Jpn. J. Cancer Res. 81, 793–798, August 1990

Induction of Growth Factor-receptor and Metalloproteinase Genes by Epidermal Growth Factor and/or Transforming Growth Factor-α in Human Gastric Carcinoma Cell Line MKN-28

Kazuhiro Yoshida,[1,2] Tetsuhiro Tsujino,[1] Wataru Yasui,[1] Takashi Kameda,[1] Toshiaki Sano,[1] Hirofumi Nakayama,[1] Tetsuya Toge[2] and Eiichi Tahara[1,3]

[1]*First Department of Pathology, Hiroshima University School of Medicine, and* [2]*Department of Surgery, Research Institute for Nuclear Medicine and Biology, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734*

We examined the effects of epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) on EGF receptor (EGFR) phosphorylation and the expression of mRNAs for oncogenes, growth factors, their receptors and metalloproteinase genes by MKN-28 gastric carcinoma cells which express EGF, TGF-α and EGFR genes. Both EGF and TGF-α stimulated EGFR phosphorylation. EGF and TGF-α induced FOS, MYC and ERBB-2 oncogene expression. Interestingly, EGF increased the expression of mRNAs for TGF-α and EGFR. On the other hand, TGF-α increased TGF-α mRNA but decreased the expression of mRNAs for EGFR and TGF-β. Furthermore, mRNAs for interstitial collagenase, stromelysin and procollagen type I genes were also enhanced after treatment with EGF and TGF-α. These results indicate that EGF and TGF-α successively evoke cascade phenomena which favor tumor progression, invasion and extracellular matrix formation, acting as autocrine growth regulators for gastric carcinomas.

Key words: Autocrine growth factor — Metalloproteinase — Cancer invasion and metastasis — Gastric carcinoma

Recent investigations have focussed much attention on autocrine growth factors and malignant characteristics of tumor cells.[1,2] In the process of tumor progression, tumor cells might acquire genetic alterations[3] and exhibit aberrant expression of several growth factors.[4] Furthermore, complicated interactions between tumor cells and normal cells might regulate the formation and degradation of the extracellular matrix. Extracellular degenerative enzymes are assumed to play an important role in tumor invasion and metastasis.[5,6] Recently, several metalloproteinases have been identified, such as interstitial collagenase,[7] stromelysin[8] and type IV collagenase,[9] which are closely associated with malignant potential of tumor cells. Kerr *et al.*[10] have demonstrated that transin RNA is induced by EGF,[4] PDGF and the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) in mouse NIH3T3 fibroblasts, suggesting the modulation of proteolysis by several growth factors.

On the other hand, we have previously demonstrated immunohistochemically that expression of EGF, EGFR and TGF-α is correlated with tumor invasion and prognosis of gastric carcinoma patients.[2,11,12] Moreover, we have detected mRNAs for EGF, TGF-α, EGFR genes[13] and other growth factor-receptor systems in gastric carcinoma cell lines and tissues[14] and suggested that EGF and/or TGF-α might act as autocrine growth factors in MKN-28 gastric carcinoma cells.[13] Regarding this point, the question arose as to why tumor cells increase the expression of EGF, TGF-α and EGFR genes as they invade more deeply into the stomach wall.

In order to resolve this question, we examined the effects of EGF and TGF-α on the expression of mRNAs for growth factors, their receptors, oncogenes, metalloproteinases and procollagen type I genes by MKN-28 cells.

MATERIALS AND METHODS

Cell culture MKN-28 cell line (human gastric well differentiated adenocarcinoma), kindly provided by Dr. T. Suzuki (Fukushima Medical University, Fukushima), was routinely grown in RPMI-1640 (Nissui, Tokyo) supplemented with fetal bovine serum (M.A. Bioproducts, Walkersville, MD). MKN-28 cells have 21.4×10^3 sites/cell EGF binding capacity, 14.7 pg/10^6 cells of membrane-bound TGF-α and an EGF content of about 218 pg/10^7 cells as described previously.[13] They were grown to subconfluence in the above medium. After 48 h of pre-incubation in serum-free medium, 1 n*M* EGF or TGF-α was added. Human recombinant EGF and

[3] To whom requests for reprints should be addressed.

