Korean J. of Pharmacology
Vol. 29, No. 1, pp. 139-148, 1993

Differential Responses to TGF Alpha in between Invasive Squamous Cell Carcinoma Cell Line and Noninvasive One

Youngsook Son*[1], Myoung-Jae Chey, Elaine Fuchs[2], Myung Hee Chung[1] and Chan Woong Park[1]

[1]*Department of Pharmacology, Seoul National University College of Medicine*
28 Yongon-dong, Chongro-ku, Seoul 110-799, Korea
[2]*Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute*
The University of Chicago, 5841 S. Maryland Ave., Chicago IL 60637, USA

ABSTRACTS

Both SCC 12 and SCC 13 cell lines were derived from squamous cell carcinoma (SCC) of the skin (Wu and Rheinwald, 1981). In the present study, we compared the inherent invasive activity in their raft cultures where most in vivo characteristics of epidermis can be reproduced by cell culture method. The raft culture of SCC 12 cell line produced many invading colonies within the collagen lattice and basal-like cells in the middle of differentiating cell layers, but no invasive activity was observed in the SCC 13 raft culture. We investigated which factors are implicated in inherent invasive activity of SCC 12 cell line by examining basal levels of type I collagenase, EGF receptor, fibronectin, and its receptor in two cell lines. Among them, only type I collagenase was significantly higher in invasive SCC 12 cells than in non-invasive SCC 13 cells. Furthermore, we tried to investigate mechanisms underlying between SCC 12 cell's inherent invasive activity and its high basal level of type I collagenase. As one of them, discrepancy in TGF alpha mediated responses between two cell lines was observed. In SCC 13 cells, TGF alpha initially stimulated type I collagenase at 12 h after TGF alpha treatment and then its down regulation was followed from 24 h even though TGF alpha was continuously present in the medium. However in SCC 12 cells, TGF alpha continuously stimulated type I collegenase up to 48 h. We propose that defect in EGF receptor's down-regulation may be involved in lack of type I collagenase's down-regulation and its possible connection to invasive activity of SCC 12 cell line.

Key Words: TGF alpha/Tumor invasion/Squamous cell carcinoma (SCC)/ Type I collagenase

Abbreviations; a.a.; amino acid, EDTA; ethylene diamine tetra acetic acid, EGF; epidermal growth factor, Fn; fibronectin, INV; involucrin, PBS; phosphate buffered saline, TGF; transforming growth factor

INTRODUCTION

Each tissue compartment is separated from neighboring tissue by basement membrane, whose major components are collagens, proteoglycans, and laminin. For tumor invasion/metastasis to occur, degradation of basement membrane and stromal collagens are required at early stage of this event. Major extracellular metalloproteases responsible for collagen turnover are type I collagenase and type IV collagenase (Emonard

*To whom correspondence should be addressed.

and Grimaud, 1990; Liotta *et al.*, 1991). They are rate limiting enzymes for degradation of type I and IV collagens respectively, which constitute a major barrier for epidermal cell's invasion into dermis where a large supply of vascular access is available for expansion of tumor cells. As previously reported, many human tumorigenic cell lines showed elevated levels of interstitial collagenase (similar to type I collagenase) and type IV collagenase (Brown *et al.*, 1990; Levy *et al.*, 1991). Therefore, genetic changes in tumor cells facilitating degradation of basement membrane and stromal collagens can be an essential step for invasive transition of carcinoma in situ.

TGF alpha is one of major autocrine growth factors of epidermis. It is single polypeptide composed of ~50 a.a. that is structurally and functionally related to epidermal growth factor (EGF), and binds to the ~170 kD, cell surface EGF receptor possessing intrinsic protein tyrosine kinase activity (Ullrich and Schlessinger, 1990). Activation of tyrosine kinase mediate a series of biological responses culminating in cell growth. EGF receptor down regulation has been reported under the condition where its ligand such as TGF alpha or EGF is continuously supplied (Miller *et al.*, 1986). Major localization of EGF receptor in normal epidermis is basal cell layer where mitotically active cells are residing (Green *et al.*, 1983; King *et al.*, 1990; Nanney *et al.*, 1984). As epidermal cells differentiate, its expression is diminished. Therefore it has been proposed that EGF receptor's down regulation may be crucial for entering terminal differentiation of epidermis.

Even though TGF alpha's mitogenic effect was well known, its other functions have not been investigated in detail. Recently it was reported that TGF alpha or EGF induces dermal invasion of epidermal cells in their raft cultures where in vivo characteristics of epidermis can be reproduced by cell culture method, and proposed that invasive activity given by TGF alpha is probably due to its ability to stimulate type I collagenase (Turksen *et al.*, 1991). In the present study, we compared inherent invasive activity in raft cultures of SCC 12 and SCC 13 cell lines derived from squamous cell carcinoma of the skin (Wu and Rheinwald, 1981). SCC 12 cell line produced many cells infiltrating collagen matrix in its raft culture but SCC 13 cell line did not. To evaluate the significance of type I collagenase for invasive activity of SCC 12 cells, we compared basal levels of type I collagenase, EGF receptor, and also molecules necessary for cell migration such as fibronectin and its receptor between inherently invasive SCC 12 cell line and non-invasive SCC 13 cell line. Only type I collagenase was significantly different between invasive and noninvasive cells. Furthermore, as an attempt to investigate mechanisms underlying in inherent invasive activity and high basal level of type I collagenase in SCC 12 cell line, we examined kinetics of type I collagenase induction after TGF alpha treatment in two cell lines.

