J. Biochem. 120, 1196-1202 (1996)

Differential Expression of Laminin-5/Ladsin Subunits in Human Tissues and Cancer Cell Lines and Their Induction by Tumor Promoter and Growth Factors[1]

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Received for publication, July 31, 1996

We previously reported a new laminin variant containing laminin $\gamma 2$ (or B2t) chain, ladsin, which exerted prominent cell-scattering, cell-adhesion, and cell-migration activities. In the present study, this laminin was further characterized, and gene expression of its three subunits in various human tissues and cancer cell lines was examined by Northern blotting. cDNA cloning of the largest subunit of ladsin and partial amino acid sequencing of its β (or B1) subunit revealed that ladsin was identical to laminin-5 (kalinin/epiligrin/nicein). Among various human tissues, placenta, lung, and fetal kidney expressed high levels of mRNAs for the three subunits of laminin-5 (laminin $\alpha 3_{EpA}$, $\beta 3$, and $\gamma 2$ chains). Most gastric and squamous carcinoma cell lines constitutively expressed all of the three subunit mRNAs, while other types of carcinoma cell lines expressed one or two of them. The tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and epidermal growth factor (EGF) strongly enhanced the gene expression of the three subunits, increasing 2 to 8- fold the secretion of laminin-5 from carcinoma cells into culture medium. However, TPA treatment did not increase the secretion of laminin $\beta 1$ chain, a subunit of laminins-1, -3, and -6. The unique properties and inducibility by TPA and EGF of laminin-5 suggest that it is associated with growth and migration of cancer cells.

Key words: cell adhesion, epidermal growth factor, gastric cancer, laminin-5, tumor promoter.

Some steps of tumor invasion and metastasis require the ability of tumor cells to adhere to and migrate on surrounding extracellular matrix components such as laminin and fibronectin (*1*). Laminin is a major component of basement membranes, which was first purified from Engelbreth-Holm-Swarm tumor (*2*). Laminin is composed of disulfide bond-linked heterotrimeric chains, α (or A), β (or B1), and γ (or B2) (*3-6*). The involvement of laminin (laminin-1) in tumor metastasis was first shown by Iwamoto *et al.* (*7*). Simultaneous injection of melanoma cells with a laminin-derived synthetic peptide capable of binding to the laminin receptor effectively inhibited experimental metastasis in mice. Since the first identification of laminin, seven laminins (laminin-1 to laminin-7), which have different chain combinations and tissue distributions, have been found in humans (*3*). Recent studies of the laminin variants have suggested that they exert specialized physiological functions (*8-10*).

We recently purified a new laminin variant containing laminin B2t subunit ($\gamma 2$ chain) from conditioned medium (CM) of a human gastric cancer cell line STKM-1 (*11*). Because of its large molecular size and prominent cell-adhesive and cell-scattering activities, it was tentatively named ladsin. The cell-scattering activity was secreted by various cultured cell lines including gastric carcinoma cells, squamous carcinoma cells, epithelial cells, and endothelial cells (*11*). Compared with laminin-1, fibronectin, and vitronectin, ladsin exhibited the highest stimulation of both cell adhesion and migration for rat liver cell line BRL (*12*). The cell adhesion to ladsin is mediated by integrin $\alpha 3\beta 1$ (*12*). Ladsin is very similar to the recently identified, keratinocyte-derived laminin variant "laminin-5" (kalinin/epiligrin/nicein), which is composed of laminin $\alpha 3_{EpA}$, $\beta 3$, and $\gamma 2$ chains and binds to integrin $\alpha 3\beta 1$ (*13-15*). Pyke *et al.* (*16*) have shown that laminin-5 is specifically expressed by invading tumor cells in colon and some other cancers.

In the present cDNA and mRNA analyses, we demonstrate that ladsin is identical to laminin-5, and that the three laminin-5 subunits, laminin $\alpha 3_{EpA}$, $\beta 3$, and $\gamma 2$ chains, are differently expressed in various human tissues

[1] This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, and Special Coordination Funds from the Science and Technology Agency of Japan. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number D83973.

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Abbreviations: CM, conditioned medium; EGF, epidermal growth factor; MMP, matrix metalloproteinase; M_r, molecular weight; PKC, protein kinase C; RT-PCR, reverse transcription-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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and cancer cell lines and up-regulated by a tumor promoter and growth factors.