[4] The abbreviations used are: EGF, epidermal growth factor; TGF-α, transforming growth factor-α; EGFR, epidermal growth factor receptor; poly(A)$^+$, polyadenylated; cDNA, complementary DNA; kb, kilobase.

TGF-α were kindly provided by Wakunaga Pharm. Co., Hiroshima. RNAs were extracted after treatment at 0 (control) time, 10 min, 30 min, 1 h, 3 h, 6 h and 12 h and the expression of mRNAs was examined.

EGFR phosphorylation by EGF and TGF-α The membrane fraction of MKN-28 cells was prepared by the method of Usui *et al.*[15] The final precipitate was suspended in 20 m*M* hepes pH 7.4 at a concentration of 2–5 mg protein/ml. Phosphorylation was carrried out as described by Carpenter *et al.*[16] with some modifications. The mixture (60 μl) containing 20 m*M* Hepes pH 7.4, 50 μg of membrane protein, 1 μM $MnCl_2$, 10 n*M* $NaVO_3$ and 15 μM [γ-^{32}P]ATP (3×10^3 cpm/pmol) was incubated in the presence or absence of 10 n*M* and 100 n*M* EGF or TGF-α for 10 min at 0°C. The reaction was terminated by boiling for 3 min in SDS-sample buffer containing 5 m*M* EDTA. The sample was subjected to 7.5% SDS-PAGE followed by autoradiography.

RNA preparation and Northern blot analysis RNA was extracted by the guanidine isothiocyanate/cesium chloride method.[17] Ten μg of poly(A)$^+$ selected RNA was electrophoresed on 1.0% agarose-formaldehyde gel and blotted onto Zeta-Probe nylon filter membrane (Bio-Rad Laboratories). Hybridization and washing were performed as described previously.[18]

DNA probes The 1.9 kb human EGF cDNA insert from phEGF 15 was kindly provided by Dr. G. I. Bell,[19] 1.4 kb human TGF-α cDNA[20] and 1.0 kb human TGF-β cDNA[21] by Dr. Rik Derynck, 2.4 kb human EGFR cDNA in pE7,[22] 0.4 kb human MYC cDNA and 2.1 kb human FOS cDNA by the Japanese Cancer Research Resources Bank (JCRB), human ERBB-2 cDNA in pCER204[23] by Dr. T. Yamamoto, and interstitial collagenase[7] and pro-stromelysin cDNA[8] by Dr. G. I. Goldberg. β-Actin probe was purchased from Oncor Gaithersburg, MD.

RESULTS

Effects of EGF and TGF-α on their receptor phosphorylation In order to confirm receptor phosphorylation of MKN-28 cells by EGF and TGF-α, 10 n*M* EGF or TGF-α was added to the membrane fraction of MKN-28 cells and the level of phosphorylation was examined as shown in Fig. 1. The 170 kDa EGFR was evidently phosphorylated by EGF in a dose-dependent manner. The EGFR phosphorylation by TGF-α was almost equal to that by EGF.

Induction of mRNA for oncogenes and growth factor-receptor system by EGF and TGF-α We have previously demonstrated that EGF induced its receptor synthesis and FOS, MYC oncogene expression by TMK-1 cells.[24] In the present study, the effect of TGF-α on the expression of mRNA for oncogenes and growth factor-receptor system was examined. TGF-α induced FOS and MYC genes; FOS gene was stimulated almost 8-fold and MYC gene expression was induced about 9-fold after 1 h of TGF-α treatment (Fig. 2A). EGF also stimulated FOS and MYC gene expressions, as reported previously in the case of TMK-1 cells.

