ARTICLE

Tumor Collagenase Stimulatory Factor (TCSF) Expression and Localization in Human Lung and Breast Cancers

Myriam Polette, Christine Gilles, Veronique Marchand, Marianne Lorenzato, Bryan Toole, Jean-Marie Tournier, Stanley Zucker, Philippe Birembaut

INSERM U 314, IFR 53, CHU Maison Blanche, Reims, France (MP,VM,J-MT,PB); Vincent T. Lombardi Cancer Research Center, University Medical Center, Washington, DC (CG); Laboratoire Pol Bouin, CHU Maison Blanche, Reims, France (MP,ML,PB); Department of Anatomy and Cellular Biology, Tufts University Health Science School, Boston, Massachusetts (BT); and Departments of Research and Medicine, Veterans Affairs Medical Center, Northport, Massachusetts (SZ)

SUMMARY Tumor cell-derived collagenase stimulatory factor (TCSF) stimulates in vitro the biosynthesis of various matrix metalloproteinases involved in tumor invasion, such as interstitial collagenase, gelatinase A, and stromelysin 1. The expression of TCSF mRNAs was studied in vivo, using in situ hybridization and Northern blotting analysis, in seven normal tissues and in 22 squamous cell carcinomas of the lung, and in seven benign proliferations and in 22 ductal carcinomas of the mammary gland. By in situ hybridization, TCSF mRNAs were detected in 40 of 44 carcinomas, in pre-invasive and invasive cancer cells of both lung and breast cancers. TCSF mRNAs and gelatinase A mRNAs were both visualized in the same areas in serial sections in breast cancers, and were expressed by different cells, tumor cells, and fibroblasts. The histological results were confirmed by Northern blot analysis, which showed a higher expression of TCSF mRNAs in cancers than in benign and normal tissues. These observations support the hypothesis that TCSF is an important factor in lung and breast tumor progression. **(J Histochem Cytochem 45:703–709, 1997)**

Tumor invasion is a multistep process that involves the degradation of basement membrane and interstitial matrix components by proteolytic enzymes. Many data actually support an important role for the matrix metalloproteinases (MMPs) in this proteolytic event. High levels of MMPs have been described in many cancer cell lines that display high invasive capacity (Gilles et al. 1994; Monsky et al. 1994; Taniguchi et al. 1992,1994; Bernhard et al. 1990; Bonfil et al. 1989). Such an observation has been recently extended to a newly discovered member of the MMPs, membrane type matrix metalloproteinase 1 (MT-MMP-1), which has also been shown to be correlated with in vitro invasiveness (Sato et al. 1994; Okada et al. 1995; Gilles et al. 1996). In vivo, MMPs have also been associated with the metastatic progression of many human cancers (Davies et al. 1993; Clavel et al. 1992; Levy et al. 1991; Monteagudo et al. 1990). However, recent in

vivo data obtained by in situ hybridization (ISH) have shown that interstitial collagenase, gelatinase A, stromelysins, and MT-MMP-1 are mostly synthesized by fibroblasts localized near tumor cell clusters (Okada et al. 1995; Pyke et al. 1993; Poulsom et al. 1992,1993; Polette et al. 1991,1993,1996; Basset et al. 1990).

The specific detection of MMPs in peritumoral fibroblasts has led to the hypothesis that tumor cells might induce the synthesis of these enzymes implicated in cancer dissemination. In agreement with such an idea, several investigators have demonstrated cooperation between tumor cells and fibroblasts in vitro in the regulation of several MMPs, such as interstitial collagenase (Noël et al. 1993; Hernandez et al. 1985; Biswas 1984,1985; Bauer et al. 1979) and gelatinase A (Ito et al. 1995; Noël et al. 1994). Furthermore, a tumor cell-derived collagenase stimulatory factor (TCSF) also present in tumor cell-conditioned media, was isolated and purified from the plasma membranes of a human lung carcinoma cell line (Ellis et al. 1989). This factor is a glycoprotein of 58 kD and was recently

identified as a member of the immunoglobulin super-family (Biswas et al. 1995). In addition to enhancing interstitial collagenase synthesis (Nabeshima et al. 1991), purified TCSF stimulates gelatinase A and stromelysin 1 expression by fibroblasts (Kataoka et al. 1993). Immunohistochemical studies employing a monoclonal antibody directed against TCSF have shown that TCSF is localized to the outer surface of cultured lung cancer cell lines (Ellis et al. 1989). The same distribution was seen in tumors of urinary bladder, in which TCSF was detected at the periphery of cancer cells but not in surrounding stromal cells (Muraoka et al. 1993). Furthermore, TCSF was also localized in tumor cells by immunohistochemistry in invasive and in situ ductal breast cancers (Zucker and Biswas 1994).

