·Basic Research·

Expression of matrix metalloproteinase-26 in multiple human cancer tissues and smooth muscle cells

Yun-Ge Zhao,[1] Ai-Zhen Xiao,[1] Jian Ni,[2] Yan-Gao Man[3] and Qing-Xiang Amy Sang[1]

[1]Department of Chemistry and Biochemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida, 32306, USA; [2]Human Antibodomics (SIP)Inc., Suzhou, Jiangsu, 215125, P. R. China; [3]Department of Gynecology & Breast Pathology, The Armed Forces Institute of Pathology, Washington, DC, 20306, USA

【Abstract】 Background and Objective: Elevated expression of matrix metalloproteinases (MMPs) has been found in multiple carcinoma tissues. MMP-26 is highly expressed in prostate and breast cancer tissues, and promotes the invasion of human prostate cancer cells not only through the cleavage of fibronectin and type IV collagen but also by the activation of pro-MMP-9, a powerful gelatinase. This study was to present a comprehensive protein expression profile of MMP-26 in multiple human cancer tissues. **Methods:** The protein expression pattern of MMP-26 was examined using immunohistochemistry and multiple-tissue microarray. MMP-26 mRNA expression in coronary artery smooth muscle cells was detected by reverse transcription-polymerase chain reaction (RT-PCR). **Results:** The expression of MMP-26 in breast, colon, lung, brain, head and neck, prostate cancer, and melanoma tissues was significantly elevated when compared with parallel normal tissues ($P<0.05$), while not significantly elevated in kidney cancer, ovarian cancer, and non-Hodgkin´s lymphoma ($P>0.05$). MMP-26 was also detected in gastric, rectal, thyroid, esophageal, and pancreatic cancers. MMP-26 protein was expressed in smooth muscle cells of the prostate and associated blood vessels. MMP-26 mRNA was also detected in human coronary artery smooth muscle cells. **Conclusions:** MMP-26 expression may be associated with multiple human carcinomas, and it may serve as a molecular marker for the early diagnosis of these carcinomas. MMP-26 may also contribute to smooth muscle function in the human prostate and cardiovascular system.

Key words: matrix metalloproteinase-26, tissue microarray, integrated morphometry analysis, human neoplasms, smooth muscle cell

Introduction

Matrix metalloproteinases (MMPs) are a group of secreted or membrane-associated enzymes that are dependent upon zinc for their catalytic activity, and as a whole, they can cleave virtually every component of the extracellular matrix (ECM). For this reason, MMPs are likely to play key roles in tissue remodeling, tumor invasion, and metastasis.[1,2] Endometase/matrilysin-2 (MMP-26) is a member of the MMP family that was cloned and characterized by our group[3] and others.[4-6] MMP-26 and MMP-7 are the two smallest members of this family, exhibiting minimal domain structure. MMP-26 cleaves multiple components of ECM, including fibronectin (FN), type IV collagen, vitronectin, gelatins and fibrinogen, as well as non-ECM proteins such as insulin-like growth factor binding protein 1 (IGFBP-1) and alpha 1-protease inhibitor.[3-7] We have demonstrated that MMP-26 can activate pro-MMP-9, thereby facilitates the efficient cleavage of ECM components and promotes the invasion of highly invasive and metastatic androgen-repressed prostate cancer (ARCaP) cells.8 These results suggest that MMP-26 may possess critical roles in the processes of tumor invasion, angiogenesis, and metastasis.

MMP-26 is primarily of epithelial origin, and the majority of cancers in glandular tissues are of the carcinoma type, arising from epithelial cells, suggesting that MMP-26 may serve as a useful biomarker for the detection and diagnosis of carcinomas. MMP-26 mRNA has been observed in multiple cancers of epithelial origin, such as endometrial carcinoma,[3,9] prostate carcinoma,[9] lung carcinoma,[9] their corresponding cell lines,[3-6,8] and in a malignant choriocarcinoma cell line (JEG-3).[10] However, the expression of MMP-26 mRNA is rare in normal adult tissues, and largely limited to the uterus,[3,5] placenta[4,5] and kidney.[6] MMP-26 expression is significantly elevated in cancerous tissues of the human prostate when compared with prostatitis, benign prostate hyperplasia, and normal prostate tissues.8 Interestingly, the expression of MMP-26 in human breast ductal carcinoma in situ (DCIS) is much higher than that observed in infiltrating ductal carcinoma (IDC), atypical intraductal hyperplasia, and normal