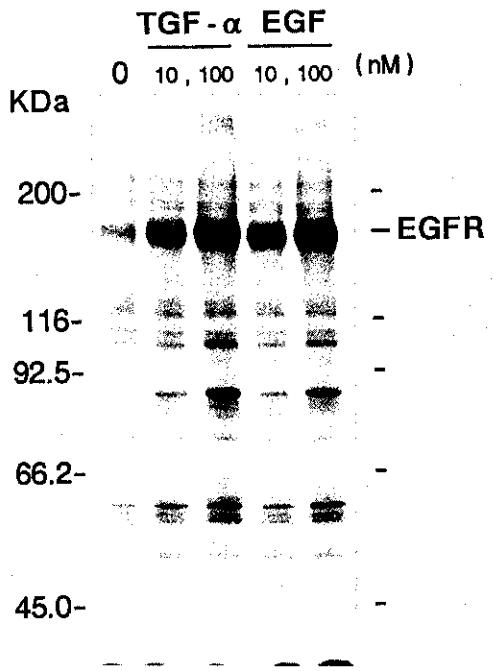

Fig. 1. The effects of TGF-α and EGF on the auto-phosphorylation of EGFR. The 170 kDa EGFR is markedly phosphorylated almost equally by TGF-α and EGF.

EGF gradually increased the expression of EGFR mRNA and 2.8-fold induction was observed at 3 h after treatment (Fig. 2B). Interestingly, the induction of ERBB-2 gene expression preceded the induction of EGFR expression. The maximal induction was observed to be about 3-fold at 1 h compared to the control level. This result was consistent with the data obtained in the case of TMK-1 cells (data not shown). Moreover, TGF-α gene expression was gradually increased and induction of about 2.7-fold was detected at 12 h after EGF treatment. TGF-β expression was not evidently changed by EGF. The level of expression was determined by densitometry and the level of autoradiographic signals was compared to that in the case without treatment. On the other hand, TGF-α did not stimulate EGFR mRNA. The 10.6 kb transcript was almost comparable to the control levels, but the 5.8 kb transcript gradually decreased after treatment (Fig. 2B). TMK-1 cells gave

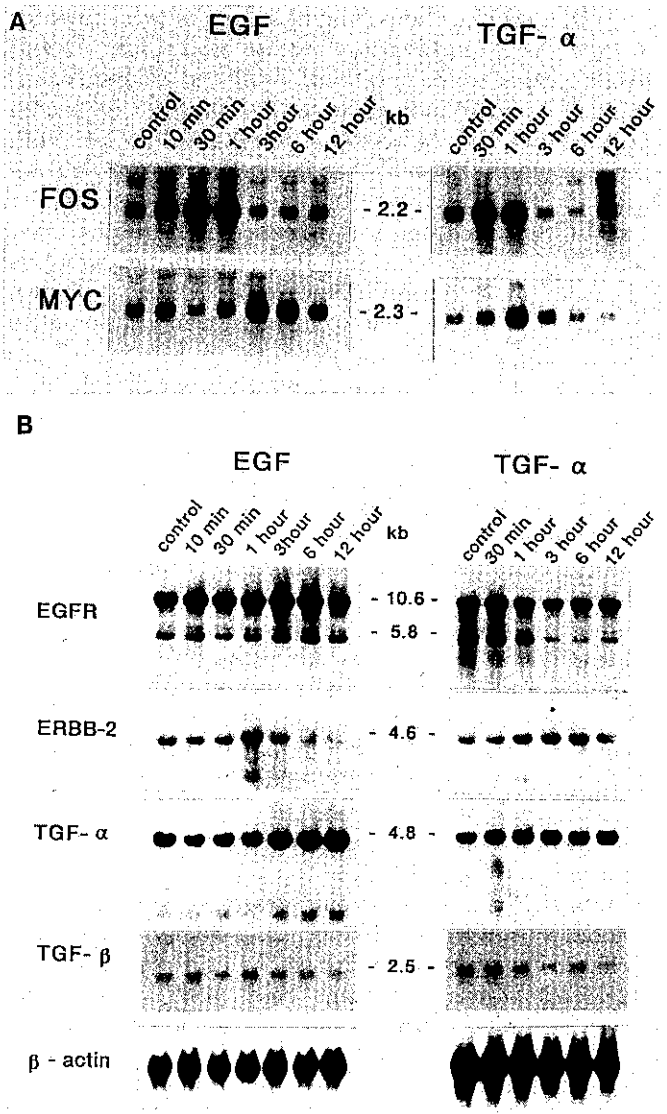

Fig. 2. The time course changes of mRNA levels of (A) FOS and MYC (B) EGFR, ERBB-2, TGF-α and TGF-β genes in MKN-28 cells after treatment with EGF and TGF-α. Ten μg of poly(A)$^+$ RNA was applied and the amount of mRNA was measured in MKN-28 cells without treatment at 0 h and 10 min, 30 min, 1 h, 3 h, 6 h and 12 h after EGF or TGF-α addition. The intensities of autoradiographic signals were determined by densitometry. β-Actin probe was employed as an internal control. The exposure times for detecting autoradiographic signals were 4 h for FOS and MYC, 16 h for EGFR and TGF-α, 4 h for ERBB-2 and TGF-β and 2 h for β-actin.