On the basis of limited information concerning TCSF localization in cancer tissue, the role of TCSF in tumor progression remains unclear. In the present study, to clarify the cell origin of TCSF and to study its role in cancer invasion, we performed in situ hybridization and Northern blot analysis on human lung and breast carcinomas as well as on normal tissues.

Materials and Methods

Source of Tissue

The tissue was obtained from 22 lungs resected for squamous cell carcinomas of Stages I (10 cases), II (eight cases), and III (four cases) according to the TMN classification, from seven normal lung samples, from 22 ductal breast cancers of Grade 1 (four cases), Grade 2 (14 cases), and Grade 3 (four cases) according to the Scarf and Bloom classification, and from seven benign breast proliferations (two fibrocystic disease and five fibroadenoma).

Tissue Preparation

Part of the samples were frozen in liquid nitrogen for Northern blot analysis and the remainder were fixed in formalin and embedded in paraffin for in situ hybridization.

In Situ Hybridization Localization

Tissue sections (5 μm) were deparaffinized, rehydrated, and treated with 0.2 M HCl for 20 min at room temperature, followed by 15 min in 1 μg/ml proteinase K (Sigma Chemical; St Louis, MO) in Tris–EDTA–NaCl, 37C, to remove basic proteins. The sections were washed in 2 $\times$ SSC (sodium saline citrate), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min, and hybridized overnight with ^{35}S-labeled (50C) anti-sense RNA transcripts. TCSF cDNA (1700 bp) and gelatinase A (1500 bp) (a gift from G. Murphy; Cambridge, UK) were subcloned into pBluescript II SK+/− plasmid and pSP64, respectively, and used to prepare ^{35}S-labeled RNA probes. Hybridizations were followed by RNAse treatment (20 μg/ml, 1 h, 37C) to remove unhybridized probe and two stringent washes (50% formamide–2 $\times$ SSC, 2 hr at 60C) before autoradiography using D 19 emul-

sion (Kodak; Rochester, NY). Slides were exposed for 15 days before development. The controls were performed under the same conditions, using ^{35}S-labeled sense RNA probes. All slides were counterstained with HPS (hematoxylin–phloxin–safran), mounted, and examined under a Zeiss Axiophot microscope.

In Situ Hybridization Quantitation by Image Cytometry

Quantitation of the number of hybridization grains/μm^2 was performed with the help of an automated image analyzer, the DISCOVERY system (Becton–Dickinson; Mountain View, CA). After thresholding, the number of grains are counted automatically on at least six fields at high magnification ($\times$ 500). At this magnification, one field measures 12,688 μm^2. We performed these measurements on six different samples (three lung and three breast carcinomas) in which we found normal, in situ, and invasive areas on the same tissue section. Statistical analyses of TCSF mRNA expression levels were compared using the non-parametric Mann–Whitney U-test. Data were expressed as mean of dots/μm^2 $\pm$ SEM. p values equal to or less than 0.05 were considered significant.

Northern Blot Analysis

Extraction of total RNA from tissues was performed by RNAzol treatment (Biogenesis; Bournemouth, UK). Ten μg of each RNA was analyzed by electrophoresis in 1% agarose gels containing 10% formaldehyde and transferred onto nylon membranes (Hybond-N; Amersham, Poole, UK). The membrane was hybridized with the cDNA probe encoding TCSF (1700 bp) labeled with ^{32}P using random priming synthesis (5 $\times$ 108 cpm/μg) (Dupont de Nemours; Bruxelles, Belgium). The filters were exposed for 1 day. Membranes were rehybridized to a ubiquitous 36B4 gene probe, which served as a control. Signal intensities were recorded using a CD 60 Desaga (Heidelberg, Germany) laser-scanning densitometer and TCSF levels (in arbitrary units) were standardized with their corresponding 36B4 levels to obtain values independent of RNA quantities deposed onto gels. Statistical analyses of TCSF expression levels were compared using the non-parametric Mann–Whitney U-test. Data were expressed as mean $\pm$ SEM. Differences or similarities between two populations were considered significant when confidence intervals were <95% ($p<0.05$).

Results

Lung Lesions

By Northern blotting, TCSF transcripts were detected in 18 of 22 carcinomas. Quantitative analysis showed significantly higher ($p<0.05$) TCSF mRNA expression in lung carcinomas than in peritumoral lung tissues (Figures 1 and 2A). However, no significant differences between the TCSF mRNA levels were found in accordance with the TNM stage (Figure 2A).

