Increased Expression and Activation of Gelatinolytic Matrix Metalloproteinases Is Associated with the Progression and Recurrence of Human Cervical Cancer

Bor-Ching Sheu, Huang-Chun Lien, Hong-Nereng Ho, Ho-Hsiung Lin, Song-Nan Chow, Su-Cheng Huang, and Su-Ming Hsu

 abstract

Cancer-derived matrix metalloproteinases (MMPs) are proposed to be essential for tumor stromal invasion and subsequent metastasis. To explore the role of MMPs in cancer progression, we examined the expression of various MMPs and tissue inhibitors of MMPs in precancerous and cancerous lesions of the uterine cervix. Immunohistochemical studies demonstrated that MMP-2 and MMP-9 were expressed in >90% of squamous cell carcinomas (SCC) and 83–100% of high-grade squamous intraepithelial lesions (HSIL), but were less frequently expressed in low-grade squamous intraepithelial lesions and normal squamous epithelium (13%). MMP-1, MMP-14, and MMP-15 were detected in 55–81% of SCC cases, and MMP-1 was detected in 39% of HSIL. The tissue inhibitors of MMPs were weakly expressed in SCC (10–61%). By direct analysis of enzyme activities in microdissected specimens, we found that the gelatinolytic activity of MMP-9 was significantly higher in HSIL and SCC than in normal cervix (P < 0.01). The levels of active-form MMP-2 increased progressively from HSIL to SCC of stage I and more advanced stages (P < 0.01). The gelatinolytic activity of MMP-9 and active-form MMP-2 in SCC were strongly correlated with lymphovascular permeation and subsequent lymph node metastasis (P < 0.02). Moreover, the gelatinolytic activity and immunoreactive percentage of both MMP-2 and MMP-9 were significantly higher in SCC cases who had a recurrence than in those who remained free of disease (P < 0.001). Thus, our data demonstrate progressively up-regulated expression of MMP-2 and MMP-9 with SCC progression, and significant associations among their gelatinolytic activity and stage, nodal metastasis, and recurrence.

INTRODUCTION

Stromal invasion and subsequent metastasis are key steps in the progression of malignant tumors, involving the degradation, remodeling, and release of growth factors from the ECM (1). This is the result of complex, multifactorial processes that include the transcriptional control and activation of proenzymes, as well as production of their natural inhibitors (1, 2). Although several classes of proteolytic enzymes are involved in ECM degradation, MMPs (3) are thought to play a central role in these processes, in view of their ability to degrade many ECM components and other substrates (2). At least 20 different MMP family members have been identified, including collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3 and -10), and MT-MMPs (i.e., MMP-14, -15, -16, and -17; Ref. 2).

Cervical cancer is one of the most common cancers among women worldwide. Cervical cancer progression is currently thought to represent a continuum of neoplastic transitions from LSIL (CIN1) to HSIL (including CIN2, CIN3, and carcinoma-in situ) and then invasive SCC. Several investigators have proposed the possible association among selected MMPs (i.e., MMP-2, MMP-9, and MMP-14) and the malignant potential of human cervical neoplasia (3–5). The major drawbacks in these studies were that, whereas the expression of an enzyme is regulated at multiple steps, including transcription, translation, and post-translational modification, the levels of mRNA by in situ hybridization and the staining intensity by immunohistochemistry do not necessarily reflect the activity of the expressed protein. Moreover, no antibody is available currently for distinguishing between the latent (pro) form and the functional activated form of MMPs by immunohistochemistry.

In the present study, we investigated the spectrum of MMP and TIMP expression in human cervical neoplastic tissues, including LSIL, HSIL, and SCC. To address the possible functional role of MMPs in cervical cancer progression, we additionally used microdissection and zymography to analyze the expression and activity of MMPs that are thought to be involved in the pathogenesis of cervical cancer.