the same result concerning the expression of EGFR mRNA after TGF-α treatment (data not shown). ERBB-2 and TGF-α gene expression was slightly increased after TGF-α treatment as shown in Fig. 2B.

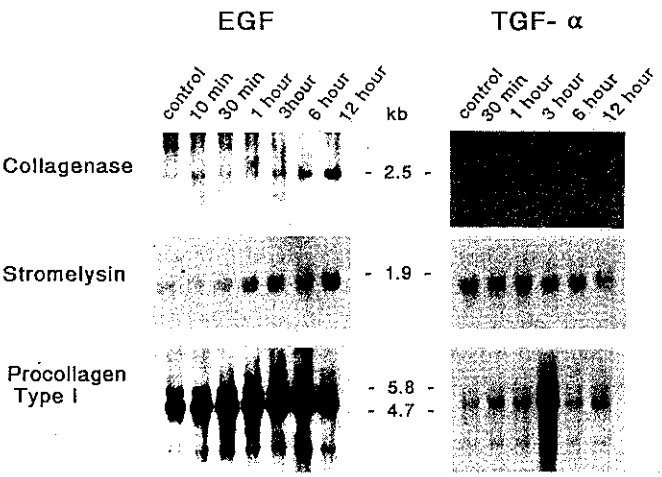

Fig. 3. The time course of effects of EGF and TGF-α on the expression of mRNA for collagenase, stromelysin and procollagen type I genes. Autoradiographic exposure time was 16 h.

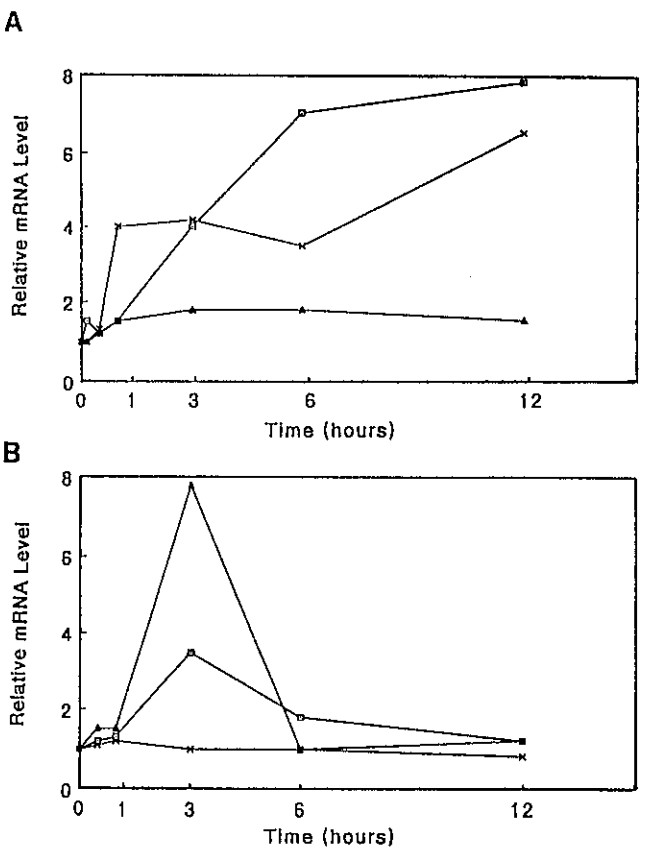

Fig. 4. The time course of changes of autoradiographic signals for collagenase (□), stromelysin (×) and procollagen type I (▲) genes determined by densitometry. (A) EGF, (B) TGF-α.

Interestingly, TGF-β expression was gradually decreased 0.3-fold at 12 h after treatment with TGF-α.

