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MICA-129 Met/Val Variant as Possible Biomarker of Diagnosis and Prognostic of Gastro-Intestinal Tract Carcinomas

Ayari Fayza[1#*], Ben Chaaben Arij[1#], Baroudi Olfa[2], Ouni Nesrine[1], Abaza Hajer[1], Harzallah Latifa[1], Elgaaeid-Benammar Amel[2], Charron D[3], Guemira Fethi[1] and Tamouza Ryad[3]

[1]*Clinical Biology Department, Saint-Louis Hospital, Paris, France*
[2]*Department of Biology, Laboratory of Immunogenetics, University of Tunis-El-Manar, Tunis, Tunisia*
[3]*Jean Dausset Laboratory, CIH-HOG, AP-HP, IUH and INSERM, Saint-Louis Hospital, Paris, France*
[#]*Fayza A and Arij BC are co-authors and equally contributed on this work*

Abstract

Background: The major histocompatibility complex class I-related chain A (*MICA*) molecules play a pivotal role in the modulation of anti-tumor immune responses. A polymorphic change from methionine (Met) to valine (Val) at amino acid position 129 of the alpha 2 heavy-chain categorize *MICA* alleles into strong and weak binders for the NKG2D receptor. We investigated here whether *MICA-129* alleles are associated with gastro-intestinal tract (GI-tract) carcinomas in Tunisian affected patients as compared to healthy controls (HC).

Material and methods: 181 patients affected by colorectal cancer (CRC) and 61 patients affected by gastric cancer (GC) along with 203 healthy controls (HC) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure.

Results: We found that the *MICA-129* Val/Val genotype was statistically more prevalent in patients affected both by CRC and GC as compared to HC. After stratification with poor prognostic parameters, we observed that *MICA-129* Val/Val genotype is significantly associated with advanced tumor extension (T3-T4), lymph node metastasis (N+), and distance metastasis (M+). In both cases, the *MICA-129* Val/Val genotype seems to behave as a risk genotype and a poor prognostic biomarker in our population.

Conclusion: Our findings suggest a potential tumor escape possibly due to an inability to activate NK cells and/or to stimulate specific T lymphocytes subsets particularly active in the GI-tract.

Keywords: Colorectal cancer; Gastric cancer; Prognostic; MICA; Polymorphism

Abbreviations: CRP: C Reactive Protein; CRC: Colorectal Cancer; GC: Gastric Cancer; GI-tract: Gastro-Intestinal Tract; HC: Healthy Controls; Met: Methionine; MICA: Major Histocompatibility Complex Class I-Related Chain A; NK: Natural Killer; Val: Valine

Introduction

Colorectal and gastric cancers are the most common forms of malignancies often presenting with a poor prognosis and are leading cause of cancer-related death in the world [1]. In Tunisia, the incidence of these types of cancers was increasing during the period of 1999-2003 according to the register of cancer. The incidence rate is 5.4 cases per 10^5 in males, however, in females the incidence rate is 4.8 cases per 10^5 [2]. The development of these complex multifactorial malignancies is under the influence of the patient genetic background [3] and environmentally related factors [4]. Among the genetic loci that could constitute a potential link between the genetic and the environmental components of immune response, the major histocompatibility complex (MHC) class I chain-related A (*MICA*) gene is an attracting candidate. The *MICA* gene is at the centromeric end of the classical class I region approximately 46.4 Kb from HLA-B [5]. *MICA* (11.7 Kb) is transcribed into an mRNA of 1382 bp, giving rise to a 383-amino acid polypeptide of 43 kDa [6]. The MICA protein comprises a transmembrane MHC-I alpha-like chain and is not associated to the β-2-microglobulin and does not bind to peptides [7]. In humans, the expression of *MICA* is restricted to gastro-intestinal epithelium, endothelial cells and fibroblasts [7,8]. Under pathological conditions, *MICA* expression is induced by factors of cellular stress and can be up-regulated by viral and bacterial infections [9,10]. It is expressed in certain epithelial tumors and lymphoproliferative malignancies including multiple myeloma, lung, kidney, prostate, breast and colon [11-14]. The cognate receptor of *MICA* is a type II C-lectin-like protein designated as NKG2D. This receptor is present on natural killer (NK) cells, most γ/δ T cells and CD8+ α/β T cells but is absent on CD4+ α/β T cells. The engagement of MICA with NKG2D strongly activates NK cells and provides costimulatory signals to T cells, enhancing their cytolytic activity and cytokine production [15,16]. Consequently, the expression of MICA on tumor cells has been proposed to play a critical role in tumor immune surveillance. Whereas the expression of MICA induces a strong tumor antigen specific immune response, its absence, or down-regulation results in tumor escape from NK and T cell attack [17-19].

