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Matrix metalloproteinase-2 and -9 expression in sinonasal inverted papilloma*

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SUMMARY

Statement of problem: *Inverted papilloma (IP) is a proliferative lesion of the epithelium lining the sinonasal tract, characterized by marked propensity for recurrence and association with carcinoma. To determine a putative role of matrix metalloproteinase-2 (MMP-2) and MMP-9 in the establishment of IP, their expression was studied in IP.*

Methods: *Archived surgical specimens from 15 IPs were studied using immunohistochemistry and compared to 12 nasal polyps (NP), a model of chronic respiratory mucosal inflammation, and to 6 control nasal mucosa (CM) samples obtained from snorers during turbinectomy. Within IP, MMP-2 and -9 expression was compared between tumoral areas with hyperplastic epithelium and non tumoral areas with nonhyperplastic epithelium.*

Results: *In IP, MMP-2 and MMP-9 epithelial expression was not different compared to CM and NP. MMP-9 expression in submucosal inflammatory cells was not different between IP and CM or NP. However, within IP, a significantly increased number of MMP-9 positive inflammatory cells in the lamina propria adjacent to the hyperplastic epithelium was observed compared to the lamina propria adjacent to nonhyperplastic epithelium.*

Conclusion: *Our findings suggest that MMP 9 expressing inflammatory cells may be involved in the pathophysiology of IP.*

Key words: metalloproteinase, gelatinase, inverted papilloma, nose, epithelium

INTRODUCTION

Inverted papilloma (IP), a particular form of sinonasal papillomas, which are locally aggressive benign tumors of the nasal cavities and paranasal sinuses, are characterized by endophytic growth [(1)]. IP represent about 0.5 to 4% of all nasal tumors [(2)]. Although the morphological features, clinical behavior and genetic alterations of IP have been well described [(3,4)], its pathogenesis is poorly understood. IP could be a manifestation of chronic inflammation of the sinuses [(4)], in the same way as nasal polyps (NP), but some authors consider them to be premalignant tumors [(3)].

Microscopically, IP are characterized by downward (i.e. inverted) expansive growth of epithelial cells into the underlying stroma with no disruption of the basement membrane [(1)]. The epithelium is composed of 5 to 30 layers of squamous, ciliated, columnar or transitional cells. Stroma of IP ranges from dense and fibrous to loose and myxoid with inflammatory cell infiltration, suggesting remodeling of extracellular matrix (ECM). ECM not only provides structural support to tissues, but also has an important influence on biological activities such as cell proliferation, differentiation and migration. Matrix metalloproteinases (MMPs) are key enzymes involved in tissue remodeling events because of their capacity to cleave structural proteins such as collagens and elastin [(5)]. Among the MMPs, MMP-2 and MMP-9 are of particular interest because they can cleave various matrix components, including basement membrane collagen [(6)]. Other possible functions of MMP-2 and MMP-9 include migration of regenerating epithelial cells and leukocytes, release of cytokines and activation of latent growth factors [(7,8)].

Although increased MMP expression has been demonstrated in NP [(9)], nothing is known about MMP expression in IP. Considering the specific effects of MMP-2 and MMP-9 and the histological features of IP, we hypothesized, that MMP could play a role in IP development. We therefore designed this study to quantify and compare the expression of MMP-2 and MMP-9 in IP, NP and control nasal mucosa (CM).

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MATERIALS AND METHODS

Study materials

We reviewed archived samples of 15 IP, 12 NP and 6 CM surgically removed between January 2000 and January 2002. There were no differences between the three groups with respect to age, sex and smoking habits. Hematoxylin-eosin stained sections for each patient were reviewed by the same pathologist to confirm the diagnosis of IP or NP. IP with concurrent squamous cell carcinoma were excluded from the study. Because of their special features, NP from patients with cystic fibrosis or primary ciliary dyskinesia were also excluded from the study. CM was obtained from the inferior turbinate removed in snorers because of turbinate hypertrophy related to vasomotor dysfunction in absence of any allergic or inflammatory rhinitis. This study was approved by the Review Board of our institution.

