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Association of *GEMIN4* gene polymorphisms with the risk of colorectal cancer in the Polish population

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Manuscript Preparation
E – Literature Search
F – Funds Collection

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ABSTRACT:

Aim: Gem-associated protein 4 (*GEMIN4*), a member of the *GEMIN* gene family, is a key compound of the regulating factors responsible for miRNA biogenesis. Genetic variability within this gene can alter the risk for development of colorectal cancer (CRC) as was shown for other genes involved in miRNA biogenesis. Therefore, presented study was intended to identify genetic variants of three single nucleotide polymorphisms (SNPs) in the *GEMIN4* gene (rs1062923, rs2740348 and rs910925) and their relationship with CRC.

Methods: The study comprised 203 patients and 179 age and sex matched controls. Genotyping of *GEMIN4* gene variants was done using Taqman® assay. The association of *GEMIN4* variants with CRC was done by odds ratio analysis. Haplotype analysis was done to see the combined effect of studied variants on CRC.

Results: Patients carrying all variant genotypes for *GEMIN4* rs1062923 (odds ratio [OR]= 0.205; 95% confidence interval [CI] = 0.1034–0.4065 for CC variant and [OR] = 0.1436; [CI] = 0.0869–0.2373 for CT variant, respectively) and GEMIN4 rs2740348 (odds ratio [OR] = 0.4498; 95% confidence interval [CI] = 0.2342–0.8637 for CC variant and [OR] = 0.3986; [CI] = 0.2043–0.7776 for CG variant, respectively) showed significant association in lower occurrence of cancer, whereas in case of *GEMIN4* G/C rs910925 variant genotype, no significance correlation was found.

Conclusion: Our study gives a substantive support for the association between the GEMIN4 gene variants/miRNA biogenesis and CRC risk.

KEYWORDS: cancer susceptibility, colorectal cancer (CRC), *GEMIN4* gene, microRNA, single nucleotide polymorphism (SNP)

ABBREVIATIONS

Ago1-4 – Argonaut proteins
CRC – colorectal cancer
HDI – Human Development Index
HWE – Hardy-Weinberg Equilibrium
miRNA – MicroRNAs
mRNA – messenger RNA
OR – odds ratio
RISC – RNA-induced silencing complex
RT-PCR – real time polymerase chain reaction
SNPs – single nucleotide polymorphisms
TNM – tumor-node-metastases
UTR – untranslated region

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant neoplasm occurring mainly in countries of High or Very-High Human Development Index (HDI). In accordance with GLOBOCAN 2018, CRC ranks second in terms of mortality. In 2018 over 1.8 million new cases and 881000 deaths of CRC were estimated to occur in the world [1]. It is believed that the increase of CRC incidence in high-income countries is the result of aging of the population. Moreover, modifiable risk factors such as: inappropriate nutritional habits, lack of exercise, obesity, alcohol consumption and smoking also have a strong impact on the increase in the incidence of CRC. Some of hereditary genetic factors (e.g., the Lynch syndrome or familial adenomatous polyposis) as well as inflammatory bowel diseases, which include Crohn's disease and ulcerative colitis, have been also associated with colorectal cancer, however they represent sporadic cases [2].

CRC can be classified on the basis of the location within the lower gastrointestinal tract, histological characteristics and molecular features. One of the major clinical challenges in CRC treatment is late diagnosis. The basic prognostic marker for CRC clinical outcomes is based on the tumor-node-metastases (TNM) describing the degree to which the tumor has invaded the bowel wall and spread to the regional lymph nodes as well as to distant organs. However, TNM has many disadvantages resulting in inadequately therapeutic approach for patients with histologically identical cancers but with different genetic background [3].

Currently, there is growing interest in the search for proper biomarkers for the CRC development. Epigenetic studies suggested that microRNAs (miRNAs) can meet all biomarker requirements for early detection, classifying the subtypes, prediction of the outcomes and treatment of CRC [4, 5]. MicroRNAs (miRNA) are an abundant class of small noncoding molecules ~ 22 nucleotides in length, which play a significant role in post-transcriptional regulation of messenger RNA (mRNA). They can mediate either its degradation or translational repression by binding to the 3' untranslated region (UTR) of the target mRNA [6]. miRNAs are able to manipulate

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multiple gene expressions and initiate signalling pathways [4], therefore any deregulation in post-transcriptional events caused by incorrect miRNA's structure can lead to the abnormal protein expression. The process of assembling mature miRNA involves complex modification steps, which can be disturbed at four different stages: transcription, nuclear processing, cytoplasm processing and RNA-induced silencing complex (RISC) formation and loading [7]. It has been suggested that single nucleotide polymorphisms (SNPs) may affect miRNA biogenesis pathway, becoming one of significant components of the silencing machinery [8].