With in situ hybridization, pre-invasive and invasive cancer cells were labeled in 18 of 22 tumors examined (the same positive samples as those found by
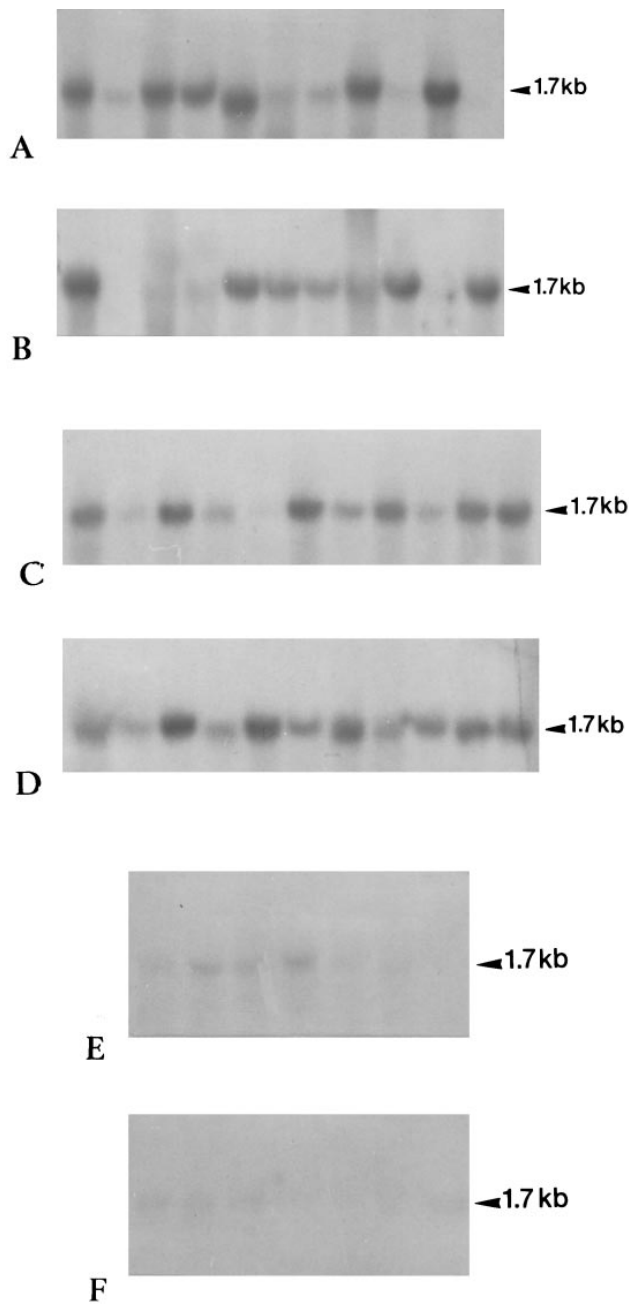
Figure 1 Northern blotting analysis of total RNA extracted from 22 lung carcinomas (**A,B**), 22 breast carcinomas (**C,D**), seven peritumoral normal lung tissue samples (**E**), and seven benign breast proliferations (**F**). A TCSF transcript of 1.7 κʙ is detected in tumor samples (40/44), whereas it is very weak or undetectable in normal tissue or benign proliferations.

Figure 2 Comparison of TCSF mRNA levels (arbitrary units) according to tissue pathology and to the TNM stage and Scarf and Bloom grading in lung (**A**) and breast (**B**) samples. (**A**) Lung tumor samples (T) expressed significant higher TCSF mRNA levels than non-tumor samples (N). However, no significant differences were found according to the TNM stage of lung carcinomas. (**B**) Breast tumor samples (T) expressed high TCSF mRNA levels whereas no signal was detected in benign breast tissues (N). Statistical analysis did not find any significant differences according to the Scarf and Bloom grade of breast carcinomas.