The *MICA* gene exhibits a high rate of polymorphism, with 93 alleles so far described [20]. Alleles of *MICA* can be categorized into strong and weak binders of NKG2D based on the (rs1051792) A>G polymorphism at position 454 in the third exon of the *MICA* gene, which corresponds to amino acid 129 in the β 2-heavy chain domain of the MICA protein [MICA-129 Met (methionine) → Val (valine)] [8].

Subsequently, several authors have recently shown that the *MICA-*

***Corresponding author:** Ayari Fayza, Clinical Biology Department, Saint-Louis Hospital, Paris, France, Tel: 21671796679; E-mail: fayzaayari4@gmail.com

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Characteristics			Colorectal cancer	Gastric cancer	Healthy controls
Total number			181	61	203
Gender (Male/Female)			100/81	43/18	100/103
Age at onset (year)			58 (16-82)	56 (20-89)	54 (16-93)
			[16-50]=45 (27%)	[20-50]=15 (25%)	
			[50-93]=124 (73%)	[50-89]=45 (75%)	
Geographic Origin	North		78%	88%	
	Center		12%	6%	
	South		10%	6%	
TNM classification Edge et al. [30]	Tumor extension	T1-T2	n=145	n=30	
			14 (10 %)	14 (47 %)	
		T3-T4	131 (90 %)	16 (53%)	
	Lymph Node metastasis	N0	49 (34%)	5 (17%)	
		N+	87 (60%)	19(63%)	
	Distant Metastasis	M0	78 (54 %)	12 (40%)	
		M+	27 (19 %)	16 (53%)	
	Not available	Tx	0	0	
		Mx	40 (27 %)	3 (7%)	
		Nx	9 (6%)	6 (20%)	
n=number, T: Tumor extension, N: Lymph Node metastasis, M: Distant Metastasis; ADK: adenocarcinoma; n=number					

Table 1: Demographic and clinical characteristics of gastrointestinal patients and healthy controls.

Prognostic factors and treatments	Colorectal cancer n=123	Gastric cancer n=34
Histological type		
ADK	37 (30%)	11(32%)
ADK liberkhunien	39 (32%)	1(3%)
ADK infiltrant	19 (15%)	3(9%)
ADK Signet Ring Cell	2 (2%)	12 (35%)
Other type of ADK	26 (21%)	8 (23%)
Histological grade		
Low differentiated	4 (3%)	9 (26%)
Modernly differentiated	37 (30%)	8 (24%)
Well differentiated	68 (56%)	6 (18%)
Unknown	14 (11%)	11 (32%)
Progression		
Yes	19 (15%)	5 (15%)
No	104 (85%)	29 (85%)
Neoadjuvant chemotherapy		
Yes	26 (21%)	10 (30%)
No	97(79%)	24 (70%)
Kind of Neoadjuvant chemotherapy		
Capecitabine (Xeloda)	12(46%)	2(20%)
Folfox/Folfiri	13(50%)	0
LV5-FU2	1(4%)	2(20%)
ELF	0	5(50%)
ECF	0	1(10%)
Adjuvant chemotherapy		
Yes	88(72%)	19(56%)
No	35(28%)	15(44%)
Kind of Adjuvant chemotherapy		
Capecitabine	6(7%)	0
Folfox/Folfiri	68(77%)	4(21%)
LV5-FU2	14(16%)	10(53%)
ELF	0	5(26%)
ECF	0	0
Surgery		
NO	19(15%)	12(35%)
Palliative	80(6519%)	9(26%)
Radical	24(%)	13(38%)

Table 2: Characteristics of prognostic factors, surgery and chemotherapy treatment in patients with gastrointestinal cancer.