GEMIN4 protein is a pivotal member of *GEMIN* protein family that is connected with multiple pathological processes. Together with *GEMIN3* and Argonaut proteins (Ago1-4), *GEMIN4* is a part of a complex that selectively incorporates to miRNA, creating an RNA-induced silencing complex. RISC is responsible for recognising the binding site in 3'-Untranslated Region (3' –UTR) of the target mRNA [9]. Thus, changes in *GEMIN4* may impact the expression of some miRNAs which have been previously associated with certain malignant tumours [10–12]. Polymorphisms in the *GEMIN4* gene were associated with the clinical outcome of several types of cancer described by Wu et al. [13], yet there are still few papers focusing on the role of *GEMIN4* gene polymorphisms in CRC. Therefore, the aim of the study is to determine the relationship between the variability in the *GEMIN4* gene encoding the *GEMIN4* protein and the incidence of colorectal cancer in the Polish population.

MATERIAL AND METHODS

Study population

The study population consisted of patients with diagnosed colorectal cancer and the control group of people who did not suffer from CRC and were recruited from unrelated individuals. All participants included in the research were Caucasians. The stage of colorectal cancer of each patient was classified with TNM, Astler-Costler's and Dukes' staging systems. Five millilitres of peripheral blood were collected from every patient with 0.5 M EDTA as an anti-coagulant and stored at – 80°C for further analysis. The material was obtained from the Department of General and Colorectal Surgery of the University Clinical Hospital WAM – Central Veteran's Hospital. Permission to conduct research was granted by the Bioethics Committee of the Medical University of Lodz. The basic details and characteristics of patients are demonstrated in Tab. I.

SNPs selection

Three functional SNP loci in the *GEMIN4* gene occurring in the exons, were identified in GTexPortal (https://gtexportal.org/home/snp) and dbSNP (www.ncbi nlmnihgov/projects/SNP) databases. *GEMIN4* A/G rs1062923, *GEMIN4* G/C/T rs2740348 and *GEMIN4* G/C rs910925 were selected for the study. Details of selected SNPs are presented in Tab. II.

DNA extraction and genotyping

Blood DNA was extracted using QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen, Chatsworth, CA, USA). Polymorphisms in *GEMIN4* rs1062923, *GEMIN4* rs2740348 and *GEMIN4* rs910925 were genotyped using TaqMan SNP genotyping assay with 96-well plate Real time polymerase chain reaction (RT-PCR) according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). The hydrolysis reaction of the TaqMan probes was performed using a thermocycler BioRad CFXConnect.

Statistical analysis

Statistical analysis was performed using Statistica 13.1 software. The Chi-square test was used to analyse the distribution of genotypes of individual SNPs in CRC patients and the control group. The calculation of odds ratio (OR) and 95% confidence interval (95% CI) was conducted with the risk option of crosstabs to estimate the risk of colorectal cancer. The threshold of statistical significance for CRC risk test was $P \leq 0.05$. The Hardy-Weinberg Equilibrium (HWE) was determined to compare the observed frequency with the expected frequency in both cases and controls.

RESULTS

As many as 203 patients with diagnosed colorectal cancer (M/F; mean age: 58.37 ± 9.19/58.14 ± 9.54) and the control group of 179 people (M/F; mean age: 58.15 ± 9.79/58.17 ± 9.55) were enrolled in the study. There was no significant difference between the patients' and controls' age and gender. Approximately 4% of the patients were in T classification stage I, 20%, 74% and 2% were found to be in stage II, III and IV, respectively. Genotype distributions for all the SNPs in the control group were in accordance with the HWE ($P = 0.0005$).

Genotyping and Association of *GEMIN4* T/C rs1062923, *GEMIN4* G/C rs2740348 and *GEMIN4* C/G rs910925 with CRC

The genotype distribution and allelic frequency of the studied *GEMIN4* polymorphisms are presented in Tab. III. Significant differences between cases and controls were observed in two SNPs: rs1062923 (OR = 0.2, 95%CI 0.1–0.41 for C/C and OR = 0.14, 95%CI 0.09–0.24 for C/T) and rs2740348 (OR = 0.45, 95%CI 0.23–0.86 for C/C and OR = 0.4, 95%CI 0.20–0.78 for C/G). Therefore, both of them were considered to be associated with a decreased risk of colorectal cancer. No significant correlation with the colorectal cancer risk was found for rs910925.

We also estimated the risk of cancer progression for studied SNPs. For this purpose, we utilized the T cancer staging system to differentiate the group of patients in non-invasive first stage (I) and others (II, III and IV) (Tab. I.). We used first stage as control group to compare it with stage II, III and IV. We found no statistical significance for all studied gene polymorphisms (data not shown).

DISCUSSION

GEMIN4 is a protein-coding gene located on chromosome 17p13.3, a hotspot for various melanomas, playing an important role in miRNA machinery. Abnormalities in the miRNA assembly could lead to altered miRNA transcription, splicing, and transcriptional regulation of cell proliferative and apoptotic genes contributing to cancer progression [11]. In this study a significant correlation was found between two SNPs of *GEMIN4* and the risk of CRC: rs1062923 and rs2740348, showing a relatively lower probability of cancer morbidity.

Tab. I. Demographic details of colorectal cancer patients and healthy controls.