MATERIALS AND METHODS

Raft culture of SCC 12 and SCC 13 cell lines

Raft culture was conducted as described in Fig. 1. Human SCC 12 or SCC 13 cell lines derived from squamous cell carcinomas of the skin (Wu and Rheinwald, 1981), were seeded on the collagen lattice prepared from Cell Matrix (Nitta gelatin, Tokyo, Japan) embedded with 3T3 mouse fibroblast (from Prof. Fuchs at The University of Chicago). Cells were grown submerged in medium for 7 d and floated at air-liquid interface for 10 d on stainless steel grids. Cultures were maintained with growth medium consisting of Dulbecco's modified Eagle's medium and Ham's nutrient mixture F12 at a 3:1 ratio. Medium was suplimented with 15% fetal bovine serum (Hyclone, Logan, UT), 1×10^{-10}M cholera toxin, 0.4 mg/ml hydrocortisone, 5 mg/ml insulin, 5 mg/ml transferrin, and 2×10^{-11}M triiodothyronine. Medium was changed every 2 d and TGF alpha (Collaborative Res., Arlington, IL, USA) was added to culture medium at 30 μg/ml as indicated in the text. TGF alpha was stored at −70℃ as 1,000X stock in 1 mg/ml bovine serum albumin. Control cultures received a 1:1,000 dilution of 1 mg/ml bovine serum albumin.

Immunocytochemistry

Paraffin-embedded sections (5 μm) were deparaffinized and hydrated before immunocytochemical staining. Staining was carried out as described by Choi and Fuchs (1991). Antisera used

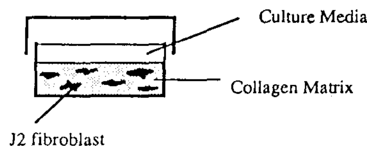
were rabbit polyclonal anti-human fibronectin receptor purified from human placenta (Chemicon Int. Inc., Temecula, CA), rabbit polyclonal anti-human fibronectin (Chemicon Int. Inc., Temecula, CA), and rabbit polyclonal anti-human Involucrin (Biomedical Technologies, Inc., Stoughton, MA). After incubation with primary antisera, slides were subjected to immunogold enhancement as described by Kopan and Fuchs (1989).

Cell labelling with [^{35}S] methionine and immunoprecipitation

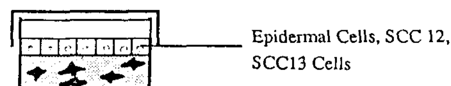
For immunoprecipitation of type I collagenase, raft cultures of SCC 12 or SCC 13 cells were labelled for 12 h with 50 μCi/ml [^{35}S]-methionine (Amersham Corp., Arlington Height, IL) at indicated time after TGF alpha treatment and culture supernatants were subjected to centrifugation at 10,000×g for 10 min to remove any cell debris. Supernatants were adjusted to 0.5% Triton X100 and incubated with rabbit polyclonal antisera against type 1 collagenase (kindly donated by Dr. G. P., Stricklin, Vandervilt University, Nashiville, USA) for 2 h at 4℃. Rabbit IgGs were precipitated with protein A-sepharose-4B (Sigma Chemical Co., St Louis, MO) and washed 3× with immunoprecipitation buffer (150 mM NaCl, 50 mM Tris pH 7.4, 1% Triton X100, 0.2% SDS, 0.5% Nadeoxycholate), 1 × with high salt buffer (0.5M NaCl, 50 mM Tris pH 7.4), and 1× with 10 mM Tris pH 7.4. The samples were separated by 10% SDS PAGE. For fluorography, gel was soaked in 2M sodium salicylate for 3 min, dried, and exposed to X-OMAT-AR film (Eastman Kodak Rochester, NY) at −70℃.

For immunoprecipitation of fibronectin, fibronectin receptor, and EGF receptor, SCC 12 cells or SCC 13 cells were cultured on plastic dishes without any fibroblast feeder layer up to confluency and labelled for 16 h with 50 μCi/ml [^{35}S]-methionine (Amersham Corp., Arlington Height, IL) at indicated time after TGF alpha treatment. Cells were washed with phosphate buffered saline (PBS) three times and incubated in extraction buffer containing 1% Triton X100, 0.5% Nadeoxycholate, 0.2% SDS, 50 mM Hepes pH 7.4, 2 mM phenyl methyl sulfonyl fluoride (PMSF), 10 mM EDTA, and 1 mM iodoacetic acid for 10 min on ice. Soluble proteins were prepared by centrifugation at 10,000×g and protein amount

I Collagen Matrix (artificial dermal layer)



II Monolayer Culture of Epidermal Cells or SCC Cell Line (Submerged Culture)



