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Laminin-5 γ2 Chain and Matrix Metalloproteinase-2 Expression in the Neoplastic Changes of Uterine Cervical Squamous Epithelium

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Laminin-5 is an essential component of epithelial basal membranes. Matrix metalloproteinase-2 (MMP-2) changes laminin-5 from static to migratory substrate by cleaving the short arm of the γ2 subunit of the laminin-5. Recently, the expression of laminin-5 γ2 chain has been reported to be a marker of invasiveness of epithelial malignant tumors. We investigated the expression of laminin-5 γ2 chain and MMP-2 in the neoplastic changes of uterine cervical squamous epithelium. Tissue samples of normal uterine cervix, cervical intraepithelial neoplasia-1, -3 (CIN-1, -3) and squamous cell carcinoma keratinizing (SCC-K) and non-keratinizing (SCC-NK) were analyzed for expression of the laminin-5 γ2 chain and MMP-2 by immunohistochemistry. In normal uterine cervix and CIN-1, laminin-5 γ2 chain was immunolocalized only in the basal membrane. In CIN-3, expression of laminin-5 γ2 chain and MMP-2 became apparent at the epithelial-stromal interface of epithelial cells. In SCC-K and SCC-NK, laminin-5 γ2 chain and MMP-2 expression were abundant at the epithelial-stromal interface of tumor clusters. These results suggest that the expression of laminin-5 γ2 chain and MMP-2 may be involved in the process of neoplastic changes of uterine cervical squamous epithelium.

INTRODUCTION

The group of laminins is composed of extracellular matrix adhesion molecules localized to the basement membrane in most tissues (6,16). Laminin-5 consists of three subunits; one heavy α3 and two light, β3 and γ2 chains (5,27,36). The laminin-5 isoform, which is an essential component of epithelial basement membrane, is involved in cell anchorage, tissue remodeling, wound healing and cancer invasion (7,20,28,30,32). Like other laminins, laminin-5 interacts with cell surface receptors. Laminin-5 is a recognized ligand for integrins α6β4, α3β1 and α6β1 (5,21). Several studies have shown that laminin-5 is frequently expressed at the invasive margin (tumor budding) of colorectal (25,26,32), gastric (14), pancreatic (31), breast adenocarcinomas (25), uterine cervical (25,26,30) and oral (9,15,23) squamous cell carcinomas. Laminin-5 promotes the adhesion, migration, and scattering of various types of cultured cells more strongly than other known extracellular matrix proteins (10,11,19). These activities are mediated mainly by integrin α3β1 (5,11,38). However, the regulatory mechanism for the overexpression of laminin-5 in cancer is currently unclear.

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Matrix metalloproteinase-2 (MMP-2) which cleaves a number of substrates including native types I, IV, V, VII, and XI collagen, denatured collagens, elastin, proteoglycans, laminin and fibronectin, is a member of the MMP family of zinc-dependent endopeptidases (3,4,18,34,37). MMP-2 activation is associated with the expression of tissue-invasive phenotypes (1,4,22,35). MMP-2 changes laminin-5 from static to migratory substrate by cleaving the short arm of the γ2 subunit of the laminin (7). MMP-2 is thought to play an important role in the degradation of extracellular matrix, which is an essential step in the cascade of metastasis and angiogenesis.

In the present study, we immunohistochemically examined the expression of laminin-5 γ2 chain and MMP-2 in the neoplastic changes of uterine cervical squamous epithelium.

MATERIAL AND METHODS

Tissue samples

After informed consent was obtained from each patient, stored tissues from the Department of Pathology, Kobe University Hospital, Kobe, Japan were used in this study. The specimens of patients diagnosed between 1999 and 2002 were formaldehyde fixed, paraffin embedded and diagnosed on hematoxylin-eosin (H-E)-stained tissue sections. Tumors were classified according to the WHO classification.