Northern blot analysis). Stromal cells surrounding labeled invasive cancer cells, were always negative (Figure 3A). Normal (Figure 3C) or squamous metaplastic epithelium and bronchial glands did not express any TCSF transcripts. Moreover, in the normal or emphysematous adjacent lung, pulmonary alveolar macro-
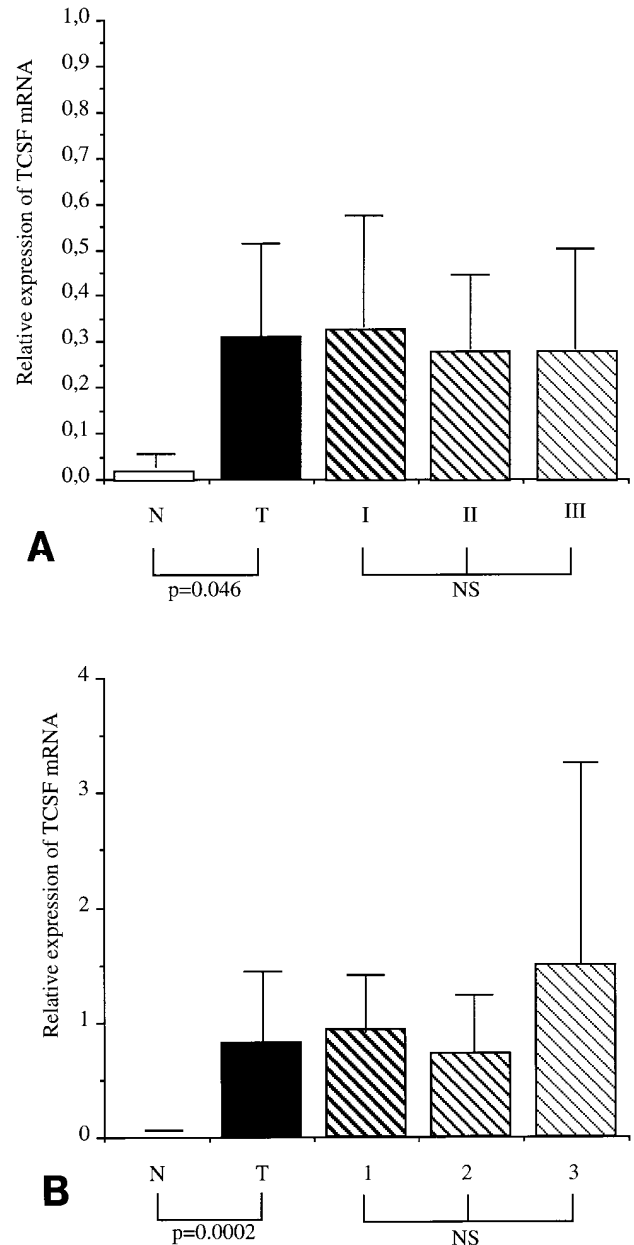
phages identified by the CD68 monoclonal antibody (Dako; Carpinteria, CA) on serial sections (not shown) were particularly rich in TCSF mRNAs (Figure 3D). In the three cases analyzed by image cytometry, TCSF mRNAs were significantly expressed in tu-
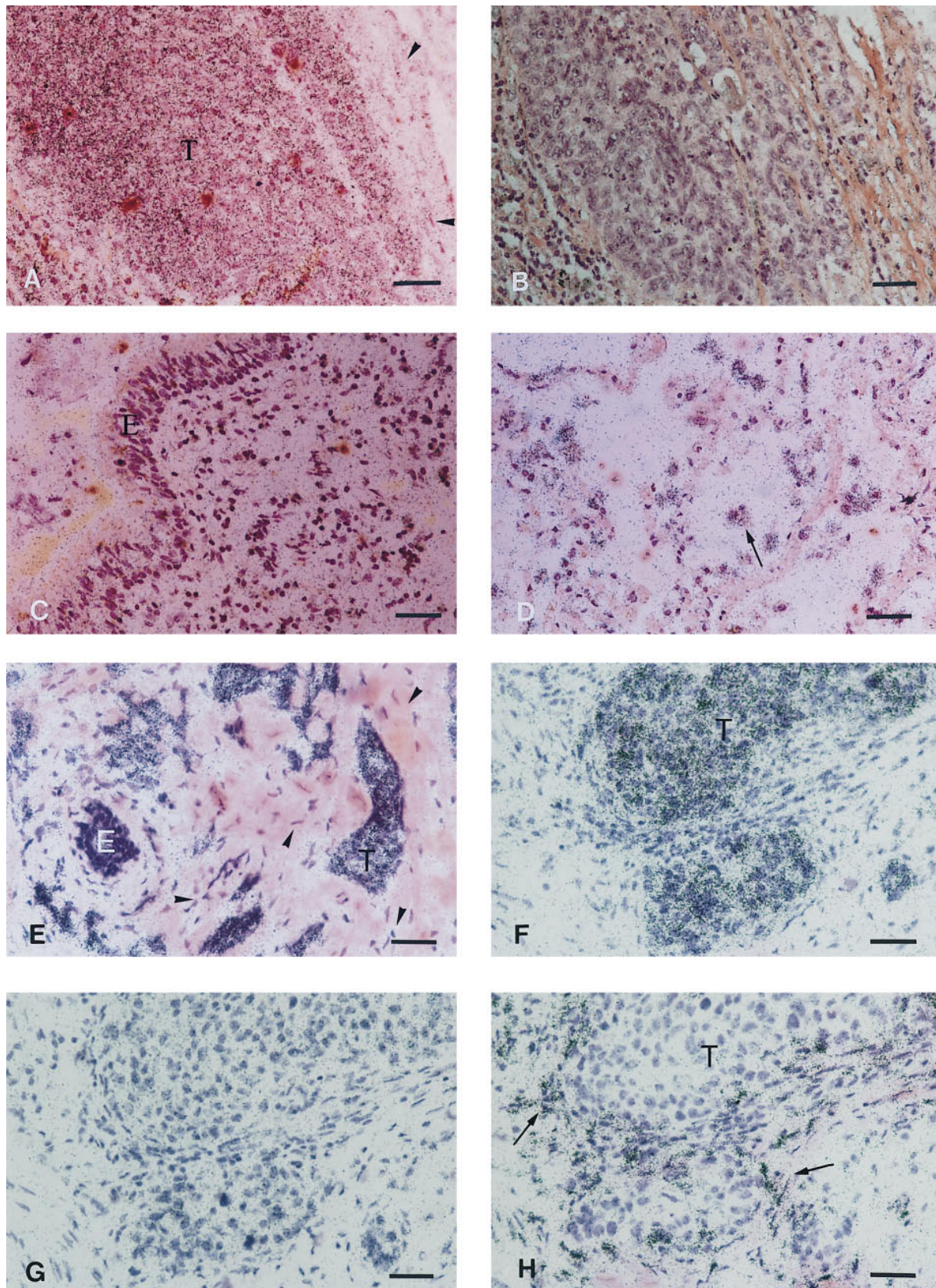
Figure 3 Localization of TCSF in lung cancers and breast cancers. (**A**) TCSF mRNAs are detected in invasive cancer cells (T) in lung carcinoma using an anti-sense probe, whereas stromal cells (arrowheads) are negative. Bar = 70 μm. (**B**) Same area treated with TCSF sense RNA probe. Bar = 70 μm. (**C**) Epithelial cells (E) of normal lung tissue do not express TCSF mRNA. (Bar = 70 μm). (**D**) TCSF mRNA is also present in many alveolar macrophages (arrow). Bar = 70 μm). (**E**) Intraductal breast tumor cells (T) express TCSF mRNAs, whereas epithelial adjacent cells (E) and stromal cells (arrowheads) do not show any hybridization grains. Bar = 70 μm. (**F**) Invasive tumor cells (T) express TCSF mRNAs, whereas stromal cells are negative. Bar = 70 μm. (**G**) Same area treated with TCSF sense RNA probe. Bar = 70 μm. (**H**) Gelatinase A mRNAs are localized in fibroblasts (arrow) in close contact to tumor clusters (T) in serial sections of breast carcinoma. Bar = 70 μm.