EGF and TGF-α induce metalloproteinase and procollagen type I gene expression In order to examine whether EGF and TGF-α stimulate expression of collagenase and stromelysin, which are closely related to tumor cell invasion and metastasis, the same filter was rehybridized with the probes. As shown in Fig 3, EGF increased the interstitial collagenase mRNA of 2.5 kb in a time-dependent manner. The induction of its expression reached about 7.8-fold at 12 h after EGF addition. Stromelysin mRNA of 1.9 kb transcript was also gradually increased to about 6.5-fold at 12 h after EGF treatment. Moveover, expression of collagenase gene was increased 3.5-fold after 3 h of treatment with TGF-α but stromelysin was not affected dramatically. On the other hand, expression of procollagen type I mRNA was gradually increased 1.8-fold with EGF stimulation; TGF-α stimulated its expression remarkably to 7.8-fold at 3 h but the level fell rapidly to the control level thereafter. The changes of expression are summarized in Fig. 4.

DISCUSSION

Autocrine growth regulation and production of growth factors by carcinoma cells have recently attracted much interest. EGF and TGF-α are assumed to act through a common receptor and to share many biological activities such as receptor phosphorylation, as described in the present study. However, we have observed a different effect of EGF and TGF-α on the expression of EGFR mRNA, that is, EGF increased EGFR mRNA expression, while TGF-α decreased the expression. The mechanism of this different effect remains to be elucidated.

EGF plays an important role in mammary tumorigenesis.[25] It increases synthesis of its receptor[24,26] and stimulates the expression of TGF-α mRNA in MDA 468 mammary carcinoma cells.[27] These reports are consistent with our present data. Fernandez-Pol *et al.*[28] also reported ERBB-2 gene induction by EGF. ERBB-2 gene was also increased after EGF and TGF-α treatment of MKN-28 cells. Interestingly, the increase of ERBB-2 mRNA level preceded that of EGFR mRNA level, as found in TMK-1 cells (data not shown). Kokai *et al.*[29] found that EGF stimulated normal and transformed cells in an EGFR-dependent manner, resulting in increased tyrosine phosphorylation of p185 c-*neu* with a concomitant increase in the respective tyrosine activities. Moreover, overexpression of p185 c-*neu* and EGFR together results in transformation of NIH3T3 cells.[30] Therefore, it is likely that interaction or cross-talk between the two may contribute to the growth and progression of gastric carcinomas.

TGF-α also functions as an autocrine growth regulator in malignant melanomas,[31] breast carcinomas,[32] and esophageal carcinomas,[33] as well as gastric carcinomas,[13] and it induces its own expression in human keratinocytes.[34] Moreover, overexpression of both TGF-α and EGFR genes can induce transformed phenotype in NIH3T3 cells.[35] In breast carcinomas, TGF-α and EGFR expression was increased after phorbol ester and EGF treatment[27] and their expressions are also regulated by estrogen.[36] However, it is not yet known whether this effect is due to enhanced transcription or to mRNA stabilization.

Growth factors might also influence the growth characteristics of cells by affecting the structure of the extracellular matrix and thus disturb or enhance the interaction of cells with their growth substratum. Modulation of proteolysis and formation or extracellular matrix involve very complex processes such as interaction between carcinoma cells and stromal cells, or between proteolytic enzymes and their inhibitors. Recently, various authors[37–39] have reported the opposite regulatory action of TGF-β on the extracellular proteolytic activities by carcinoma cells and fibroblasts. TGF-β also stimulates the synthesis of mRNA for procollagen type I, fibronectin and thrombospondin by fibroblasts.[40] Moreover, high expression levels of TGF-β mRNA were detected in scirrhous gastric carcinomas and TGF-β also stimulated the synthesis of procollagen type I mRNA by TMK-1 gastric carcinoma cells.[41]

What is interesting in our present study was the induction of mRNA for collagenase, stromelysin and procollagen type I genes by exogenous EGF and TGF-α in MKN-28 gastric carcinoma cells. Kerr *et al.*[10] and Matrisian *et al.*[42] demonstrated the induction of transin gene in rat and mouse fibroblasts by growth factors. FOS gene was also induced by EGF and TGF-α in the present study as demonstrated by Kerr *et al.*[10] The increased FOS protein or FOS associated protein with transcription factor JUN/AP-1 binds to the promotor region of collagenase and stromelysin genes, which increase the transcription of these genes.[10,43] Further examination is needed to elucidate the relation between carcinoma cells and fibroblasts in the degradation and formation of extracellular martrixes and how the gene expressions are regulated.

