

ORIGINAL PAPERS

Matrix metalloproteases expression in different histological types of colorectal polyps

Noemí Eiró¹, Luis O. González^{1,2}, Sandra Cid¹, Alejandro Andicoechea^{1,3} and Francisco J. Vizoso^{1,3}

¹Research Unit, ²Department of Anatomic Pathology and ³Department of General Surgery. Fundación Hospital de Jove. Gijón, Spain

ABSTRACT

Introduction: Colorectal carcinoma (CC) may begin as benign polyps, which may be classified in different histological types with a different risk to develop cancer. Matrix metalloproteases (MMPs) are able to degrade all components in the extracellular matrix and are important tissue-remodeling enzymes and key elements in tumor invasion and metastasis. The aim of this study was to investigate the expression and clinical relevance of MMPs in different histological types of colorectal polyps.

Methods: The expression levels of MMP-1, 2, 7, 9, 11, 13 and 14 were analyzed by real-time PCR, Western-blot and immunohistochemistry in 50 patients with different histological types of colorectal polyps, 28 of which developed CC.

Results: The results indicate that hyperplastic polyps had the lowest levels of MMP-1 and MMP-7, tubular polyps showed higher levels of both MMP-7 and MMP-14, and tubulovillous adenoma showed higher levels of MMP-1, MMP-7 and MMP-14.

Conclusion: MMP expression was decreased in hyperplastic, tubular and tubulovillous adenoma polyps from patients who developed CC. Our findings suggest that MMP expression may be a pathological marker of colorectal polyps and for cancer susceptibility, which may improve strategies for CC prevention based on screening colonoscopy.

Key words: Colorectal polyps. MMPs. Colorectal cancer. Prognosis. Real-time PCR.

INTRODUCTION

Colorectal carcinoma (CC) is one of the most common cancers in the world, accounting for approximately one million new cases per year in the world (1). CC may begin as polyps that grow from the mucosa and are often non-cancerous, but some can develop into cancer. There are different histological types of colorectal polyps, some of them associated with a higher risk of CC development (serrated, villous adenoma or tubulovillous villous) compared with those associated with a lower cancer risk (hyperplastic and tubular). Colorectal polyps are proliferative lesions

which have been established as the precursor of CC in the general population, although only 5% of adenomas are in danger of becoming malignant (2). Consequently, the rationale for CC screening is that the detection and removal of adenomas interrupts the progression from adenoma to carcinoma and thus prevents cancer (3). Nevertheless, overuse of colonoscopy has a significant cost, and serious complications are estimated to occur in approximately one to five of every 1,000 colonoscopies (4).

Certain pathologic features of polyps have been found to correlate with the risk of finding cancer in polyps. However, it is not yet possible to determine which adenomas will progress to cancer (5), and the molecular mechanisms underlying colorectal carcinogenesis are poorly understood. Therefore, new pathologic markers of risk for colorectal polyps could be of clinical interest. In this line, recent data from our group suggested a possible protective role of toll-like receptor (TLRs) expression against malignant transformation from the colorectal mucosa (6), showing that patients who developed CC had lower TLR7 and/or TLR9 expression in colorectal polyps, regardless of the polyp type, compared with control patients. TLRs are considered to be a link between innate (non-specific) and adaptive (specific) immunity and contribute to the immune system's capacity to efficiently combat pathogens (7), triggering different signaling pathways that induce the release of various immune and inflammatory cytokines such as TNF and IL6, excellent targets in inflammatory diseases (8). Also, it has been described that cytokines can regulate the expression of various proteins, such as matrix metalloproteases (MMPs) (9,10). MMPs are proteolytic enzymes implicated in the degradation of the stromal connective tissue and basement membrane components, which are key aspects in tumor invasion and metastasis (11). MMPs are able to affect tumor cell behavior *in vivo* as a consequence of their ability to cleave growth factors, cell surface receptors, cell adhesion molecules, or chemokines/

Received: 26-08-2016

Accepted: 14-01-2017

Correspondence: Francisco J. Vizoso. Research Unit. Fundación Hospital de Jove. Av. Eduardo Castro, s/n. 33290 Gijón, Asturias. Spain
e-mail: investigacion@hospitaldejove.com

Eiró N, González LO, Cid S, Andicoechea A, Vizoso FJ. Matrix metalloproteases expression in different histological types of colorectal polyps. *Rev Esp Enferm Dig* 2017;109(6):414-420.

DOI: 10.17235/reed.2017.4551/2016

cytokines (12-15). Furthermore, by cleaving proapoptotic factors, MMPs are able to produce a more aggressive phenotype generating apoptosis-resistant cells (16).

Therefore, we consider the analysis of the expression of other molecular factors associated with inflammation and tissue-remodeling pathways in colorectal polyps to be relevant, such as MMPs, which are produced by different cells in the gastrointestinal tract mucosa and have been related to prognosis in CC (17).

The purpose of the present study was to explore MMPs expression in different histological types of colorectal polyps. To address these questions, we used real-time PCR technology and Western-blot analysis. Our findings suggest that MMP expression may be a pathological marker of colorectal polyps and for cancer susceptibility.