Correspondence to: Yun-Ge Zhao; Tel: +1-434-924-5836; Fax: +1-434-924-1218 or: Qing-Xiang Amy Sang; Tel: +1-434-924-8683; Fax: +1-850-644-8281; Email: yz5u@virginia.edu; Email: sang@chem.fsu

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breast epithelia adjacent to DCIS and IDC.[11, 12] These results suggest that MMP-26 is involved in the initial invasion of human breast DCIS, and in the spread of prostate carcinoma into surrounding stroma.

Previous reports indicated that MMP-26 might serve as a new molecular marker for the diagnosis of human prostate and breast cancer at early stages.[8,11,12] This present study expanded upon those findings through the quantification of MMP-26 protein expression in tissues from ten common human cancers, in addition to several less-conventional cancers that remain insufficiently explored.

Materials and Methods

The multi-cancer tissue microarray samples

Multi-cancer tissue microarray slides were obtained from Cybrdi, Inc. (Cat #: CC00-01-001, Gaithersburg, Maryland) and Chemicon International (Chemicon Select Tissue Arrays, TMA1010-4, Temecula, CA). All microarray slides were prepared by neutral formalin-fixing and paraffin-embedding procedures, and consequently, no living organisms were present on any single paraffin-embedded array. Section thickness was uniform at 5 m. Each slide obtained from Cybrdi contained 48 array dots (cancer tissues) from 48 individual patients. All samples were selected and confirmed by certified pathologists according to WHO published standards for diagnosis and classification, and pathologic grades were assigned to every tissue spot on each array slide. A pathologic re-confirmation report was then generated and digital images were captured. The cancer tissue origins were the brain, esophagus, stomach, liver, colon, rectum, lung, kidney, bladder, breast, ovary, uterus, thyroid gland, skin, prostate, and pancreas. Each of the 16 cancer types was represented by three samples, for a total of 48 spots. The Chemicon Select Tissue Arrays contained ten common human cancer tissues, including breast, colon, lung, brain, head and neck, kidney, ovarian, prostate cancers, melanoma, and non-Hodgkin's lymphoma (NHL). Each of the ten cancer types was represented by six duplicate samples (120 spots), and three duplicate samples of normal tissues from each of the ten tissue types (60 spots) were provided on the same slide as control samples, for a total of 180 spots.

Immunohistochemical staining (IHC) of tissue microarray

The polyclonal rabbit anti-human MMP-26-metallodomain IgGs were generated as described previously.[8,10,12] The DAKO EnVision Double-stain System was purchased from DAKO Corporation (Carpinteria, CA), and IHC was performed according to the manufacture's instructions, though slightly modified according to our previous reports.[8,12] Briefly, the paraffin-embedded samples were dewaxed, rehydrated by ethanol gradient, and boiled for 15 min in 0.1 mol/L citric acid for antigen unmasking. The samples were then blocked with DAKO blocking solution for 15 min and incubated with anti-human MMP-26 antibodies (25 μg/mL) at room temperature for 45 min. Sections were then incubated with alkaline phosphatase-conjugated or horseradish peroxidase (HRP)-conjugated secondary anti-rabbit antibodies at room temperature for 30 min. Positive signals were detected with Fast-Red or 3, 3'-diaminobenzidine (DAB). Purified pre-immune IgGs from rabbit were used as negative controls.

Densitometric and statistical analysis

Six pictures were taken from each cancerous tissues or normal control tissues after immunohistochemical staining. Quantification of the immunostaining signals was performed using the Metamorph System (version 4.6r8, Universal Imaging Corporation, Inc., West Chester, PA) according to our previous description.[8,12] Briefly, an appropriate color threshold was determined (Color Model: 70–255 for red, 0–255 for green, 80–255 for blue). The cancerous or normal tissues from each image were isolated into closed regions and the selected area was obtained by region measurement. The areas of staining were then measured by integrated morphometry analysis and expressed in terms of optical density (OD), integrated optical density (IOD), and area in pixels. The ratio of the IOD or pixel area of positive signals to the total area of closed region was determined for each image, and the average ratios from each sample were then calculated and used for statistical analysis. Statistical analysis of all samples was performed with the least significant difference correction of analysis of variance (ANOVA) for multiple comparisons. Data represent the mean ± standard error, and $P < 0.05$ was considered significant.