129 polymorphism was associated with several pathologies [21-28]. However, MICA-129 polymorphism in gastric and colorectal cancer susceptibility has not been studied so far.

In this case-control study, we sought to determine whether or not the *MICA-129* polymorphism is associated with CRC and GC in the Tunisian population. Besides, we have tested the possible association of this polymorphism with clinical features.

Material and Methods

Patients and control groups

In this study, 181 consecutive CRC patients (100 men and 81 women, age, 16-82 years) and 61 GC patients (43 men and 18 women, age, 20-89 years) were enrolled from the Institute of Cancer "Salah Azaiz" in Tunis, Tunisia. The average age of all cases at diagnosis was 57 years with a range of 16 to 89 years. Demographic and clinico-pathological characteristics of the study subjects are given in Table 1. The data collected included stage, lymph node status, differentiation and histological type of tumor, besides we collected data concerning chemotherapy and surgery treatment. Some patients received FOLFOX treatment as well as FOLFIRI combination, whereas other received monotherapy treatment such as XELODA and LV5-FU2. Patients were assessed before the initiation of chemotherapy and every 2 weeks during treatment. In addition, a total of 203 age and sex-matched healthy controls were recruited in this study from "Clinical Biology Department, Salah Azaiz Institute". These controls were considered without any history of malignancy, the sex ratio of the group was 0.97 (100 men and 103 women), and the average age was 54 years with a range from 16 to 93 years [29,30]. The patients and healthy controls were not consanguineous. In our study, written informed consent was obtained from all participants before their participation and is approved by the Ethical Committee of Salah Azaiz institute.

Genotyping

Genomic DNA was extracted from EDTA tube from peripheral blood leukocytes samples using chloroform/phenol assay, and the

MICA-129 polymorphism	Controls (n=203)	Patients (n=181)	P	pc	OR	95% IC
CRC Alleles						
MICA-129 Val	224 (55%)	235 (65%)	0.006	0.012	1.5	[1.11-2.01]
MICA-129 Met	182 (45%)	127 (35%)	0.006	0.012	0.67	[1.49-0.90]
Genotypes						
MICA-129 Val/Val			0.002	0.006	2.09	[1.28-3.43]
MICA-129 Met/Val	39 (19%)	60 (33%)	0.06	0.18	NS	
MICA-129 Met/Met	146 (72%)	114 (63%)	0.04	0.12	NS	
	18 (9%)	7 (4%)				
		(n=61)				
GC Alleles	(n=203)					
MICA-129 Val	224 (55%)	84 (69%)	0.007	0.014	1.8	[1.17-2.78]
MICA-129 Met	182 (45%)	38 (31%)	0.007	0.014	0.56	[0.35-0.87]
Genotypes						
MICA-129 Val/Val	39 (19%)	25 (41%)				
MICA-129 Met/Val	146 (72%)	34 (56%)	$5.2\ 10^{-4}$	$1.56\ 10^{-3}$	2.92	[1.49-5.65]
MICA-129 Met/Met	18 (9%)	2 (3%)	0.02	0.06	0.49	[0.26-0.93]
			NS	NS	NS	

Table 3: MICA-129 allele and genotype frequencies among patients and controls.

MICA-129 polymorphism were explored at the DNA level (A-to-G change in exon 3, at nucleotide position 454) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure using an automated thermal cycler. *MICA* exon was amplified with the following primers 5'F-GGGTCTGTGAGATCCATGA3' and 5'R-TGAGCTCTGGAGGACTGGGGTA'3. The presence of *MICA-129* Val allele was identified by the presence of a restriction site for Rsa I. The 127 bp PCR product was cleaved into 104 bp and 23 bp then electrophoresed on 3% agarose gels. The explored MICA variants are designated *MICA-129* Val and *MICA-129* Met.

Statistical analysis

Statistical analysis was performed using the compare V.2.1 statistical software package. Both allele and genotype frequencies of *MICA-129* polymorphism were compared between cases and controls using the χ^2 test or Fisher exact test for a low number of cases. Corrected P value (Pc) was obtained by multiplying the P value by the number of alleles tested according to Bonferroni's correction. The odds ratios (OR) were calculated as estimates of relative risk for disease, and 95% confidence intervals (CI) were calculated for all observed allele frequencies. P<0.05 was considered to be statistically significant.