PATIENTS AND METHODS

Patient selection, characteristics and tissue specimen handling

This study included a retrospective and selected series of 50 patients with colorectal polyps, who underwent polypectomy by

colonoscopy, from January 1993 until December 2004, in the Department of Surgery of the Fundación Hospital de Jove in Gijón (Spain). Many of these patients had been included in a prior study from our group (6). We excluded patients who had a history of inflammatory bowel disease, hereditary polyposis syndrome, antibiotic use or non-steroidal anti-inflammatory drug use. From patients fulfilling these criteria, we selected a sample size of 50 patients stratified with regard to their polyp type (hyperplastic, tubular, serrated, villous adenoma or tubulovillous) and the development of CC.

The patient's characteristics included in the study are listed in table I. In a total of 28 cases, polyps were obtained from patients who were diagnosed with CC; in ten patients, polypectomy was performed before a diagnosis of CC (median [range] 46 [9-112] months), in 16 patient during diagnosis, and in two patients after the diagnosis (7.5 [6-9] months), as tumors were excised during emergency surgery. In all cases with cancer, polyps were at least 2 cm from the tumor. The control group was constituted by 22 patients who did not develop CC and who underwent a median follow-up period of 7.42 years (range: 5-10 years). Removed polyps (one from each patient) were analyzed. In addition, normal colorectal tissue samples from five patients without colorectal polyps or any other colorectal disease were included in the present study as a control.

The study adhered to national regulations and was approved by our Institution's Ethics and Investigation Committee.

Table I. Basal characteristics of 50 patients with colorectal polyps

<i>Characteristics</i>	<i>Patients without tumor development n (%)</i>	<i>Patients with tumor development n (%)</i>
Number	22 (44)	28 (56)
<i>Mean age (years)</i>		
< 76	13 (59)	13 (46.4)
≥ 76	9 (41)	15 (53.6)
<i>Sex</i>		
Male	18 (81.8)	21 (75)
Female	4 (18.2)	7 (25)
<i>Localization of the polyp</i>		
Sigmoid	7 (31.8)	2 (7.1)
Ascending colon	2 (9.1)	5 (17.9)
Descending colon	1 (4.6)	4 (14.3)
Transversal	3 (13.6)	2 (7.1)
Recto	6 (27.3)	3 (10.7)
Unknown	3 (13.6)	12 (42.9)
Mean number of polyps (range)	2 (1-6)	2.1 (1-6)
<i>Polyp size</i>		
< 1cm	13 (59.1)	18 (64.3)
≥ 1 cm	9 (40.9)	10 (35.7)
<i>Histological grade</i>		
Hyperplastic	4 (18.2)	6 (21.4)
Tubular adenoma	4 (18.2)	6 (21.4)
Serrated adenoma	6 (27.2)	5 (17.9)
Villous adenoma	4 (18.2)	5 (17.9)
Tubulo-villous adenoma	4 (18.2)	6 (21.4)
High grade of dysplasia	2 (9.1)	7 (25)

RNA extraction, cDNA synthesis and real-time PCR

Total RNA was isolated from formalin-fixed, paraffin embedded (FFPE) tissue blocks using the NucleoSpin® FFPE RNA (Macherey-Nagel, Düren, Germany), including DNase treatment, as described previously (6). The integrity of the eluted total RNA was checked by agarose gel electrophoresis and the RNA concentration was determined by spectrophotometry. The ratio of absorbance at 260 and 280 nm, measured by the NanoDrop ND-1000 spectrophotometer (Thermo Scientific), was used as a parameter to quantify and evaluate the quality of the total RNA extracted (values ranging 2.02 and 2.22). First strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cheshire, UK) following the manufacturer's instructions. The reverse transcription step was performed using the following program: 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 sec. Expression levels of MMP-1, 2, 7, 9, 11, 13 and 14 were assessed by real-time PCR using the ABI Prism 7900 HT thermocycler (Applied Biosystems, Cheshire, UK), 200 ng of cDNA and Fast SYBR Green Master Mix (Applied Biosystems, Cheshire, UK) with the following cycling conditions: 95 °C for 20 sec, 40 cycles of 95 °C for 1 sec and 60 °C for 20 sec. The primers used are described in table II. All real-time PCR were performed in triplicate and the amplification signal from the target was normalized using β -actin as a control. The SDS RQ Manager Program (Applied Biosystems) was used to analyze the results. The PCR products were separated on 2% agarose gels containing ethidium bromide (0.5 μ g/ml).