MATERIALS AND METHODS

Cells and Culture—Gastric adenocarcinoma cell line STKM-1 (*17*) was a generous gift from Dr. Yanoma (Kanagawa Cancer Center, Yokohama). Other cell lines were obtained from Japanese Cancer Resources Bank (JCRB). Types of these cell lines are as follows: MKN-1, MKN-28, MKN-45, MKN-74, and NUGC-3, gastric adenocarcinoma; SCH, stomach chorionic carcinoma; KATO III, stomach signet ring cell carcinoma; EJ-1 and T24, bladder carcinoma; HT1080, fibrosarcoma; KB, oral epidermoid carcinoma; HSC-2 and HSC-4, tongue squamous adenocarcinoma; CaSki and C-4I, cervix epidermoid carcinoma; A431, vulva epidermoid carcinoma; HLE, HLF, PLC/PRF/5 and HuH-7, hepatoma; Chang Liver, normal hepatocyte-derived cell line. All kinds of cells were cultured in Dulbecco's modified Eagle's medium plus Ham's F12 medium (1 : 1), DME/F12, supplemented with 10% fetal calf serum, penicillin, and streptomycin sulfate.

Probes—Purified ladsin was digested with lysyl endopeptidase, and N-terminal amino acid sequences of resultant peptide fragments were determined. One of the fragments had the sequence SQLQGLSASAGLLEQMRHMEXQAK. To amplify a cDNA fragment encoding the amino acid sequence, we designed two degenerate oligonucleotide primers, 5′-GCGAATTCAG(C/T)CA(A/G)(C/T)TICA-(A/G)GGA-3′ and 5′-GCGAATTC(C/T)TCCAT(A/G)TG-IC(G/T)CAT-3′, in which underlines indicate *Eco*RI sites, I indicates dITP, and bracketed nucleotides are degenerated ones. Reverse transcription and polymerase chain reaction (RT-PCR) was performed using poly(A)-RNA obtained from STKM-1 cells. A fragment with an expected size of 76 bp was specifically amplified (data not shown).

cDNA probes for Northern blotting analysis of laminin β3 (B1k) and γ2 (B2t) chains were obtained by RT-PCR. Nucleotide sequences of primers were as follows: laminin β3-5′, 5′-TCTCAGCAGCCCCGCAGTCACAG-3′ (nt. no. 1854-1876); laminin β3-3′, 5′-CTTGGGGACAGCTCAC-CAGGGC-3′ (nt. no. 2345-2358); laminin γ2-5′, 5′-CATG-AAGGAGTCGAAGCGGAAGC-3′ (nt. no. 2509-2532); and laminin γ2-3′, 5′-TGCTTGCTGGGTCTTGTCACTG-GC-3′ (nt. no. 3072-3096). The nucleotide numbering of cDNAs for these laminin β3 and γ2 chains was derived from the reports by Gerecke *et al.* (*18*) and Kallunki *et al.* (*19*), respectively. Nucleotide sequences of RT-PCR products were confirmed as described below. A *Pst*I cDNA fragment of laminin $\alpha 3_{EpA}$ chain corresponding to the sequence from nt. no. 684 to 1264 was used as a laminin α3 chain probe (*20*).

Construction and Screening of cDNA Library—cDNA was synthesized with poly(A)-RNA purified from STKM-1 cells by priming with random 9-mer or oligo-dT oligonucleotides, and ligated into an *Eco*RI site of λgt11 vector (Stratagene, La Jolla, CA, USA). About 1×10^5 independent clones were screened with the [α-^{32}P]dCTP-labeled probe (1×10^6 cpm/ml). Hybridization was performed in a low stringent hybridization buffer containing 40% formamide at 42°C for 24 h. Hybridized membranes were washed twice with 2×SSC-0.1% SDS, once with 0.5× SSC-0.1% SDS, each time at 65°C for 30 min, then exposed to Kodak XAR-1 films (Eastman Kodak, Rochester, NY, USA) with an intensifying screen at −80°C. Obtained clones were subcloned into a plasmid vector. These clones were digested with various restriction enzymes, and resultant fragments were subcloned for sequencing. For large fragments, a series of deletion mutants was constructed by using Exonuclease III. Nucleotide sequencing was performed on both strands with an automatic DNA sequencer.

Northern Blotting Analysis—Total RNA was prepared from cultured cells with ISOGEN (Wako, Tokyo) according to the manufacturer's protocol. Twenty micrograms of total RNA was electrophoresed on a formamide-containing 0.8% agarose gel and blotted onto a nylon membrane. Multiple Tissue Northern Blot membranes were obtained from CLONTECH Laboratories (Palo Alto, CA, USA). The membranes were hybridized with [α-^{32}P]dCTP-labeled probes (1×10^6 cpm/ml) in the hybridization buffer containing 50% formamide at 42°C for 20 to 24 h. The membranes were washed twice with 2×SSC-0.1% SDS, once with 0.1×SSC-0.1% SDS, each time at 65°C for 30 min, then exposed to Kodak XAR-1 films (Eastman Kodak) with an intensifying screen at −80°C.