Results

Characteristics of prognostic factors, surgery and chemotherapy treatment in patients with gastrointestinal cancer

A total of 181 cases with CRC and 61 cases with GC were included in this study. Stratified phenotypic characteristics of the studied cohort are summarized in Table 1. At the time of diagnosis, the vast majority of the patients with CRC presented an advanced tumor extension T3-T4 (90%) with lymph node metastasis (60%). Besides, we observed that the vast majority of the patients with GC presented only lymph node metastasis (63%).

About 123 CRC and 34 GC were followed and we have grouped some clinico-pathological information listed in Table 2, such as histological type, evolution, surgery, and chemotherapy treatment.

We observed that for CRC, the majority of the histological types were adenocarcinoma (30%) and adenocarcinoma Lieberkuhnien (32%) and were most frequently of a well differentiated histological grade (56%). As for GC cases, the majority of patients had an adenocarcinoma (32%) and adenocarcinoma Signet Ring Cell type (35%). In addition, they had low and moderate histological grade (24%, 26% respectively). Furthermore, the majority of both patients did not have a progressive disease (85% for CRC and GC). Moreover, for CRC, most of the patients have received adjuvant chemotherapy based on biotherapy treatment combination FOLFOX (5FU Oxaliplatine) and FOLFIRI (5FU Irinitecan) (50%). However, for GC, most of the patients have received adjuvant chemotherapy LV5-FU2 (53%).

MICA-129 alleles and genotypes frequencies among patients and controls

The *MICA-129* alleles and genotypes frequencies were determined by PCR amplification. We observed that the *MICA-129* Val allele was significantly more frequent in CRC and GC patients than controls (for CRC patients: 65% vs. 55% for the controls, p=0.006, pc=0.012, OR=1.5, and 95% CI (1.11-2.01); for GC patients: 69% vs. 55% for the controls, p=0.007, pc=0.014, OR=1.8, and 95% CI (1.17- 2.78). Whereas *MICA-129* Met allele was significantly more frequent in control than in patient groups (for CRC: 35% vs. 45%, pc=0.012, OR=0.67, and 95% CI (0.49-0.90); for GC: 31% vs. 45%, pc=0.014, OR=0.56, and 95% CI (0.35 to 0.87)) (Table 3).

The analysis of the distribution of the different genotypes between the three groups revealed that the *MICA-129* Val/Val genotype is significantly associated with CRC and patients rather than controls (for CRC: 33% vs. 19%, p=0.006; pc=0.018, OR=2.09, and 95% CI=(1.28 to 3.43); for GC: 41% vs. 19%, pc=$1.56\ 10^{-3}$, OR=2.92, and 95% CI=(1.49-5.65)).

Nevertheless, we did not find any association of MICA-129 Met/Met genotype with the risk of GIC (P> 0.05).

Interaction between MICA-129 polymorphism and prognostic factors

We have presented in Table 4 the interaction between *MICA-129* Val/Val and *MICA-129* Met/Met genotypes with poor prognosis factors such as age >50 years old, advanced tumor extension (T3-T4), lymph node metastasis (N+), distance metastasis (M+), differentiation and evolution.

