Int J Clin Exp Med 2014;7(9):2804-2811
www.ijcem.com /ISSN:1940-5901/IJCEM0001298

Original Article

Role of human neutrophil gelatinase associated lipocalin (NGAL) and Matrix Metalloproteinase-9 (MMP-9) overexpression in neoplastic colon polyps

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Received July 3, 2014; Accepted August 16, 2014; Epub September 15, 2014; Published September 30, 2014

Abstract: To explore the role of Human neutrophil gelatinase associated lipocalin (NGAL) and Matrix Metalloproteinase-9 (MMP-9) overexpression in neoplastic polyps and might used as a marker to separate those from non-noeplastic polyps. The study was performed on total 65 cases, 40% (n = 26) of them females and 60% (n = 39) of them males, in Haydarpasa Numune Education and Research Hospital between March 2012 and June 2012. The assessment of immunostained sections was performed by a random principle by one experinced pathologists to the clinico-pathological data. NGAL expression was based on the presence of cytoplasmic and membranous staining. The NGAL intensities of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). The NGAL prevalences of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). The NGAL ID scores of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). We could hypothesize that NGAL and MMP-9 overexpression in neoplastic polyps might be used as a marker to separate those from non-noeplastic polyps. However, in this study, we determined that NGAL overexpression could not distinguish dysplasia from adenocancer. Finally, we suggest NGAL and MMP-9 as an immunohistochemical marker for colonic dysplasia. To determine dysplasia in early steps of colorectal adenoma-carcinoma sequence, it could help to determine new targets in preventive cancer therapy for colorectal cancer. We suggest development of standards for study method, introduction to routine practice by investigating in future studies including many patients.

Keywords: NGAL, MMP-9, colorectal polyps

Introduction

Colorectal Cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females and rates are significantly higher in males than in females [1]. Although death rates from CRC have declined progressively since the mid-1980s in many western countries, mortality rates continue to increase in devoloping countries [2, 3]. This outcome improvement in western countries can be attributed partially to detection and removal of colonic polyps [4]. Therefore, screening and surveillance strategies become very important in devoloping countries like Turkey [5].

Adenomatous polyps are neoplastic polyps [6]. About two-thirds of all colonic polyps are adenomas [7]. Mostly all colorectal cancers arise from adenomas, but nearly 5% percent of adenomas progress to cancer [8]. Adenomas are by definition dysplastic and their malignant potential changes according to be with advanced pathology or not [9]. Advanced adenoma is an adenoma with high-grade dysplasia, an adenoma that is > 10 mm in size, or an adenoma with

Table 1. Distribution of descriptive characteristics

		n	%
Age	≤ 70 years	52	80.0
	> 70 years	13	20.0
Gender	Female	26	40.0
	Male	39	60.0
Primary Tumor Site	Right Colon	19	29.2
	Left Colon	46	70.8
Tumor Size	*Min-Max*	3.0	70.0
	Mean±SD	14.77	16.15
Pathological Results	Dysplasia	36	55.4
	Hyperplasia Polyp	14	21.5
	Adenocarcinoma	15	23.1
Biopsy Grade (n = 15)	Good	2	13.3
	Poor	13	86.7
N Stage (n = 15)	Present	9	60.0
	Absent	6	40.0
Tumor Depth (n = 15)	T1	2	13.3
	T2	2	13.3
	T3	6	40.0
	T4	5	33.4
LVI (n = 15)	Present	9	60.0
	Absent	6	40.0
PNI (n = 15)	Present	8	53.3
	Absent	7	46.7
Anemia (n = 15)	Present	12	80.0
	Absent	3	20.0
Malignancy (n = 15)	Present	2	13.3
	Absent	13	86.7

a villous component [10]. However, there is insufficient data about the malignant transformation of adenoma into carcinoma.

Human neutrophil gelatinase associated lipocalin (NGAL), also known as 24p3, human neutrophil lipocalin (HNL) was isolated by activated human neutrophils in 1994 [11]. NGAL is a new member of the lipocalin family which are small secreted proteins that act as carriers, transporting mainly small lipophilic molecules, has a key role in cell hemosteosis [12]. Recent studies determined that NGAL plays major role in cancerogenesis by reducting in the amount of intracellular iron which is known as a key process in cell apoptosis [13-15].

