* (*original magnification,* ×*630; cf* A). *In addition, stromal fibroblasts and endothelial cells showed some cytoplasmic positivity.* D: *ISH analysis for HGFR mRNA showing IDC surrounding a benign duct. Intensity of cytoplasmic staining is similar in both benign and malignant epithelium, regardless of whether the malignant epithelium forms well defined ductular structures* (*original magnification,* ×*400*).

found to varying degrees in adipocytes, endothelial cells, and stromal fibroblasts (Figure 1B). Ductal carcinoma *in situ* and IDC cells in all tissues examined showed a heterogeneous pattern of expression of both HGFR and HGF mRNA (Figure 2). In 10 of 10 IDCs examined, intense expression of HGFR mRNA was not restricted to ductular structures but was seen also in non-duct-forming carcinoma cells (Figure 2D). There was no apparent difference in patterns of HGF mRNA and HGFR mRNA in IDCs of different grades. The same zones of a tumor that were strongly positive for HGFR mRNA were commonly also strongly positive for HGF mRNA expression, suggesting a possible autocrine loop effect (eg, Figure 2, A and C). Where heterogeneity of expression for HGF and HGFR mRNA was apparent within a tumor, strongest positivity for both was usually seen at or near the advancing margins of the tumor (Figure 2, A and C), whereas expression in more central regions of the tumor was less intense (Figure 2B). This was true for 9 of 10 tumors, which included sampling of the advancing margin. This effect was seen most commonly in the absence of morphological evidence of ischemia/necrosis in more central regions of the tumor, such that higher level expression of mRNA for both HGF and HGFR at the advancing margins is unlikely a result of the ischemia effect alone.

Pretreatment of slides with RNAse A eliminated all positive signals. Likewise, riboprobes generated from the parent vector lacking the HGF or HGFR insert showed no positivity (not shown). ISH of skin sections for HGFR mRNA showed the expected pattern of strong positivity in immature layers of the epidermis and in the adnexal epithelium,[5,39] and expression of HGF mRNA in placental tissue showed the expected pattern of strong positivity in the trophoblast[40] (not shown). These controls verify that