mor cell nests of both pre-invasive (1.68 ± 0.22 /μm^2) and invasive areas (2.14 ± 0.35 /μm^2) compared to the extracellular control compartment (0.19 ± 0.02 /μm^2) and normal tissue (0.23 ± 0.04 /μm^2) ($p<0.05$).

Breast Lesions

By Northern blotting, TCSF mRNAs were detected in the 22 breast carcinomas. Quantitative analysis showed significantly higher ($p<0.05$) expression of TCSF mRNAs in breast carcinomas than in benign breast lesions (Figures 1 and 2B). No significant differences between the TCSF mRNA levels were found in accordance with the Scarf and Bloom staging (Figure 2B).

With in situ hybridization, benign proliferations and normal mammary areas mixed with cancer cells or adjacent to cancer areas did not show any hybridization grains (Figure 3E), whereas the TCSF mRNAs were detected in cancer cells in pre-invasive and invasive areas (Figure 3F) of all 22 tumors examined. Stromal cells did not contain any hybridization grains. In the three cases studied by quantification, the density of markers in both pre-invasive (2.14 ± 0.48/μm^2) and invasive (2.95 ± 0.92/μm^2) areas was significantly higher than in the extracellular control compartment (0.23 ± 0.12/μm^2) and the normal areas (0.28 ± 0.10/μm^2) ($p<0.05$). On serial sections, TCSF mRNAs were localized in cancer cells, whereas fibroblasts close to tumor clusters expressed mRNAs encoding gelatinase A (Figures 3F and 3H) in the same areas.