Prognostic factors	Met/Met	Met/Val	Val/Val	P for interaction	Pc	O.R	IC 95%
CRC (n=123)							
Age							
[16-50]	3/45 (6%)	25/45(56%)	17/45 (38%)				
[50-93]	3/124 (2%)	80/124(65%)	41/124(33%)	$5\ 10^{-11}$	$1\ 10^{-10}$	19.9	[5.98-102.8]
Tumor extension							
T1T2	2/14 (14%)	6/14(43%)	6/14(43%)				
T3T4	4/131 (3%)	82/131 (63%)	45/131(34%)	$1.7\ 10^{-11}$	$3.4\ 10^{-11}$	16.6	[5.7-65.3]
Lymph node metastasis							
N0	4/49(8%)	31/49 (63%)	14/49 (29%)	$4.6\ 10^{-11}$	$1.21\ 10^{-10}$	55.17	[8.57-2267]
Nx	1/9 (11%)	7/9(78%)	1/9 (12%)				
N+	1/87(1%)	52/87(60%)	34/87 (39%)				
Distant metastasis							
M0	3/78(4%)	44/78 (56%)	31/78 (40%)	0.011	0.033	13	[1.5-591]
Mx	0	29/40 (73%)	11/40 (27%)				
M+	1/27 (4	17/27 (63%)	9/27 (33%)				
Differentiation Low differentiated	0	2/4 (50%)	2/4 (50%)	$2.9\ 10^{-4}$	8.710^{-4}	8.4	[2.25-46.1]
Modernly differentiated	1/37(3%)	25/37(68%)	11/37(30%)				
Well differentiated	3/68(4%)	46/68(68%)	19/68(28%)				
Progression							
Yes	3/19(16%)	13/19(68%)	3/19 (16%)	1	–	–	–
No	1/104(10%)	74/104(%)	29/104 (28%)				
GC (n=34)							
Age							
[20-50]	1/15 (6%)	7/15 (47%)	7/15 (47%)	$1.1\ 10^{-5}$	$2.2\ 10^{-5}$	29.3	[4.76-1249]
[50-89]	1/45 (2%)	26/45 (58%)	18/45 (40%)				
Tumor extension							
T1T2	0	8/14 (57%)	6/14 (43%)	0.08		–	–
T3T4	1/16 (6%)	9/16 (56%)	6/16 (38%)				
Lymph node metastasis							
N0	1/5 (20%)	2/5 (40%)	2/5(40%)	0.4	–	–	–
Nx	0	11/19 (56%)	8/19 (42%)				
N+	0	4/6 (67%)	2/6 (33%)				
Distant metastasis							
M0	1/12(8%)	6/12(50%)	5/12 (42%)	0.002	0.006	169	[3.9-7303]
Mx	0	1/3 (25%)	2/3 (75%)				
M+	0	0	6/6 (100%)				
Histological grade Low differentiated	0	6/9(67%)	3/9(33%)	0.3	–	–	–
Modernly differentiated	1/8(13%)	5/8(62%)	2/8(25%)				
Well differentiated	0	4/6(67%)	2/6(33%)				
Progression							
Yes	0	3/5(60%)	2/5(40%)	NS	–	–	–
No	1/29(3%)	16/29(55%)	12/29(42%)				

CRC: colorectal cancer, GC: gastric cancer, n=number, P for interaction was calculated between MICA-129 Met/Met and MICA-129 Val/Val genotypes in poor prognosis factors, Pc Bonferroni test correction, 95% CI confidence interval, NS no significant

Table 4: Interaction between MICA-129 polymorphism and prognostic factors.

Interestingly, In CRC patients, we have found that the frequency of the *MICA-129* Val/Val genotype was higher in patients with advanced age (50-93) than the *MICA-129* Met/Met genotype (33% vs. 2%; p=5 10^{-11}, OR=19.9, CI 95% (5.98-102.8)). Similarly, we found that *MICA-129* Val/Val genotype is significantly associated with advanced Tumor extension (T3-T4), lymph node metastasis (N+) and distance metastasis (M+) (p=1.7 10-11, OR=16.6, CI 95% (5.7-65.3); p=4.6 10-11, OR=55.17, CI 95% (8.57-2267); p=0.011, OR=13, CI 95% (1.5-591) respectively).

Furthermore, we observed a higher frequency of *MICA-129* Val/Val genotype in patients with moderate and well differentiated histological grade (58% vs. 7%; p=2.9 10-4, OR=8.4, CI 95% (2.25-46.1)).

For GC patient, we found that *MICA-129* Val/Val was higher in patient with age >50 years old (p=1.1

10^{-5}, OR=29.3, CI 95% (4.76-1249)) and with Metastasis (M+) (p=0.002, OR=169, CI 95% (3.9-7303)). However, no correlations were found between TNM classification, histological grade, evolution, and *MICA-129* polymorphism.