Immunohistochemistry

Immunohistochemistry was performed on 4 μm sections of formaldehyde-fixed and paraffin-embedded samples. In situ expression of MMP-2 and MMP-9 was evaluated in samples using monoclonal mouse IgG1 kappa antibodies that recognize both the latent and activated forms of either MMP-2 [9] (CA-4001; Neo Markers, Union City, CA, USA) or MMP-9 [9] (56-2A4; Oncogene Research Products, Cambridge, MA, USA). For MMP-9 immunodetection, antigen retrieval was first performed in citrate buffer solution (pH 6) (Dako, France) and processed in a microwave oven (750 W, 5 minutes, three cycles) [9]. After washing in phosphate saline, endogenous peroxidase activity was blocked with 3% (v/v) hydrogen peroxide in phosphate buffer solution containing 0.025% Tween 20. Sections were subsequently incubated in blocking serum (Dako) for 3 h at 37°C, then with anti-MMP-2 or anti-MMP-9 monoclonal antibodies at 1/30 and 1/40, respectively, for 1 h at room temperature. Antigen detection was performed using peroxidase LSAB kit (labeled streptavidin-biotin method, Dako). A 3-amino-9-ethylcarbazole (AEC) chromogen substrate was used, which generates a red-brown reaction product. Tissue sections were then conterstained with hemalun. Negative controls were obtained by replacing the primary antibody with an irrelevant antibody of similar isotype. Samples of breast carcinoma known to produce MMP-2 and MMP-9 were used as positive controls [10].

Epithelial and inflammatory cell expression of MMP-2 and MMP-9 were evaluated separately and compared in IP, NP and CM samples. In IP, only tumoral areas with hyperplastic epithelium were considered for these counts. In each sample, the epithelial labeling index was estimated as the percentage of positive surface epithelial fields over the total number of surface epithelial fields present on each slide (final magnification x500). In each sample, the inflammatory cell index was expressed as the total number of positive cells counted in ten randomly selected fields of stroma or epithelium (final magnification x500). Since MMP-2 is not constitutively expressed in inflammatory cells [11], the inflammatory cell index was not evaluated for MMP-2.

Focusing on IP, we compared in each sample, the results obtained in tumoral areas to MMP-2 and MMP-9 expression in non tumoral areas with nonhyperplastic epithelium. In these two areas, we firstly evaluated MMP-2 and MMP-9 epithelial labeling indices, and secondly three different MMP-9 inflammatory cell indices, corresponding to MMP-9 positive inflammatory cells infiltrating either epithelium, lamina propria or deep stroma. All counts were performed by two independent investigators and the mean count was calculated for each sample.

Statistical analysis

Results were expressed as mean ± SD or as percentages for comparison of quantitative data. Epithelium and inflammatory indices were compared between IP, NP and CM using the Anova or Mann-Whitney test. Within IP, epithelium indices were compared between tumoral hyperplastic and nonhyperplastic epithelium, and inflammatory cell indices in the epithelium were compared between tumoral hyperplastic and nonhyperplastic epithelium. Finally, within IP, inflammatory cell indices in the lamina propria and deep stroma were compared between lamina propria or deep stroma adjacent to tumoral hyperplastic epithelium and adjacent to nonhyperplastic epithelium. The data for IP were compared using the Wilcoxon test. A $p < 0.05$ was considered significant.

RESULTS

Qualitative features of MMP-2 and MMP-9 expression in IP, NP and CM

Areas of hyperplastic epithelium and of nonhyperplastic respiratory epithelium were observed in all IP samples. Tumoral areas with hyperplastic epithelium extended deeply into the underlying stroma, forming cords and nests, and epithelial-lined duct-like structures that always maintained a connection with the surface epithelium. No areas of squamous cell carcinoma were observed in any of the IP samples. IP stroma consisted of dense stroma infiltrated by inflammatory cells, also infiltrating the epithelium. Most of these inflammatory cells were polymorphonuclear cells looking like neutrophils.