Protein extraction

Protein extraction from FFPE samples was carried out as described previously (18). Five FFPE tissue sections (50 μ m each) were deparaffinized by adding 0.5 ml of xylene and incubated for eight minutes at 60 °C. Xylene was removed and sections were rehydrated in successive washes in 100%, 96%, and 70% ethanol and water. Water was removed by centrifugation, and the residuum dried in a hood for 2-3 minutes. After that, 200 μ l of 20 mM Tris-HCl buffer (pH 9) containing 2% sodium dodecyl sulfate (SDS) were added to the dewaxed FFPE tissue sections, followed by heating at 100 °C on a heat block (Fisher Scientific; Pittsburgh, PA) for 20 minutes, and then incubated at 60 °C in an Eppendorf Thermomixer (shaking at 650 rpm) for two hours (19). Protein concentrations were

determined performing the Bradford method and using bovine serum albumin (BSA) as a standard (Sigma, St. Louis, MO). The samples were treated according to the manufacturer's "microwell" protocol and the absorbance was read at 495 nm on a plate reader (Bio-Rad, Munich, Germany).

Western-blot analysis

Protein samples (40 μ g/lane) were separated by SDS-PAGE using 10% acrylamide resolving and 4% stacking gels. After electrophoresis, proteins were blotted onto 0.45 μ m nitrocellulose membranes (BioRad Laboratories). Nonspecific binding sites were blocked by incubating the nitrocellulose membranes for one hour with 5% nonfat milk in Tris-buffered saline containing 0.05% Tween-20 (TBS-T). The membranes were then washed twice with TBS-T and incubated overnight with antibodies against human MMP-1 (1/400), MMP-7 (1/300), MMP-14 (1/600) and β -actin (1/700) (Santa Cruz Biotechnology, Inc.) diluted in 5% nonfat milk in TBS-T. The membranes were subsequently washed with TBS-T and incubated for one hour with protein A-peroxidase (Sigma) in 5% nonfat milk in TBS-T. Protein bands were detected using the Immobilon Western Chemiluminescent HRP substrate (Millipore, Bedford, MA) with subsequent exposure to X-ray film.

Immunohistochemistry

All specimens were routinely fixed in 10% neutral buffered formalin and stored after being embedded in paraffin at room temperature before further testing was performed. Serial 5-mm sections were consecutively cut in a microtome (Leica Microsystems GmbH, Wetzlar, Germany) and transferred to adhesive-coated slides. Immunohistochemistry was performed on these sections using a TechMate TM50 autostainer (Dako, Glostrup, Denmark). For MMP7 and MMP14 staining, tissue sections were deparaffinized in xylene, and then rehydrated in graded concentrations of ethyl alcohol (100%, 96%, 80%, 70%, and water). The dilution for each antibody was 1/50 for MMP7 (MAS-14215, ThermoScientific) and 1/100 for MMP14 (GTX61603, Genetex). All the dilutions were made in antibody diluent (Dako), and incubated for 60 minutes at room temperature.

Endogenous peroxidase activity was blocked by incubating the slides in peroxidase-blocking solution (Dako) for five min. The

Table II. Real-time PCR primers

Gene product	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
MMP1	CAGTGGTGATGTTCTAGCTAGCTCA	GCCGATGGGCTGGACA
MMP2	GAGGACTACGACCGCGACAA	CTTCACTTTCTGGGCAACAA
MMP7	GTGGGAACAGGCTCAGGACTATCTCAA	CACATCTGGGCTTCTGCATTATTACTA
MMP9	CCTGGAGACCTGAGAACCAATC	GATTTCGACTCTCCACGCATCT
MMP11	GAGCAGGTGCGGCAGACGA	GAGCAGGTGCGGCAGACGA
MMP13	ATCCCTTGATGCCATTACCA	AAGAGCTCAGCCTCAACCTG
MMP14	GCCCAATGGGAAGACCTACT	AGGGTACTCGCTGTCCACTG
β -actin	GGCACCCAGCACAATGAAG	CCGATCCACACGGAGTACTTG

EnVision Detection Kit (Dako) was used as the staining detection system. Sections were counterstained with hematoxylin, dehydrated with ethanol, and permanently covered with a cover slip. For each antibody preparation tested, the stained cell type was determined. We distinguished stromal cells from epithelial cells because the latter are larger in size and are closely packed and arranged in one or more layers. In addition, fibroblasts are spindle shaped whereas mononuclear inflammatory cells are rounded.

Data analysis and statistical methods

All values are expressed as means $\pm$ standard deviation (SD). Significant differences among experimental groups of $p \leq 0.05$ are indicated. We analyzed the distribution of variables by the Kolmogorov-Smirnov test. On the basis of this analysis, parametric methods (unpaired Student's t-test and ANOVA test) or non-parametric methods (Mann-Whitney or Kruskal-Wallis analysis) were used for comparison between groups. Student's t-test and one way ANOVA was used to determine significant differences in mRNA expression between the different groups. Bonferroni post-hoc test was performed. Differences in relative RNA levels were calculated with the Mann-Whitney or Kruskal-Wallis analysis. The SPSS 18.0 program was used for all calculations.

RESULTS

In the present study, we analyzed the expression levels of different MMPs in 50 patients with colorectal polyps. We found several significant differences depending on the histological type of colorectal polyp (hyperplastic, tubular, serrated, villous adenoma or tubulovillous).