III Air Liquid Interface Culture

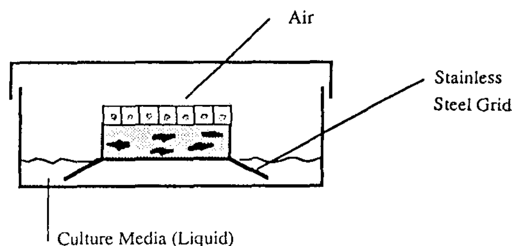


Fig. 1. Raft cultures of SCC 12 cell line and SCC 13 cell line. I. 3T3 mouse fibroblasts selected by their contact inhibition ability were embedded in type I collagen matrix and cultured for one or two days until fibroblasts are nicely spreading in the matrix, which serves as in vitro substitute of dermis (artificial dermis). II. Epidermal cells derived from normal fore skin, or SCC 12 cells, or SCC 13 cells were plated on top of collagen matrix and submerged-cultured until confluency. III. After cells reach confluency, cells and collagen matrix was cut from culture dish with spatula and transfered on to stainless steel grid which was designed for culture media to reach epidermal cells only through transdermal diffusion (air-liquid interface culture). Growth factors or drugs can be applied to the media.

was measured by standard BCA method (Sigma Chemical Co., St Louis, MO). Same amount of protein was immunoprecipitated with anti-human EGF receptor antibody (Cambridge Research Bi-

ochemicals, Cambridge, UK), or rabbit polyclonal anti-human fibronectin. Polyclonal sera were precipitated with protein A-sepharose 4B, samples were analyzed by 8% polyacrylamide gel electrophoresis, and the least of procedures were followed as described above.

RESULTS

SCC 12 cells in the raft culture were able to invade collagen matrix without exogenous addition of EGF or TGF alpha

When SCC 12 cells or SCC 13 cells were cultured at air-liquid interface (raft culture) for 10 days, two cell lines showed quite different morphological characteristics (Fig. 2). SCC 12 cells gave rise to many invading colonies within layer of collagen matrix mainly composed of type 1 collagens corresponding to in vivo dermis. In SCC 13 raft culture, two or three bottom cell layers consisting of mitotically active basal cells as previously confirmed (Kopan and Fuchs, 1989) were nicely demarcated from collagen matrix, which represents obvious increase in basal cell population but no activity in invasive behavior. However when raft culture of non-invasive SCC 13 cells or normal keratinocytes was performed in the presence of 30 ng/ml EGF or TGF alpha, they generated similar invading colonies in the collagen matrix (Turksen *et al.*, 1991; Son, 1993). Thus hyperproliferation in epidermal basal compartment per se is not sufficient for invasive activity, but EGF receptor pathway probably is involved in this process.

Another interesting feature of SCC 12 raft culture is appearance of circular region clustered with basal-like cells in the middle of differentiating cell layers (refered as foci in this paper). Upper layers of SCC 12 raft culture were well stratified as judged by presence of many flattened and enucleated differentiating cells but those of SCC 13 raft culture were filed up with less-differentiated nucleated cells. In terms of epidermal differentiation, SCC 12 cells are able to differentiate further than SCC 13 cells do.

Reappearance of basal cells in the middle of differentiating cell layers of SCC 12 raft culture and its possible implication of subsequent migration center

To determine biochemical features of invading cell colonies and basal-like cells identified in the middle layer of SCC 12 raft cultures, levels of biochemical marker proteins for epidermal differentiating cells (involucrin) and basal cells (fibronectin and its receptor) (Adams and Watt, 1990) were measured by immunocytochemical staining with specific antibodies (Fig. 3). Invading cells and basal cells were not expressing involucrin, but cells on top of single basal cell layer (at certain location, two basal cell layers) were heavily stained with involucrin antibody

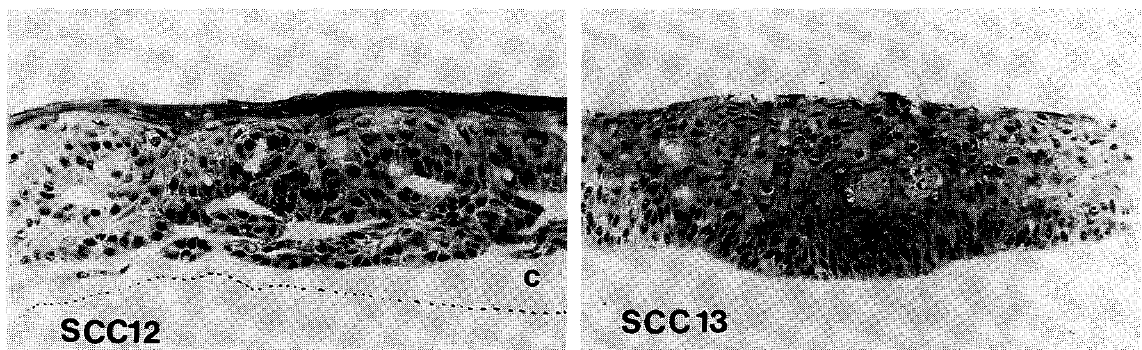


Fig. 2. Morphological examination of SCC 12 and SCC 13 raft cultures. SCC 12 cells or SCC 13 cells were cultured at air-liquid interface for 10 d, fixed in Carnoy's solution, and embedded in paraffin. Sections (5 μM) were stained with hematoxylin and eosin.

