

Expression of matrix metalloproteinase-2 in preinvasive and invasive carcinoma of the uterine cervix

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Summary

Purpose: To study the immunohistochemical expression of matrix metalloproteinase-2 (MMP-2) in preinvasive and invasive carcinoma of the uterine cervix so as to demonstrate whether the expression of MMP-2 is an early or late event in the process of dedifferentiation and cancer progression.

Methods: A total number of 50 samples of cervical tissue were studied for MMP-2 immunoreactivity. The cases were selected to include ten normal cases used as a control group, 20 CIN cases and 20 cervical carcinoma cases. The CIN group was subdivided into CIN1 (n = 7), CIN2 (n = 6) and CIN3 (n = 7), while the carcinoma group was represented by squamous cell carcinoma (n = 16) and adenocarcinoma (n = 4).

Results: MMP-2 expression was totally absent in control cervixes and low-grade squamous intraepithelial lesions, while high-grade squamous intraepithelial neoplasia and invasive carcinoma showed up-regulation of MMP-2 expression with no significant difference concerning the type of carcinoma. This overexpression of MMP-2 points to the possibility that it is an early marker of tumor progression in cervical carcinoma.

Conclusion: MMP-2 has a key role in extracellular matrix degradation and invasion in carcinoma of the uterine cervix. Its expression in high-grade squamous intraepithelial lesions may denote a potential risk for invasion and metastasis.

Key words: Metastasis; MMP-2; CIN; Cervical carcinoma.

Introduction

Invasion and metastasis are the greatest obstacles to successful tumor treatment. Although advances have been made in conventional tumor therapies and surgical techniques, most cancer deaths still result from metastatic disease. The lack of understanding of the molecular mechanisms involved in tumor cell invasion and metastasis has hindered the development of effective antimetastatic therapies; however, recent discoveries in this field are leading to potential therapeutic strategies [1].

Tumor invasion and metastasis require extensive degradation of the basement membrane and interstitial extracellular matrix (ECM). Therefore, in order to initiate the metastatic process, carcinoma cells must first attach to and penetrate the epithelial basement membrane, and then invade the interstitial stroma which requires active proteolysis of the dense matrix of type IV collagen, glycoproteins, and proteoglycans [2, 3]. None of these functions is unique to tumor cell behavior. Attachment, proteolysis, and migration are steps of trophoblast implantation, mammary gland development, embryonic morphogenesis, and tissue remodeling. The difference between normal processes and the pathogenic nature of tumor cell invasion must therefore be one of regulation. Thus, an understanding of the controlling factors in the processes of cellular adhesion, matrix proteolysis, and cell migration should allow the identification of new targets for therapeutic disruption of metastatic spread [4].

Matrix metalloproteinases (MMPs) are a group of

calcium dependent, zinc-containing enzymes able to degrade all components of the extracellular-matrix (ECM) and type IV collagen, the major component of the basement membrane. It appears to have a key role in the sequence of events that lead to local invasion and metastasis [5, 6].

The aim of the current study was to study the expression of the immunoreactive protein of matrix metalloproteinase-2 (MMP-2) in preinvasive and invasive cervical cancer so as to demonstrate whether the expression of MMP-2 is an early or late event in the process of dedifferentiation and cancer progression.

Materials and Methods

The present work is a retrospective study of 50 cervical specimens obtained from cases undergoing hysterectomy or cervical biopsies at the Gynecologic Oncology Unit, Ain Shams University Maternity Hospital. These cases were classified into three groups as follows:

- Group 1: Ten normal cases used as a control.
- Group 2: Twenty cervical intraepithelial neoplasia (CIN) cases of variable degrees.
- Group 3: Twenty cases of cervical carcinoma.

Five micrometer (5 µm) paraffin-embedded tissue sections had been deparaffinized and rehydrated through graded alcohol series and treated in a microwave oven for two to five minutes at 700W in ready to use antigen retrieval citra (Biogenex cat. no IIK087-SK). The sections were left to cool, and incubated with 3% hydrogen peroxide for ten minutes followed by a wash for two to five minutes with phosphate buffer saline (PBS). Non-specific background is eliminated by adding two drops of ready to use 10% non-immune goat serum to each section for ten minutes, and then, drain or blot off the solution.