We first analyzed the expression of seven MMPs (MMP-1, 2, 7, 9, 11, 13 and 14) by real-time PCR in colorectal polyps compared to normal colorectal tissue, but only MMP-1, 7 and 14 could be detected in our colorectal polyp samples. As shown in figure 1, MMPs expression is increased at least two fold in colorectal polyps compared with normal colon tissue, excluding MMP-1 expression

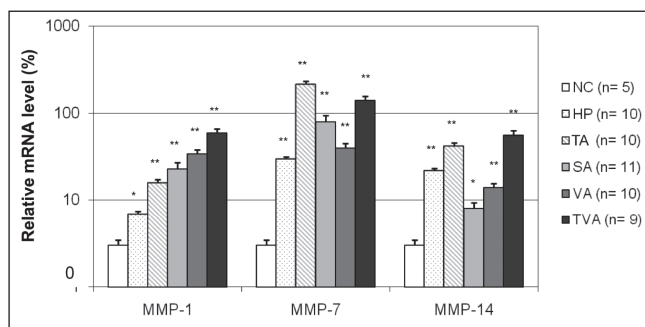


Fig. 1. MMP-1, MMP-7 and MMP-14 gene expression measured by real-time PCR in normal colon tissue (NC) or in different types of colorectal polyps: hyperplastic polyps (HP), tubular adenoma (TA), serrated adenoma (SA), villous adenoma (VA) and tubulo-villous adenoma (TVA). * $p \leq 0.05$; ** $p \leq 0.01$.

in hyperplastic polyps. The MMP expression profile differed depending on the histological type of polyps. The results showed that hyperplastic polyps had lower levels of MMP-1 and 7 than the other polyp types; moreover, a higher MMP-1 expression was found in histological types of polyps associated with cancer risk (serrated, villous or tubulovillous adenomas) compared with polyps associated with a lower cancer risk (both hyperplastic and tubular). Tubular adenomas showed high levels of both MMP-7 and 14, and tubulovillous adenomas showed high levels of MMP-1, 7 and 14.

Our results showed no significant relationship between MMPs expression and different characteristics of polyps, such as location, size or grade of dysplasia (data not shown).

In the present study we also consider of interest the analysis of possible changes in MMPs expression in polyps from patients who developed CC. Thus, as shown in figure 2, we compared MMP expression by real-time PCR between polyps corresponding to patients with and those without associated CC, according to each type of colorectal polyp. MMP-1, 7 and 14 expressions were decreased in hyperplastic, tubular and villous adenoma polyps from patients who developed CC, compared to patients who did not develop CC (NCC). With regard to patients with serrated adenoma polyps, patients who developed CC had lower MMP-7 expression but higher MMP-14 expression, whereas the expression of MMP-1 was not altered. We found no significant differences in MMPs expression in tubulovillous adenoma polyps between patients with or without CC.

The differences on MMP expression levels between the various histological types of polyps from patients who did not develop CC and patients who developed CC were subsequently analyzed by Western-blot analysis (Fig. 3). These analyses confirm the previous results obtained by real-time PCR, as MMP protein levels were lower in hyperplastic, tubular and villous adenoma polyps from patients who developed CC. Also, in patients with serrated polyps, patients who developed CC showed decreased MMP-7 expression but higher MMP-14 expression. We found that MMP-7 and MMP-14 were mainly expressed by epithelial cells (76% and 82% of positive cases, respectively); less frequently by MICs (34% and 37% of positive cases, respectively), and no expression by fibroblasts (Fig. 4). No significant correlation was found between expression by cell type and histological type or tumor development (data not shown).

DISCUSSION

This study is, to the best of our knowledge, the first analyzing a wide range of MMPs expression in different histological types of colorectal polyps. We found differences in MMPs expression levels depending on the histological type of polyps, but also related to CC development.