(panel INV). Cells at the foci (marked as arrow) in the middle layer which were noted as basal-like cells by morphology in Fig. 2 were not expressing involucrin. Absence of differentiation associated protein at the foci implies that cells at the foci are basal cells probably regenerated dur-

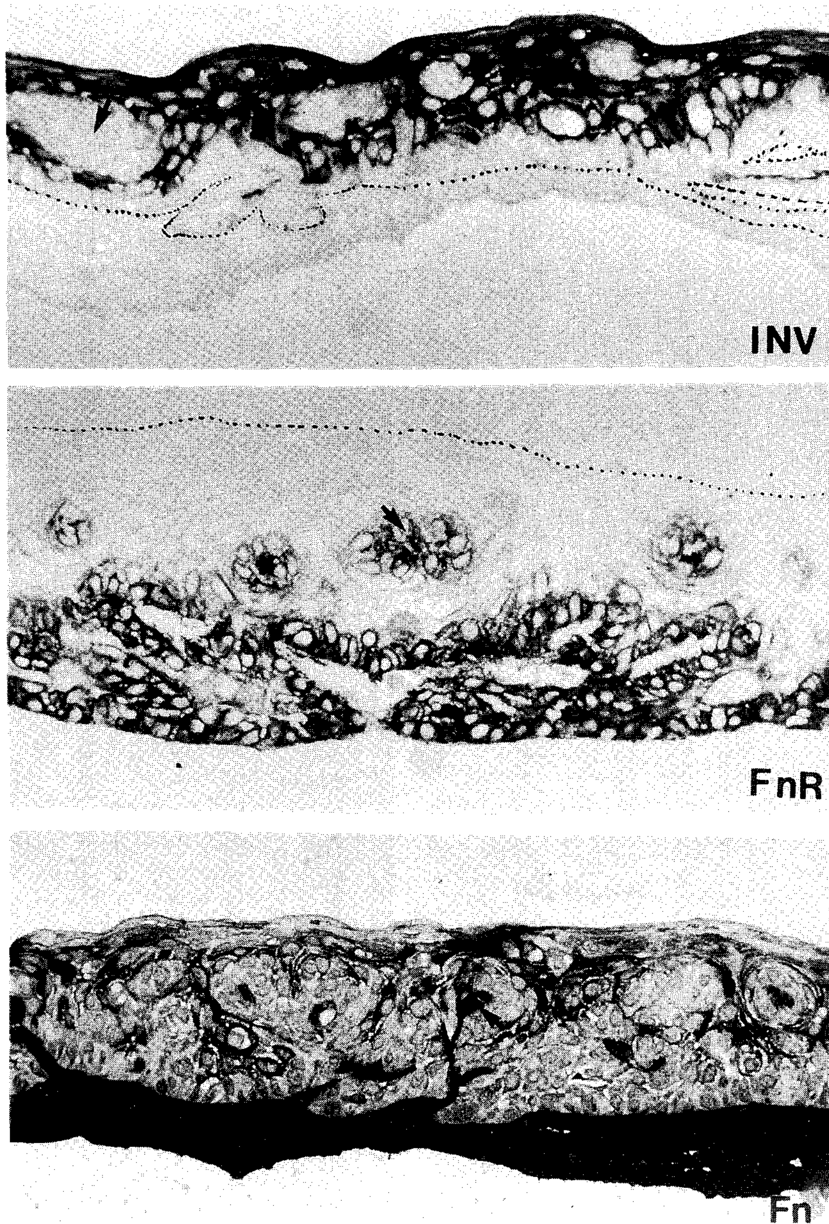


Fig. 3. Immunocytochemistry of SCC 12 raft culture. Carnoy fixed sections were deparaffinized and stained with antibodies against involucrin as epidermal differentiation marker protein (INV), and fibronectin (Fn) and its receptor (FnR) as basal cell marker proteins. Arrows indicate basal cell foci regenerated in the middle of differentiating cell layer.

ing differentiation process.

To localize basal cell population within layers of SCC 12 raft culture, sections were stained with polyclonal antibodies against fibronectin (Fn) receptor ($\alpha_5\beta_1$ integrin) purified from human placenta (panel FnR). Cells located at periphery of invading colonies were stained with Fn receptor antibodies and clusters of Fn receptor positive cells were also observed at foci in the middle layer (arrow), whose staining was mainly localized at basolateral domain of plasma membrane. Staining pattern of Fn receptor was in contrast to that of involucrin (compare panels INV and FnR).

To determine which cells are expressing Fn within architectures of SCC 12 raft culture, sections were stained with antibodies against purified human Fn. Intense Fn staining was observed in the collagen matrix and nicely lined along basal cell layer, which clearly represents massive depostion of Fn secreted by either fibroblasts embedded in collagen matrix or SCC 12 cells themselves. At center of the foci (arrow) was dense plaque of Fn formed, which was presumably secreted by surrounding basal-like cells at the foci. Reappearance of basal-like cells confirmed by expression of biochemical basal marker proteins in the middle of SCC 12 raft culture implicates that some kind of differentiation block or dedifferentiation has proceeded at this foci.