Cell culture and RT-PCR

To confirm the expression of MMP-26 in human smooth muscle cells, coronary artery smooth muscle cells were cultured and harvested, and the total RNA was extracted for reverse transcription-polymerase chain reaction (RT-PCR). cDNA was prepared using SuperScript™ First-Strand Synthesis Systems for RT-PCR (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions. For MMP-26, the forward primer was 5'-CAGCTCGTCATCTTAAGAGTTAC-3' and the reverse primer was 5'-AGGTATGTCAGATGAACATTTTTC-3'; for GAPDH, the forward primer was 5'-ACGGATTTGGTCGTATTGGG-3' and the reverse primer was 5'-TGATTTTGGAGGGATCTCGC-3'. PCR reactions were performed using a Biometra Personal Cycler (Biometra, Germany) with 35 thermal cycles of denaturing at 94℃ for 10 s, annealing at 60℃ for 30 s, and elongation at 72℃ for 1 min. The presence of MMP-26 mRNA expression was confirmed by electrophoresis of the resultant samples in a 1% agarose gel.

Results

Validation of the tissue microarray

About 10-12 tissue spots per slide were missing during the staining process, leaving about 170 spots available for scanning and analysis on each slide. For the tissue microarray slides from Cybrdi, Inc. (Cat #: CC00-01-001), about 45 spots were available on each slide after IHC staining, representing a loss of about 3 spots per slide.

Expression of MMP-26 in normal and cancerous colon, lung, breast, prostate, kidney and ovarian tissues

Weak staining for MMP-26 was observed in normal human colon (Fig. 1A), breast, prostate, kidney and ovarian epithelia. There was no significant difference in signal intensity between MMP-26 antibody and pre-immune IgG in these normal epithelia (P>0.05), except for lung epithelia (Fig.1B), which exhibited positive staining for MMP-26 that was significantly higher than that observed for pre-immune IgG. Strong positive signals for MMP-26 were observed in human colon (Fig. 1E), lung (Fig. 1F), breast, and prostate carcinomas. The intensity of MMP-26 signals was significantly increased in the human colon (P< 0.001), lung (P=0.009), breast (P<0.001), and prostate (P= 0.040) carcinomas when compared with the intensity of pre-immune IgG signals. MMP-26 signals were localized to the apical regions of colon carcinoma cells, but were evenly distributed within lung, breast and prostate carcinoma cells. However, the expression of MMP-26 was not significantly elevated in kidney carcinoma (P=0.189) (Fig. 1G) and ovarian carcinoma (P=0.095) (Fig. 1H), when compared with relevant normal tissues (Fig. 1C, 1D).