In all IP samples, epithelium and stroma showed positive immunostaining for MMP-2 and MMP-9. In IP epithelium, MMP-9 immunolabeling was preferentially observed in tumoral hyperplastic areas. In the stroma, numerous elongated cells showed strong MMP-2 positivity, while inflammatory cells showed intense MMP-9 positivity (Figure 1). Interestingly, numerous clusters of MMP-9-positive inflammatory cells were observed just underneath the basement membrane, i.e. the lamina propria, close to invaginated hyperplastic epithelium (Figure 2). In all NP and CM samples, surface epithelium and stroma showed positive immunostaining for MMP 2 and MMP-9. In stroma, numerous elongated cells showed strong MMP-2 positivity. Scarce inflammatory cells in CM and numerous inflammatory cells in NP showed intense MMP-9 positivity (Figure 1).

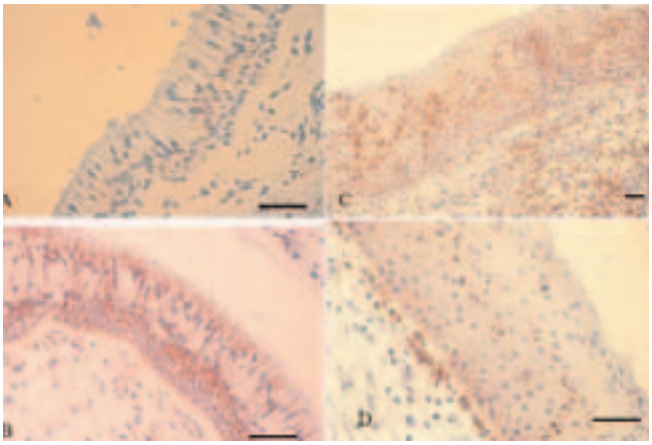

Figure 1. MMP-2 and MMP-9 immunolabeling in nasal specimens. In CM, MMP-2 (A) and MMP-9 (B) positivity was present in basal cells. In all IP samples, epithelium showed positive immunostaining for MMP-2 (C) and MMP-9 (D). In IP stroma and epithelium, inflammatory cells showing intense MMP-9 positivity were preferentially observed in the hyperplastic epithelium (D). Bar = 40 μm.

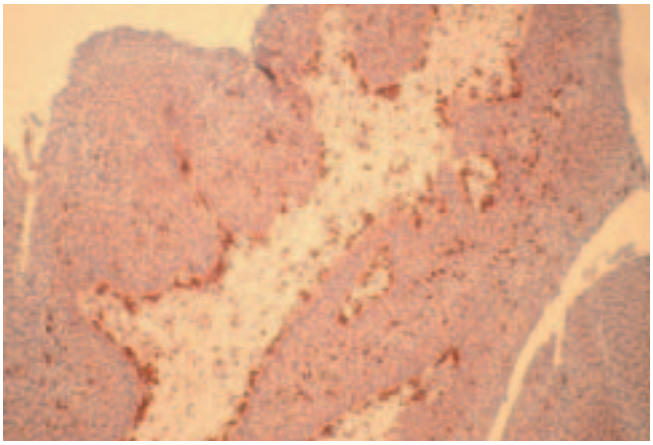

Figure 2. Representative micrograph of MMP-9-positive inflammatory cell clusters observed just underneath the basement membrane in tumoral areas with invaginated hyperplastic epithelium (bar = 40 μm).

Comparison of quantitative features of MMP-2 and MMP-9 expression between IP, NP and CM

MMP-2 epithelial labeling indices were not significantly different in IP, NP and CM. Although MMP-9 epithelial labeling indices tended to be higher in IP than in NP, and in NP than in CM, these differences were not significant (p=0.06) (Figure 3).

In stroma, MMP-9 inflammatory cell labeling indices were not different between IP and NP or CM (Figure 4).

Since the distribution of positive cells in IP was different between hyperplastic tumoral areas and nonhyperplastic areas, we therefore decided to compare these distinct areas within IP.

Comparison of quantitative features of MMP-2 and MMP-9 expression within IP

MMP-2 and MMP-9 epithelial labeling indices were similar between hyperplastic and nonhyperplastic epithelium (data not shown). MMP-9 inflammatory cell labeling indices were significantly (p=0.001) higher in hyperplastic than in nonhyperplastic epithelium (Figure 5). Interestingly, there was a significant (p=0.004) increase of MMP-9 inflammatory cell indices in the lamina propria underlying invaginating hyperplastic epithelium compared to lamina propria underlying nonhyperplastic epithelium (Figure 5). In the deep stroma, inflammatory cell labeling indices were similar underneath nonhyperplastic and hyperplastic epithelium (data not shown).