MATERIALS AND METHODS

Case Recruitment. Sixty-two cases of primary cervical neoplasia were enrolled in this study. These included 8 cases of LSIL, 23 cases of HSIL, and 31 cases of SCC. Tissue specimens were obtained via biopsy, conization, and simple or radical hysterectomy. Cervical tissues from 5 cases of uterine fibroids were included as normal controls. Each case of SCC was evaluated for clinical parameters including grade, lymphatic or vascular permeation, lymph node metastatic status, and clinical stage. Histological grades of SCC included well, moderately, and poorly differentiated, based on the degree of differentiation toward keratinization with the formation of squamous pearls. All of the patients had been followed regularly at our clinics for at least 2 years. Clinical staging of each patient was defined according to the 1995 modification of the International Federation of Gynecologists and Obstetricians staging of carcinoma of the cervix uteri (6).

Immunohistochemistry. Five-μm sections of paraffin-embedded tissue were dewaxed and rehydrated before being transferred to 0.5% H2O2 for blocking of endogenous peroxidase before washing with PBS buffer. An avidin-biotin-peroxidase complex method (Vector Laboratory, Burlingame, CA) was used for immunostaining. Monoclonal antibodies against MMP-1, -2, -3, -7, -8, -9, -13, -14, and -15, as well as TIMP-1, -2, and -3, purchased from Chemicon Inc. (Temecula, CA), were used. Titrated concentrations of individual antibodies were prepared, and controls were performed as recommended by the reagent supplier. Quantitative evaluation was performed by counting the percentage of positively stained cells by use of a 10 × 10 square grid placed over the eyepiece of the microscope. The staining intensity was scored as negative (−), faint (+), moderate (2+), or intense (3+) as compared with the background staining intensity. Immunostaining results for MMPs/TIMPs were scored by two independent observers.

Microdissection. Fresh tissue fragments were obtained from specimens after conization, simple or radical hysterectomy. Fresh uterine cervical tissues obtained from patients who underwent hysterectomy for myoma were included as controls. The samples were embedded without fixation in Tissue-Tek OCT compound (Miles Scientific, Naperville, IL) and snap-frozen. The remaining tissues were formalin-fixed and evaluated immunohistochemically for MMPs, and the findings were correlated with the results of zymography. Tumor cell nests were carefully separated and scraped from underlying stroma along contiguous margins with a fine needle (30G15G) from frozen sections. The removed tissues were homogenized

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2 The abbreviations used are: ECM, extracellular matrix; MMP, matrix metalloproteinase; MT, membrane-type; HPV, human papilloma virus.

3 The abbreviations used are: ECM, extracellular matrix; MMP, matrix metalloproteinase; MT-MMPs (-13), gelatinases (MMP-2 and -9), stromelysins (MMP-3 and -10), and other MMPs.

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5 Cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; TIMP, tissue inhibitor of metalloproteinase; MT, membrane-type; HPV, human papilloma virus.
with sample lysis buffer containing glycerol (10%), Triton X-100 (1%), sodium PP, (1 mm), NaCl (137 mm), EDTA (5 mm), sodium orthovanadate (1 mm), NaF (10 mm), and Tris (pH 7.9; 20 mm). The cell lysates were centrifuged, and protein concentrations were measured by a dye-binding method according to the manufacturer's instructions (Bio-Rad Inc., Hercules, CA).

Gelatin Zymography. The gelatinolytic activity was determined by gelatin-substrate gel electrophoresis. With this method, we also detected the inactive proforms of gelatinases, because SDS causes activation of the enzymes without proteolytic cleavage of the NH2-terminal propeptide sequence (7). Aliquots of tissue lysates (15 μg protein/lane) were applied without heating or reduction to a 10% SDS-polyacrylamide gel containing 1 mg/ml of gelatin. After electrophoresis, gels were washed for 1 h at room temperature in a 2% Triton X-100 solution before being transferred to a reaction buffer containing 50 mM Tris-HCl (pH 7.4), 0.15 M NaCl, and 10 mM CaCl2, and incubated at 37°C for overnight. The gel was stained with 0.1% Coomassie blue in 50% methanol/10% acetic acid and destained in 20% methanol/10% acetic acid. Clear zones of gelatin lysis against a blue background stain blue in 50% methanol/10% acetic acid. Quantitative analysis of gelatinolytic activity was achieved by scanning densitometry of the zymograms (IS-1000 Digital Imaging System; α Innotech Corp., San Leandro, CA) as described previously (8). Values were expressed as lysis per μg protein. Each sample was processed in triplicate, and the mean levels were recorded.