Interaction between MICA-129 Val/Val genotype and treatment

Concerning the treatment presented in Table 5, we have only shown an association between *MICA-129* Val/Val genotype and chemotherapy

Treatment	Met/Met	Met/Val	Val/Val	P for interaction	Pc	O.R	CI95%
	CRC (n=123)						
Surgery							
No	0(%)	14/19(74%)	5/19(26%)	1			
Palliative	2/80(2%)	52/80(65%)	26/80(33%)				
Radical	2/24(8%)	21/24(88%)	1/24(4%)				
Neoadjuvant Chemotherapy							
Yes	1/26(4%)	19/26(73%)	6/26(23%)	0.8			
No	3/97(3%)	68/97(70%)	26/97(27%)				
Preoperatory radiation							
Yes	3/104(3%)	71/104(78%)	30/104(29%)	0.1		–	
No	1/19(5%)	16/19(84%)	2/19(11%)				
Adjuvant Chemotherapy							
Yes	1/87(1%)	64/87(74%)	22/87(25%)	0.8			
No	3/36(8%)	23/36(64%)	10/36(28%)				
Post-operatory radiation							
Yes	4(100%)	0	0	0.2		–	
No	5/121(4%)	87/121(72%)	29/121(24%)				
	GC (n=34)						
Surgery							
NO	0	5/12(42%)	7/12(58%)	0.1		–	
Palliative	0	6/9(67%)	3/9(34%)				
Radicale	1/13(7%)	8/13(54%)	4/13(39%)				
Neoadjuvant Chemotherapy							
Yes	0	3/10(30%)	7/10(70%)	0.02	0.04	6.67	[1.07-48.5]
No	1/24(4%)	16/24(64%)	7/24(29%)				
Preoperatory radiation							
Yes	0	0	2/2 (100%)	0.1			
No	1/32(31%)	19/32(60%)	12/32(9%)				
Adjuvant Chemotherapy							
Yes	1/19 (5%)	/19 (68%)	5/19 (27%)	0.05	0.1		
No	0	6/15 (40%)	9/15 (60%)				
Preoperatory radiation							
Yes	1/9(11%)	6/9(67%)	2/9(22%)	0.2			
No	0	13/25(52%)	12/25(48%)				

CRC: colorectal cancer, GC: gastric cancer, ADK: adenocarcinoma, P for interaction was calculated between MICA-129 Val/Val genotype and prognosis factors, Pc Bonferroni test correction, 95% CI confidence interval

Table 5: Interaction between treatment and MICA-129Val/Val genotype.

neoadjuvant treatment for GC patients (70% vs. 29%; p=0.02, OR=6.67; 95% CI=(1.07-48.80)).

However, in patients with CRC, we did not find any interaction between surgery, chemotherapy, radiotherapy and *MICA-129* Val/Val genotype (Table 5).

Discussion

MICA is constitutively expressed within the gastro-intestinal tract and is upregulated in response to stress. It is a ligand for the NKG2D receptor on both CD8+ T cells and NK cells, reducing the threshold for lysis. Tumors may up-regulate *MICA* in response to physical stress such as anoxia; however, this may make them susceptible to immune attack, resulting in control of tumor growth and a more favorable prognosis.

In particular, we focused our attention on functionally relevant characteristics of MICA polymorphism. This SNP (rs1051792) of *MICA* gene resulting in the *MICA-129* Met/Val dimorphism was the first *MICA* polymorphism for which a functional consequence was and categorizes that *MICA-129* Met variants as high and *MICA-129* Val variants as low avidity NKG2D ligands [28,29].

In our study, we shed the light for the first time on the crucial role of *MICA-129* polymorphism in gastro-intestinal cancer and its association with clinical features in Tunisian population. We found that the frequency of the *MICA-129* Val allele and *MICA-129* Val/Val genotype were increased in patients with gastro-intestinal cancer. These observations suggested that the *MICA-129* Val is an allele dose-dependent manner that increased the risk of CRC and GC, and the effect is recessive. Our findings and interpretations were in disagreement with the results published by Gong et al. where they not found an association between this dimorphism and the risk of development of colorectal cancer [24]. This discrepancy could be explained by the small size of the sample in an important genetic background like Chinese population (117 colorectal cancer patients and 113 healthy individuals) or by the