The tissue sections were incubated for 60 minutes with the monoclonal primary antibody anti MMP-2 protein (Zymed Laboratories, cat. no. 33-7000). The antibody is supplied as a 50 µl aliquot of 1 mg/ml in PBS and used at a concentration of 5 µg/ml. Sections were then washed with PBS for three times and incubated for ten minutes in two drops of biotinylated secondary antibody (supersensitive immunodetection system from Biogenex Laboratories, cat. no. QD 000-5L), and then washed as above and incubated for ten minutes in two drops of streptavidin peroxidase. Lastly, sections were washed again for three times with PBS.

Slides were then incubated in two drops of diamino-benzidine tetra-hydrochloride (DAB) chromogen for three to ten minutes, rinsed with distilled water, counterstained with Harris hematoxylin and finally mounted and covered with cover slips.

The positive controls in this study were taken from tissue sections of normal colonic mucosa and negative controls were prepared by replacing the primary antibody by the negative control serum.

Examination of the control slides was first done to assess non-specific staining. Staining variables included percentages of cells stained and intensity. MMP-2 reactivity was arbitrarily defined with more than 10% as positive, and less than 10% as negative of 500 cells examined. The following scoring system was devised:

- Percentage of cells stained in 500 cells counted: 0 = < 10%, 1 = 10-25%, 2 = 26-50%, 3 = > 50%.
- Intensity: 1 = weak, 2 = moderate, 3 = strong.
- Heterogeneity score: -1 = marked, 0 = moderate, +1 = mild.

The final score was calculated by the score of percentage of cells stained multiplied by intensity score and then adding heterogeneity score: Final score = percentage of positive cells x intensity score + heterogeneity score.

Data were collected, revised then analyzed structurally using SPSS statistical package software version 12.0. The following tests were done: X (Mean), SD (standard deviation), ANOVA (analysis of variance), X² (chi-square) and post-hoc tests.

Results

In this study, a total of 50 cervical paraffin-embedded tissue specimens were studied for MMP-2 immunoreactivity. The specimens were obtained from cases who had had hysterectomies (n = 17) or cervical biopsies (n = 33). The first group included ten normal cases and were used as a control, the second group comprised 20 cervical intraepithelial neoplasia (CIN) cases [CIN1 (n = 7), CIN2 (n = 6), CIN3 (n = 7)] while the third group included 20 cervical carcinoma cases; 16 were squamous cell carcinoma and four were adenocarcinoma.

Of the 20 carcinoma cases, 12 (60%) were well differentiated (9 squamous, 3 adenocarcinomas), six (30%) were moderately differentiated (5 squamous, one adenocarcinoma) and two (10%) were poorly differentiated squamous carcinomas.

The mean age for the studied groups was 39.80 ± 7.84 for the control group, 32.85 ± 7.42 for CIN1, 40.16 ± 9.68 for CIN2, 46.71 ± 17.99 for CIN3, 49.25 ± 9.84 for squamous cell carcinoma and 53.0 ± 17.0 for the adenocarcinoma group.

The immunohistochemical expression of MMP-2 in all control groups and CIN1 cases was found to be negative. In CIN 2/3 cases, positive expression was observed in only seven out of 13 cases (53.8%). Uniform expression was observed in four cases (57.14%), while focal expression was observed in three cases (42.86%), with mild to moderate staining intensity.

High scores of MMP-2 were observed in different types of cervical carcinomas. All types revealed positive expression in tumor cells with variable stromal cell staining for MMP-2. The staining intensity in tumor cells was variable ranging from mild to severe and appeared enhanced in 10% of tumors at the invasive edge at the tumor-stroma interface (Figures 1, 2).