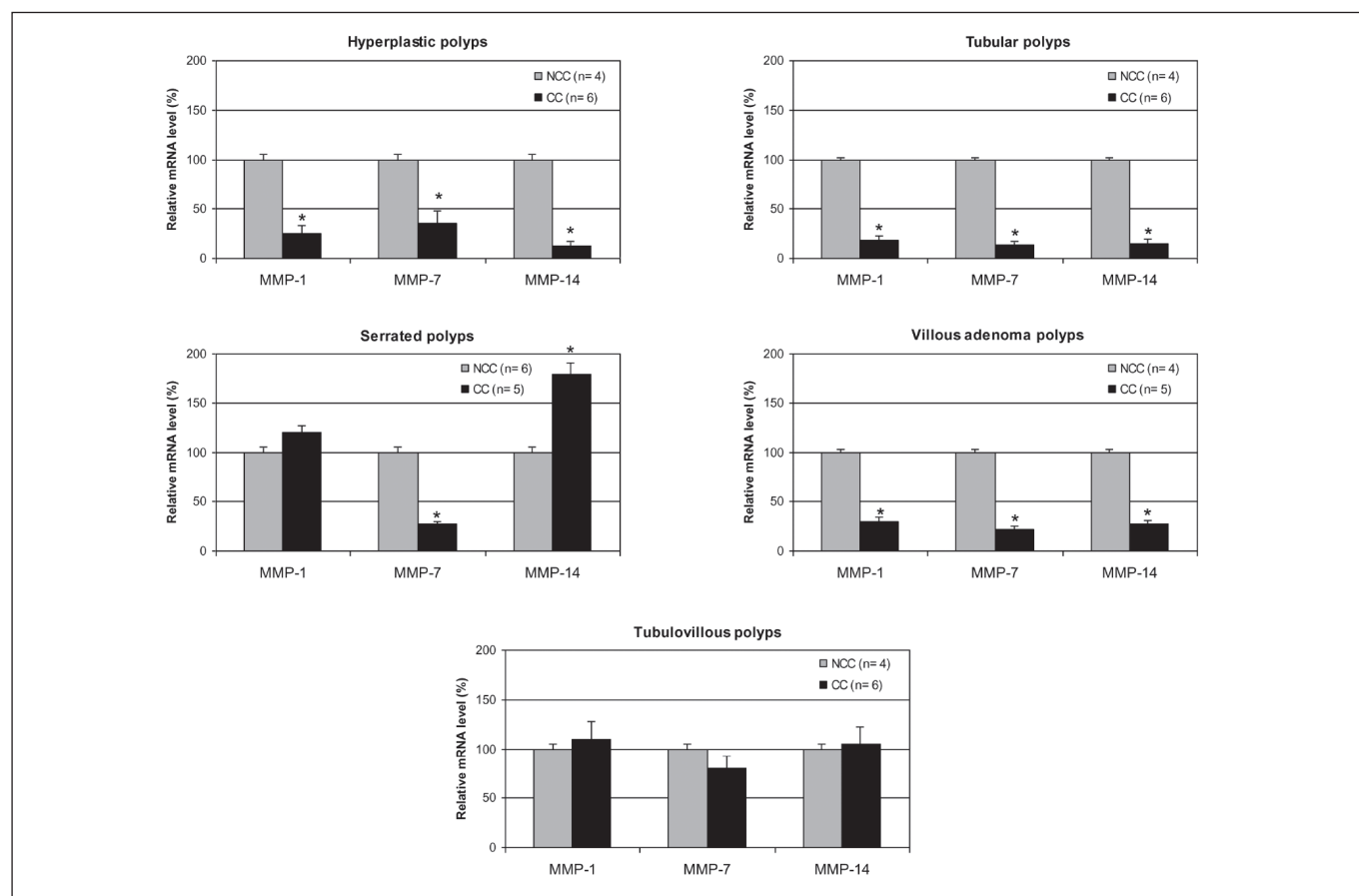


Fig. 2. MMP-1, MMP-7 and MMP-14 expression measured by real-time PCR in the different polyp types from patients who developed colorectal cancer (CC) compared to patients who did not develop CC (NCC). * $p \leq 0.05$ was considered as significant.

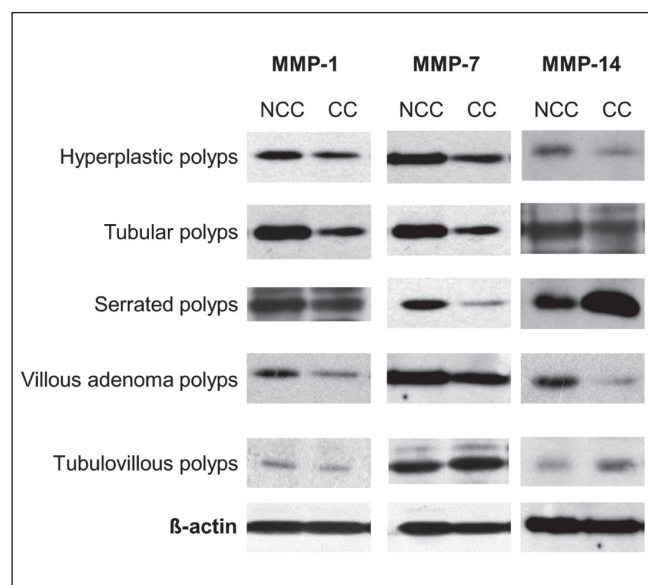


Fig. 3. Expression of MMP1, MMP7 and MMP14 analyzed by Western-blot in the different polyp types from patients who developed colorectal cancer (CC) and from patients who did not develop CC (NCC). β-actin was used as internal loading control.