Immuno-Blotting Analysis—Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose membranes. The membranes were probed with an anti-laminin-α3 chain monoclonal antibody, which was prepared in our laboratory, or an anti-laminin-β1-chain monoclonal antibody (Chemicon International, Temecula, CA, USA). Purified human laminin-1 was purchased from CosmoBio (Tokyo).

RESULTS

Primary Structure of Ladsin—We previously identified one of the three subunits of ladsin as laminin γ2 (or B2t) chain (*11*). To further characterize ladsin, its whole molecule was digested with lysyl endopeptidase, and many proteolytic fragments were obtained. One of the fragments had the N-terminal amino acid sequence SQLQGLSASAG-LLEQMRHME. A mouse monoclonal antibody prepared against a synthetic peptide having the same sequence recognized the longest subunit of ladsin, that of molecular weight (M_r) 160,000, in Western blotting analysis (see below). In an initial database search, no similar sequences were found in any of the proteins listed. Using degenerate oligonucleotide primers designed for the peptide sequence, we cloned a cDNA encoding the M_r 160,000 chain of ladsin from an STKM-1 cell cDNA library (Accession No. D83973 in DDBJ, EMBL, and GenBank Data Bases). The nucleotide sequence of the cDNA was identical to that of epiligrin A chain (laminin $\alpha 3_{EpA}$ chain), which was reported during the present study by Ryan *et al.* (*20*). There was no difference in the nucleotide sequence of the coding region between the STKM-1- and keratinocyte-derived cDNAs. The 5′ and 3′ untranslated regions of our sequence extended 59 and 54 bp from the reported sequence, respectively (data not shown). The extended 3′ 54-bp sequence contains a polyadenylation signal [nt. no. 5464 to 5469, according to the numbering of Ryan *et al.* (*20*)] followed by poly(A) tail 18-bp downstream, while the polyadenylation in the reported sequence begins at nt. no. 5434. The meaning of this difference is not clear, but it might affect the stability or translational

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efficiency of the mRNA.

Direct sequencing of the N-terminal amino acid sequence of the ladsin M_r 140,000 subunit showed the sequence GYHPPSAYYAVCQLRQ. This sequence corresponded to the sequence of laminin $\beta 3$ (laminin B1k) chain from residue 214 to residue 229 (*18*), suggesting that the M_r 140,000 subunit had been cleaved by a trypsin-like enzyme between residues Arg^{213} and Gly^{214}. In addition, we obtained another internal sequence of laminin $\beta 3$ chain by analyzing proteolytic fragments of the M_r 140,000 subunit. From these results, we concluded that ladsin was identical to laminin-5.

Gene Expression of Three Laminin-5 Subunits in Human Tissues—Laminin-5 is expressed by epithelial basal cells in skin and some other tissues. We examined gene expression of the three subunits (laminin $\alpha 3$, $\beta 3$, and $\gamma 2$) in various human tissues by Northern blotting (Fig. 1). Expression of three laminin-5 mRNAs, $\alpha 3$ (5.5 and 8.3 kb), $\beta 3$ (3.7 kb), and $\gamma 2$ (5.0 and 4.2 kb), was the highest in adult placenta (lane 3) and lung (lane 4) and fetal lung (lane 18) and kidney (lane 20), moderate in adult thymus, prostate, testis, and ovary (lanes 10–13), and low in many other adult tissues. Relative levels of the three mRNAs varied from one tissue to another. For example, laminin $\alpha 3$ mRNA was dominant in the placenta and ovary, while laminin $\beta 3$ mRNA was dominant in the prostate.

Two kinds of laminin $\alpha 3$ chains, the short form ($\alpha 3_{EpA}$ or $\alpha 3A$) and the long form ($\alpha 3_{EpB}$ or $\alpha 3B$), also showed distinct expression patterns. Laminin $\alpha 3_{EpA}$ mRNA (5.5 kb) was dominant in the placenta, thymus and testis (lanes 3, 10, and 12, respectively), whereas laminin $\alpha 3_{EpB}$ mRNA (8.3 kb) was dominant in the lung, ovary, and fetal lung and kidney (lanes 4, 13, 18, and 20, respectively). The specific expression of $\alpha 3_{EpB}$ mRNA in fetal lung and kidney is consistent with the result of *in situ* hybridization of fetal mouse tissues (*21*). The Northern blot for laminin $\gamma 2$ also showed an alternatively spliced transcript of 4.2 kb in addition to the major transcript of 5.0 kb (*19*).