Statistical Analysis. Data were expressed as mean ± SD unless stated otherwise. Data analysis was performed with SPSS software (R.9.0.1; SPSS Inc., Chicago, IL). One-way ANOVA with a multiple comparison test (Bonferroni t test) was used for data analysis. Correlation and linear regression analyses were used for identifying potentially causal associations between variables. Statistical significance was defined by a P < 0.05.

RESULTS

The clinical stages of the SCC group included 5 stage Ia, 13 stage Ib, 7 stage Ia, 2 stage Ia, and 4 stage III cancers. The mean age of the patients diagnosed with SCC was 50.8 ± 13.5 years, compared with 41.7 ± 12.8 and 38.2 ± 10.8 years in the HSIL and LSIL patient groups, respectively. All of the patients treated for HSIL and LSIL are currently free of disease for at least 2 years of follow-up. In the SCC group, 21 cases (stage Ia to IIa) underwent radical hysterectomy and pelvic lymphadenectomy. The remaining 10 cases (stage IIa to III) underwent radiotherapy. Seven patients had recurrent/persistent disease after treatment during 2-year follow-up. Histological grading in the SCC group showed well, moderately, and poorly differentiated grades in 11 (35.5%), 14 (45.1%), and 6 (19.4%) cases, respectively.

Immunohistochemical Staining in Tumor Cells. As summarized in Table 1, 62 cervical tissues from patients with primary uterine cervical lesions (31 SCC, 23 HSIL, and 8 LSIL) were evaluated for the expression and tissue localization of MMPs and TIMPs.

Both MMP-9 and MMP-2 were found in almost all of the SCC and HSIL cases. MMP-9 was immunoreactive within tumor cells in 30 of 31 cases (97%) of SCC and in all of the cases of HSIL. The percentage of tumor cells that were positive for MMP-9 varied from 50% to 95% (average, 71% and 68% in SCC and HSIL, respectively). MMP-2 was detected in 28 of 31 (90%) of SCC cases, and weak to moderate MMP-2 staining was noted in 83% of HSIL cases. The percentage of MMP-2+ tumor cells in SCC (average, 40%) was significantly greater than in HSIL (average, 20%, P = 0.006). The staining intensity of the cells was similar in most cases, but appeared to be enhanced at the invasive edges of SCC or the basal layers of the tumor in some cases of HSIL. Both MMP-2 and MMP-9 were absent or minimally expressed in LSIL and normal cervical epithelium.

MMP-14, MMP-15, and MMP-1 were detected in 81% (25 of 31), 65% (20 of 31), and 55% (17 of 31) of SCC cases, respectively, with the percentage of immunoreactive tumor cells ranging from 5% to 70%. The staining also had a tendency to be stronger at the tumor periphery than in the center of the tumor. Cases with HSIL were occasionally positive for MMP-1 (39%), but were rarely positive for MMP-14 (9%) or MMP-15 (9%). Cells in LSIL and normal cervical epithelium were generally negative or faintly stained for MMP-1, -14, and -15. Carcinoma cells that stained for MMP-3, -7, -8, and -13 were found in various regions of 10–35% of SCC cases, 0–9% of HSIL, and 0–13% of LSIL. The staining intensity for these MMPs was weaker than for MMP-2 and MMP-9. TIMP-1 and TIMP-2 were expressed in 32% and 61% of SCC cases, respectively, but were rarely seen in HSIL. Only 3 (10%) of the 31 cases of SCC showed weak staining for TIMP-3. Representative photomicrographs of histological features are shown in Fig. 1.

To elucidate potential interactions between the various MMPs and TIMPs, we also analyzed their relative levels of correlated expression. In this regard, MMP-2 tended to be expressed with MMP-14 (MT1-MMP), and MMP-3 was usually expressed with MMP-15 (MT2-MMP). The remaining MMPs and TIMPs were not significantly correlated with one another in terms of their expression.