Biochemical differences between invasive SCC 12 cells and noninvasive SCC 13 cells

SCC 12 cells possess inherent ability to invade collagen matrix as shown in Fig. 2 but non-invasive SCC 13 cells could acquire this property only after exogenous addition of EGF or TGF alpha to the media (Son, 1993). TGF alpha overexpression or some sort of defects in regulation of EGF receptor mediated responses might take place in SCC 12 cells, which eventually render SCC 12 cells invasive in collagen matrix. Thus biochemical differences between invasive SCC 12 and non-invasive SCC 13 cells may reveal why SCC 12 cells are capable of invading collagen matrix without TGF alpha or EGF treatment. In this regard, basal biosynthetic rates of TGF alpha, EGF receptor, Fn and Fn receptor implicated in cellular locomotive activity, and type I collagenase facilitating collagen degradation were measured in invasive SCC 12 cells and non-invasive SCC 13 cells by immunoprecipitation of [^{35}S]-methionine labelled cell extracts or supernatants prepared as described in materials and methods with specific antibodies (Fig. 4). No significant differences at basal level of EGF receptor, Fn, and Fn receptor were observed between two cell lines. It was not

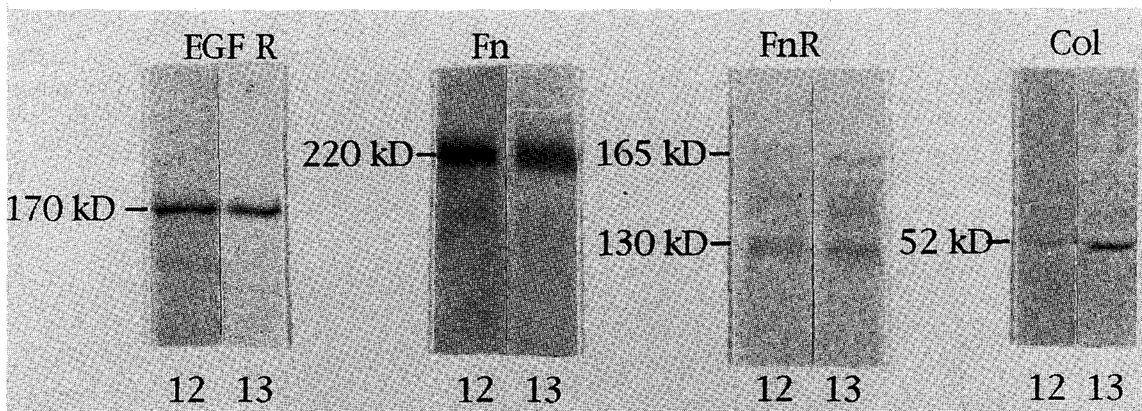


Fig. 4. Comparison of basal levels of EGF receptor, Fn, Fn receptor, and type I collagenase in between SCC 12 cells and SCC 13 cells. [^{35}S]-methionine labelled cell extracts or culture supernatants of two cell lines cultured on plastic dishes were immunoprecipitated with specific antibodies as described in materials and methods. EGF receptor polypeptide was indicated as 170 kD band. Fn polypeptide was noted as 220 kD band. FnR($\alpha_5\beta_1$) is composed of ~160 kD α polypeptide and ~135 kD β polypeptide. Active form of type I collagenase was indicated as 52 kD.