Above all, EGF and TGF-α stimulate the expression of the growth factor-receptor system and metalloproteinase by tumor cells themselves and thus they may play an important role in tumor cell growth and progression as autocrine growth regulators in human gastric carcinomas.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan. We are grateful to Miss M. Ito, Miss S. Yasuda and Miss M. Obata for their excellent technical assistance.

(Received February 7, 1990/Accepted April 27, 1990)

REFERENCES

1) Sporn, M. B. and Roberts, A. B. Autocrine growth factors and cancer. *Nature,* **313,** 745–747 (1985).
2) Tahara, E., Sumiyoshi, H., Hata, J., Yasui, W., Taniyama, K., Hayashi, T., Nagae, S. and Sakamoto, S. Human epidermal growth factor in gastric carcinomas as a biological marker of high malignancy. *Jpn. J. Cancer Res.,* 77, 145–152 (1986).
3) Tsujino, T., Yoshida, K., Nakayama, H., Ito, H., Shimosato, T. and Tahara, E. Alteration of oncogenes in metastatic tumours of human gastric carcinomas. *Br. J. Cancer* (1990), in press.
4) Tahara, E. Growth factors and oncogenes in human gastrointestinal carcinomas. *J. Cancer Res. Clin. Oncol.,* **116,** 121–131 (1990).
5) Liotta, L. A. Tumor invasion and metastasis — Role of the extracellular matrix: Rhoads memorial award lecture. *Cancer Res.,* **46,** 1–7 (1986).
6) Laiho, M. and Keski-Oja, J. Growth factors in the regulation of pericellular proteolysis: a review. *Cancer Res.,* **49,** 2533–2553 (1989).
7) Goldberg, G. I., Wilhelm, S. M., Kronberger, A., Bauer, E. A., Grant, G. A. and Eisen, A. Z. Human fibroblast collagenase. *J. Biol. Chem.,* **261,** 6660–6605 (1986).
8) Wilhelm, S. M., Collier, I. E., Kronberger, A., Eisen, A. Z., Marmer, B. L., Grant, G. A., Bauer, E. A. and Goldberg, G. I. Human skin fibroblast stromelysin: structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. *Proc. Natl. Acad. Sci. USA,* **84,** 6725–6729 (1987).
9) Collier, I. E., Wilhelm, S. M., Eisen, A. Z., Marmer, B. L., Grant, G. A., Seltzer, J. L., Kronberger, A., He, C., Bauer, E. A. and Goldberg, G. I. H-ras oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. *J. Biol. Chem.,* **263,** 6579–6587 (1988).
10) Kerr, L. D., Holt, J. T. and Matrisian, L. M. Growth factors regulate transin gene expression by c-*fos*-dependent and c-*fos*-independent pathways. *Science,* **242,** 1424–1427 (1988).
11) Yasui, W., Hata, J., Yokozaki, H., Ochiai, A., Ito, H. and Tahara, E. Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. *Int. J. Cancer,* **41,** 211–217 (1988).
12) Yamamoto, T., Hattori, T. and Tahara, E. Interaction between transforming growth factor-alpha and c-Ha-*ras* p21 in progression of human gastric carcinoma. *Pathol. Res., Pract.,* **183,** 663–669 (1988).
13) Yoshida, K., Kyo, E., Tsujino, T., Sano, T., Niimoto, M. and Tahara, E. Expression of epidermal growth factor, transforming growth factor-α and their receptor genes in human gastric carcinomas; implication for autocrine growth. *Jpn. J. Cancer Res.,* **81,** 43–51 (1990).
14) Tsuda, T., Yoshida, K., Tsujino, T., Nakayama, H., Kajiyama, G. and Tahara, E. Coexpression of platelet-derived growth factor (PDGF) A-chain and PDGF receptor genes in human gastric carcinomas. *Jpn. J. Cancer Res.,* **80,** 813–817 (1989).
15) Usui, H., Yoshikawa, K., Imazu, M., Tsukamoto, H. and Takeda, M. Comparison of tyrosine phosphorylation of proteins by membrane fractions from mouse liver, Ehrlich ascites tumor and MH134 hepatoma. *FEBS Lett.,* **184,** 60–64 (1985).
16) Carpenter, G., King, L. and Cohen, S. Rapid enhacement of protein phosphorylation in A431 cell membrane preparation by epidermal growth factor. *J. Biol. Chem.,* **254,** 4884–4891 (1979).
17) Maniatis, T., Fritsch, E. F. and Sambrook, J. "Molecular Cloning: A Laboratory Manual," 8th Ed., pp. 187–210 (1984). Cold Spring Harbor Laboratory, New York.
18) Yoshida, K., Tsuda, T., Matsumura, T., Tsujino, T., Hattori, T., Ito, H. and Tahara, E. Amplification of epidermal growth factor receptor (EGFR) gene and oncogenes in human gastric carcinomas. *Virchows Arch. B Cell Pathol.,* **57,** 285–290 (1988).
19) Bell, G. I., Fong, N. M., Stempien, M. M., Wormsted, M. A., Caput, D., Ku, L., Urdea, M. S., Rall, L. B. and Sanchez-Pescador, R. Human epidermal growth factor precursor: cDNA sequence, expression *in vitro* and gene organization. *Nucleic Acids Res.,* **14,** 8427–8446 (1986).
20) Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y. and Goeddel, D. V. Human transforming growth factor-α: precursor structure and expression in *E. coli. Cell,* **38,** 287–297 (1984).
21) Derynck, R., Jarrett, J. A., Chen, E. Y., Eaton, D. H., Bell, J. R., Assoian, R. K., Roberts, A. B., Sporn, M. B. and Goeddel, D. V. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. *Nature,* **316,** 701–705 (1985).
22) Xu, Y. H., Ishii, S., Clark, A. J. L., Sullivan, M., Wilson, R. K., Ma, D. P., Roe, B. A., Merlino, G. T. and Pastan, I. Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells. *Nature,* **309,** 806–810 (1984).
23) Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Miyajima, N., Saito, T. and Toyoshima, K.