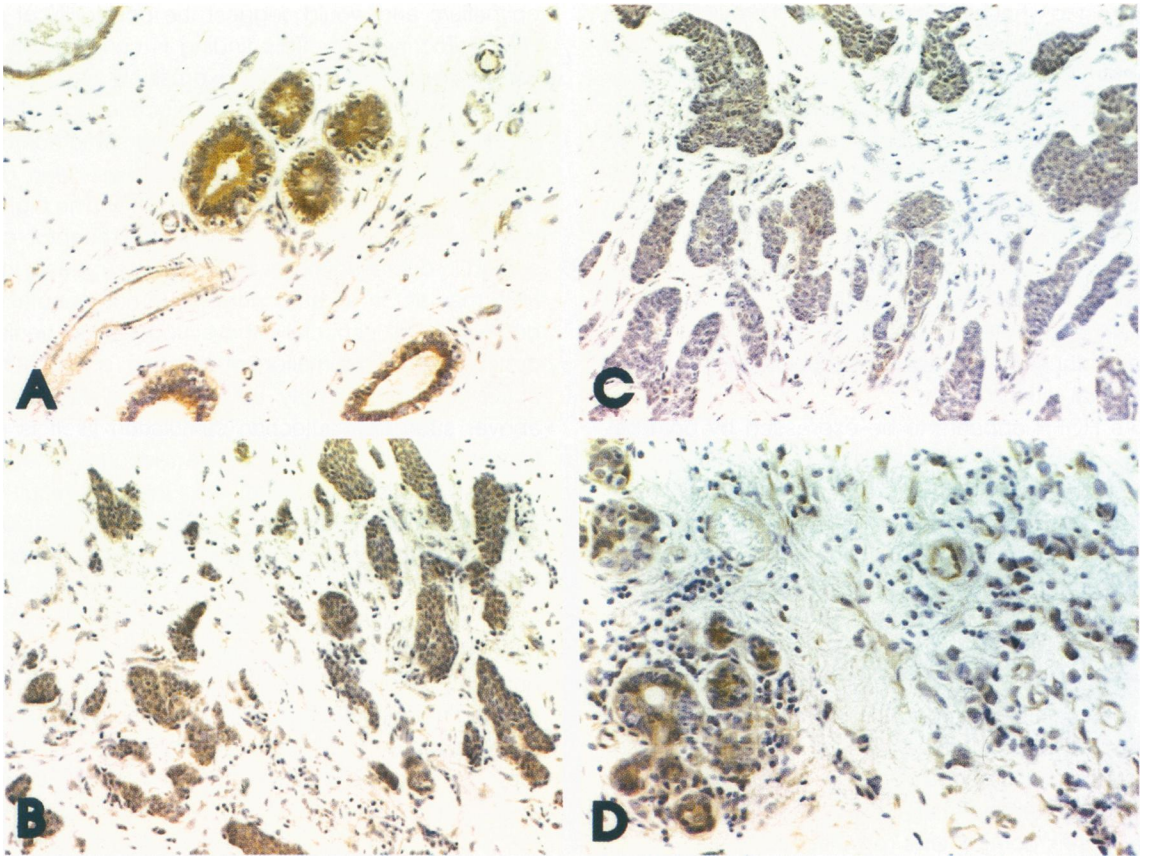

Figure 3. *Immunoperoxidase localization of HGFR protein expression.* A: *Benign ducts showing strong cytoplasmic/membrane positivity (brown; original magnification, ×250).* B: *Moderately differentiated IDC showing strong cytoplasmic/membrane positivity at the advancing margin (original magnification, ×250).* C: *Same tumor as in* B, *showing gradient of staining intensity: strongest closer to the advancing margin* (top) *and weakest in more central regions (bottom) of the tumor (original magnification, ×250).* D: *Poorly differentiated IDC (right) adjacent to nonmalignant ducts (left) showing comparable staining intensity in both malignant (non-duct-forming) and nonmalignant (ductal) epithelium (original magnification, ×400).*

HGF and HGFR probes were in fact binding to the corresponding mRNA. Analysis of breast tissue with a control antisense platelet-derived growth factor α-receptor riboprobe showed the predicted pattern of weak positive expression in stromal fibroblasts, endothelial cells, and inflammatory cells, with no expression in benign or malignant ductal epithelial cells (not shown). Similarly, analysis of skin sections for platelet-derived growth factor α-receptor expression showed positivity in dermal fibroblasts and endothelial cells, with no expression in the epidermis, as reported previously by Pontén et al[41] (not shown). Collectively, these controls confirmed the specificity of the ISH procedure.

Immunoperoxidase analysis of HGFR protein expression showed a pattern of staining closely reflecting that seen for expression of the corresponding mRNA (Figure 3, A–D). In 25 of 25 cases examined, strong expression of HGFR protein was seen in both benign and malignant epithelium and was not restricted to ductal or acinar structures. Most intense positive staining for HGFR protein was apparent at or near the advancing margins of invasive carcinomas (Figure 3B).

Discussion

The ISH data reported here show that HGF mRNA is expressed by benign and malignant mammary ductal epithelium, as well as variable expression by stromal cells (fibroblasts, adipocytes, and endothelial cells), in a manner similar to that described by Wang et al.[16] Also in agreement with Wang et al,[16] we found intense expression of HGF mRNA in benign ductal epithelium in regions of ductal epithelial hyperplasia as well as in malignant epithelium of different histological grades.