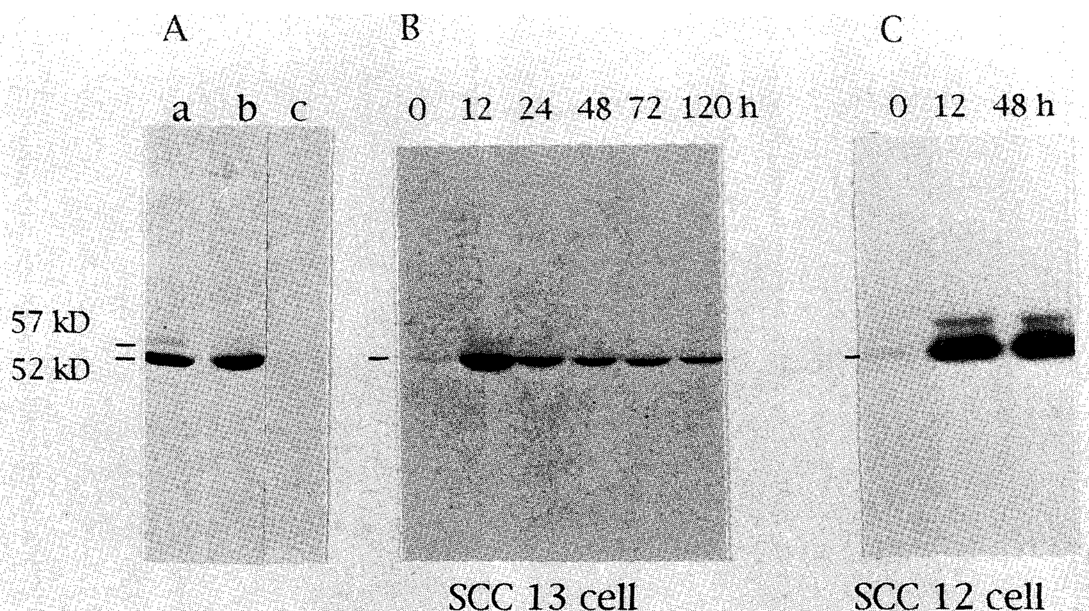


Fig. 5. Kinetics of type I collagenase induction after TGF alpha treatment in raft cultures of SCC 12 cells or SCC 13 cells. A. Specificity of type I collagenase antibody. Purified type I collagenase was separated by 10% SDS PAGE (lane a). Culture supernatant of SCC 12 raft culture was labelled with [^{35}S]-methionine and immunoprecipitated with type I collagenase antibody (lane b) and immunoprecipitation was followed by preincubation of the antibody with purified type I collagenase (lane c). Precursor form of type I collagenase was indicated as 57 kD band, and active form was as 52 kD band. B. Kinetic of type I collagenase induction in SCC 13 cells after TGF alpha treatment. SCC 13 raft cultures were treated with 30 ng/ml TGF alpha at 10 d air-liquid interface culture. Culture supernatants labelled with [^{35}S]-methionine for last 12 h before harvest were collected at indicated time after TGF alpha treatment and followed by immunoprecipitation with type I collagenase antibody. C. Kinetic of type I collagenase induction in SCC 12 cells after TGF alpha treatment. Same methods were applied as described in section B except that SCC 12 cells were used instead of SCC 13 cells.

possible to detect TGF alpha peptide under our experimental conditions since it is persent at very low level in culture media. However, basal level of type I collagenase was higher in invasive SCC 12 cells than in non-invasive SCC 13 cells. From this data, higher basal level of type I collagenase in SCC 12 cells may be considered as a contributing factor for their invasive activity in collagen matrix.

Reappearance of basal population on top of differentiating cells shown in Fig. 3 may reflect delayed or incomplete down-regulation of EGF receptor which might happen to SCC 12 cells. To investigate mechanisms involved in higher basal level of type I collagenase and reappearance of basal cells in the differentiating layers in SCC 12 raft culture, and their possible link to defects in EGF receptor pathway, kinetics of type I collagenase induction in response to 30 ng/ml TGF alpha in SCC 12 and SCC 13 cell lines were measured by immunoprecipitation of culture supernatant from [^{35}S]-methionine labelled cells (Fig. 5).

Specificity of type I collagenase antibody was determined (Fig. 5A). Purified type I collagenase (lane a) showed weak band of 57 kD precursor and intense band of 52 kD active form. Polyclonal antibody precipitated both 57 kD and 52 kD polypeptide (lane b) from [^{35}S]-methionine labelled culture supernatant of SCC 13 raft culture, and

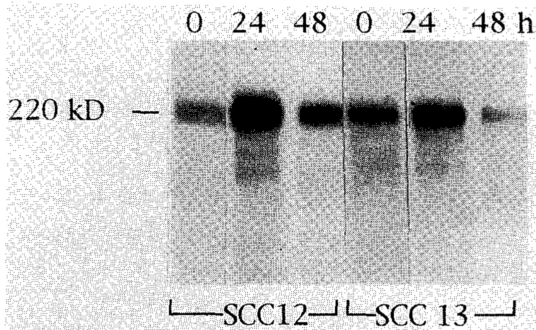


Fig. 6. Fn induction kinetics in SCC 12 and SCC 13 cells after TGF alpha treatment. SCC 12 or SCC 13 cells were cultured on plastic dishes until confluency, treated with 30 ng/ml TGF alpha for indicated time, and labelled with [^{35}S]-methionine for last 16 h before extraction with detergent mixtures as described in materials and methods. Fn polypeptide was indicated as a 220 kD band.

both bands became undetectable after preincubation of the antibody with purified type I collagenase (lane c). Most collagenases secreted by SCC 13 cells were in the active form.

When SCC 13 raft culture was treated with 30 ng/ml TGF alpha, within first 12 h, marked stimulation of type I collagenase was observed and then its down-regulation was seen from 24 h after TGF alpha treatment (Fig. 5B), which shows similar kinetic pattern to that observed in normal keratinocyte (Turksen *et al.*, 1991). In contrast to non-invasive SCC 13 cells, SCC 12 cells continuously stimulated type I collagenase biosynthesis up to 48 h after TGF alpha treatment (Fig. 5C). This implicates that invasive SCC 12 cell does not possess capacity to suppress type I collagenase expression stimulated by TGF alpha.