DISCUSSION

To our knowledge, this is the first study of MMP expression in sinonasal IP. We showed that epithelial cells expressed MMP-2 and MMP-9 in IP as in normal airway tissues and NP. Moreover, within IP, we observed a significantly increased number of MMP-9-positive inflammatory cells in hyperplastic epithelium and lamina propria adjacent to invaginated epithelial areas.

In the present study, we confirmed that normal airway epithelial cells (CM) expressed both MMP-2 and MMP-9, as already shown in normal upper and lower airways [9,12]. In IP, MMP-2 epithelial expression was not different compared to NP and CM. Although not statistically significant, epithelial MMP-9 expression in IP tended to be more intense than in the epithelium of NP and CM. More precisely, MMP-9 immunolabeling appeared to be preferentially observed in the epithelial areas of IP exhibiting hyperplasia, as already reported in colorectal adenoma [13]. In addition, cholesteatoma of the middle ear, a locally aggressive, benign epithelial cell proliferation, appears to overexpress MMP-9, while MMP-2 expression is similar to that of control tissue [14].

Global MMP-9 inflammatory cell indices were not different between IP and NP or CM. In NP, while inflammatory cells expressing MMP-9 were homogeneously distributed in the mucosa, the inflammatory cell indices varied widely between patients. In IP, the inflammatory cell indices were quite constant between patients but inflammatory cells with intense MMP-9 expression were heterogeneously distributed within the mucosa i.e. in the lamina propria and hyperplastic epithelium. This feature was never observed in NP or CM. Most of the inflammatory cells infiltrating IP were polymorphonuclear cells looking like neutrophils. Tissue infiltration by numerous inflammatory cells, especially neutrophils transmigrating through the epithelium, is a classical feature of IP [4]. While the presence of neutrophils expressing MMP-9 is frequently described in various chronic inflammatory diseases such as chronic bronchitis [15], asthma [16] or NP [9], MMP-9 expression has not been previously reported in neutrophils infiltrating

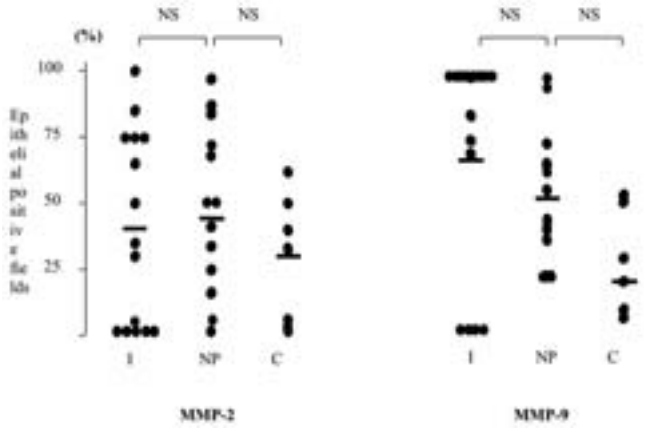

Figure 3. Quantification of epithelial MMP-2 and MMP-9 expression in IP, NP and CM. In IP, MMP-2 and MMP-9 epithelial labeling indices were not significantly different from those observed in NP and CM. Values are expressed as percentage of positive fields.

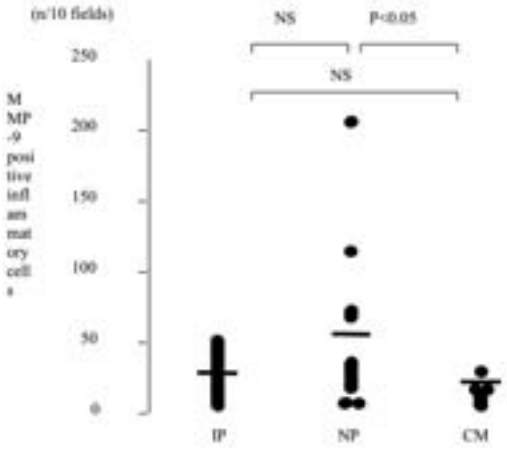

Figure 4. Quantification of inflammatory cell MMP-9 expression in stroma of IP, NP and CM. In stroma, inflammatory cell indices were significantly different only between NP and CM ($p<0.05$). Values are expressed as the number of positive cells in 10 fields.