Expression of HGFR mRNA was detected in both benign and malignant ductal epithelium, consistent with the demonstration by Tsarfaty et al[2] of HGFR protein expression in similar tissues. However, in

contrast to what has been described previously,[2] we have found that expression of HGFR mRNA and protein levels are not decreased uniformly in malignant epithelium, and expression of HGFR is not limited to cells lining ductules or tubules. In fact, we have found that both *in situ* and invasive ductal carcinomas of all grades show a heterogeneous pattern of HGFR expression. Individual malignant cells or groups of cells of poorly differentiated IDCs were commonly found to stain strongly for both HGFR mRNA and protein. Level of expression of HGFR in itself is thus not necessarily associated with degree of ductal differentiation.

As HGFR appears to be expressed by both benign and malignant epithelium and is not restricted to duct-forming structures (suggesting that the potential for HGF/HGFR binding is maintained in malignancy), it may be that functional differences exist between benign and malignant breast tissue relating to HGF/HGFR. Such differences could include the activity of the ligand or receptor itself and post-receptor signal transducer/effector systems. For example, cellular responsiveness to HGF has been shown to be influenced by factors (eg, urokinase) regulating HGF activation[42] or other extracellular mediators (transforming growth factor-β or extracellular matrix components (eg, heparan sulfate)).[43,44] Alternatively, HGFR activation in benign epithelium may, as suggested by Tsarfaty et al[2] result in activation of a morphogenic program leading to duct formation. In malignancy, defective regulation of, or alteration of, components of this program (for example, loss of proper cell-cell adhesion) may result in an incomplete or dysfunctional program. In fact, scatter activity of HGF on invasive carcinomas has been found to be influenced by the integrity of the cadherin system.[45–47] Decreased expression of E-cadherin in more poorly differentiated carcinomas may render them more susceptible to the scatter activity of HGF. Thus, whereas HGF may induce ductal morphogenesis in the presence of an intact cadherin system in areas of well differentiated carcinoma, disruption of cadherin expression in more poorly differentiated areas may instead result in disaggregation, scatter, and more invasive behavior. Such a difference in pattern of response to HGF in different areas within a given tumor or by a given tumor at different times (during progression) could also explain what appears at first glance to be contradictory evidence in the literature for tumor-suppressing *versus* tumor-promoting effects of HGF ligand and receptor.

Zonal coexpression of HGF ligand and receptor mRNA was also found in both benign and malignant epithelium and would suggest the possibility of an HGF autocrine loop. This finding is consistent with recent evidence that HGFR expression may be upregulated by HGF.[48] A similar autocrine loop effect for HGF and HGFR in malignant (and some nonmalignant, proliferative) epithelial cells has been described previously in other systems.[13–15] The higher levels of coexpression of ligand and receptor observed in ductal epithelial hyperplasia suggests that HGF may act in an autocrine role in non-neoplastic ducts as well, especially those undergoing benign proliferation. In the malignant situation, a normal reactive mechanism may be altered as suggested above, such that autocrine stimulation itself is no longer properly regulated or the response to autocrine stimulation is altered, with the end result of invasion and metastasis (malignant conversion) rather than morphogenesis and ductular differentiation (tumor suppression). Also in keeping with this hypothesis is the finding that, in the majority of the tumors examined, highest levels of expression of both HGF and HGFR mRNA were found at the advancing margins of the tumor, where active invasion is taking place. This pattern of expression has been reported for other peptides, eg, transforming growth factor-β,[49] platelet-derived growth factor β-receptor,[50] cathepsin B,[51] and plasminogen activator,[52] which may be involved in invasiveness at the tumor front. Interestingly, urokinase-type plasminogen activator is also an activator of HGF.[42]

Additional work (both *in vivo* and *in vitro*) is necessary to elucidate the role of HGF in the control of these aspects of epithelial cell behavior and to study possible alterations of the response to HGF in malignancy. The recent evidence in the literature for a strong potential prognostic role for the level of HGF expression[32] adds an extra level of significance and urgency to unraveling the role of HGF in human breast carcinoma.