We examined whether TGF alpha induces change in biosynthesis of Fn or not, and furthermore any discrepancy in this response exists between SCC 12 and SCC 13 cell lines. Since fibroblasts embedded in raft culture can secrete a large amount of Fn, we cultured SCC 12 and SCC 13 cells on plastic dishes without any fibroblast feeder and labelled with [^{35}S]-methionine. Cell extracts at indicated time after TGF alpha treatment were immunopreciptated with antibody to Fn (Fig. 6). In SCC 12 cells, prominent stimulation of ~220 kD Fn polypeptide was observed at 24 h after TGF alpha treatment and went back down to basal level at 48 h after TGF alpha treatment. However, Fn induction in SCC 13 cells was not as prominent as in SCC 12 cells. Thus responses to TGF alpha in SCC 12 cells are more enhanced than those in SCC 13 cells.

DISCUSSION

In this paper, we report that SCC 12 cells in the raft culture showed inherent activity in dermal invasion and its biochemical responses to TGF alpha was quite distinct from those observed in non-invasive SCC 13 cell line. At least in terms of type I collagenase induction, noninvasive SCC 13 cells have retained the ability to down-regulate TGF alpha mediated responses in a similar kinetic to normal keratinocyte (Turksen *et al.*, 1991), but invasive cell line SCC 12 cells lost this type of down-regulation. Lack of type I collagenase down-regulation in response to TGF alpha observed in SCC 12 cells could contribute their inherent invasive activity in some degree and furthermore it suggests that longer duration of EGF receptor activation may provide environment favorable for invasive transition of squamous cell carcinomas. What mechanisms are involved in down-regulation of type I collagenase in non-invasive cells or what is defective in invasive cells remains to be elucidated.

Fn induction kinetic in response to TGF alpha is different from that observed in type I collagenase. Probably different regulation mechanisms may be involved in two responses. Suppression of type I collagenase in non-invasive SCC 13 cells may be due to EGF receptor down regulation which has been demonstrated in other systems (Miller *et al.*, 1986), or other feed back regulation system yet to be identified. EGF receptor down regulation can be obatined by decreasing numbers of responsive EGF receptor at cell surface either through receptor internalization (Miller *et al.*, 1986) or functional down regulation probably mediated by protein kinase-C pathway (Hunter *et al.*, 1984), or by decreasing its synthesis at transcriptional level. It is under study to examine the kinetic difference of EGF receptor

internalization after TGF alpha treatment in two cell lines as a model system.

Significance of collagen degradation in tumor invasion has been reported by many groups including this paper (Brown *et al.*, 1990; Levy *et al.*, 1991; Liotta *et al.*, 1991). It is generally accepted that net degradation of extracellular matrix proteins is dependent on the balance of existing proteases and their inhibitors at certain time (Emonard and Grimaud, 1990). Therefore both TIMPs and collagenases expression should be considered for estimation of collagen destruction even though many regulators act on TIMPs or collagenase inversely (Clark *et al.*, 1987). It was not possible to examine whether TGF alpha changes TIMPs expression or not in this study. However, future experiments such as TIMPs cDNA transfection into SCC 12 cells or application of recombinant TIMPs to culture media can answer how much type I collagenase has contributed to invasive activity of SCC 12 cells.

Formation of basal-like cells in the middle of differentiating cell layer could play an important role in generation of invading colonies in SCC 12 raft culture. Since Fn receptor serves as one of necessary apparatus for attachment of migrating cells to the substratum mainly consisting of Fn (Clark, 1990), reappearance of FnR expressing basal cell population and Fn depostion at center of the foci appeared in the middle of differentiating layers strongly suggests that this foci can be utilized as next migration center. How are basal-like cells regenerated on top of differentiating cells? can not be answered at this point. It awaits more informations about roles of growth factors in controlling epidermal differentiation program. However, appearance of the foci only in invasive SCC 12 cells or EGF treated SCC 13 cells (Son. 1993) suggests that there exists certain interrelationship between foci formation and enhanced TGF alpha mediated responses as shown in invasive SCC 12 cells.

ACKNOWLEDGEMENT

Authors greatly appreciate Prof. Yong Sik Kim at the department of pharmacology, Seoul National University (SNU) for helpful discussion and providing tissue culture facilities and supplies, Prof. Seung Tae Hong at the department of parasitology for providing microscope and technical advices, Dr. James Rheinwald (Biosurface Technology, USA) for donating SCC 12 and SCC 13 cell lines, and Dr. George P. Stricklin (Vandervilt University, USA) and Prof. Zena Werb (University of California, San Francisco, USA) for donating antibodies against type I collagenase. This work was supported by grants from SNU research funds (92-#3) and by Clinical Research Grant from SNU Hospital (1993).