Discussion

In this study we clearly showed the presence of mRNA encoding TCSF in epithelial tumor cells of lung and breast carcinomas. In agreement with our observations, previous immunohistochemical studies detected TCSF in cancer cells in breast (Zucker and Biswas 1994) and in bladder carcinoma (Muraoka et al. 1993). In the latter study, no staining was found in epithelial cells in non-neoplastic urothelium, except in superficial umbrella cells. However, Zucker and Biswas (1994) reported that TCSF is also present in normal breast ductules and lobules near in situ carcinoma areas, describing a more extensive distribution of TCSF compared with our data obtained by in situ hybridization (ISH). No TCSF transcripts were detected in normal epithelial cells in both non-neoplastic breast and lung lesions. It is therefore likely that normal epithelial cells express low levels of the TCSF mRNAs that we could not detect by ISH but that could produce amounts of the TCSF proteins detectable by immunohistochemistry. The presence of TCSF mRNAs in benign and normal tissues was confirmed by our Northern blot analysis, identifying weak expression of TCSF mRNAs in those samples. Furthermore, our

Northern blot analysis strengthened our ISH data because a higher significant abundance of TCSF transcripts was found in both breast and lung carcinomas than in benign and normal samples. The detection of TCSF mRNAs in intraepithelial cancer areas of the lung and mammary gland indicates that TCSF mRNA overexpression is an early event in carcinogenesis. These data, taken together, suggest that the expression of TCSF mRNA can be correlated with tumor progression. However, despite its implication in cancer progression, TCSF might play a role in some other pathological processes, such as inflammation and emphysema, because it has also been detected in alveolar macrophages in our study and it has been proposed as a factor in arthritis (Karinrerk et al. 1992).

Even though the precise function of TCSF is not known, some recent in vitro studies have shown that TCSF is able to stimulate the production of several MMPs by fibroblasts. Recent experimental data have demonstrated that TCSF stimulates the production of interstitial collagenase, stromelysin 1 and gelatinase A but not stromelysin 3 in fibroblasts (Kataoka et al. 1993). However, stromelysin 3 is known to have a poor proteolytic activity against collagen-like substrates (Murphy et al. 1994). Moreover, these authors have also demonstrated that TCSF increases activation of gelatinase A. These results are of particular interest regarding the implication of TCSF in cancer progression, because in many carcinomas in vivo the stromal cells have been demonstrated to be the principal source of several MMPs. A variety of studies have indicated that fibroblasts adjacent to the malignant tumors clusters produce interstitial collagenase (Urbanski et al. 1992; Hewitt et al. 1991; Polette et al. 1991), stromelysins 1, 2, 3 (Polette et al. 1991; Basset et al. 1990), and MT-MMP1 (Polette et al. 1996; Okada et al. 1995). More precisely, in both lung and breast carcinomas, mRNAs encoding gelatinase A, which degrades basement membrane collagens (Tryggvason et al. 1993), have also been localized by ISH in the stromal cells surrounding invasive carcinomas (Polette et al. 1993,1994; Poulsom et al. 1992,1993; Soini et al. 1993). In addition to their specific localization at the tumor–stromal interface, MMPs were not or were only weakly found in normal tissues and benign lesions. These studies have therefore demonstrated an association between MMP expression and the invasive process in cancers. Using serial sections in breast carcinomas, we showed that there is an obvious expression of TCSF mRNAs by tumor cells and gelatinase A mRNAs by fibroblasts in the same areas.

It therefore appears that some MMPs, as well as TCSF, are expressed selectively in both pre-invasive and invasive carcinoma but by different cell types, peritumoral fibroblasts and tumor cells, respectively. Relating our in vivo data to the observation that TCSF

enhances the production of some particular MMPs in fibroblasts in vitro, it can be postulated that the TCSF produced by tumor cells in vivo stimulates the expression of some MMPs by peritumoral fibroblasts. However, in vivo, interstitial collagenase and stromelysin 1, which are induced by TCSF in fibroblasts in vitro, are infrequently observed in stromal cells in breast carcinomas (Polette et al. 1991), whereas they are present at high levels in lung cancers (Muller et al. 1991), despite the presence of TCSF in both types of carcinoma. Moreover, in four of 22 of our lung carcinomas, we failed to detect any TCSF transcripts. These findings are consistent with the involvement of other factors in the regulation of those MMPs, such as the origin of the tissue, the extracellular matrix environment, and genetic rearrangements.

In conclusion, our observations on lung and breast cancers strongly support the hypothesis that TCSF is an important factor in tumor progression. More precisely, TCSF produced by tumor cells could play a role in the degradation of extracellular matrix associated with tumor invasion by stimulating the synthesis of some MMPs by peritumoral fibroblasts.