Acknowledgments

We thank Dr. Iain Young for helpful suggestions regarding ISH with digoxigenin-UTP. We thank Mr. Nader Rahimi for helpful discussions in preparation of the manuscript. We also thank Mr. Lloyd Kennedy for artistic and photographic assistance and Ms. Marg Morrow for help with the immunohistochemistry.

References

1. Iyer A, Kmiecik TE, Park M, Daar I, Blair D, Dunn KJ, Sutrave P, Ihle JN, Bodescot M, Vande Woude GF:

Structure, tissue-specific expression, and transforming activity of the mouse *met* proto-oncogene. Cell Growth Differ 1990, 1:87–95
2. Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL, Vande Woude GF: The Met proto-oncogene receptor and lumen formation. Science 1992, 257:1258–1261
3. Gherardi E, Stoker M: Hepatocyte growth factor-scatter factor: mitogen, motogen, and Met. Cancer Cells 1991, 3:227–232
4. Rubin JS, Bottaro DP, Aaronson SA: Hepatocyte growth factor/scatter factor and its receptor, the *met* proto-oncogene product. Biochim Biophys Acta 1993, 1155:357–371
5. Zarnegar R, Michalopoulos G: Purification and biological characterization of human hematopoietin A, a polypeptide growth factor for hepatocytes. Cancer Res 1989, 49:3314–2230
6. Weidner KM, Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Rieder H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y, Birchmeier W: Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci USA 1991, 88:7001–7005
7. Rosen EM, Goldberg ID, Kacinski BM, Buckholz T, Vinter DW: Smooth muscle releases an epithelial scatter factor which binds to heparin. In Vitro Cell Dev Biol 1989, 25:163–173
8. Seki T, Ihara I, Sugimura A, Shimonishi M, Nishizawa T, Asami O, Hagiya M, Nakamura T, Shimizu S: Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocytes. Biochem Biophys Res Commun 1990, 172:321–327
9. Noji S, Yashiro K, Koyama E, Nohno T, Ohyama K, Taniguchi S, Nakamura T: Expression of hepatocyte growth factor gene in endothelial and Kupffer cells of damaged rat livers, as revealed by *in situ* hybridization. Biochem Biophys Res Commun 1990, 173:42–47
10. Rubin JS, Chan AM-L, Bottaro D, Burgess W, Taylor WJ, Cech AC, Hirschfield DW, Wong J, Miki T, Finch P, Aaronson SA: A broad-spectrum human lung fibroblast-derived mitogen is a variant of hepatocyte growth factor. Proc Natl Acad Sci USA 1991, 88:415–419
11. Sonnenberg E, Weidner KM, Birchmeier W: Expression of the Met-receptor and its ligand, scatter factor/hepatocyte growth factor, during mouse development. Hepatocyte Growth Factor/Scatter Factor and the Met Receptor. Edited by ID Goldberg. Birkhäuser Verlag, Basel, 1993, pp 381–394
12. Birchmeier C, Birchmeier W: Molecular aspects of mesenchymal-epithelial interactions. Annu Rev Cell Biol 1993, 9:511–540