Gene Expression of Three Laminin-5 Subunits in Human Cancer Cells—We previously detected cell-scattering activity of ladsin in culture media of many gastric carcinoma and squamous carcinoma cell lines (*11*). In the present study, expression of laminin $\alpha 3$, $\beta 3$, and $\gamma 2$ mRNAs in human cancer cell lines was examined by Northern blotting.

As expected from the secretion of cell-scattering activity, high expression of the three laminin-5 mRNAs was detected in most cell lines of gastric carcinoma (*e.g.*, lane 1/STKM-1, lane 4/MKN-45, lane 6/NUGC-3, and lane 8/KATO III) and squamous carcinoma (*e.g.*, lane 14/HSC-4, lane 15/CaSki, and lane 16/C-4I) (Fig. 2A). Interestingly, we could not detect cell-scattering activity in the culture medium of signet ring cell carcinoma cell line KATO III (*11*), in spite of the significant expression of the three laminin-5 messages (Fig. 2A, lane 8).

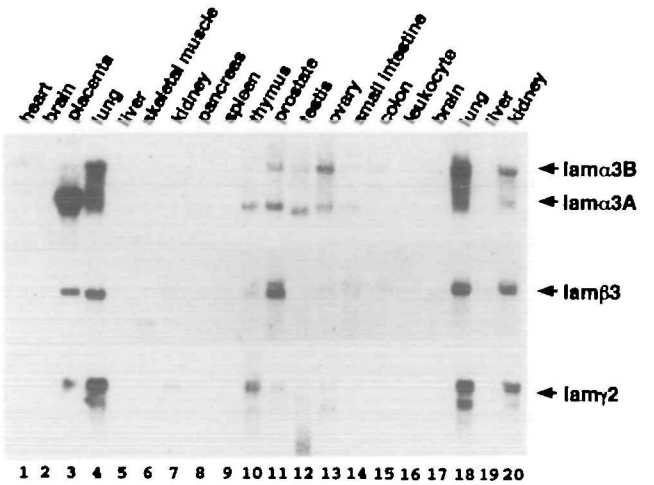

Fig. 1. **Northern blotting of mRNAs for three laminin-5 subunits in adult and fetal human tissues.** Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas; 9, spleen; 10, thymus; 11, prostate; 12, testis; 13, ovary; 14, small intestine; 15, colon; 16, peripheral blood leukocyte; 17, brain; 18, lung; 19, liver; 20, kidney. Lanes 1 to 16, adult tissues; lanes 17 to 20, fetal tissues. Each lane contains 2 μg of poly(A)-RNA. Arrowheads indicate laminin $\alpha 3_{EpA}$ (lamα3A; 5.5 kb), $\alpha 3_{EpB}$ (lamα3B; 8.3 kb), $\beta 3$ (lamβ3; 3.7 kb), or $\gamma 2$ (lamγ2; 5.0 and 4.2 kb) chain mRNA.