MMP-1, 7 and 14 were the proteases which were variably expressed among the different histological types of colorectal polyps, showing a different pattern of expression between them. Higher MMP-1 (interstitial collagenase) expression was found in histological types of polyps associated with cancer risk (serrated, villous or tubulovillous adenomas). In addition, higher MMP-7 (matrilysin) and MMP-14 (type I membrane MMP) expression was found in both tubular and tubulovillous polyp types, indicating that MMPs likely play important roles in tissue pathophysiology in the gastrointestinal tract. Our finding about the relationship between low MMP expression by hyperplastic polyps and the development of CC is consistent with recent studies that associate some hyperplastic polyps with an increased risk of CC development (20-22). Serrated colorectal polyps comprise a family of lesions with some histological similarities (23), and have been suggested as precursor lesions for CC (23,24). Our data indicate that both MMP-7 and MMP-14 expression may represent new pathologic markers of cancer risk in this colorectal polyp type. MMPs mediate cell migration, tissue damage, remodeling and repair (25). Therefore, these different MMP expression patterns among the different

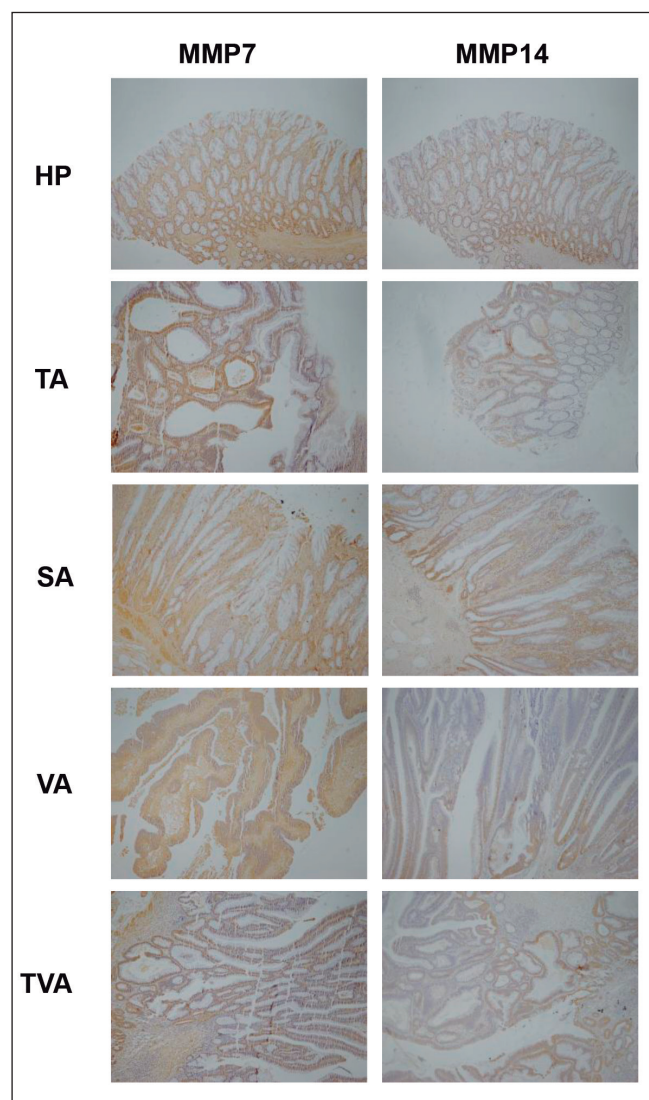


Fig. 4. Examples of immunohistochemical staining for MMP7 and MMP14 in colorectal polyps samples: hyperplastic polyps (HP), tubular adenoma (TA), serrated adenoma (SA), villous adenoma (VA) and tubulovillous adenoma (TVA). All were positive cases.

histological types of colorectal polyps may be due to a diverse pathophysiology among them, and thus implicate different matrix degrading activities.

Considering the clinical difficulty to explore the relationship between the expression of molecular factors by one colorectal polyp and its subsequent CC transformation, we consider it reasonable to assess this predisposition by investigating the possible relationship between MMPs expression by polyps and the occurrence of CC in these patients. With this perspective, our results also indicated that MMP-1, 7 and 14 expression by hyperplastic, tubular or villous adenoma polyps was lower in patients with associated cancer compared to patients without associated cancer, suggesting a possible role of MMPs against cancer development. These findings are in apparent contradiction with previous studies

showing that MMPs are over-expressed in CC. However, these studies were performed in the colorectal mucosa (26,27) instead of in the colorectal polyps. Some studies have shown the prognostic relevance of these proteins in CC, such as MMP-1, 7, 9, 11, 13 and 14, TIMP-1 and 2 (17,28-32). Based on these data in CC, we could raise the question of whether high MMP expression levels in colorectal polyps may influence carcinogenesis from these epithelial lesions. However, several experimental evidences have demonstrated roles for MMPs at all stages of tumor development in both pro- and anti-tumorigenic roles (33-35).

On the basis of our results, we can speculate that MMPs may have an anti-tumorigenic role in benign colorectal polyps. Continuous renewal of the intestinal epithelium requires coordinated regulation between different signaling pathways and molecules to maintain the balance between proliferation and differentiation of epithelial stem cells and immature progenitor cells. In this context, MMPs have been shown to be pro-apoptotic while in other cases they may be anti-apoptotic (14,36), contradictory results that may be partially explained by the different cell and tissue systems under examination. It is also of note that several MMPs, such as MMP-3, 9, 11 or 19, which were originally described as pro-tumorigenic enzymes, appear to play dual roles in cancer and exhibit protective roles in some specific circumstances (33). In the case of MMP-9, the pro-tumorigenic role was demonstrated by Sinnamon et al., since APC-Min mice genetically deficient in MMP-9 expression had fewer tumors than their littermate controls (37); likewise, the anti-tumorigenic role was confirmed by Garg et al., since they reported a protective role and a tumor suppressor function of MMP-9 in colitis-associated colon cancer by modulation of Notch activation (38).