Expression of MMPs and TIMPs in Stromal Cells and Inflammatory Cells. MMP-1 and MMP-2 were universally expressed in stromal cells close to SCC cell nests, but were almost completely absent in stromal cells that were remote from the carcinoma. Faint MMP-2 immunostaining was also occasionally observed in stromal cells of HSIL, but not in LSIL or normal cervical epithelium. The remaining MMPs and TIMPs (MMP-3, -9, -14, and -15, and TIMP-1 and -2) were less frequently immunoreactive in stromal cells surrounding SCC, but were entirely absent in those surrounding HSIL, LSIL, and control cervix.

The immune cells within the cancer milieu expressed a variety of MMPs. Stromal macrophages were frequently immunoreactive for MMP-1 and less commonly immunoreactive for MMP-2, -3, and -9. In contrast, polymorphonuclear inflammatory cells were predominantly immunoreactive for MMP-8. Plasma cells were generally negative for MMPs or TIMPs.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Staining intensity</th>
<th>% of cases expressing MMPs and TIMPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC (n = 31)</td>
<td>+++</td>
<td>MMP-1 10%</td>
</tr>
<tr>
<td>HSIL (n = 23)</td>
<td>++</td>
<td>MMP-1 26%</td>
</tr>
<tr>
<td>LSIL (n = 8)</td>
<td>+</td>
<td>MMP-1 19%</td>
</tr>
</tbody>
</table>

* ++++, strongly immunoreactive; ++, moderately immunoreactive; +, faintly immunoreactive; --, negatively immunoreactive.
Staining patterns vary from case to case. Note the increased gelatinase activity in CC. Staining in the peritumoral stromal cells is also discernible, as shown for MMP-13, MMP-14, and MMP-15. Staining in the peritumoral stromal cells is also discernible, as shown for MMP-13 (G), MMP-14 (H), and MMP-15 (I). Staining patterns vary from case to case.

Associations between Gelatinolytic MMPs and Cancer Progression. Gelatinolytic activity was assessed by zymography in 31 cases of SCC and 11 cases of HSIL. Five cases of normal cervical squamous epithelial tissues were also used as controls. The $M_r$ 92,000 latent form of MMP-9 appeared as a broad band in all cases of SCC (Fig. 2), whereas its $M_r$ 84,000 activated form could not be clearly distinguished in the gels, as has been reported previously (9–11). In contrast to MMP-9, the gelatinolytic bands of latent proform MMP-2 ($M_r$ 72,000) and active-form MMP-2 ($M_r$ 66,000) were clearly separated in the gels. Gelatinolytic bands at $M_r$ 200,000 and $M_r$ 140,000 were also observed and may represent dimer forms of MMP-9 and MMP-2, respectively (Ref. 11; Fig. 2). Table 2 and Fig. 3 show the normalized densitometric levels of the different gelatinolytic species in normal, HSIL, and staged SCC tissue specimens.

Mean MMP-9 levels were relatively low in normal squamous epithelium, ∼4-fold higher in HSIL and stage Ia SCC ($P < 0.01$), and 27–37-fold higher in more advanced stages of SCC ($P < 0.001$; Fig. 3C). The levels of total (pro- + active-form) MMP-2 were approximately 4–6-fold higher in HSIL and SCC than in normal control tissues ($P < 0.01$; Fig. 3B). In addition, the levels of active-form MMP-2 showed a progressive stage-dependent increase, with each stage having an increased average level of activated MMP-2 and advanced stage III cancers having ∼19 times more active enzyme than normal cervix ($P < 0.01$; Fig. 3A). Thus, not only was the level of MMP-2 protein increased, but so was its level of activation.

Consistent with a stage-dependent increase in gelatinolytic MMP

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**Table 2 Levels of gelatinolytic activities (lumi/μg protein)**