Similarity of protein encoded by the human c-*erb*-B-2 gene to epidermal growth factor receptor. *Nature,* **319,** 230–234 (1986).
24) Yoshida, K., Takanashi, A., Kyo, E., Ito, M., Ito, H., Niimoto, M., Hattori, T. and Tahara, E. Epidermal growth factor induces the expression of its receptor gene in human gastric carcinoma cell line TMK-1. *Jpn. J. Cancer Res.,* **80,** 743–746 (1989).
25) Kurachi, H., Okamoto, S. and Oka, T. Evidence for the involvement of the submandibular gland epidermal growth factor in mouse mammary tumorigenesis. *Proc. Natl. Acad. Sci. USA,* **82,** 5940–5943 (1985).
26) DePalo, L. and Das, M. Epidermal growth factor-induced stimulation of epidermal growth factor-receptor synthesis in human cytotrophoblasts and A431 carcinoma cells. *Cancer Res.,* **48,** 1105–1109 (1988).
27) Bjorge, J. D., Paterson, A. J. and Kudlow, J. E. Phorbol ester or epidermal growth factor (EGF) stimulates the concurrent accumulation of mRNA for the EGF receptor and its ligand transforming growth factor-α in a breast carcinoma cell line. *J. Biol. Chem.,* **264,** 4021–4027 (1988).
28) Fernandez-Pol, J. A., Hamilton, P. D. and Klos, D. J. Transcriptional regulation of proto-oncogene expression by epidermal growth factor, transforming growth factor β1, and triiodothyronine in MDA-468 cells. *J. Biol. Chem.,* **264,** 4151–4156 (1989).
29) Kokai, Y., Dobashi, K., Weiner, D. B., Myers, J. N., Nowell, P. C. and Greene, M. I. Phosphorylation process induced by epidermal growth factor alters the oncogenic and cellular *neu (NGL)* gene products. *Proc. Natl. Acad. Sci. USA,* **85,** 5389–5393 (1988).
30) Kokai, Y., Myers, J. N., Wada, T., Brown, V. I., LeVea, C. M., Davis, J. G., Dobashi, K. and Greene, M. Synergistic interaction of p185c-*neu* and the EGF receptor leads to transformation of rodent fibroblasts. *Cell,* **58,** 287–292 (1989).
31) Imanishi, K., Yamaguchi, K., Suzuki, M., Honda, S., Yanaihara, N. and Abe, K. Production of transforming growth factor-α in human tumor cell lines. *Br. J. Cancer,* **59,** 761–765 (1989).
32) Ciardiello, F., Kim, N., Liscia, D. S., Bianco, C., Lidereau, R., Merlo, G., Callahan, R., Greiner, J., Szpak, C., Kidwell, W., Schlom, J. and Salomon, D. S. mRNA expression of tranforming growth factor alpha in human breast carcinomas and its activity in effusions of breast cancer patients. *J. Natl. Cancer Inst.,* **81,** 1165–1171 (1989).
33) Yoshida, K., Kyo, E., Tsuda, T., Tsujino, T., Ito, M., Niimoto, M. and Tahara, E. EGF and TGF-α, the ligands of hyperproduced EGFR in human esophageal carcinoma cells, act as autocrine growth factors. *Int. J. Cancer,* **45,** 131–135 (1990).