Studies that focus on human colon tissues, demonstrated that NGAL expression is upregulated in patients with colorectal cancer [16, 17]. Altough these studies determined that the normal colon does not express NGAL, its expression appears during low grade dysplasia and increases progressively through advanced adenoma to cancer. Therefore they suggest that NGAL may play a role in the progression of colorectal cancer. The relationship between NGAL expression and cancerogenesis also may be attributed to the forming of the NGAL/MMP-9 complex [18]. Therefore, NGAL can protect MMP-9 from proteolytic degradation which is a normal physiological mechanism of key importance in controlling the activity of this protein. Consequently, the enzymatic activity of MMP-9 would be potencialized and this can explain the accelerating tumoral invasiveness associated with NGAL overexpression [19]. The purpose of our current study was to explore the role of NGAL in the malignant transformation of adenoma into carcinoma and also NGAL and MMP-9 overexpression in neoplastic polyps might be used as a marker to separate those from non-noeplastic polyps.

Material and methods

The study was performed on total 65 cases, 40% (n = 26) of them females and 60% (n = 39) of them males, in Haydarpasa Numune Education and Research Hospital between March 2012 and June 2012. The ages of the cases range between 34 and 87 years and the mean age is 62.03 ± 10.76 years.

While 80.0% (n = 52) of the cases are ≤ 70 years old, 20.0% (n = 13) of the cases are > 70 years old. When the primary tumor sites are investigated; 29.2% (n = 19) of them is located in the right-hand side of the colon and 70.8% (n = 46) of them is located in the left-hand side of the colon. When the pathological results are investigated; 55.4% (n = 36) of them are dysplasia, 21.5% (n = 14) of them are control and 23.1% (n = 15) of them are adenocarcinomas. While 60.0% (n = 9) of the cases have N stage, 40.0% (n = 6) of them do not have N stage. When the depth of tumor invasion is investigated; 13.3% (n = 2) of the tumors are T1, 13.3% (n = 2) of them are T2, 40.0% (n = 6) of them are T3 and 33.4% (n = 5) of them are T4. Lymphovascular invasion (LVI) is seen in 60.0% (n = 9) of the cases, perineural invasion (PNI) is seen in 53.3% (n = 8) of them, anemia is seen in 80.0% (n = 12) of them and malignancy is seen in 13.3% (n = 2) of them. The distribution of the descriptive characteristics of the cases included in the study is shown in **Table 1**.

Immunohistochemistry

In our study, paraffin blocks consisting suitable areas were selected by evaluating H&E stained archival biopsy specimens. Four-micron-thick sections were taken from the blocks on "poly-l-lysine" coated slides. Slides were deparaffinized by leaving in the incubator at 56°C overnight. Deparaffinization procedure was continued with leaving in xylene for 3 × 10 minutes each and then in alcohol for 3 × 10 minutes each. Slides were washed in the distilled water. Solution prepared by the addition of 10 ml EDTA buffer solution to 90 ml distilled water was used for antigen retrieval. The sections were placed in this solution and boiled in the pressure cooker. And then it was allowed to wait outside to reach room temperature for 20 minutes. To prevent the overflow of the reagents out of the section, the slides was washed with distilled water and around tissue sections on them was drawn by using the "DAKO Pen". Hydrogen peroxide was instilled for 10 minutes to block endogenous peroxidase activity. To block antibodies, Ultra V Block ("Blocking Reagent-ultra v block, Lab Vision) was instilled on the slides allowed to wait in PBS solution for 10 minutes and allowed to wait for 10 minutes. Then the solution was removed from the slide. The slides were incubated with primary polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA; w.d. 1:100) and MMP-9 antibodies (Dako Cytomation, Glostrup, Denmark; w.d. 1:50) in a humidified container at room temperature for 60 minutes. The slide was allowed to wait in PBS solution for 10 minutes. Secondary antibody solution ("Biotinylated goat Anti-polyvalent, Labvision") was instilled and allowed to wait for 20 minutes and washed with PBS. "Streptavidin peroxidase" (Labvision) was instilled and allowed to wait for 20 minutes and washed with PBS. It was incubated with chromogen (UltraVision Detection System Large Volume AEC Substrate System (RTU)). It was allowed to wait for 15 minutes. The slides were washed with distilled water and counterstained by holding it in Mayer's haematoxylin solution for 30 seconds. They were were rinsed in tap water. The slides were impregnated with "Aqueus medium".