VARIABLES	PATIENTS N = 203 (%)	CONTROLS N = 179 (%)
Sex		
Female	104 (51.23)	93 (51.96)
Male	99 (48.77)	86 (48.04)
Age (years)		
Mean age ± SD	58.26 ± 9.35	58.16 ± 9.64
Stage		
T1	7 (3.61)	NA
T2	39 (20.10)	
T3	144 (74.23)	
T4	4 (2.06)	
Grade		
G1	13 (6.84)	NA
G2	173 (91.05)	
G3	4 (2.11)	
Astler-Costler		
A	3 (1.54)	NA
B1	35 (17.95)	
B2	64 (32.82)	
C1	14 (7.18)	
C2	78 (40.00)	
D	1 (0.51)	
Dukes		
A	3 (1.55)	NA
B	98 (50.52)	
C	92 (47.42)	
D	1 (0.52)	

No association between the third polymorphism – rs910925 and the CRC risk was observed in the study, suggestive of either no role in CRC pathogenesis or limited sample size. This is the first epidemiological study evaluating the effects of these SNPs on CRC risk in the Polish population. Obtained values correlate fairy well with those presented by Lin et al. [14] and further support the role of genetic variability of genes coding proteins involved in miRNA biogenesis pathway and CRC. They selected, using data mining of several SNP datasets and an miRNA prediction algorithm, 41 SNPs located in eleven genes related to miRNA biogenesis, and 15 in pri-, pre-, or mature miRNA sequences, of which *GEMIN4*/2740348 was found to show a highly significant correlation with recurrence-free survival in patients of stage III of CRC. It seems that the impact of SNPs located within the *GEMIN4* gene is not limited to CRC. *GEMIN4* SNPs have been investigated in various cancers. In a study performed by Liu et al. in order to evaluate the role of miR-SNPs of *GEMIN4* in prostate cancer a high-resolution melting method was used to genotype seven polymorphisms in the *GEMIN4* gene. Patients carrying the variant heterozygote GC genotype in rs2740348 were at a 36% decreased risk of prostate cancer (OR = 0.64; 95% CI: 0.42–0.99) [10]. In two other studies, two SNPs in the *GEMIN4* gene were significantly associated with an altered renal cell carcinoma risk. The variant-containing genotypes of rs2740348 and rs7813 presented a significantly reduced risk, with odds ratios of 0.67 (95% CI: 0.47–0.96). Haplotype analysis showed that a common haplotype of *GEMIN4* was associated with a significant reduction in the risk of renal cell carcinoma (OR = 0.66; 95% CI:0.45–0.97).

Tab. II. The characteristics of the chosen polymorphisms of *GEMIN4* gene.

SNP ID	POSITION	MAJOR/MINOR ALLELE	MAF (%)
rs1062923	Ile739Thr	T/C	7.0
rs2740348	Gln450Glu	G/C	11.0
rs910925	Ala579Gly	C/G	29.0

SNP – single-nucleotide polymorphism; MAF – minor allele frequency.

Tab. III. The polymorphisms of the *GEMIN4* gene and the risk of colorectal cancer.

POLYMORPHISM	GENOTYPE/ALLELE	PATIENTS	CONTROL	OR	95% CI	P
rs1062923	T/T	148	55	Ref.		
	C/C	**16**	**27**	**0.2**	**0.1–0.41**	**<0.0001**
	C/T	**34**	**88**	**0.14**	**0.09–0.24**	**<0.0001**
	T	330	198	Ref.		
	C	**66**	**142**	**0.28**	**0.2–0.63**	**<0.0001**
rs2740348	G/G	38	16	Ref.		
	C/C	**94**	**88**	**0.45**	**0.23–0.86**	**<0.05**
	C/G	**71**	**75**	**0.4**	**0.20–0.78**	**<0.01**
	G	147	107	Ref.		
	C	259	251	0.75	0.55–1.02	0.06
rs910925	C/C	77	65	Ref.		
	G/G	53	47	0.95	0.57–1.59	0.84
	C/G	66	61	0.91	0.56–1.47	0.71
	C	220	191	Ref.		
	G	172	155	0.96	0.72–1.29	0.80

The most significant associations were SNPs in *GEMIN4* with the variant alleles of both rs7813 and rs910925 associated with 1.74-fold (95% CI: 1.15–2.62) increased risk of death [12, 15].

How can GEMIN gene polymorphisms reduce/increase cancer risk and what is the molecular background of this process? It has been suggested that *GEMIN4* SNPs may have a causal physiological role. The different variant forms of the *GEMIN4* protein have been connected with increased cellular proliferation and reduced apoptosis and DNA repair in hepatocellular carcinoma [16]. Thus, a similar phenotypic effect could be responsible for CRC development as DNA repair abnormalities are characteristic for CRC [17–19].

In summary, we have obtained accurate results proving that the *GEMIN4* gene can alter the risk of CRC. Certainly, these findings should be further reconfirmed by further large-scale studies to better understand the essence of *GEMIN4* and its role in cancer biogenesis.

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