<table>
<thead>
<tr>
<th>Diagnosis/ Stages</th>
<th>Sample number</th>
<th>Total MMP-9 ($M_r$ 92,000 + 84,000)</th>
<th>Total MMP-2 ($M_r$ 72,000 + 66,000)</th>
<th>Proform MMP-2 ($M_r$ 72,000)</th>
<th>Active-form MMP-2 ($M_r$ 66,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>$4.1 \pm 1.8^b$</td>
<td>$6.5 \pm 4.7^b$</td>
<td>$4.9 \pm 4.4^b$</td>
<td>$1.6 \pm 0.4^b$</td>
</tr>
<tr>
<td>HSIL</td>
<td>11</td>
<td>$14.7 \pm 5.6^b,c$</td>
<td>$7.5 \pm 2.7^b$</td>
<td>$4.3 \pm 2.8^c$</td>
<td>$3.2 \pm 0.9^b,c$</td>
</tr>
<tr>
<td>Ia</td>
<td>5</td>
<td>$17.8 \pm 6.4^b$</td>
<td>$13.5 \pm 2.9^b$</td>
<td>$9.2 \pm 3.2^b$</td>
<td>$4.3 \pm 1.4^b$</td>
</tr>
<tr>
<td>Ib</td>
<td>13</td>
<td>$113.2 \pm 74.5^b,c$</td>
<td>$27.0 \pm 14.8^b,c$</td>
<td>$18.0 \pm 6.9^b,c$</td>
<td>$10.1 \pm 12.5^b$</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>$148.1 \pm 66.2^b,c$</td>
<td>$44.1 \pm 11.8^b,c$</td>
<td>$29.5 \pm 5.9^b,c$</td>
<td>$20.1 \pm 17.8^b,c$</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>$1541.1 \pm 61.0^b,c$</td>
<td>$45.7 \pm 13.2^b,c$</td>
<td>$29.2 \pm 3.3^b,c$</td>
<td>$31.0 \pm 4.9^b,c$</td>
</tr>
<tr>
<td>All stages</td>
<td>31</td>
<td>$113.3 \pm 76.6^b,c$</td>
<td>$33.7 \pm 17.9^b,c$</td>
<td>$21.4 \pm 9.4^b,c$</td>
<td>$14.8 \pm 13.4^b,c$</td>
</tr>
</tbody>
</table>

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All data are expressed as mean ± SD.

$^b$ $P < 0.01$ by ANOVA and multiple comparison tests when the levels of gelatinolytic activities were compared to those in control cervix.

$^c$ $P < 0.01$ by ANOVA and multiple comparison tests when the levels of gelatinolytic activities were compared to those in HSIL.

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Fig. 1. Representative immunostaining results of MMPs and TIMPs in uterine SCC and HSIL. A, MMP-1; B, MMP-2; C, MMP-3; D, MMP-7; E, MMP-8; F, MMP-9; G, MMP-13; H, MMP-14; I, MMP-15; J, TIMP-1; K, TIMP-2; and L, TIMP-3. Arrows indicate nests of cancer cells. Diffuse intense immunoreactivity is noted in carcinoma and/or precancerous cells for MMP-1 (A), MMP-2 (B), and MMP-9 (F). Focal and weak reactivity is seen with MMP-13 (G), MMP-14 (H), and TIMP-3 (L). Staining in the peritumoral stromal cells is also discernible, as shown for MMP-13 (G), MMP-14 (H), and MMP-15 (I). Staining patterns vary from case to case.

Fig. 2. Representative zymographic profiles of gelatinase activities in tissue homogenates of control cervix (Lane N) and three cases with in situ SCC and invasive SCC. Invasive carcinoma cells (CC) and in situ cancer cells (CIS) are dissected separately from tissue sections, as described in “Materials and Methods.” Equal amounts of proteins are loaded. Note the increased gelatinase activity in CC.
expression and activation in SCC, we also noted an association between gelatinolytic activity and lymphatic spread. Microscopic lymphovascular invasion was detected in 11 (stage Ib: 3 cases; stage IIa: 4 cases; stage IIb: 2 cases; and stage III: 2 cases) of 31 SCC cases, and of the 21 stage Ia-IIa SCC cases who underwent surgical treatment, 6 (stage Ib: 3 cases and stage IIa: 3 cases; 28.6%) had lymph node metastases. Significantly higher levels of total MMP-2, active-form MMP-2, and total MMP-9 were observed in the 11 SCC cases with lymphovascular invasion than in the remaining cases without lymphovascular permeation (50.6 ± 13.4 versus 23.4 ± 11.0, 27.3 ± 10.9 versus 4.9 ± 1.9, and 186.9 ± 56.6 versus 66.2 ± 50.9, respectively; P < 0.001; Fig. 4A). Moreover, as compared with the metastasis-free stage Ia-IIa cases who had undergone surgery, the cases with positive lymph node metastases had significantly higher levels of total MMP-2 (47.4 ± 16.7 versus 29.6 ± 16.3; P = 0.013), active-form MMP-2 (26.4 ± 14.9 versus 9.6 ± 7.2; P < 0.01), and total MMP-9 (215.3 ± 40.0 versus 83.5 ± 62.1; P < 0.001; Fig. 4B).