Quantification of parameters

The assessment of immunostained sections was performed by a random principle by one experinced pathologists to the clinico-pathological data. NGAL expression was based on the presence of cytoplasmic and membranous staining. The intensity of staining (IS) was graded as (0) negative, (1) weak, (2) moderate, (3) strong. The area of staining positivity (ASP), recorded as percentage of neoplastic positive cells, was assessed as follows: 0 (≤ 10%), 1 (11-25%), 2 (26-50%), 3 (51-75%), 4 (> 75%), as described previously [7, 11]. Then, an intensity-distribution (ID) score was generated for each case by multiplying the values of IS and ASP. Cases displaying an ID score 0 were considered as negative for NGAL.

For the statistical analyses, samples were subdivided into negative and positive tumors according to the median NGAL ID score, which was 0. Staining scores for MMP-9 were established semi-quantitatively as already proposed [10]. Specifically, an intensity distribution (ID) score for each antibody was calculated by optical analysis using the sum of the percentage of positive cells (0: none; 1: 1-30%; 2: 31-60%; 3: 61-100%) and staining intensity graded from 1 to 3 (1, weak; 2, moderate; and 3, strong). ID scores of 4 or greater were considered to show high expression, and those of 3 or lower to indicate low expression.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) program was used for the statistical analysis. During the evaluation of the study data, Mann Whitney U test was used for the intergroup comparisons of quantitative data as well as descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum), Kruskal Wallis test was used for the intergroup comparisons of the groups more than 3 and Mann Whitney U test was used for the determination of the group causing difference. Pearson's Chi-Square test, Fisher's Exact test and Yates's Continuity Correction test (Yates-corrected Chi-squared test) were used for comparison of qualitative data. Spearman's Correlation Analysis was used for evaluation of the relationships between the parameters. Significance was evaluated at the levels of $p < 0.01$ and $p < 0.05$.

Results

Assessments regarding pathological results

There is no statistically significant difference in the distribution of the cases by age and gender according to the pathological results ($p > 0.05$). But there is a statistically significant difference in the distribution of the cases by primary tumor sites according to the pathological results ($p < 0.05$). According to pairwise comparisons performed to determine the group causing difference; tumor incidence rate in the left-hand side of the colon of the cases with High Dysplasia is significantly higher than the cases with adenocarcinoma and Low Dysplasia ($p = 0.042$; $p = 0.005$; $p < 0.05$). No statistically significant difference was determined between the other groups ($p > 0.05$).

A highly statistically significant difference was determined between the tumor sizes of the cases according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; tumor sizes of the cases with adenocarcinoma are significantly higher than the tumor sizes of the cases with Low Dysplasia, High Dysplasia and Hyperplasia ($p = 0.001$; $p = 0.001$; $p = 0.001$; $p < 0.01$). While the tumor sizes of the cases with High Dysplasia are significantly higher than the tumor sizes of the cases with Low Dysplasia and Hyperplasia ($p = 0.001$; $p = 0.001$; $p < 0.01$); also the tumor sizes of the cases with Low Dysplasia are significantly higher than the tumor sizes of the cases with Hyperplasia ($p = 0.001$; $p < 0.01$).

The NGAL intensities of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the NGAL intensities of the cases with High Dysplasia are significantly higher than the NGAL intensities of the cases with adenocarcinoma, Low Dysplasia and Hyperplasia ($p = 0.003$; $p = 0.001$; $p = 0.001$; $p < 0.01$). The NGAL intensities of the cases with adenocarcinoma and Low Dysplasia are also significantly higher than the NGAL intensities of the cases with Hyperplasia ($p = 0.001$; $p = 0.001$; $p < 0.01$). There was no significant difference between the NGAL intensities of the cases with adenocarcinoma and Low Dysplasia ($p > 0.05$).