The tissue samples used consisted of 5 normal uterine cervical tissues, 5 CIN-1, 5 CIN-3, 5 SCC-K and 5 SCC-NK. Five-micron-thick paraffin sections were used for immunohistochemical staining. Invasiveness was graded according to the International Federation of Gynecology and Obstetrics (FIGO) classification system as follows: carcinoma in situ (FIGO 0), microinvasive cancer (FIGO IA), and frankly invasive cancer (FIGO IB). The carcinoma samples included FIGO stages in a range IB1 to IIB SCC-K and age at diagnosis ranged from 27 to 68. The samples of SCC-NK included FIGO stages IA2 to IIB and age at diagnosis ranged from 45 to 75. All specimens were obtained before anti-tumor therapy was started.

Immunohistochemistry

Immunohistochemical staining was carried out using the avidin–biotin immunoperoxidase technique (24) with the polyvalent immunoperoxidase kit (Omnitag, Lipshaw, MI, USA with DAB Chromogen). In order to improve the immunostaining efficacy, the antigen retrieval method was used (24). The sections were digested with Trypsin Sigma 0.2% for 30 minutes at 37°C (for laminin-5). Before deparafinization, sections were heated for 1 hour at 37°C (for MMP-2). The sections were incubated with each antibody at 37°C for 90 minutes. Mouse anti-laminin 5 (laminin γ2 chain) monoclonal antibody (Chemicon Int.) at 5 μl/ml dilution and anti-hMMP-2 purified mouse IgG monoclonal antibody F-68 (Daiichi Fine Chemical Co. L.T.D. against human) at 4 μl/ml dilution were used as the primary antibody, respectively. The sections were counterstained with Mayer's Hematoxylin. The slides were dehydrated and mounted with a xylene-soluble mounting medium and examined microscopically. Control sections were subjected to the same technique except that the primary antibody was replaced by non-immune murine IgG at the same dilution as the specific antibody. No significant staining was observed in the negative controls.

Immunohistochemistry was analyzed as follows: negative (-) for no detectable immunostaining, (±) for weak but definitely detectable immunostaining, (+) for moderate immunostaining, (++) abundant immunostaining. The immunostaining intensity was evaluated by two independent observers (R.M., S.H.).

RESULTS

Immunohistochemical expression of laminin-5 γ2 chain in normal uterine cervical tissue and CIN-1 was noted only in the basement membrane. Basement membrane showed intense staining for the laminin-5 γ2 chain in a linear pattern. Peribasal and basal cells showed no positive immunostaining for laminin-5 γ2 chain (Fig.1-A, 1-B).

In CIN-3, weak to moderate immunostaining for laminin-5 γ2 chain was preferentially apparent in the cytoplasm of epithelial cells at the epithelial-stromal interface, whereas the basal membrane was negative for laminin-5 γ2 chain immunostaining (Fig.1-C).

SCC-K (Fig.1-D) showed a comparable expression for laminin-5 γ2 chain with SCC-NK (Fig.1-E). Immunostaining for laminin-5 γ2 chain was abundant in the cytoplasm of tumor cells localized at the cancer-stromal interface and in the tumor cells invading into the stroma, but negative in basal membrane in SCC-K and SCC-NK. The characteristics of neoplastic changes and results of the immunohistochemical analysis were summarized in Table 1**.**