On the other hand, although increased MMPs expression has been reported at early stages of CC (39), our results led us to consider that these different patterns in MMPs expression may be related to the pathophysiological tissue removal in each polyp type or to a specific signaling pathway related to TLR activation. In this regard, deregulation of TLRs has also been associated with the development of CC (6). TLRs trigger different signaling pathways that induce the release of various cytokines (8), which can regulate the expression of various proteins, such as MMPs.

We consider that our findings, although clinically preliminary, demonstrate significant differences in MMPs expression in various colorectal polyp types. With regard to the development of CC, this may lead to further studies to explore the possible clinical interest of MMPs as pathological markers of colorectal polyps and for cancer susceptibility, which may improve strategies for CC prevention based on screening colonoscopy.

ACKNOWLEDGMENTS

This work was supported by grants from: Fondo de Inversión Sanitaria del Instituto de Salud Carlos III

(FIS-PI070306) and FICEMU. Sandra Cid was a recipient of a fellowship financed by the Gobierno del Principado de Asturias "Severo Ochoa" PhD Program.

REFERENCES

- Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60(5):277-300. DOI: 10.3322/caac.20073
- Bujanda L, Cosme A, Gil I, et al. Malignant colorectal polyps. *World J Gastroenterol* 2010;16(25):3103-11. DOI: 10.3748/wjg.v16.i25.3103
- Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: Clinical guidelines and rationale. Update based on new evidence. *Gastroenterol* 2003;124(2):544-60. DOI: 10.1053/gast.2003.50044
- Levin TR, Zhao W, Conell C, et al. Complications of colonoscopy in an integrated health care delivery system. *Ann Intern Med* 2006;145(12):880-6. DOI: 10.7326/0003-4819-145-12-200612190-00004
- Markowitz AJ, Winawer SJ. Screening and surveillance for colorectal carcinoma. *Hematol Oncol Clin North Am* 1997;11(4):579-608. DOI: 10.1016/S0889-8588(05)70452-4
- Eiro N, González L, González LO, et al. Study of the expression of toll-like receptors in different histological types of colorectal polyps and their relationship with colorectal cancer. *J Clin Immunol* 2012;32(4):848-54. DOI: 10.1007/s10875-012-9666-3
- Kawai T, Akira S. TLR signaling. *Cell Death Differ* 2006;13(5):816-25. DOI: 10.1038/sj.cdd.4401850
- O'Neill LA, Bryant CE, Doyle SL. Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer. *Pharmacol Rev* 2009;61(2):177-97. DOI: 10.1124/pr.109.001073
- Ries C, Petrides PE. Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction in disease. *Biol Chem Hoppe Seyler* 1995;376(6):345-55.
- Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 2004;16(5):558-64. DOI: 10.1016/j.ccb.2004.07.010
- Nelson AR, Fingleton B, Rothenberg ML, et al. Matrix metalloproteinases: Biologic activity and clinical implications. *J Clin Oncol* 2000;18(5):1135-49.
- Manes S, Llorente M, Lacalle RA, et al. The matrix metalloproteinase-9 regulates the insulin-like growth factor-triggered autocrine response in DU-145 carcinoma cells. *J Biol Chem* 1999;274(11):6935-45. DOI: 10.1074/jbc.274.11.6935
- Noe V, Fingleton B, Jacobs K, et al. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci* 2001;114(Pt 1):111-8.
- Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002;2(3):161-74. DOI: 10.1038/nrc745
- Türk V, Kos J, Türk B. Cysteine cathepsins (proteases) - On the main stage of cancer? *Cancer Cell* 2004;5(5):409-10.
- Fingleton B, Vargo-Gogola T, Crawford HC, et al. Matrilysin (MMP-7) expression selects for cells with reduced sensitivity to apoptosis. *Neoplasia* 2001;3(6):459-68. DOI: 10.1038/sj.neo.7900190
- González L, Eiro N, González LO, et al. Effect of the expression of matrix metalloproteinases and their tissue inhibitors on survival of patients with resectable colorectal cancer. *Dig Dis Sci* 2012;57(8):2063-71. DOI: 10.1007/s10620-012-2154-z
- Eiro N, González-Reyes S, González L, et al. Duodenal expression of Toll-like receptors and interleukins are increased in both children and adult celiac patients. *Dig Dis Sci* 2012. DOI: 10.1016/j.neuron.2012.05.018