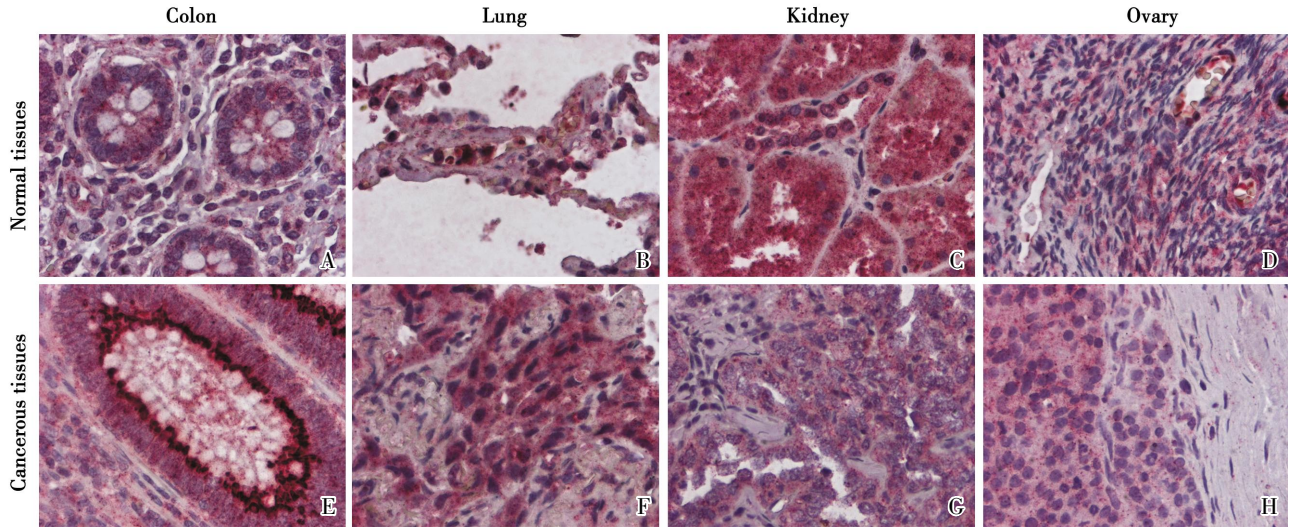

Figure 1 Expression of matrix metalloproteinase-26 (MMP-26) protein in normal and cancerous human colon, lung, kidney, and ovarian tissues (IHC ×40)

A, normal colon tissue; B, normal lung tissue; C, normal kidney tissue; D, normal ovarian tissue; E, well-differentiated colon adenocarcinoma (tubulovillous adenocarcinoma) tissue; F, lung cancer tissue; G, renal cell cancer tissue; H, endometrioid type poorly differentiated ovarian adenocarcinoma tissue. MMP-26-positive cells are stained in red.

Expression of MMP-26 in normal and cancerous brain, head and neck, lymphocytic and skin tissues

Weak staining for MMP-26 was observed in normal human brain (Fig. 2A), head and neck (Fig. 2B), lymphocytic (Fig. 2C), and skin tissues (Fig. 2D). There was no significant difference in signal intensity between MMP-26 antibody and pre-immune IgG in these normal cells. Strong positive staining for MMP-26 was seen in human brain tumor (P=0.012) (Fig. 2E), head and neck tumor (P=0.040) (Fig. 2F), and melanoma (P=0.021) (Fig. 2H). The difference in signal intensity between MMP-26 antibody and pre-immune IgG was significant in these cancerous samples. However, the expression of MMP-26 in NHL (P=0.163) (Fig. 2G) had no significant difference when compared with normal lymphocytic tissue (Fig. 2C). Relative ratios of signals are presented in Figure 3.

Expression of MMP-26 in some other human cancerous tissues

MMP-26 was also highly expressed in carcinomas of the esophagus (Fig. 4A), pancreas (Fig. 4B), rectum (Fig. 4C), and stomach (Fig. 4D), but not expressed in the remaining samples.

Expression of MMP-26 in circulation systems, smooth muscle cells of blood vessels and prostate tissues

MMP-26 was highly and evenly expressed in the smooth muscle cells of arteries in the lung (Fig. 4E), and in the smooth muscle cells of the prostate (Fig. 4F). Strong signals for MMP-26 were also observed in blood cells (Data not shown). RT-PCR identified a single 780 bp MMP-26 cDNA band in coronary artery smooth muscle cells (Fig. 5). MMP-1, -2, -3, -9, and -14 cDNA bands were also observed in coronary artery smooth muscle cells, while MMP-13 was not detected (Data not shown).

Discussion

MMP-26 in human cancerous tissues

Unlike other MMPs, which are primarily expressed by stromal cells, the expression of MMP-26 is restricted to the epithelia and to tumor cells themselves.[3-6] Thus, MMP-26 may serve as a potential biomarker for carcinoma/adenocarcinoma diagnoses. Here, we have demonstrated that MMP-26 is highly expressed in multiple types of carcinoma tissues, including those of the colon,