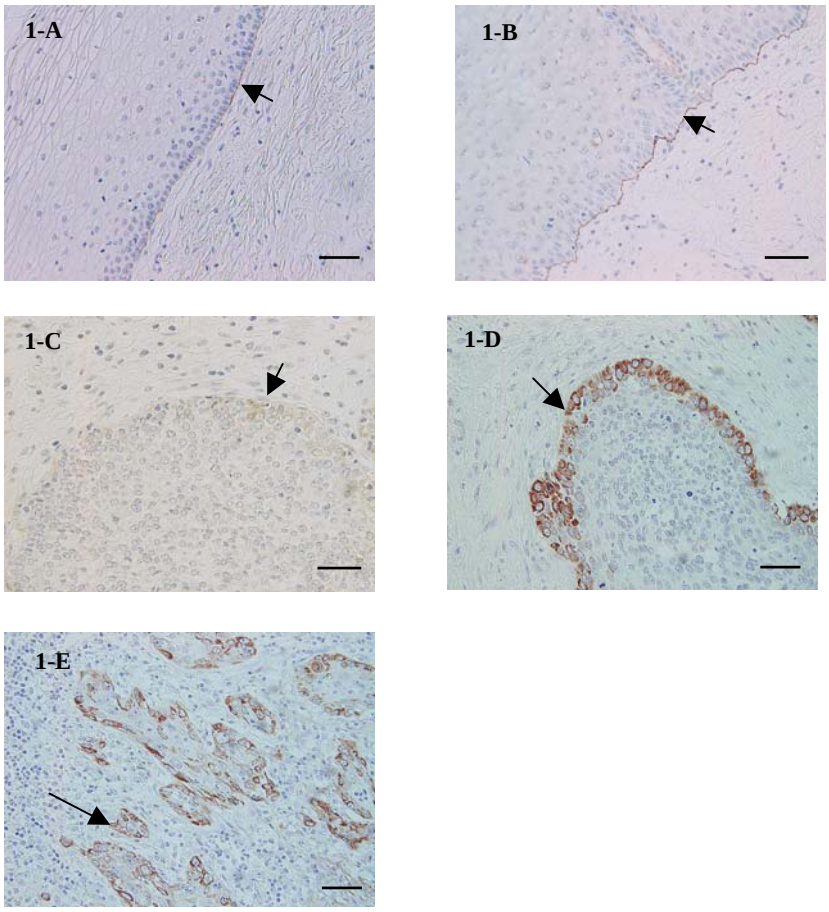

Figure 1. Immunostaining for laminin-5 γ2 subunit in the uterine cervix. The basal membrane was positive for laminin-5 γ2 immunostaining in normal uterine cervix (Fig.1-A) and CIN-1 (Fig.1-B). In CIN-3, immunostaining for laminin-5 γ2 chain was weak to moderate in the epithelial cells at the epithelial-stromal interface, but negative in the basal membrane (Fig.1-C). In SCC-K (Fig.1-D) and SCC-NK (Fig.1-E), abundant cytoplasmic staining for laminin-5 γ2 chain was apparent at the epithelial-stromal interface and in invading tumor cells (tumor budding cancer cells), whereas the basal membrane was negative for the immunostaining. Arrows indicate the areas of positive immunostaining. (Bars represent 100 μm. Original magnification, x200)

Table 1. Immunohistochemical staining for laminin-5 γ2 chain in the uterine cervix

	Basal Membrane	Epithelial-stromal Interface	Neoplastic Lesion
Normal	+	-	No neoplastic cells
CIN 1	+	-	Neoplastic cells involve less than 1/3 of the thickness of epithelium, koilocytotic changes
CIN 3	-	±	2/3 to full thickness epithelium replaced by neoplastic cells
SCC-K	-	++	Carcinoma with keratinization
SCC-NK	-	++	Carcinoma with no keratinization

Immunohistochemistry was analysed as follows: negative(-); weak(±); moderate(+); strong(++). Neoplastic cells show increased nuclear-cytoplasmic ratio, irregularity of nuclear size and nucl

The same specimens were subjected to immunohistochemical analysis with anti-MMP-2 monoclonal antibody. In normal cervical tissue (Fig. 2-A) and CIN-1 (Fig.2-B) immunostaining for MMP-2 was negative at the basal membrane and basal cells, but positive in the suprabasal cells of the squamous epithelium. In CIN-3, moderate immunostaining for MMP-2 became apparent in the neoplastic cells (Fig. 2-C).

In invasive carcinomas, each subgroup of SCC-K and SCC-NK showed a distinct expression for MMP-2. SCC-K (Fig.2-D) showed diffuse staining pattern of MMP-2 in cytoplasm of cancer cells. In SCC-NK (Fig.2-E), the immunostaining for MMP-2 was abundant in the invasive front at the epithelial-stromal interface. The characteristics of neoplastic changes and results of the immunohistochemical analysis were summarized in Table 2.