13. Adams JC, Furlong RA, Watt FM: Production of scatter factor by ndk, a strain of epithelial cells, and inhibition of scatter factor by suramin. J Cell Sci 1991, 98:385–394
14. Amano O, Matsumoto K, Nakamura T, Iseki S: Expression and localization of hepatocyte growth factor in rat submandibular gland. Growth Factors 1994, 10:145–151.
15. Tsao M-S, Zhu H, Giaid A, Viallet J, Nakamura T, Park M: Hepatocyte growth factor/scatter factor is an autocrine factor for human normal bronchial epithelial and lung carcinoma cells. Cell Growth Differ 1993, 4:571–579
16. Wang Y, Selden AC, Morgan N, Stamp GWH, Hodgson HJF: Hepatocyte growth factor/scatter factor expression in human mammary epithelium. Am J Pathol 1994, 144:675–682
17. Kan M, Zhang GH, Zarnegar R, Michalopoulos G, Myoken Y, McKeehan WL, Stevens JI: Hepatocyte growth factor/hematopoietin A stimulates the growth of rat kidney proximal tubule epithelial cells (RPTE), rat nonparenchymal liver cells, human melanoma cells, mouse keratinocytes and stimulates anchorage-independent growth of SV-40 transformed RPTE. Biochem Biophys Res Commun 1991, 174:331–337
18. Rahimi N, Lall R, Saulnier R, Nakamura T, Park M, Elliott B: Role of hepatocyte growth factor (HGF) in breast cancer: a novel mitogenic factor secreted by adipocytes. DNA Cell Biol 1994, 13:1189–1197
19. Rosen EM, Meromsky L, Setter E, Vinter DW, Goldberg ID: Smooth muscle-derived factor stimulates mobility of human tumor cells. Invasion Metastasis 1990, 10: 49–64
20. Stoker M: Effect of scatter factor on motility of epithelial cells and fibroblasts. J Cell Physiol 1989, 139:565–569
21. Bhargava M, Joseph A, Knesel J, Halaban R, Li Y, Pang S, Goldberg I, Setter E, Donovan MA, Zarnegar R, Michalopoulos GA, Nakamura T, Faletto D, Rosen EM: Scatter factor and hepatocyte growth factor: activities, properties, and mechanism. Cell Growth Differ 1992, 3:11–20
22. Rosen EM, Knesel J, Goldberg ID, Jin L, Bhargava M, Joseph A, Zitnik R, Wines J, Kelley M, Rockwell S: Scatter factor modulates the metastatic phenotype of the EMT6 mouse mammary tumor. Int J Cancer 1994, 57: 706–714
23. Birchmeier W, Weidner KM, Behrens J: Molecular mechanisms leading to loss of differentiation and gain of invasiveness in epithelial cells. J Cell Sci Suppl 1993, 17:159–164
24. Watanabe M, Matsumoto K, Nakamura T, Takeichi M: Effect of hepatocyte growth factor on cadherin-mediated cell-cell adhesion. Cell Struc Func 1993, 18:117–124
25. Montesano R, Matsumoto K, Nakamura T, Orci L: Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell 1991, 67:901–908
26. Tsarfaty I, Rong S, Resau JH, Rulong S, Pinto da Silva P, Vande Woude GF: The *met* proto-oncogene mesenchymal to epithelial cell conversion. Science 1994, 263:98–101
27. Johnson M, Koukoulis G, Matsumoto K, Nakamura T, Iyer A: Hepatocyte growth factor induces proliferation