The NGAL prevalences of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the NGAL prevalences of the cases with adenocarcinoma are significantly higher than NGAL prevalences of the cases with Hyperplasia ($p = 0.002$; $p < 0.01$). The NGAL prevalences of the cases with High Dysplasia and Low Dysplasia are also significantly higher than the NGAL prevalences of the cases with Hyperplasia ($p = 0.002$; $p = 0.001$; $p < 0.01$). The NGAL prevalences of the cases with High Dysplasia are also significantly higher than the NGAL prevalences of the cases with Low Dysplasia ($p = 0.001$; $p < 0.01$). No statistically significant difference was determined between the NGAL prevalences of the other groups ($p > 0.05$).

The NGAL ID scores of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the NGAL ID scores of the cases with adenocarcinoma are significantly higher than NGAL ID scores of the cases with Hyperplasia ($p = 0.001$; $p < 0.01$). The NGAL ID scores of the cases with High Dysplasia and Low Dysplasia are also significantly higher than the NGAL ID scores of the cases with Hyperplasia ($p = 0.001$; $p = 0.001$; $p < 0.01$). The NGAL ID scores of the cases with High Dysplasia are also significantly higher than the NGAL ID scores of the cases with Low Dysplasia ($p = 0.001$; $p < 0.01$). No statistically significant difference was determined between the NGAL ID scores of the other groups ($p > 0.05$). A highly statistically significant difference was also determined between NGAL ID score groups of the cases according to the pathological results ($p < 0.01$). While there is no case with positive NGAL ID score among the cases with Hyperplasia, all (100%) of the cases with Low and High Dysplasia are positive for NGAL ID score and 93.3% of the cases with adenocarcinoma are positive for NGAL ID score.

The MMP intensities of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the MMP intensities of the cases with High Dysplasia are signifi-

Table 2. Assessments of descriptive characteristics according to pathological results

	Pathological Results				
	Adenocarcinoma (n = 15)	Low Dysplasia (n = 14)	High Dysplasia (n = 22)	Hyperplasia Polyp (n = 14)	*p*
Age					
≤ 70 years	9 (60.0%)	11 (78.6%)	19 (86.4%)	13 (92.9%)	[a]*0.123*
> 70 years	6 (40.0%)	3 (21.4%)	3 (13.6%)	1 (7.1%)	
Gender					
Female	6 (40.0%)	2 (14.3%)	11 (50.0%)	7 (50.0%)	[a]*0.147*
Male	9 (60.0%)	12 (85.7%)	11 (50.0%)	7 (50.0%)	
Primary Tumor Site					
Right Colon	6 (40.0%)	8 (57.1%)	2 (9.1%)	3 (21.4%)	[a]*0.013**
Left Colon	9 (60.0%)	6 (42.9%)	20 (90.9%)	11 (78.6%)	
Tumor Size					
Min-Max	10-70	3-9	5-25	3-5	[b]*0.001***
Median	40.0	6.0	9.0	4.0	

[a]Pearson's Chi-square Test. [b]Kruskal Wallis Test. *$p < 0.05$; **$p < 0.01$.

cantly higher than the MMP intensities of the cases with adenocarcinoma, Low Dysplasia and Hyperplasia ($p = 0.008$; $p = 0.001$; $p = 0.001$; $p < 0.01$). The MMP intensities of the cases with adenocarcinoma are also significantly higher than the MMP intensities of the cases with Low Dysplasia and Hyperplasia ($p = 0.048$; $p = 0.001$; $p < 0.05$). There was no statistically significant difference between the MMP intensities of the cases with Low Dysplasia and Hyperplasia ($p > 0.05$).

The MMP prevalences of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the MMP prevalences of the cases with adenocarcinoma and High Dysplasia are significantly higher than MMP prevalences of the cases with Hyperplasia ($p = 0.010$; $p = 0.001$; $p < 0.05$). The MMP prevalences of the cases with High Dysplasia are also significantly higher than the MMP prevalences of the cases with Low Dysplasia ($p = 0.001$; $p < 0.01$). No statistically significant difference was determined between the MMP prevalences of the other groups ($p > 0.05$).