Table 2. Immunohistochemical staining for MMP-2 in the uterine cervix

	Basal Membrane	Epithelial-stromal Interface	Neoplastic Lesion
Normal	-	-	No neoplastic cells
CIN 1	-	-	Neoplastic cells involve less than 1/3 of the thickness of epithelium, koilocytotic changes
CIN 3	-	+	2/3 to full thickness epithelium replaced by neoplastic cells
SCC-K	-	+	Carcinoma with keratinization
SCC-NK	-	++	Carcinoma with no keratinization

Immunohistochemistry was analysed as follows: negative(-); weak(±); moderate(+); strong(++). Neoplastic cells show increased nuclear-cytoplasmic ratio, irregularity of nuclear size and nuclear density.

DISCUSSION

In the present study, immunohistochemical expression of laminin-5 γ2 chain and MMP-2 was found to increase according to the degree of neoplastic changes of uterine cervical epithelium. In normal uterine cervical tissues and CIN-1, the laminin-5 γ2 chain was

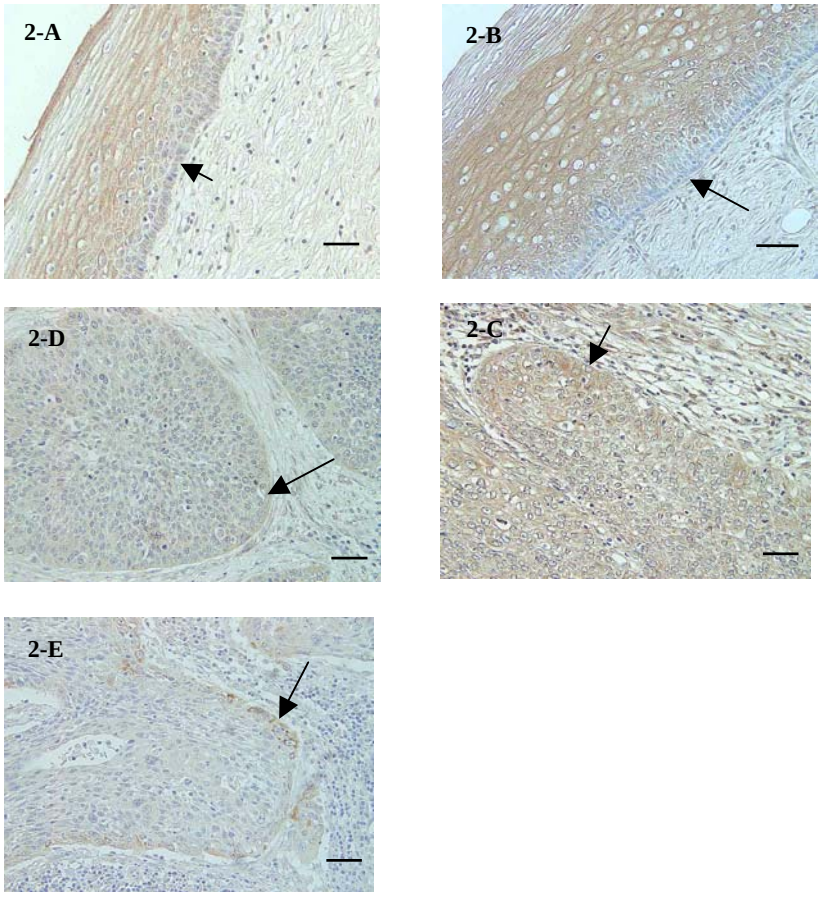

Figure 2. Immunostaining for MMP-2 in the uterine cervix. The basal membrane in normal uterine cervix (Fig.2-A) and CIN-1 (Fig.2-B) was negative for immunostaining for MMP-2, whereas the suprabasal cells were positive for immunostaining for MMP-2. In CIN-3 (Fig.2-C), moderate immunostaining for MMP-2 was apparent in the neoplastic cells at the epithelial-stromal interface, but the basal membrane was negative for signal for MMP-2. In SCC-K (Fig.2-D), diffuse immunostaining for MMP-2 was noted in the tumor cells. In SCC-NK (Fig.2-E), cytoplasmic staining for MMP-2 was positive in the tumor cells at the epithelial-stromal interface and invading tumor cells (tumor budding cancer cells), whereas the basal membrane was negative for the immunostaining. Arrows indicate areas of positive immunostaining. (Bars represent 100 μm. Original magnification, x200)