When comparing the MMP-2 scoring in the three

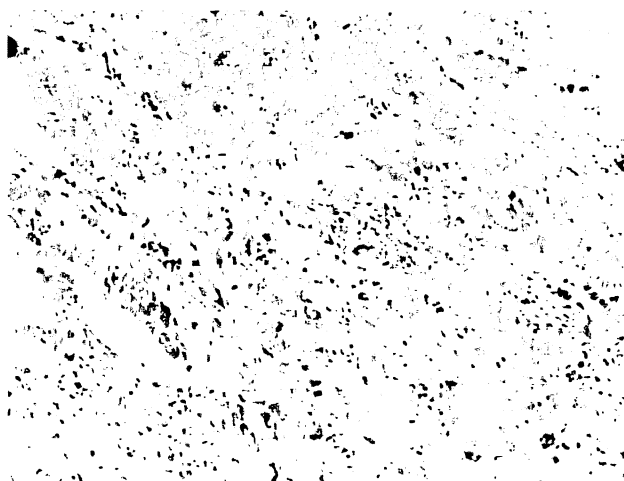


Fig. 1

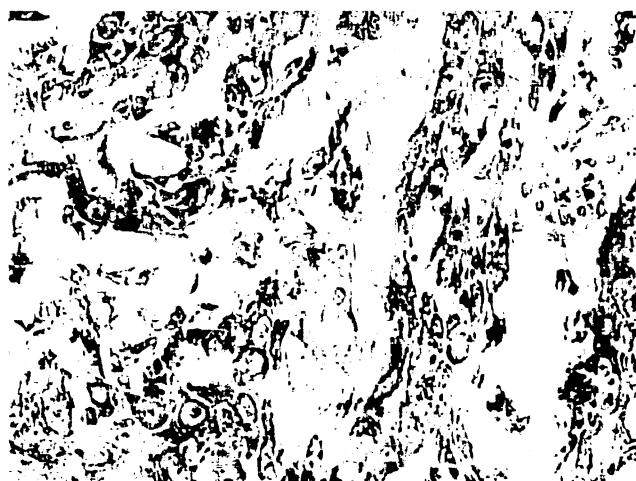


Fig.

Figure 1. — Moderately differentiated cervical adenocarcinoma with the cytoplasm of the malignant cells showing moderate heterogeneous staining for MMP-2 with positive expression in the stromal cells as well (x 250).

Figure 2. — Poorly differentiated squamous cell carcinoma with the malignant cells showing intense cytoplasmic immunoreactivity for MMP-2 and the stromal cells inbetween are negatively stained (x 400).

studied groups, it was found that the mean score and standard deviation of the control group was zero, 1.50 ± 1.50 for the CIN group and 4.70 ± 2.18 for the carcinoma group (Table 1).

Table 1. — Comparison of the MMP-2 score in the three main groups.

	N	Mean	SD	Minimum	Maximum
Normal	10	0.00	0.00	0.00	0.00
CIN	20	1.50	1.50	0.00	4.00
Invasive	20	4.70	2.18	2.00	9.00

When comparing the MMP-2 staining scores of squamous cell carcinoma and adenocarcinoma, the mean staining score and standard deviation for squamous cell carcinoma was 4.94 ± 2.35 and 3.75 ± 1.00 for adenocarcinoma. There was no statistical significant difference ($p > 0.05$) between the mean staining score for both groups (Table 2).

Table 2. — The mean and standard deviation for MMP-2 scores in the two types of cervical carcinoma.

	N	Mean	SD	Minimum	Maximum	p
Squamous cell carcinoma	16	4.93	2.35	2.00	9.00	> 0.05
Adenocarcinoma	4	3.75	1.00	3.00	5.00	

Further studying of the MMP-2 expression scores among the different grades of differentiation of invasive cervical carcinoma was done. It was found that inside the well differentiated group, two cases (16.7%) had score 2, six cases (50%) had score 3, and four cases (33%) had score 4. While inside the moderately differentiated group, one case (16.7%) had score 5, two cases (33.3%) had score 6, three cases (50%) had score 9. On the other hand, all poorly differentiated cases had score 9 (Table 3). Using the χ^2 test, a highly significant difference was found among the three grades of differentiation ($p < 0.001$).

Table 3. — MMP-2 expression according to grade of differentiation.

	Well differentiated carcinoma	Moderately differentiated carcinoma	Poorly differentiated carcinoma	
MMP-2 scores	Score 2	2 (16.7%)		
	Score 3	6 (50%)		
	Score 4	4 (33%)		
	Score 5		1 (16.7%)	
	Score 6		2 (33.3%)	
	Score 7			
	Score 8			
	Score 9		3 (50%)	2 (100%)

The post-hoc test was done to detect the least significant difference between the mean staining scores of the three different studied groups. It was found that there was a significant difference between high-grade CIN and the control group ($p < 0.05$). The staining values were of high significant difference in invasive carcinomas when compared to control cases as well as high-grade CIN ($p < 0.001$).

Discussion

The spread of malignant neoplasms is closely associated with matrix and basement membrane degradation, mediated by various classes of MMPs [5, 7]. It has been demonstrated that MMP-2 is involved in the spread of carcinomas having a high degree of affinity for type IV collagen, a major component of epithelial basement membranes which are lysed during metastatic invasion. In addition, MMP-2 lyses fibrillar collagen as well as elastin [6].