The MMP ID scores of the cases show highly statistically significantly difference according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the MMP ID scores of the cases with High Dysplasia are significantly higher than the MMP ID scores of the cases with adenocarcinoma, Low Dysplasia and Hyperplasia ($p = 0.019$; $p = 0.001$; $p = 0.001$; $p < 0.05$). The MMP ID scores of the cases with adenocarcinoma are also significantly higher than the MMP ID scores of the cases with Low Dysplasia and Hyperplasia ($p = 0.016$; $p = 0.001$; $p < 0.05$). The MMP ID scores of the cases with Low Dysplasia are also significantly higher than the MMP ID scores of the cases with Hyperplasia ($p = 0.023$; $p < 0.05$). A highly statistically significant difference was also determined between MMP ID score groups of the cases according to the pathological results ($p < 0.01$). According to pairwise comparisons performed to determine the group causing difference; the rate of MMP ID score to be ≥ 4 in the cases with High Dysplasia is significantly higher than the rate of MMP ID score to be ≥ 4 in the cases with adenocarcinoma, Low Dysplasia and Hyperplasia ($p = 0.006$; $p = 0.001$; $p = 0.001$; $p < 0.01$). The rate of MMP ID scores of the cases with adenocarcinoma to be ≥ 4 is significantly higher than the rate of MMP ID scores of the cases with Hyperplasia to be ≥ 4 ($p = 0.035$; $p < 0.05$). No statistically significant difference was determined between the other groups ($p > 0.05$). The detailed clinic and immunhistochemical characteristics of patients summarized in **Tables 2** and **3**.

Discussion

NGAL has been shown to be overexpressed in inflammatory bowel diseases and colorectal

Table 3. Assessments regarding NGAL and MMP parameters according to pathological results

		Pathological Results				
		Adenocarcinoma (n = 15)	Low Dysplasia (n = 14)	High Dysplasia (n = 22)	Hyperplasia Polyp (n = 14)	*p*
NGAL Intensity	1	1 (6.7%)	0 (0%)	0 (0%)	12 (85.7%)	
	2	6 (40.0%)	9 (64.3%)	1 (4.5%)	2 (14.3%)	
	3	7 (46.7%)	4 (28.6%)	15 (68.2%)	0 (0%)	
	4	1 (6.7%)	1 (7.1%)	6 (27.3%)	0 (0%)	
	Min-Max	1-4	2-4	2-4	1-2	[b]*0.001***
	Median	3.0	2.0	3.0	1.0	
NGAL Prevalence	< 10	4 (26.7%)	3 (21.4%)	0 (0%)	11 (78.6%)	
	11-25	1 (6.7%)	9 (64.3%)	6 (27.3%)	3 (21.4%)	
	26-50	4 (26.7%)	2 (14.3%)	13 (59.1%)	0 (0%)	
	51-75	5 (33.3%)	0 (0%)	3 (13.6%)	0 (0%)	
	> 75	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	
	Min-Max	0-4	1-2	1-3	0-1	[b]*0.001***
	Median	2.0	1.0	2.0	0.0	
NGAL ID Score	*Min-Max*	0-9	1-6	1-9	0-0	[b]*0.001***
	Median	4.0	2.0	6.0	0.0	
NGAL ID Score Group	Negative	1 (6.7%)	0 (0%)	0 (0%)	14 (100%)	[a]*0.001***
	Positive	14 (93.3%)	14 (100%)	22 (100%)	0 (0%)	
MMP Staining	Absent	0 (0%)	0 (0%)	0 (0%)	1 (7.1%)	
	Weak	4 (26.7%)	8 (57.1%)	0 (0%)	11 (78.6%)	
	Moderate	8 (53.3%)	6 (42.9%)	10 (45.5%)	2 (14.3%)	
	Strong	3 (20.0%)	0 (0%)	12 (54.5%)	0 (0%)	
	Min-Max	1-3	1-2	2-3	0-2	[b]*0.001***
	Median	2.0	1.0	3.0	1.0	
MMP Prevalence	0	0 (0%)	0 (0%)	0 (0%)	5 (35.7%)	
	1	6 (40.0%)	9 (64.3%)	2 (9.1%)	5 (35.7%)	
	2	5 (33.3%)	5 (35.7%)	19 (86.4%)	4 (28.6%)	
	3	4 (26.7%)	0 (0%)	1 (4.5%)	0 (0%)	
	Min-Max	1-3	1-2	1-3	0-2	[b]*0.001***
	Median	2.0	1.0	2.0	1.0	
MMP ID Score	*Min-Max*	1-9	1-4	2-6	0-4	[b]*0.001***
	Median	3.0	2.0	6.0	1.0	
MMP ID Score Group	0-3	8 (53.3%)	12 (85.7%)	2 (9.1%)	13 (92.9%)	[a]*0.001***
	≥ 4	7 (46.7%)	2 (14.3%)	20 (90.9%)	1 (7.1%)	