expressed along the basal membranes in a linear pattern. By contrast, the cytoplasmic immunoreactivity for laminin-5 γ2 chain was observed to be related with the degree of cervical intraepithelial neoplasia. These findings were partially different from previous study by Kohlbreger et al. (12) who showed no basal membrane staining in normal uterine cervix. A possible explanation could be the use of different immunohistochemical procedure with different sensitivity to laminin-5 γ2 chain. Skyldberg et al. (30) demonstrated no significant difference among expression of laminin-5 γ2 chain in CIN-1 and CIN-2 and consistent positive cytoplasmic expression of laminin-5 γ2 chain in invasive carcinomas. MMP-2 expression in normal uterine cervix and CIN-1 was positive in

suprabasal cells. This pattern of expression was similar to that in dysplastic epithelium of esophagus (29). Several studies reported that MMP-2 and laminin-5 γ2 chain were involved in adhesion, migration and scattering of tumor cells and were associated with tumor budding (17). In invading human cancer cells, cytoplasmic staining for laminin-5 was apparent. The prognostic significance of this phenomenon was reported in colon adenocarcinoma. Hase et al. (8) found a positive correlation between the degree of tumor budding and recurrence of tumors following curative surgery. We have shown that laminin-5 γ2 chain and MMP-2 were expressed at the cancer-stromal interface and at the invasive front of uterine cervical tumors.

The mechanism of the preferential expression of laminin-5 γ2 chains at the invasive margin in cancer is not known yet. Koshikawa et al. found that γ2 chain of laminin 5 without a signal for α3 and β3 was strongly expressed at the invasive margin of gastric carcinoma cells (14). These results suggest that laminin-5 γ2 chain monomer may have a function different from the laminin-5 trimmer. On the other hand, Sordat et al. reported co-expression of the laminin β3 chain and γ2 chain in colorectal cancers (33). Predominant expression of the laminin γ2 chain at the invasive front has been reported in carcinomas of the colon, pancreas and stomach. Laminin-5 may act as a ligand for invading carcinoma cells. Gianneli et al. (7) have shown that MMP-2 changes laminin-5 from static to migratory substrate by cleaving the short arm of the γ2 subunit of the laminin-5. In the present study, increased expression of laminin-5 γ2 chain and MMP-2 were noted at the cancer- stromal interface in SCC-NK, whereas in SCC-K, diffuse expression of MMP-2 was noted in the tumor cells.

The membrane-type 1 matrix metalloproteinase also plays an important role in cell migration on laminin-5 and cleaves laminin-5 γ2 chain more efficiently than MMP-2 (13). It may be a reason why we found a different pattern of expression of MMP-2 between SCC-K and SCC-NK. Previous studies showed that MMP-2 was concentrated along the basement membrane at the sites of tissue remodeling (2) and at the leading edge of invading tumors (7). Masaki et al. (17) showed that although tumor cells might produce MMP-2, tumor cell budding was not associated with MMP-2 expression in the tumor cells, but it was associated with MMP-2 expression in stromal cells in colorectal carcinoma. Interactions of carcinoma cells with stromal cells or with the extracellular matrix surrounding cells at the invasive front may result in an accumulation of laminin-5 γ2 chains and MMP-2 at the invasive front, where they may play a direct role in tumor invasion processes. Taking these findings into account, it is likely that laminin-5 and MMP-2 may be involved in the initial stage of tumorigenesis of uterine cervical squamous epithelium and thus the detection of laminin-5 and MMP-2 expression may be useful as a marker of invasiveness in uterine cervical lesions.

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