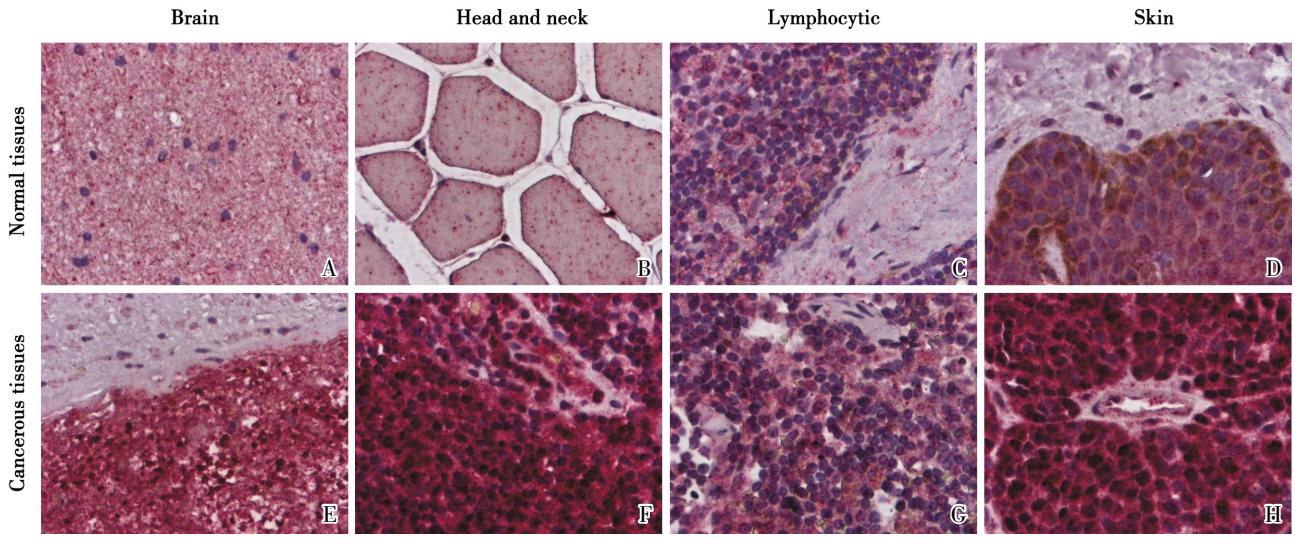

Figure 2 Expression of MMP-26 protein in normal and cancerous human brain, head and neck, lymphcytic, and skin tissues (IHC ×40)

A, normal brain tissue; B, normal head and neck tissue; C, normal lymphcytic tissue; D, normal skin tissue; E, brain astrocytoma (glial cell) tissue; F, follicular lymphoma (mixed cellularity) tissue; G, non-Hodgkin's lymphoma tissue; H, melanoma (small cell type) tissue. MMP-26-positive cells are stained in red.

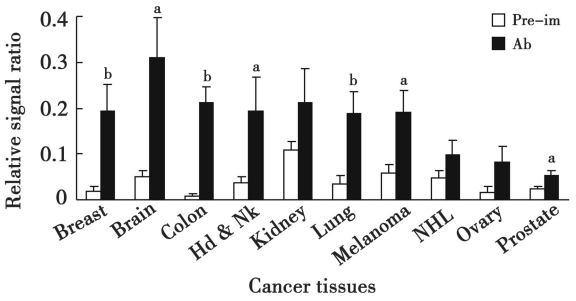

Figure 3 Densitometric quantification analyses of MMP-26 protein expression in ten types of common human cancerous tissues using the Metamorph System

Each value represents the mean ± standard error from three repeat experiments. $^{a}P<0.05$; $^{b}P<0.01$. Ab, antibody; Pre-Im, pre-immune IgG; Hd & Nk, head and neck; NHL, non-Hodgkin's lymphoma.

lung, brain, head and neck, skin, esophagus, rectum, stomach, pancreas, prostate, and breast, further buttressing the potential role for MMP-26 as a tool in cancer diagnoses, particularly in diagnosing cancers of the digestive system. MMP-26 expression in prostate and breast tissues were consistent with our previous finding.[8,12]

Among the cancerous tissues, MMP-26 signals were very strong in some cases and weak in others, which may indicate a variation in expression corresponding to subtypes or grades among these samples, as we have previously demonstrated that MMP-26 was highly expressed in early-stage breast carcinomas, with decreasing expression during the later stages of the disease.[11,12] Our findings in normal and cancerous ovarian tissues also confirmed the recent report that MMP-26 was expressed in both normal and cancerous ovarian tissues.[13] Our results revealed that MMP-26 expression was not elevated in several types of carcinomas, which included renal carcinoma, NHL and ovarian carcinoma. Normal kidney, lymphocytic and ovarian tissues also expressed high level of MMP-26.