upper airway tumors. An interesting finding, reported here for the first time, was the significantly increased number of MMP-9-positive inflammatory cells in the lamina propria adjacent to hyperplastic epithelium compared to lamina propria adjacent to nonhyperplastic epithelium. The exact reason for this feature is not known but it is has been recently suggested that the concentration of neutrophils in localized areas of tissues could result from detection of specific MMP substrates via cell surface receptors, leading to selective MMP activation at these sites [11].

Taken as a whole, these results suggest that MMP-9 expressing inflammatory cells could play a role in the pathophysiology of IP. MMP-9, which is able to protect tumor cells from apoptosis [17], could participate in the increased epithelial cell proliferation observed in IP [18]. Moreover, MMP-9 and probably other MMP are involved in regulation of cell proliferation via local release from the extracellular matrix of growth factor precursors such as transforming growth factor-α and insulin-like growth factors [8]. MMP-9 has also been reported to play a crucial role in the transmigration of inflammatory cells, especially neutrophils [7] and therefore, in IP, MMP-9 could participate in inflammatory cell infiltration. Infiltrating inflammatory cells, especially neutrophils, could secrete preformed MMP-9, especially after exposure to chemokines such as IL-8 [19], already detected in IP [20]. IL-8 can also act as a chemotactic factor for neutrophils [21]. Thus, via MMP-9 secretion, locally attracted neutrophils could enhance epithelial cell proliferation and degrade pericellular areas of extracellular matrix components, facilitating endophytic growth.

Clinically, IP is characterized by a high rate of post-treatment recurrence and squamous cell carcinoma is associated in about 8.9 % of cases [2]. In colorectal or prostatic tumors [22,23], MMP-9 expression has been shown to be a marker of malignant transformation and/or association. Since MMP-9 positive inflammatory cells are concentrated in areas of hyperplastic epithelium and adjacent lamina propria in IP, it could be interesting to initiate a study in order to investigate the potential correlation between MMP-9 positive inflammatory cell infiltration and recurrence and/or malignant association.

In conclusion, MMP-2 and MMP-9 are expressed in situ in sinonasal IP. While global MMP 2 and MMP-9 expressions are similar between IP, NP and CM, MMP-9-expressing inflammatory cells are concentrated in hyperplastic epithelium and lamina propria adjacent to hyperplastic epithelium of IP. A complex interaction between nasal epithelial cells and inflammatory cells could lead to tissue remodeling and participate in the specific endophytic growth of IP.

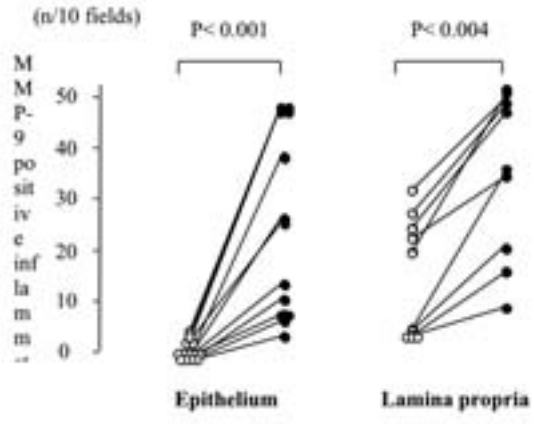

Figure 5. Quantification of inflammatory cell MMP-9 expression within IP. Significantly ($p=0.001$) more MMP-9 positive inflammatory cells infiltrated tumoral hyperplastic epithelium (black dot) than nonhyperplastic epithelium (white dot). A significant ($p=0.004$) increase of MMP-9-positive inflammatory cells was observed in the lamina propria adjacent to tumoral hyperplastic epithelium (black dot) compared to lamina propria adjacent to nonhyperplastic epithelium (white dot). Values are expressed as the number of positive cells in 10 fields.