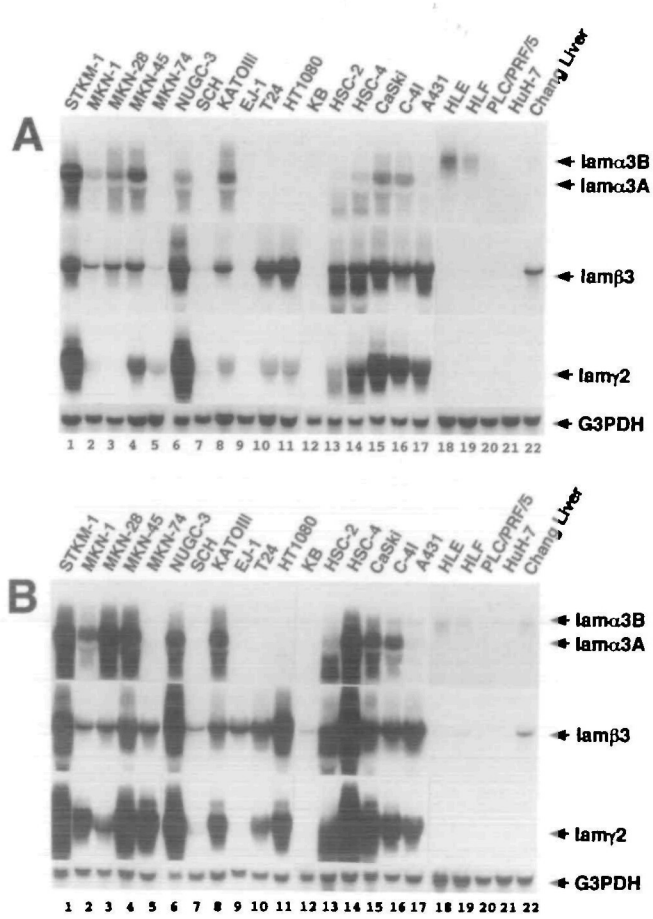

Fig. 2. **Northern blotting of three laminin-5 mRNAs in 22 human cancer cell lines.** Cells were cultured in serum-free DMEM/F12 medium for 2 days in the absence (A) or presence (B) of TPA (100 ng/ml). Arrowheads indicate laminin $\alpha 3_{EpA}$ (lamα3A), $\alpha 3_{EpB}$ (lamα3B), $\beta 3$ (lamβ3), or $\gamma 2$ (lamγ2) chain mRNA. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) is a control. Lanes: 1, STKM-1; 2, MKN-1; 3, MKN-28; 4, MKN-45; 5, MKN-74; 6, NUGC-3; 7, SCH; 8, KATO III; 9, EJ-1; 10, T24; 11, HT1080; 12, KB; 13, HSC-2; 14, HSC-4; 15, CaSki; 16, C-4I; 17, A431; 18, HLE; 19, HLF; 20, PLC/PRF/5; 21, HuH-7; 22, Chang Liver.

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Cancer cell lines other than gastric carcinoma and squamous cell carcinoma expressed some of the three laminin-5 mRNAs. Fibrosarcoma HT1080 (lane 11), from which laminin $\gamma 2$ (or B2t) cDNA was first cloned (*19*), and bladder carcinoma T24 (lane 10), as well as two gastric carcinomas (lane 5/MKN-74 and lane 7/SCH), expressed $\beta 3$ and $\gamma 2$ mRNAs, but hardly $\alpha 3$ mRNA. In contrast, three hepatoma cell lines, HLE, HLF, and PLC/PRF/5 (lanes 18, 19, 20, respectively), expressed only the 8.3 kb transcript corresponding to laminin $\alpha 3_{EpB}$ mRNA (*20*). These results indicate that the three chains of laminin-5 are also differentially expressed in laminin-5-nonproducing cancer cells. The results of Northern blotting and cell-scattering activity assay are summarized in Table I. This demonstrates that coexpression of the three laminin-5 subunits is prerequisite for the expression of cell-scattering activity.

TABLE I. **Summary of Northern blot analysis and cell-scattering activity assay.** Relative gene expression was measured by densitometric analysis. High, moderate, and low expression is indicated by +++, ++, and +, respectively. Very weak expression is expressed as ±. − denotes the absence of the expression. [a]Concentrated CM (30-fold) was serially diluted with PBS. Five microliters of each sample was added to the culture of BRL cells (7×10^3 cells in 0.5 ml of medium), and cell-scattering activity was monitored microscopically after 2 days of culture. The activity at 1 : 8, 1 : 4, 1 : 2, and 1 : 1 dilution is expressed as ++++, +++, ++, +, respectively. − indicates the absence of cell-scattering at 1 : 1 dilution. [b]Expression of laminin $\alpha 3_{EpB}$ chain.

Cell line	Laminin			Cell-scattering activity[a]
	$\alpha 3$	$\beta 3$	$\gamma 2$	
STKM-1	+++	+++	+++	++++
MKN-1	+	+	±	−
MKN-28	++	+	−	−
MKN-45	+++	++	++	+++
MKN-74	−	±	+	−
NUGC-3	+	+++	+++	+
SCH	−	±	±	−
KATO III	++	++	+	−
EJ-1	−	−	−	−
T24	−	+++	+	−
HT1080	−	+++	+	−
KB	−	−	−	−
HSC-2	+	+++	+	++
HSC-4	+	+++	+++	++
CaSki	++	+++	+++	+
C-4I	+	++	+++	+++
A431	+	+++	+++	+
HLE	+[b]	−	−	−
HLF	+[b]	−	−	−
PLC/PRF/5	±[b]	−	−	−
HuH-7	−	−	−	−
Chang Liver	−	+	−	−

Up-Regulation of Laminin-5 Subunits by Tumor Promoter and Growth Factors—To understand the regulatory mechanism of gene expression of laminin-5, the effect of TPA treatment on the expression of laminin-5 in various human cancer cell lines was examined by Northern blotting.