REFERENCES

Adams JC and Watt FM: *Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha_5\beta_1$ integrin loss from the cell surface. Cell 63: 425-435, 1990*

Brown PD, Levy At, Margulies IMK, Liotta LA and Stetler-Stevenson WG: *Independent expression and cellular processing of Mr 72,000 type collagenase and interstitial collagenase in human tumorigenic cell lines. Can Res 50: 6184-6191, 1990*

Choi Y and Fuchs E: *TGF-beta and retinoic acid: regulators of growth and modifyers of differentiation in human epidermal cells. Cell Regul 1: 791-809, 1990*

Clark RAF: *Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. J Invest Dermatol 94: 128S-134S, 1990*

Clark SD, Kobayashi DK and Welgus HG: *Regulation of the expression of tissue inhibitor of metallo-proteinases and collagenase by retinoids and glucocorticoids in human fibroblasts. J Clin Invest 80: 1280-1288, 1987*

Emonard H and Grimaud JA: *Matrix metalloproteinases. A review. Cell Mol Biol 36: 131-153, 1990*

Green NR, Basketter DA, Couchman JR and Ress DA: *Distribution and number of epidermal growth factor receptors in skin is related to epithelial cell growth. Dev Biol 100: 506-512, 1983*

Hunter T Ling N and Cooper JA: *Protein kinase C phosphorylation of the EGF receptor at a threonine residue close to the cytoplasmic face of the plasma membrane. Nature 311: 480-483, 1984*

King LE, Gates RE, Stocscheck CM and Nanney IB: *The EGF/TGF-alpha receptor in skin. J Invest Dermatol 94: 164S-170S, 1990*

Kopan R and Fuchs F: *The use of retinoic acid to relation between hyperproliferation-associated keratins and cell proliferation in normal and malignant epidermal cells. J*

Cell Biol 109: 295-307, 1989

Miller K, Beardmore J, Kanety H, Schlessinger J and Hopkins CR: *Localization of the epidermal growth factor (EGF) receptor within the endosome of EGF-stimulated epidermal carcinoma (A431) cells. J Cell Biol 102: 500-509, 1986*

Nanney LB, McKanna J, Stoscheck CM, Carpenter G and King LE: *Visualization of epidermal growth factor receptors in human epidermis. J Invest Dermatol 82: 165-169, 1984*

Levy AT, Cioce V, Sobel ME, Garbisa S, Grigioni WF and Liotta LA: *Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. Can Res 51: 439-444, 1991*

Liotta LA, Steeg PS and Stetler-Stevenson WG: *Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 64: 327-336, 1991*

Son (Choi) Y and Fuchs E: *Molecular controls of epidermal growth and differentiation: transforming growth factors. Korean J Toxicol 7: 209-229, 1991*

Son Y: *EGF stimulates invasive transition of non-invasive SCC 13 cells accompanied by increases in type I collagenase and basal cell population. Molecules and Cells 3: 35-42, 1993*

Turksen K, Choi Y and Fuchs E: *Transforming growth factor alpha induces collagen degradation and cell migration in differentiating human epidermal raft cultures. Cell Reg 2: 613-625, 1991*

Ullrich A and Schlessinger J: *Signal transduction by receptors with tyrosine kinase activity. Cell 61: 203-212, 1990*

Wu YJ and Rheinwald JG: *A new small (40 kd) keratin filament protein made by some cultured human squamous cell carcinomas. Cell 25: 627-635, 1981*

=국문초록=

침투성 상피암세포주와 비침투성 상피암세포주의 TGF alpha에 대한 반응의 차이

서울대학교 의과대학 약리학교실[1], 미국시카고대학 Howard Hughes 의학연구소[2]

손영숙[1] · 최명재[1] · Elaine Fuchs[2] · 정명희[1] · 박찬웅[1]

SCC 12와 SCC 13 세포주는 피부의 상피암 조직에서 유래되었다. 본 연구에서는, 세포배양으로 in vivo 피부의 거의 모든 특징들을 재현할 수 있는 raft 배양법을 이용하여, 두 종류의 상피암세포주의 진피 침투성 정도를 비교해 보았다. SCC 12 세포주의 raft 배양에서는, collagen matrix로 침투하는 많은 세포군들이 관찰되었고, 또한 기저세포와 유사한 세포들이 분화층 중간에 관찰되었다. 그러나 SCC 13 세포주의 raft 배양에서는 침투성 세포들이 전혀 관찰되지 않았다. 어떤 인자가 SCC 12 세포주의 침투성에 관여하였는지를 조사하기 위하여, 세포이동 또는 matrix 파괴에 관여하는 각종 단백질 즉, type 1 collagenase, Fn, Fn 수용체와 EGF 수용체 등의 기본 생합성속도를 침투성과 비침투성 세포주에서 측정하였다. 그 중 type 1 collagenase의 생합성만이 침투성 세포주에서 현저히 높게 나타났다. 침투성 SCC 12 세포주에서 관찰된 높은 type 1 collagenase 생합성과 이들 세포주의 침투성과의 연관관계 및 그 기작을 조사하기 위하여 두 세포주에서 TGF alpha에 대한 반응의 차이를 조사하였다. 비침투성 세포주에서는, TGF alpha에 의해 type 1 collagenase의 생합성이 처음 12시간 동안에 현저한 증가를 보였으나 24시간부터 그 유도가 down-regulation되는 현상을 보였다. 그러나 침투성 세포는 TGF alpha에 의해 계속적인 type 1 collagenase의 유도를 보였다. 이에 저자들은 EGF 수용체의 down-regulation으로 인해 type 1 collagenase의 down-regulation이 유도되고, 이와 같은 regulation의 결함으로 인해 SCC 12 세포주가 collagen matrix로 침투하는 현상을 나타낼 것으로 제시하였다.