The present study evaluated the role of MMP-2 in cervical neoplasia. Using immunohistochemistry, 50 cervical tissue specimens were analyzed. The study included ten normal specimens as a control group, seven low-grade cervical intraepithelial lesions, 13 high-grade cervical intraepithelial lesions, 16 cervical squamous cell carcinoma and four adenocarcinoma specimens.

The immunohistochemical analysis showed negative immunoreactivity in control cervixes and low-grade intraepithelial lesions which is in agreement with Davidson *et al.* [5]. On the contrary, Brummer *et al.* and Asha-Nair *et al.* demonstrated weak positivity for MMP-2 in normal cervical epithelium and low-grade intraepithelial lesions [2, 8]. This controversy concerning MMP-2 expression in normal and low-grade intraepithelial lesions could be explained by the fact that the expression and the effective action of MMP-2 are influenced by multiple factors which include growth factors, specific membrane activators, integrin receptor expression and matrix composition which are themselves subject to different control mechanisms [9].

As regards high-grade CIN lesions, our results revealed mild to moderate positivity in 53.8% of the cases. Uniform expression was observed in four cases (57.14%) and focal expression was observed in three cases (42.86%). It should be noted that there is a more or less general agreement with the above results concerning the positive expression of MMP-2 in high grade CIN. Nonetheless, there is disagreement about the intensity and pattern of staining as Grazetti *et al.* and Zhou *et al.* mentioned positive staining of MMP-2 in CIN lesions without commenting on the intensity and pattern of staining while Asha-Nair *et al.* showed intense cellular and stromal reactivity for MMP-2 in high-grade CIN [8, 10, 11]. Furthermore, Talvensaari *et al.* pointed out that CIN lesions revealed positive MMP-2 expression, which was localized in most of the cases to the periphery of the CIN cells [12]. In addition, Brummer and co-workers demonstrated that the high-grade CIN cells displayed a heterogeneous distribution of MMP-2 expression ranging from total absence to uniform or focal expression [2].

Concerning invasive cervical carcinoma, the study showed high scores for MMP-2 in different types of cervical carcinoma. All types revealed positive expression in tumor cells with variable stromal cell staining for MMP-2. The staining intensity varied (ranging from mild to severe) and appeared to be enhanced at the invasive edge (tumor-stromal interface) in 10% of the tumors. Similar

to these results, nearly all investigators confirmed the strong expression of MMP-2 in invasive cervical carcinoma, either in a diffuse pattern [8, 13, 14] or with intense staining at the invasive edge [5]. However, there was no significant difference between different types of carcinoma as regards MMP-2 expression ($p > 0.05$) with a mean staining score for squamous cell carcinoma of 4.94 and a mean staining score for adenocarcinoma of 3.75. This is similar to what Davidson *et al.* found but contradicts the results of Yoshida *et al.* who found significantly higher MMP-2 expression in squamous cell carcinoma when compared to adenocarcinoma [5, 14].

Pertaining to the MMP-2 expression in relation to the different grades of differentiation, the study revealed a high significant difference, being higher in poorly differentiated tumors, which is in agreement with Talvensari *et al.* [12]. On the other hand, Davidson and co-workers found no correlation with tumor grade or differentiation [5].

By doing a post-hoc test to detect the least significant difference of MMP-2 expression scores among the different groups, it was found that there was a significant difference between high-grade intraepithelial lesions and the control group ($p < 0.05$). The staining values were of high significant difference in invasive carcinoma when compared to the control group as well as high-grade intraepithelial lesions ($p < 0.001$). Similarly, Grazetti *et al.* found a significant increase in the 72-kDa metalloproteinase immunostaining with increased CIN degree and when they compared invasive cervical carcinoma to CIN, they found a high significant difference with immunostaining being higher in invasive carcinoma [10].

The current work revealed that MMP-2 expression is totally absent in control cervixes and low-grade intraepithelial lesions, while high-grade intraepithelial neoplasia and invasive carcinoma showed overexpression of MMP-2 with no significant difference concerning the type of carcinoma which points to the possibility that MMP-2 is an early marker of tumor progression in cervical carcinoma. Its expression in high-grade cervical intraepithelial lesions may denote a potential risk for invasion and metastasis. It is recommended that further larger studies should be directed to investigate the role of MMP-2 in preinvasive and invasive cervical lesions and combined with a basement membrane marker to detect any early and minor disruption heralding the metastatic process.

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