Figure 2B shows gene expression of the three laminin-5 chains in 22 cancer cell lines after 48 h of stimulation by TPA. TPA enhanced the synthesis of laminin $\alpha 3_{EpA}$, $\beta 3$, and $\gamma 2$ mRNAs in laminin-5-producing cells. However, the stimulatory effect of TPA differed among the three subunits and among cell lines. For example, expression of laminin $\alpha 3_{EpA}$ mRNA in MKN-74 (lane 5), SCH (lane 7), T24 (lane 10), and HT1080 (lane 11) was hardly detected even after the TPA stimulation, whereas the other two mRNAs were markedly induced in the same cell lines. In

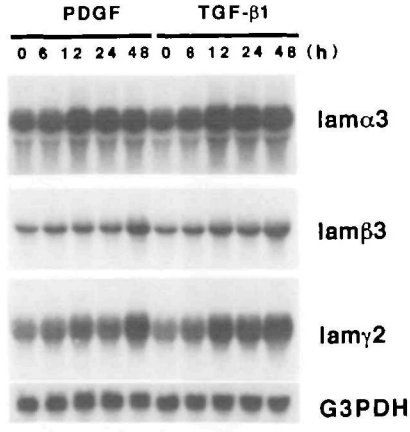

Fig. 4. **Effects of PDGF and TGF-β1 on expression of three laminin-5 mRNAs.** STKM-1 cells were incubated with PDGF (2 ng/ml) or TGF-β1 (10 ng/ml) in serum-free medium for the indicated lengths of time, and the mRNAs in the cells were analyzed by Northern blotting. lamα3, laminin α3; lamβ3, laminin β3; lamγ2, laminin γ2. G3PDH is a control.

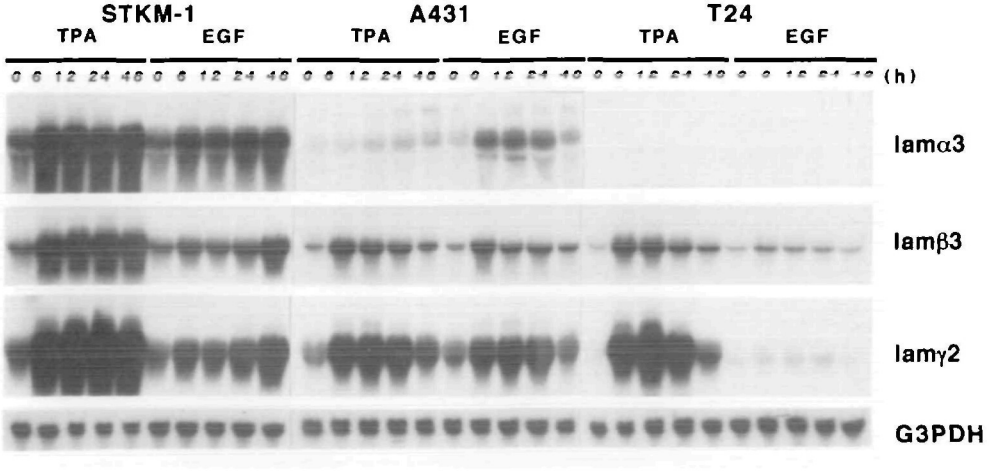

Fig. 3. **Effects of TPA and EGF on expression of three laminin-5 mRNAs.** STKM-1, A431, and T24 cells were cultured in serum-free medium for 24 h, then incubated in fresh serum-free medium containing TPA (100 ng/ml) or EGF (30 ng/ml) for the indicated lengths of time. The three mRNAs in the cells were analyzed by Northern blotting. lamα3, laminin α3; lamβ3, laminin β3; lamγ2, laminin γ2. G3PDH is a control.

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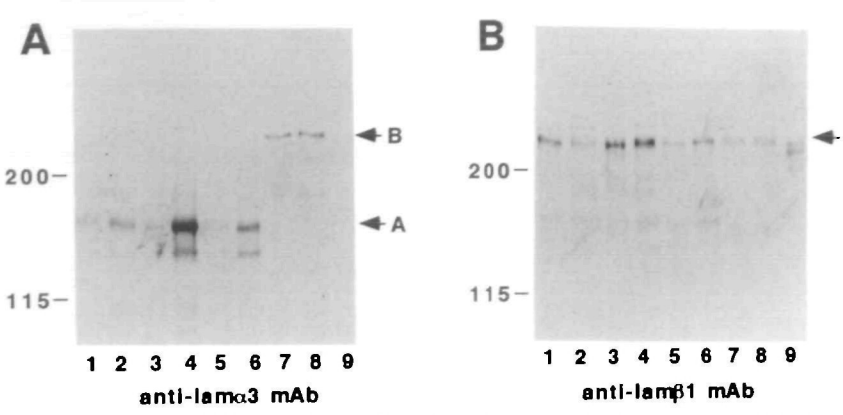