Acknowledgments

Literature Cited

Basset P, Bellocq JP, Wolf C, Stoll I, Limacher JM, Podjahcer OL, Chenard MP, Rio MC, Chambon P (1990) A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348:699–704

Bauer EA, Uitto J, Walters RC, Eisen AZ (1979) Enhanced collagenase production by fibroblasts derived from human basal cell carcinoma. J Invest Dermatol 39:4594–4599

Bernhard EJ, Muschel RJ, Hughes EN (1990) Mr 92,000 gelatinase release correlates with the metastatic phenotype in transformed rat embryo cells. Cancer Res 50:3872–3877

Biswas C (1985) Matrix influence on the tumor cell stimulation of fibroblast collagenase production. J Cell Biochem 28:39–45

Biswas C (1984) Collagenase stimulation in cocultures of human fibroblasts and human tumor cells. Cancer Lett 24:201–207

Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, Nabeshima K (1995) The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobin superfamily. Cancer Res 55:434–439

Bonfil RD, Reddel RR, Ura H, Reich R, Fridman R, Harris CC, Klein–Szanto AJP (1989) Invasive and metastatic potential of a v-Ha-ras-transformed human bronchial epithelial cell line. J Natl Cancer Inst 81:587–594

Clavel C, Polette M, Doco M, Binninger I, Birembaut P (1992) Immunolocalization of matrix metalloproteinases and their tissue inhibitor in human mammary pathology. Bull Cancer (Paris) 79:261–270

Davies B, Miles DW, Happerfiled LC, Naylor MS, Bobrow LG, Rubens RD, Balkwill FR (1993) Activity of type IV collagenases in benign and malignant breast disease. Br J Cancer 67:1126–1131

Ellis SM, Nabeshima K, Biswas C (1989) Monoclonal antibody preparation and purification of a tumor cell collagenase-stimulatory factor. Cancer Res 49:3385–3391

Gilles C, Polette M, Piette J, Birembaut P, Foidart JM (1994) Epithelial-to-mesenchymal transition in HPV-33-transfected cervical keratinocytes is associated with increased invasiveness and expression of gelatinase A. Int J Cancer 59:661–666

Gilles C, Polette M, Piette J, Munaut C, Thompson EW, Birembaut P, Foidart JM (1996) High level of MT-MMP expression is associated with invasiveness of cervical cancer cells. Int J Cancer 65:209–213

Hernandez AD, Hibbs MS, Postlethwaite AE (1985) Establishment of basal cell carcinomas in culture: evidence for a basal cell carcinoma-derived factor(s) which stimulates fibroblasts to proliferate and release collagenase. J Invest Dermatol 85:470–475

Hewitt RE, Leach IH, Powe DG, Clark IM, Cawston TE, Turner DR (1991) Distribution of collagenase and tissue inhibitor of metalloproteinases (TIMP) in colorectal tumors. Int J Cancer 49:666–672

Ito A, Nakajima S, Sasaguri Y, Nagase H, Morri Y (1995) Co-culture of human breast adenocarcinoma MCF7 cells and human dermal fibroblasts enhances the production of matrix metalloproteinases 1, 2, and 3 in fibroblasts. Br J Cancer 71:1039–1045

Karinrerk W, Fieberger E, Steffanova I, Baumruker T, Knapp W, Stockinger H (1992) Human leucocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, more basigin, and chicken HT7 molecule. J Immunol 149:847–854

Kataoka H, DeCasto R, Zucker S, Biswas C (1993) Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res 53:3154–3158

Levy AT, Cioce V, Sobel ME, Garbisa S, Grigioni WF, Liotta LA, Stetler–Stevenson WG (1991) Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. Cancer Res 51:439–444

Monsky WL, Lin CYL, Aoyama A, Kelly T, Akiyama SK, Mueller SC, Chen WT (1994) A potential marker protease of invasiveness, seprase, is localized on invadopodia of human malignant melanoma cells. Cancer Res 54:5702–5710

Monteagudo C, Merino M, San-Juan J, Liotta L, Stetler–Stevenson WG (1990) Immunohistochemical distribution of type IV collagenase in normal, benign, and malignant breast tissue. Am J Pathol 136:585–592

Muller D, Breathnach R, Engelmann A, Mill R, Bronner G, Flesch H, Dumont P, Eber M, Abecassis J (1991) Expression of collagenase-related metalloproteinase genes in human lung or neck tumors. Int J Cancer 48:550–556

Muraoka K, Nabeshima K, Murayama T, Biswas C, Koono M (1993) Enhanced expression of a tumor-cell-derived collagenase-stimulatory factor in urothelium carcinoma: its usefulness as a tumor marker for bladder cancers. Int J Cancer 55:19–26