As additional evidence of their potential prognostic utility, we noted a significant association between gelatinolytic MMP levels and SCC recurrence. Over 2 years of regular follow-up, 7 (stage Ib: 2 cases; stage IIa: 2 cases; stage IIb: 1 case; and stage III: 2 cases) of 31 SCC cases in our series developed recurrent/persistent disease, and the levels of MMP-2 and MMP-9 that were detected by zymography were significantly higher in the recurrent cases than in those who remained free of disease. As compared with the recurrence-free cases, those with recurrent SCC had significantly elevated levels of total MMP-2 (55.3 ± 4.9 versus 26.5 ± 14.3; P < 0.001), active-form MMP-2 (33.5 ± 5.1 versus 6.8 ± 5.9; P < 0.001), and total MMP-9 (207.0 ± 30.3 versus 80.5 ± 63.4; P < 0.001). Thus, gelatinolytic levels in SCC show significant associations with stage, nodal metastasis, and, perhaps most importantly, disease recurrence.

DISCUSSION

The MMPs, a family of zinc-dependent endopeptidases, are essential for embryonic development, morphogenesis, reproduction, tissue remodeling, angiogenesis, and perhaps for cancer cell invasion and metastasis (1–3). It has been proposed that cancer cells initiate disseminating environments by eliciting the expression of MMPs to break down the surrounding ECM (1, 12). The up-regulation of certain MMPs seems to be specific to tumors of different tissue origin; for example, MMP-13 in vulvar SCC (13), MMP-2 in thyroid papillary carcinoma (14), and MMP-7 in endometrial (15) and gastric adenocarcinoma (16). In addition, recent studies have shown that MMP-2 and/or MMP-9 are up-regulated in cervical SCC (3, 4, 17, 18). Although many of these studies focused on a few selected MMPs, the results seem to indicate a restrictive expression pattern of MMPs in tumors. It should also be noted that previous reports applying substrate zymography in the evaluation of MMP-2 and MMP-9 were largely based on homogenized whole-tissue lysates or cancer cell-condition culture medium (8, 9, 11, 19). However, as we observed here, MMPs, and in particular MMP-2 and MMP-9, are expressed frequently in stromal cells and inflammatory cells residing in or around tumors, which may interfere with precise measurements of these MMPs. Thus, we used microdissection to separate cancer cell nests from underlying stroma in hematoxylin-counterstained sections. By this technique, we could accurately evaluate the enzyme activity of MMPs produced by the tumor cells themselves in various stages of cancer progression.

Our results suggest that MMP-2 and MMP-9 may have prognostic value in SCC. The data clearly indicate a significant increase in MMP expression and activity from normal cervix to HSIL and SCC for MMP-2 and MMP-9. Significant positive correlations were also observed between gelatinolytic MMPs and the presence of lymphovascular invasion and nodal metastasis. Moreover, we demonstrated a strong, previously unreported, positive correlation between gelatinolytic MMP activity and cancer recurrence in SCC. Although the latter association was observed in unstratified SCC cases of all of the stages and may, thus, reflect the stage-dependent increase that we saw in MMP expression, it remains consistent with the hypothesis that MMPs promote cancer progression and recurrence and may, therefore, have prognostic value.