two genetic distant populations (Tunisian/Chinese). We can neither exclude the possibility that such genetic difference could be because of linkage disequilibrium with a yet to be identified locus implicated in the etiopathology of colorectal cancer. However, our study is in line with the previously reported in nasopharyngeal cancer in Tunisian population. Where, Douik et al. found that the homozygous state for *MICA-129* Val allele increased the risk of developing nasopharyngeal cancer [22]. Indeed, these results may emphasize the importance of *MICA* polymorphism in the development of CRC and GC or other cancer in our population. Similar associations of this variant with the malignant diseases, such as cutaneous malignant melanoma [26], hepatitis B virus-induced hepatocellular carcinoma [25], chronic and acute graft vs. host disease [22,26] and a number of autoimmune diseases, such as early-onset ankylosing spondylitis [21], rheumatoid arthritis [31], inflammatory bowel disease [32], systemic lupus erythematosus [33], Type 1 diabetes [34] and psoriatic disease [35]. Altogether, these data highlighted the potential role of *MICA-129* in tumor and auto-immune susceptibility. The functional consequence of *MICA-129* variants is investigated in many studies. In patients with ulcerative colitis, the MICA-Val/Val was associated with higher sMICA serum levels [36] as well as in patients with hepatitis B virus-induced hepatocellular carcinoma and healthy controls [25]. But, it was unclear however whether the *MICA-129* dimorphism has a direct effect on the generation of sMICA and affecting MICA shedding. Recently, Isernhagen et al. clarify whether the *MICA-129* not only affects NKG2D signaling but also directly affects plasma membrane expression and shedding and associated with high sMICA concentration [28].

Taken together, these results led us to hypothesize that the weaker binder *MICA-129* Val allele associated with high concentration of sMICA might downregulate the activation and co-stimulate NK cells or cytotoxic T cells and thereby may allow tumor escape to the immune system and that play important roles in the development of gastro-intestinal cancer in Tunisia. However, these suggestions must be confirmed by additional functional and association studies.

Therefore, after stratification with TNM, we found that *MICA-129* Val/Val genotype is strongly associated with a poor prognosis in CRC (Age >50 years old), advanced Tumor extension (T3-T4), lymph node metastasis (N+) and distance metastasis (M+), moderate and well differentiated histological grade. This association with disease progression here in observed raises the possibility that the weaker binder Val allele may have an impact on the disease extension.

Furthermore, based to previous data suggesting that *MICA* may have an unrecognized role in cancer therapy, we investigated here the relationship between *MICA* dimorphism and treatment. We found that the association between *MICA-129* Val/Val genotype and chemotherapy neoadjuvant treatment increased 5.67 fold the risk of gastric cancer in our population. In this context, our results could be explained by the recent study of Keisuke et al. where they demonstrate that chemotherapy may inhibit *MICA* expression in hepatocellular carcinoma (HCC) and suggesting that efficient activation of liver innate immunity after anti-HCC chemotherapy treatment might represent a particularly promising approach to suppress tumor growth [37]. While, this recent progress of therapy sheds light on the important implication of MICA in a good prognosis of patients and in the regression of tumor in and suggests a promising aspect for chemo-immunotherapy against human HCC [37].

A potential limitation of this study is that the number of GC subjects was relatively fewer than those in past reports on the relationships between *MICA-129*-Met/Val polymorphism and cancer. However, despite this limitation, we were able to show an association between *MICA-129* variant and gastric cancer in Tunisian population. Additionally, we have found that *MICA-129* Val/Val genotype is strongly associated with a poor prognosis correlations and treatment in GC.

Interestingly, our preliminary results should be interpreted with caution and will require confirmation in larger populations. Moreover, to draw comprehensive and more reliable conclusions, it is necessary to integrate more studies exploring the functional data (sMICA serum levels) in correlation with our genetics results.

Conclusion

These findings indicate the relevance of *MICA-129*-Met/Val polymorphism (weak/strong binders of NKG2D receptor) as a good biomarker of diagnosis and prognosis of GIC in Tunisian population. If these findings are to be confirmed with a larger patient cohort, by additional functional and association studies, the novel therapeutic intervention involving *MICA* can be envisaged.

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