34) Coffey, R., Derynck, R., Wilcox, J. N., Bringman, T. S., Goustin, A. S., Moses, H. L. and Pittelkow, M. R. Production and auto-induction of transforming growth factor-α in human keratinocytes. *Nature* **328,** 817–820 (1987).
35) Di Marco, E., Pierce, J. H., Fleming, T. P., Kraus, M. H., Molloy, C. J., Aaronson, S. A. and Di Fiore, P. P. Autocrine interaction between TGFα and EGF-receptor: quantitative requirements for induction of the malignant phenotype. *Oncogene,* **4,** 831–838 (1989).
36) Bates, S. E., Davidson, N. E., Valverius, E. M., Freter, C. E., Dickson, R. B., Tam, J. P., Kudlow, J. E., Lippman, M. E. and Salomon, D. S. Expression of transforming growth factor α and its messenger ribonucleic acid in human breast cancer: its regulation by estrogen and its possible functional significance. *Mol. Endocrinol.,* **2,** 543–555 (1988).
37) Keski-Oja, J., Raghow, R., Sawdey, M., Loskutoff, D. J., Postlethwaite, A. E., Kang, A. H. and Moses, H. L. Regulation of mRNAs for type-1 plasminogen activator inhibitor, fibronectin, and type I procollagen by transforming growth factor-β. *J. Biol. Chem.,* **263,** 3111–3115 (1988).
38) Lund, L. R., Riccio, A., Andreasen, P. A., Nielsen, L. S., Kristensen, P., Laiho, M., Saksela, O., Blasi, F. and Dano, K. Transforming growth factor-β is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. *EMBO J.,* **6,** 1281–1286 (1987).
39) Keski-Oja, J., Blasi, F., Leof, E. B. and Moses, H. L. Regulation of the synthesis and activity of urokinase plasminogen activator in A549 human lung carcinoma cells by transforming growth factor-β. *J. Cell. Biol.,* **106,** 451–459 (1988).
40) Penttinen, R. P., Kobayashi, S. and Bornstein, P. Transforming growth factor β increases mRNA for matrix proteins both in the presence and in the absence of changes in mRNA stability. *Proc. Natl. Acad. Sci. USA,* **85,** 1105–1108 (1988).
41) Yoshida, K., Yokozaki, H., Niimoto, M., Ito, H., Ito, M. and Tahara, E. Expression of TGF-β an procollagen type I and type III in human gastric carcinomas. *Int. J. Cancer,* **44,** 394–398 (1989).
42) Matrisian, L. M., Leroy, P., Ruhlmann, C., Gesnel, M-C. and Breathnach, R. Isolation of the oncogene and epidermal growth factor-induced transin gene: complex control in rat fibroblasts. *Mol. Cell. Biol.,* **6,** 1679–1686 (1986).
43) Schonthal, A., Herrlich, P., Rahmsdorf, H. J. and Ponta, H. Requirement for *fos* gene expression in the transcriptional activation of collagenase by other oncogenes and phorbol esters. *Cell,* **54,** 325–334 (1988).