Fig. 5. **Effect of TPA treatment on secretion of laminin $\alpha 3$ and $\beta 1$ chains from three cancer cell lines.** STKM-1 (lanes 1 and 2), MKN-45 (lanes 3 and 4), HSC-4 (lanes 5 and 6), and HLE (lanes 7 and 8) cells were incubated in serum-free medium without (lanes 1, 3, 5, and 7) or with (lanes 2, 4, 6, and 8) TPA (100 ng/ml) for 2 days. The resulting conditioned media were concentrated 30-fold by ammonium sulfate precipitation at 80% saturation, then subjected to immunoblotting. Samples were separated by SDS-PAGE on 5% polyacrylamide gels and blotted onto nitrocellulose membranes. The membranes were probed with the anti-laminin $\alpha 3$ chain monoclonal antibody (A) and with the anti-laminin $\beta 1$ antibody (B). Arrowheads indicate laminin $\alpha 3$ chains of M_r 160,000 and M_r 240,000 in A, and laminin $\beta 1$ chain of M_r 220,000 in B. Purified laminin-1 was also run as a control (lane 9). M_r markers are shown left in thousands.

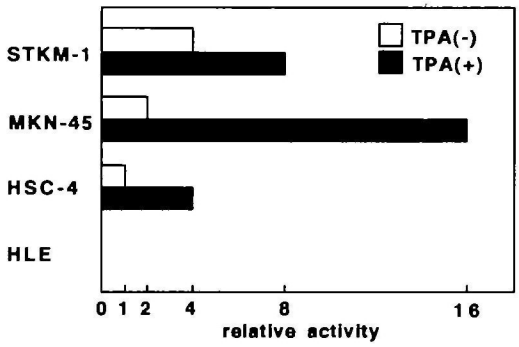

Fig. 6. **Effect of TPA treatment on secretion of cell-scattering activity from four cancer cell lines.** Cell-scattering activity of the conditioned media was assayed as described previously (*11*). STKM-1, MKN-45, HSC-4, and HLE cells were incubated in the absence (open) or presence (closed) of TPA (100 ng/ml) for 2 days as described in Fig. 3. Abscissa, relative cell-scattering activity.

MKN-28 (lane 3) and EJ-1 (lane 9) cells, laminin $\gamma 2$ and $\beta 3$ chains were strongly induced, respectively. Interestingly, the expression of laminin $\alpha 3_{EpB}$ mRNA in HLE and HLF was hardly induced or even decreased by the TPA treatment. The induction of laminin-5 subunits by TPA suggests that growth factor signals mediated by protein kinase C (PKC) regulate the gene expression. Indeed, we found that epidermal growth factor (EGF) induced the synthesis of the three laminin-5 subunit mRNAs.

Figure 3 shows the time course of expression of the three subunit mRNAs in three cell lines after treatment with TPA or EGF. When STKM-1 cells were treated with TPA, the level of laminin $\alpha 3_{EpA}$, $\beta 3$, and $\gamma 2$ mRNAs was almost maximal after 6 h, and the maximal level was maintained up to at least 48 h. When the same cells were treated with EGF, the levels of the three mRNAs gradually increased over 48 h. In A431 cells, EGF was more effective than TPA in induction of $\alpha 3_{EpA}$ mRNA. In T24 cells, neither EGF nor TPA induced laminin $\alpha 3_{EpA}$ mRNA, while TPA but not EGF was effective in induction of laminin $\beta 3$ and $\gamma 2$ mRNAs. Thus, responses to TPA and EGF differed among cell lines. Two other growth factors, platelet-derived growth factor (PDGF) and transforming growth factor-$\beta 1$ (TGF-$\beta 1$), also stimulated the transcriptional levels of the three mRNAs in STKM-1 cells, but to lower extents than TPA and EGF (Fig. 4).

Secretion of Laminin-5 Subunits—The stimulatory effect of TPA on expression of laminin-5 genes was examined at the level of the protein product. STKM-1, MKN-45, HSC-4, and HLE cells were treated with TPA for 2 days, and the levels of laminin $\alpha 3$ chain in the resultant conditioned media were analyzed by immunoblotting with an anti-laminin $\alpha 3$ monoclonal antibody (Fig. 5). The TPA treatment markedly increased the amount of laminin $\alpha 3_{EpA}$ chain of M_r 160,000 in STKM-1, MKN-45, HSC-4 (lanes 2, 4, and 6, respectively). An additional M_r 140,000 band seemed to be a product of degradation or further processing of the same chain. In HLE cells, an immuno-stained band of M_r 240,000, which seemed to correspond to laminin $\alpha 3_{EpB}$ chain, was detected, but its level was not influenced by the TPA treatment (lane 8). In contrast to laminin $\alpha 3_{EpA}$ chain, the amount of laminin $\beta 1$ chain, which was analyzed as a negative control, was not significantly increased or even decreased by TPA in the same cell lines (Fig. 5). When the cell-scattering activities of the four conditioned media were analyzed, the activity was proportional to the level of laminin $\alpha 3_{EpA}$ in STKM-1, MKN-45, and HSC-4 cells (Fig. 6). The conditioned medium of HLE cells showed no cell-scattering activity. TPA increased the secretion of laminin-5 activity 2 to 8 times in these cell lines.