According to our results, the clinical application of MMP-26 (use as a diagnostic marker) could be varied according to tissue origins. When MMP-26 is highly expressed in cancerous tissues, but not in corresponding normal tissues, it could be a diagnostic marker for these tumors, including colon, lung, breast carcinomas and gastric adrenal carcinoma. MMP-26 expression can be detected by RT-PCR or real-time PCR at mRNA level, while by immunohistochemistry or Western blot at protein level. When MMP-26 expression is elevated in cancerous tissues, but weak in corresponding normal tissues, the ratio of semi-quantitative MMP-26 expression of tumor tissues (real-time PCR for mRNA or densitometric analysis for protein) to normal tissues can be used for diagnostic application in these tumors, including brain, head and neck, esophageal, rectal, pancreatic, prostate carcinomas and melanoma. When MMP-26 is highly expressed in corresponding normal tissues, it should not be used as diagnostic marker for these tumors, including renal carcinoma, ovarian carcinoma and NHL.

MMP-26 in smooth muscle cells

Interestingly, we found that MMP-26 was also highly expressed in the smooth muscle cells of the prostate and the arteries of the lungs, in addition to blood cells. This finding indicated that MMP-26 may have an undetermined function in these tissues and cells, and because MMP-26 is a relatively new member of the MMP family, many facets of its enzymatic function are still under investigation. In blood vessels, MMPs play an important role in maintaining vessel integrity by breaking down ECM while new matrix is being synthesized, which is necessary to avoid weakening from continuous mechanical stresses. MMPs also contribute to the migration of vascular smooth muscle cells