Related research has indicated an important role for active MMP-2 in...
tumor invasiveness and metastasis in oral SCC, and adenocarcinomas of the colon and stomach (19–21). In addition to matrix degradation, MMPs may have other currently unidentified functions that are important in the pathogenesis of cancer. We demonstrated recently that MMP-9 plays a central role in the cleavage of certain cytokine receptors (i.e., interleukin 2Rα) on tumor-infiltrating lymphocytes derived from human cervical cancer (22, 23). Several other reports have also shown the ability of MMPs to activate tumor necrosis factor α or inactivate interleukin 1β, which may potentiate tumor progression by regulating the activity of these immunoregulatory cytokines at the site of tumor invasion (24, 25). The proteolytic effects on certain cytokines and/or their receptors by MMP-9 could also explain the association of MMP-9 expression with lymph node metastasis and perhaps the compromised activity of immunocytes in patients with cancer. In addition, up-regulated MMP-9 can also activate downstream MMPs, thereby additionally enhancing the degradation of ECM by cancer cells.

Unlike MMP-2 and MMP-9, other MMPs sporadically appear in cases of SCC. Among these, MMP-14 and MMP-15 serve as cell membrane receptors for the complex of pro-MMP-2 and TIMP-2, and play a critical role in the activation of pro-MMP-2 at the cell surface (2, 26). Gilles et al. (5) reported that overexpression of MT1-MMP correlated with the highly invasive behavior of cervical cancer cells via activation of MMP-2. In this study, expression of MMP-14 was noted both in stromal fibroblasts adjacent to tumor cells and in cancer cells in cases of SCC. Notably, we observed the correlated expression of MMP-14 with MMP-2 in our cases, as well as the correlated expression of MMP-15 with MMP-3. Collagenolytic MMPs (MMP-1 and MMP-13) were detected more often in our series of cases than in normal control tissues, but less often than the gelatinolytic MMPs. Expression of MMP-1 and/or MMP-13 has also been reported in a variety of human epithelial cancers, including lung carcinomas (27), colorectal adenocarcinomas (28), SCCs of head and neck (29), and vulvar SCCs (13); however, their significance remains to be determined.

Earlier studies have also demonstrated that decreased expression of TIMPs at sites of invasion and an increased MMP:TIMP ratio correlate with a poor prognosis (4). Increased expression of MMP-2,
MMP-9, and/or TIMPs also appears to correlate with a poor prognosis in a variety of human malignancies, including hepatocellular, pancreatic, renal cell, and ovarian cancers (30–32). In this study, TIMP-1 and TIMP-2 were weakly expressed in SCC, and were undetectable in precancerous lesions. TIMP-3 was rarely present in SCC and HSIL. Thus, the immunoreactive percentage of TIMP-1 and -2 was not significantly correlated with any of the clinical parameters. These contradictory results for the TIMPs may reflect their complex roles as inhibitors of MMP activity, key participants in the cell surface activation of MMP-2, and MMP-independent growth regulators (2).

The role of HPV as a possible regulator of MMPs and TIMPs also remains to be determined. Specific HPV subtypes, including HPV 16, 18, 31, and 33, are frequently associated with various grades of squamous intraepithelial neoplasia and SCC. Nuovo (33) demonstrated no significant change in the percentage of cells expressing either TIMP or HPV E6/E7 RNA in correlated cancer invasion. On the other hand, Johansson et al. (13) found that the expression of MMP-13 in cervical SCC cell lines appeared to correlate with the presence of oncogetic HPV, suggesting that HPV may play a role in the induction of MMP-13 expression. However, our study showed infrequent expression of MMP-13 by SCC and HSIL. Nevertheless, recent research has shown that EBV proteins may up-regulate MMP-1 expression in nasopharyngeal carcinoma, suggesting that other viral proteins may also regulate MMP expression (34).

Stromal remodelling, characterized by loosening and edematous changes in connective tissues adjacent to lesions of in situ and invasive carcinoma, may result from the matrix degrading effects of MMP-2, MMP-9, and other MMPs, and could pave the way for tumor cell invasion. Notably, MMP inhibitors have demonstrated antitumor activity in several clinically relevant animal models, although clinical successes have not yet followed (35). In the present study, we demonstrated the potential significance of MMP-2 and MMP-9 in the progression of cervical neoplasms. Thus, our findings suggest that these MMPs may have prognostic value in cervical SCC, and if these correlations reflect a causal role for MMP-2 or MMP-9, then their inhibition may provide therapeutic value as well.

REFERENCES


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