and morphogenesis in nonparenchymal epithelial liver cells. Hepatology 1993, 17:1052–1061
28. Zhu H, Naujokas MA, Park M: Receptor chimeras indicate that the Met tyrosine kinase mediates the motility and morphogenic responses of hepatocyte growth/scatter factor. Cell Growth Differ 1994, 5:359–366
29. Komada M, Kitamura N: The cell dissociation and motility triggered by scatter/hepatocyte growth factor are mediated through the cytoplasmic domain of the Met receptor. Oncogene 1993, 8:2381–2390
30. Dean M, Park M, Le Beau MM, Robins TS, Diaz MO, Rowley JD, Blair DG, Vande Woude GF: The human *met* oncogene is related to the tyrosine kinase oncogenes. Nature 1985, 318:385–388
31. Bièche I, Champème MH, Matifas F, Hacène K, Callahan R, Lidereau R: Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer. Lancet 1992, 339:139–143
32. Yamashita J, Ogawa M, Yamashita S, Nomura K, Kuramoto M, Saishoji T, Shin S: Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. Cancer Res 1994, 54:1630–1633
33. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugiura A, Tashiro K, Shimizu S: Molecular cloning and expression of human hepatocyte growth factor. Nature 1989, 34:440–443
34. Park M, Dean M, Kaul K, Braun M, Gonda MA, Vande Woude GF: Sequence of *met* proto-oncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. Proc Natl Acad Sci USA 1987, 84:6379–6383
35. Claesson-Welsh L, Eriksson A, Westermark B, Heldin C-H: cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. Proc Natl Acad Sci USA 1989, 86:4917–4921
36. Yang MX, Park M: Expression of the Met/hepatocyte growth factor/scatter factor receptor and its ligand during differentiation of murine p19 embryonal carcinoma cells. Dev Biol 1993, 157:308–320
37. Rodrigues GA, Naujokas MA, Park M: Alternative splicing generates isoforms of the *met* receptor tyrosine kinase which undergo differential processing. Mol Cell Biol 1991, 11:2962–2970
38. Giaid A, Hamid QA, Springall DR, Yanagisawa M, Shinmi O, Sawamura T, Kimura S, Masaki T, Corrin B, Polak JM: Expression of endothelin immunoreactivity and mRNAs in pulmonary tumours. J Pathol 1990, 162: 15–22
39. Prat M, Narsimhan RP, Crepaldi T, Nicotra MR, Natali PG, Comoglio PM: The receptor encoded by the human *met* oncogene is expressed in hepatocytes, epithelial cells and solid tumors. Int J Cancer 1991, 49: 323–328
40. Wolf HK, Zarnegar R, Oliver L, Michalopoulos GK: Hepatocyte growth factor in human placenta and trophoblastic disease. Am J Pathol 1991, 138:1035–1043
41. Pontén F, Ren Z, Nistér M, Westermark B, Pontén J: Epithelial-stromal interactions in basal cell cancer: the PDGF system. J Invest Dermatol 1994, 102:304–309
42. Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F, Comoglio PM: Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. EMBO J 1992, 11:4825–4833
43. Zarnegar R, Michalopoulos G: Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. Cancer Res 1989, 49:3314–3320
44. Santos OFP, Nigam SK: HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-β. Dev Biol 1993, 160:293–302
45. Behrens J, Weidner KM, Frixen UH, Schipper JH, Sachs M, Arakaki N, Daikuhara Y, Birchmeier W: The role of E-cadherin and scatter factor in tumor invasion and cell motility. Experientia Suppl 1991, 59:109–126
46. Birchmeier W, Weidner KM, Behrens J: Molecular mechanisms leading to loss of differentiation and gain of invasiveness in epithelial cells. J Cell Sci Suppl 1993, 17:159–164
47. Watabe M, Matsumoto K, Nakamura T, Takeichi M: Effect of hepatocyte growth factor on cadherin-mediated cell-cell adhesion. Cell Struc Func 1993, 18:117–124
48. Boccaccio C, Gaudino G, Gambarotta G, Galimi F, Comoglio PM: Hepatocyte growth factor (HGF) receptor expression is inducible and is part of the delayed-early response to HGF. J Biol Chem 1994, 269:12846–12851
49. Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA: Immunohistochemical staining for transforming growth factor β1 associates with disease progression in human breast cancer. Cancer Res 1992, 52:6949–6952
50. Sundberg C, Ljungstrom M, Lindmark G, Gerdin B, Rubin K: Microvascular pericytes express platelet-derived growth factor-β receptors in human healing wounds and colorectal adenocarcinoma. Am J Pathol 1993, 143:1377–1388
51. Sinha AA, Gleason DF, Deleon OF, Wilson MJ, Sloane BF: Localization of biotinylated cathepsin B oligonucleotide probe in human prostate including invasive cells and invasive edges by *in situ* hybridization. Anat Rec 1993, 235:233–240
52. DeVries TJ, Quax PH, Denijn M, Verrijp KN, Verheijen JH, Verspaget HW, Weidle UH, Ruiter DJ, van Muijen GN: Plasminogen activators, their inhibitors, and urokinase receptor emerge in late stages of melanocytic tumor progression. Am J Pathol 1994, 144:70–81