REFERENCES

1. Batsakis JG. Pathology consultation. Nasal (Schneiderian) papillomas. Ann Otol Rhinol Laryngol 1981; 90: 190-191.
2. Lawson W, Kaufman MR, Biller HF. Treatment outcomes in the management of inverted papilloma: an analysis of 160 cases. Laryngoscope 2003; 113: 1548-1556.
3. Califano J, Koch W, Sidransky D, Westra WH. Inverted sinonasal papilloma : a molecular genetic appraisal of its putative status as a Precursor to squamous cell carcinoma. Am J Pathol 2000; 156: 333-337.
4. Roh HJ, Procop GW, Batra PS, Citardi MJ, Lanza DC. Inflammation and the pathogenesis of inverted papilloma. Am J Rhinol 2004; 18: 65-74.
5. Shapiro SD, Senior RM. Matrix metalloproteinases. Matrix degradation and more. Am J Respir Cell Mol Biol 1999; 20: 1100-1102.
6. Collier IE, Wilhelm SM, Eisen AZ, et al. H-ras oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. J Biol Chem 1988; 263: 6579-6587.
7. Lee KS, Jin SM, Kim HJ, Lee YC. Matrix metalloproteinase inhibitor regulates inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression in a murine model of toluene diisocyanate-induced asthma. J Allergy Clin Immunol 2003; 111: 1278-1284.
8. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. J Pathol 2003; 200: 448-464.
9. Lechapt-Zalcman E, Coste A, d'Ortho MP, et al. Increased expression of matrix metalloproteinase-9 in nasal polyps. J Pathol 2001; 193: 233-241.
10. Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol 1996; 149: 273-282.
11. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 2003; 253: 269-285.
12. Yao PM, Buhler JM, d'Ortho MP, et al. Expression of matrix metalloproteinase gelatinases A and B by cultured epithelial cells from human bronchial explants. J Biol Chem 1996; 271: 15580-15589.
13. Parsons SL, Watson SA, Collins HM, Griffin NR, Clarke PA, Steele RJ. Gelatinase (MMP-2 and -9) expression in gastrointestinal malignancy. Br J Cancer 1998; 78: 1495-1502.
14. Schmidt M, Grunsfelder P, Hoppe F. Up-regulation of matrix metalloprotease-9 in middle ear cholesteatoma-correlations with growth factor expression in vivo? Eur Arch Otorhinolaryngol 2001; 258: 472-476.
15. Zheng L, Lam WK, Tipoe GL, et al. Overexpression of matrix metalloproteinase-8 and -9 in bronchiectatic airways in vivo. Eur Respir J 2002; 20: 170-176.
16. Lemjabbar H, Gosset P, Lamblin C, et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. Am J Respir Crit Care Med 1999; 159: 1298-1307.
17. Bergers G, Brekken R, McMahon G, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000; 2: 737-744.
18. Guichard C, Gilain L, Abd-Al Samad I, et al. Epithelial cell proliferation, apoptosis, and apoptosis inhibition in inverted papillomas. Laryngoscope 1998; 108: 716-720.
19. Nagaoka I, Hirota S. Increased expression of matrix metalloproteinase-9 in neutrophils in glycogen-induced peritoneal inflammation of guinea pigs. Inflamm Res 2000; 49: 55-62.
20. Yokoshima K, Ohnishi M, Takizawa R, Pawankar R, Okubo K and Okuda M. [Cytokines of nasal inverted papilloma: quantification and distribution]. Nippon Jibiinkoka Gakkai Kaiho 1995; 98: 66-70.
21. Mukaida N. Interleukin-8: an expanding universe beyond neutrophil chemotaxis and activation. Int J Hematol 2000; 72: 391-398.
22. Festuccia C, Bologna M, Vicentini C, et al. Increased matrix metalloproteinase-9 secretion in short-term tissue cultures of prostatic tumor cells. Int J Cancer 1996; 69: 386-393.
23. Tomita T, Iwata K. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in colonic adenomas-adenocarcinomas. Dis Colon Rectum 1996; 39: 1255-1264.

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