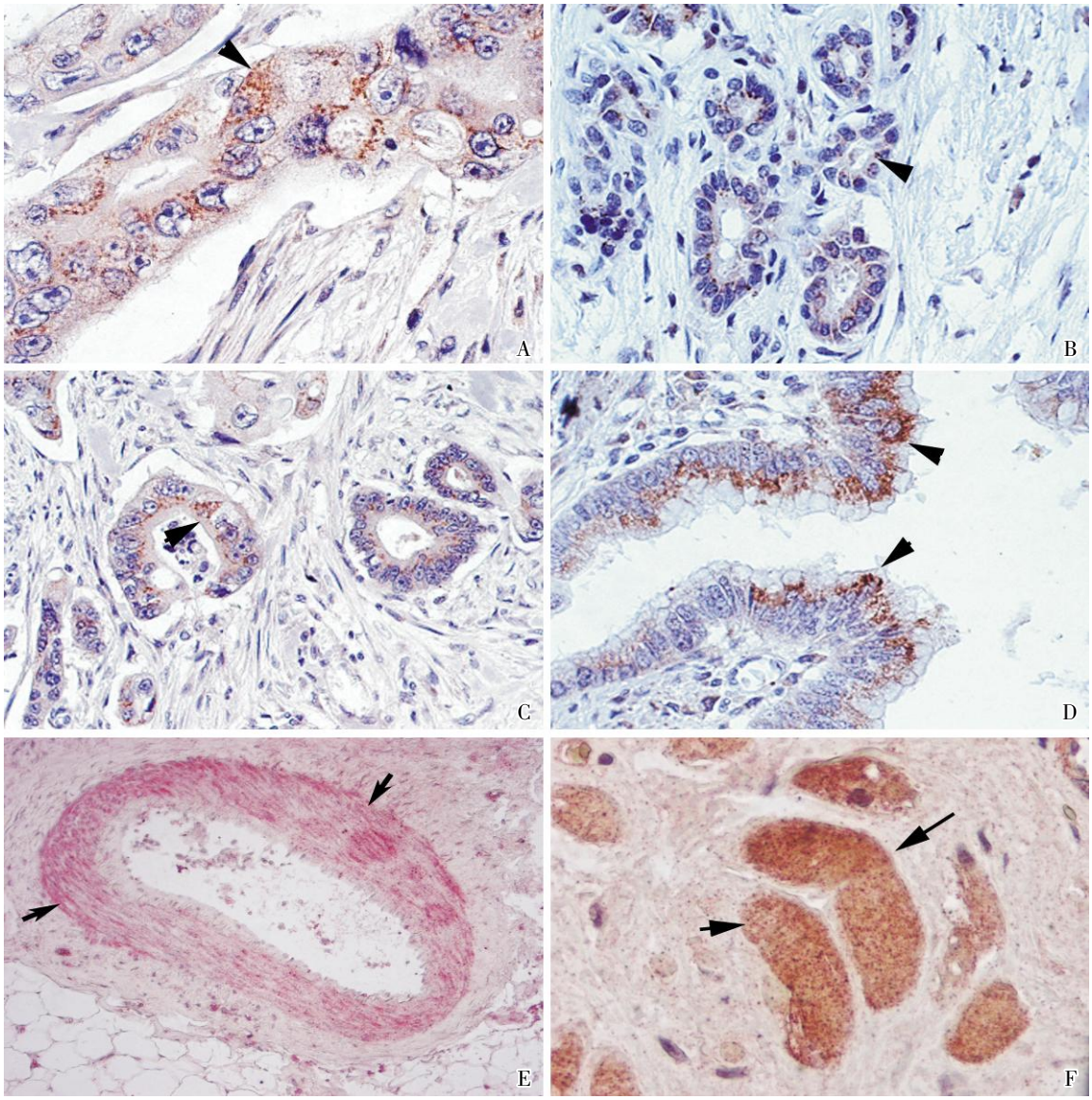

Figure 4 Expression of MMP-26 in various human cancerous tissues (IHC ×40)

A, esophageal carcinoma tissue; B, pancreatic carcinoma tissue; C, rectal carcinoma tissue; D, gastric adrenal carcinoma tissue; E, arterial smooth muscle cells of the lung; F, smooth muscle cells of the prostate gland. MMP-26-positive cells are stained in brown (A-D, F) or red (E). Arrows indicate MMP-26-positive regions.

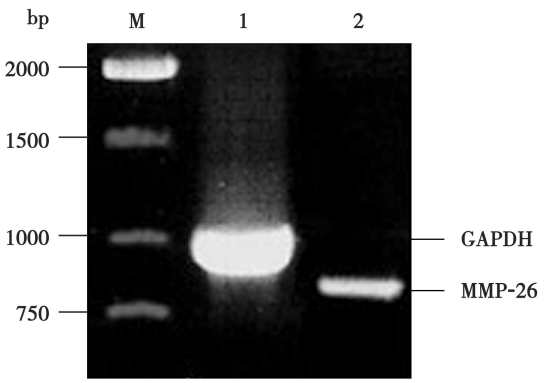

Figure 5 Expression of MMP-26 mRNA in human coronary artery smooth muscle cells

Lane M, marker; lanes 1 and 2, human coronary artery smooth muscle cells.

in vivo, abetting the processes of arteriosclerosis and restenosis after balloon injury.[14] Our results demonstrated that coronary artery smooth muscle cells express MMP-26 mRNA, in addition to several other members of the MMP family, including MMP-1, -2, -3, -9 and membrane-type-1 MMP (MT1-MMP or MMP-14). It has already been shown that MMP-2 and -9 were required for the migration of vascular smooth muscle cells through Matrigel in vitro,[15,16] and MMP-9 expression was increased in response to tumor necrosis factor-α (TNF-α) in aged mouse aortic smooth muscle cells,[17] indicating that MMP-9 expression may play a role in vascular remodeling during aging. Smooth muscle cells produce both MMP-2 and MMP-9, which contribute to smooth muscle cell migration in vitro and neointimal hyperplasia formation in vivo. However, only MMP-9, not MMP-2, is necessary for the organization of collagen by smooth muscle cells.[18] We have previously demonstrated that MMP-26 promoted

prostate cancer invasion through the activation of MMP-9,8 and it was highly possible that MMP-26 played a similar role in smooth muscle cells, contributing to the vascular integrity and remodeling mediated by MMP-9. More recently, Kato et al.19 have implicated MMP-1, a collagenase expressed in smooth muscle cells, in the modulation between a synthetic and a contractile phenotype, as this enzyme was down-regulated when cells began to exhibit an increasingly contractile phenotype. Contraction of prostate glands is modulated by smooth muscle cells, it is therefore possible that MMP-26 is involved in prostate gland contraction and secretion, and understanding the specific roles of MMP-26 in smooth muscle cells could improve the design of therapeutic interventions aimed at controlling vascular and prostate remodeling.

In summary, MMP-26 expression may serve as a molecular marker for the diagnosis of multiple carcinomas, while the enzyme itself may represent a potential new molecular target for therapeutic regimens. Additional studies are needed to identify the clinical significance of elevated MMP-26 expression, and to extract the functions of MMP-26 from the carcinoma and smooth muscle cell systems where it resides.

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