- Shi SR, Liu C, Balgley BM, et al. Protein extraction from formalin-fixed, paraffin-embedded tissue sections: Quality evaluation by mass spectrometry. *J Histochem Cytochem* 2006;54(6):739-43. DOI: 10.1369/jhc.5B6851.2006
- Liljgren A, Lindblom A, Rotstein S, et al. Prevalence and incidence of hyperplastic polyps and adenomas in familial colorectal cancer: Correlation between the two types of colon polyps. *Gut* 2003;52(8):1140-7. DOI: 10.1136/gut.52.8.1140
- Lim HK, Lee KH, Kim SY, et al. Does the amount of tagged stool and fluid significantly affect the radiation exposure in low-dose CT colonography performed with an automatic exposure control? *Eur Radiol* 2011;21(2):345-52.
- Omata F, Deshpande G, Tokuda Y, et al. Meta-analysis: Somatostatin or its long-acting analogue, octreotide, for prophylaxis against post-ERCP pancreatitis. *J Gastroenterol* 2010;45(8):885-95. DOI: 10.1007/s00535-010-0234-4
- Torlakovic E, Snover DC. Sessile serrated adenoma: A brief history and current status. *Crit Rev Oncog* 2006;12(1-2):27-39. DOI: 10.1615/CritRevOncog.v12.i1-2.30
- Jass JR. Serrated adenoma of the colorectum and the DNA-methylator phenotype. *Nat Clin Pract Oncol* 2005;2(8):398-405. DOI: 10.1038/npcn0248
- Castaneda FE, Walia B, Vijay-Kumar M, et al. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: Central role of epithelial-derived MMP. *Gastroenterol* 2005;129(6):1991-2008. DOI: 10.1053/j.gastro.2005.09.017
- Pesta M, Topolcan O, Holubec L, et al. Clinicopathological assessment and quantitative estimation of the matrix metalloproteinases MMP-2 and MMP-7 and the inhibitors TIMP-1 and TIMP-2 in colorectal carcinoma tissue samples. *Anticancer Res* 2007;27(4A):1863-7.
- Asano T, Tada M, Cheng S, et al. Prognostic values of matrix metalloproteinase family expression in human colorectal carcinoma. *J Surg Res* 2008;146(1):32-42. DOI: 10.1016/j.jss.2007.02.011
- Murray GI, Duncan ME, O'Neil P, et al. Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. *Nat Med* 1996;2(4):461-2. DOI: 10.1038/nm0496-461
- Lyall MS, Dundas SR, Curran S, et al. Profiling markers of prognosis in colorectal cancer. *Clin Cancer Res* 2006;12(4):1184-91. DOI: 10.1158/1078-0432.CCR-05-1864
- Jensen SA, Vainer B, Bartels A, et al. Expression of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinases 1 (TIMP-1) by colorectal cancer cells and adjacent stroma cells - Associations with histopathology and patients outcome. *Eur J Cancer* 2010;46(18):3233-42. DOI: 10.1016/j.ejca.2010.07.046
- Chu D, Zhao Z, Zhou Y, et al. Matrix metalloproteinase-9 is associated with relapse and prognosis of patients with colorectal cancer. *Ann Surg Oncol* 2012;19(1):318-25. DOI: 10.1245/s10434-011-1686-3
- Akishima-Fukasawa Y, Ishikawa Y, Akasaka Y, et al. Histopathological predictors of regional lymph node metastasis at the invasive front in early colorectal cancer. *Histopathol* 2011;59(3):470-81. DOI: 10.1111/j.1365-2559.2011.03964.x
- López-Otin C, Matrisian LM. Emerging roles of proteases in tumor suppression. *Nat Rev Cancer* 2007;7(10):800-8. DOI: 10.1038/nrc2228
- Martin MD, Matrisian LM. The other side of MMPs: Protective roles in tumor progression. *Cancer Metastasis Rev* 2007;26(3-4):717-24. DOI: 10.1007/s10555-007-9089-4
- Noel A, Jost M, Maquoi E. Matrix metalloproteinases at cancer tumor-host interface. *Semin Cell Dev Biol* 2008;19(1):52-60. DOI: 10.1016/j.semcdb.2007.05.011
- Jiang Y, Goldberg ID, Shi YE. Complex roles of tissue inhibitors of metalloproteinases in cancer. *Oncogene* 2002;21(14):2245-52. DOI: 10.1038/sj.onc.1205291
- Sinnamon MJ, Carter KJ, Fingleton B, et al. Matrix metalloproteinase-9 contributes to intestinal tumorigenesis in the adenomatous polyposis coli multiple intestinal neoplasia mouse. *Int J Exp Pathol* 2008;89(6):466-75. DOI: 10.1111/j.1365-2613.2008.00621.x
- Garg P, Sarma D, Jeppsson S, et al. Matrix metalloproteinase-9 functions as a tumor suppressor in colitis-associated cancer. *Cancer Res* 2010;70(2):792-801. DOI: 10.1158/0008-5472.CAN-09-3166
- Jeffery N, McLean MH, El-Omar EM, et al. The matrix metalloproteinase/tissue inhibitor of matrix metalloproteinase profile in colorectal polyp cancers. *Histopathol* 2009;54(7):820-8. DOI: 10.1111/j.1365-2559.2009.03301.x