Murphy G, Segain JP, O'Shea M, Cockett M, Ionnou C, Lefebvre O, Chambon P, Basset P (1994) The 28-kDa N-terminal domain of mouse stromelysin-3 has the general properties of a weak metalloproteinase. J Biol Chem 268:15435–15441

Nabeshima K, Lane WS, Biswas C (1991) Partial sequencing and characterization of the tumor cell-derived collagenase stimulatory factor. Arch Biochem Biophys 285:90–96

Noël A, Munaut C, Nusgens B, Lapière CM, Foidart JM (1993) Different mechanisms of extracellular matrix remodelling by fibroblasts in response to human mammary neoplastic cells. Invasion Metastasis 13:72–81

Noël A, Polette M, Lewalle JM, Munaut C, Emonard H, Birembaut P, Foidart JM (1994) Coordinate enhancement of gelatinase A mRNA and activity levels in human fibroblasts in response to breast-adenocarcinoma cells. Int J Cancer 56:331–336

Okada A, Bellocq JP, Rouyer N, Chenard MP, Rio MC, Chambon P, Basset P (1995) Membrane-type matrix metalloproteinase

(MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc Natl Acad Sci USA 92:2730–2734

Polette M, Clavel C, Cockett M, Girod de Bentzmann S, Murphy G, Birembaut P (1993) Detection and localization of mRNAs encoding matrix metalloproteinases and their tissue inhibitor in human breast pathology. Invasion Metastasis 13:31–37

Polette M, Clavel C, Muller D, Abecassis J, Binninger I, Birembaut P (1991) Detection of mRNAs encoding collagenase I and stromelysin 2 in carcinomas of the head and neck by in situ hybridization. Invasion Metastasis 11:76–83

Polette M, Gilbert N, Stas I, Nawrocki B, Noël A, Remacle A, Stetler-Stevenson WG, Birembaut P, Foidart JM (1994) Gelatinase A expression and localization in human breast cancers. An *in situ* hybridization study and immunohistochemical detection using confocal microscopy. Virchows Arch 424:641–645

Polette M, Nawrocki B, Gilles C, Sato H, Seiki M, Tournier JM, Birembaut P (1996) MT-MMP expression and localisation in human lung and breast cancers. Virchows Arch 428:29–35

Poulsom R, Hanby AM, Pignatelli M, Jeffrey RE, Longcroft JM, Rogers L (1993) Expression of gelatinase A and TIMP2 mRNAs in desmoplastic fibroblasts in both mammary carcinomas and basal cell carcinomas of the skin. J Clin Pathol 46:429–436

Poulsom R, Pignatelli M, Stetler–Stevenson WG, Liotta LA, Wright PA, Jeffrey RE, Longcroft JM, Rogers L, Stamp GWH (1992) Stromal expression of 72 kDa type IV collagenase (MMP-2) and TIMP-2 mRNAs in colorectal neoplasia. Am J Pathol 141:389–396

Pyke C, Ralfkiaer K, Dano K (1993) Messenger RNA for two type IV collagenases is located in stromal cells in human colon cancer. Am J Pathol 142:359–365

Sato H, Takino T, Okada Y, Cao J, Shinigawa A, Yamamoto E, Seiki M (1994) A matrix metalloproteinase expressed on the surface of invasive tumor cells. Nature 370:61–65

Soini Y, Pääkkö P, Autio–Harmainen H (1993) Genes of laminin B1 chain, a1 (IV) chain of type IV collagen, and 72-kd type IV collagenase are mainly expressed by the stromal cells of lung carcinomas. Am J Pathol 142:1622–1630

Taniguchi S, Iwamura T, Kitamura N (1992) Correlation between spontaneous metastatic potential and type I collagenolytic activity in a human pancreatic cancer cell line (SUIT-2) and sublines. Clin Exp Metastasis 10:259–266

Taniguchi S, Iwamura T, Kitamura N, Yamanari H, Setoguchi T (1994) Heterogeneities of attachment, chemotaxis, and protease production among clones with different metastatic potentials from a human pancreatic cancer cell line. Clin Exp Metastasis 12:238–244

Tryggvason K, Hoyhtya M, Pyke C (1993) Type IV collagenase in invasive tumors. Breast Cancer Res Treat 24:209–218

Urbanski SJ, Edwards DR, Maitland A, Leco KJ, Watson A, Kossakowska AE (1992) Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. Br J Cancer 66:1188–1194

Zucker S, Biswas C (1994) Tumor collagenase-stimulating factor: a paracrine stimulator of fibroblast production of matrix metalloproteinases in cancer. Bull Inst Pasteur 92:284–290