[a]Pearson's Chi-square Test. [b]Kruskal Wallis Test. ***$p < 0.01$.

neoplasms; however, there are few studies on NGAL expression in the colorectal adenoma–carcinoma sequence to explore the effect of NGAL expression on tumorigenesis of CRC [19, 20]. Therefore, in the present study, we investigated the prognostic value of NGAL and MMP-9 immunohistochemical expression in colon adenoma-carcinom sequence. In this study, we examined NGAL and MMP-9 immunohistochemistry staining and intensity on 65 colorectal tissue specimens. In adenocancer group, there was no positive correlation found between NGAL and MMP-9 staining and intensity. We found that NGAL and MM-9 intensities and staining of the cases with neoplasia were determined to be significantly higher than the control group. Also NGAL ID scores of the cases with neoplasia were determined to be significantly higher than the control group. However when we divide the groups as Adenocarcinoma and Dysplasia; there is no statistically significant difference between NGAL intensities and NGAL

staining of the cases according to the pathological results.

The relationship between NGAL expression and cancer cells could be attributed to the ability of this protein to accelerate activity of the matrix metallo-proteinase-9 (MMP-9) enzyme which degrades the extracellular matrix and basement membranes [21-23]. NGAL can protect MMP-9 from proteolytic degradation with forming NGAL/MMP-9 complex and enhances the effects of MMP-9 [24]. Therefore, tumoral invasiveness and diffusion associated with NGAL overexpression could be explained by this enzyme pathway. In agreement with these findings, several studies report that the experimental induction of NGAL in various cancer types enhanced MMP-9 hyperexpression [22-26]. They determined that it would lead to an increase in the levels of circulating MMP-9 and responsible for more aggressive tumoral phenotype. In the evidence of the relationship between NGAL and MMP-9 as discussed earlier, they hypothesized a role of NGAL in contributing to the tumorigenesis of CRC. Therefore, our data in the current study provide consistent evidence that NGAL overexpression plays an important role in colorectal tumorigenesis.

In 2010, Baresi et al. [22] reviewed the role of iron overload in carcinogenesis. They pointed that iron overload enhanced the oxidative DNA damage and increased cell apoptosis, hence iron chelators which inhibit cell proliferation by depletion iron levels and iron transport proteins have become new targets in cancer therapy. They also pointed out the proven NGAL role in iron metabolism and decided that NGAL cytoplasmic presence might show augmented iron requirement in the cancerogenesis of colorectal cancer. In this well designed study performed by Bolignano et al. [26], it was reported that NGAL overexpression likely contributed to the progression of CRC.

Recently, Baresi et al. [22] reported that the negative prognostic and independent value of NGAL immunoexpression in colorectal carcinoma might be independent from MMP-9 regulation [21]. They suggested NGAL overexpression in stage I colorectal carcinoma related with iron deprivation pathway [21].

With these considerations, we could hypothesize that NGAL and MMP-9 overexpression in neoplastic polyps might be used as a marker to separate those from non-noeplastic polyps. However, in this study, we determined that NGAL overexpression could not distinguish dysplasia from adenocancer. Finally, we suggest NGAL and MMP-9 as an immunohistochemical marker for colonic dysplasia. To determine dysplasia in early steps of colorectal adenoma-carcinoma sequence, it could help to determine new targets in preventive cancer therapy for colorectal cancer. We suggest development of standards for study method, introduction to routine practice by investigating in future studies including many patients.

Disclosure of conflict of interest

None.

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