DISCUSSION

In this study we identified ladsin as laminin-5, and gene expression of its three subunits in various human tissues and cancer cell lines was examined. This laminin variant was independently identified by three other groups as epiligrin, kalinin, and nicein (*13-15*), and recently these have been shown to be the same protein (*20, 24, 25*). Several lines of evidence have suggested that laminin-5 plays an important physiological role in the maintenance of skin architecture at the basement membrane of the epidermal/dermal junction (*10, 22, 23, 26*). It may also maintain epithelial structure in other tissues including stomach (*23, 27*). The present Northern blotting analysis indicated that the placenta and the lung are major organs producing laminin-5.

We previously showed that ladsin/laminin-5 was secreted by various human gastric and squamous carcinoma cell lines, and that this new laminin had potent cell-adhesion

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and cell-migration activities compared with laminin-1, fibronectin, and vitronectin (*11, 12*). This unique feature of laminin-5 suggests some special roles in expression of malignant properties of cancer cells. In this study, we found that the three laminin-5 genes were up-regulated by TPA and EGF. This finding seems important for understanding the physiological and pathological roles of laminin-5. It is clear that laminin-1 and laminin-5 have different regulatory mechanisms. The association of the relatively rapid induction of laminin-5 with cell growth is also supported by the following observations reported. First, expression of laminin-5 is restricted to basal cells in normal skin tissues. Differentiated keratinocytes do not express laminin $\alpha 3$ chain (*20*). Second, expression of laminin $\alpha 3$ chain is increased at wound healing sites (*20*). Induction of laminin-5 by growth factors seems favorable to wound repair, which requires vigorous cell proliferation. Retinoic acid, which induces laminin $\beta 1$ chain (*28, 29*), has been reported to increase a glycoprotein of M_r 600,000 (laminin-5) in keratinocytes (*15*), but the induction requires prolonged treatment of the cells. Retinoic acid or vitamin A prevents keratinization of epidermoid carcinoma cells (*30*) and normal keratinocytes (*31*) and supports their continuous growth. Increase of laminin-5 production by retinoic acid in keratinocytes seems to be related with the growth-promoting effect.

TPA induces or enhances various kinds of genes that are responsible for malignant progression of cancers through activating the PKC-mediated signal (*32-34*). It is well known that some proto-oncogene products like Jun and Fos (*35*) and matrix metalloproteinases (MMPs) are induced by TPA and EGF (*36-38*). At present, the precise mechanisms for the gene regulation of laminin-5 subunits and their high level of expression in gastric and squamous carcinoma cells remain unknown. It has recently been reported that invasive colon adenocarcinomas and some other types of tumor cells express laminin $\gamma 2$ chain (*16*). We have found that laminin-5 and some MMPs (collagenase, stromelysin, and gelatinase B) are induced by TPA and EGF in STKM-1 cells in a similar time course (data not shown). Therefore, it seems likely that the potent cell-motility factor laminin-5 and TPA-inducible MMPs cooperate in tumor invasion and metastasis. Frequent detection of high levels of EGF and its receptor in gastric (*39, 40*) and squamous cell carcinomas (*41, 42*) might support our speculation.

We found that some of the three laminin-5 mRNAs were expressed in many types of cancer cells besides gastric carcinoma and squamous cell carcinoma. The physiological meaning of the partial expression of the laminin-5 genes remains unknown. We detected laminin $\alpha 3_{EpB}$ chain in the medium of HLE cells, which expressed only the laminin $\alpha 3_{EpB}$ mRNA. In HLE cells, $\alpha 3_{EpB}$ chain might form a laminin complex with some laminin β and γ chains. On the contrary, KATO III cells, which expressed the three laminin-5 subunit mRNAs at high levels, did not secrete laminin-5. These results indicate that complex mechanisms exist for the production of laminin molecules.

In this study, we could not detect significant induction of laminin $\alpha 3_{EpB}$ chain mRNA in any cell lines by TPA and growth factors. This suggests that transcription of this mRNA is regulated by a mechanism distinct from that of laminin $\alpha 3_{EpA}$ chain. Different tissue distributions of laminin $\alpha 3_{EpA}$ and $\alpha 3_{EpB}$ mRNAs might be explained by differential inducibility by TPA and growth factors.

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