The Egyptian Journal of Medical Human Genetics (2017) 18, 53–59

HOSTED BY

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com

ORIGINAL ARTICLE

Role of *TLR4* gene polymorphisms in the colorectal cancer risk modulation in ethnic Kashmiri population – A case–control study

Saniya Nissar [a,b], Aga Syed Sameer [d,*], Roohi Rasool [b], Qurteeba Qadri [b], Nissar A. Chowdri [e], Fouzia Rashid [c]

[a] *Department of Biochemistry, University of Kashmir, Hazratbal, Srinagar, Kashmir, India*
[b] *Department of Immunology and Molecular Medicine, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India*
[c] *Department of Clinical Biochemistry, University of Kashmir, Hazratbal, Srinagar, Kashmir, India*
[d] *Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Jeddah, Kingdom of Saudi Arabia*
[e] *Department of Surgery, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India*

Received 14 March 2016; accepted 10 April 2016
Available online 26 May 2016

KEYWORDS

Colorectal cancer;
Kashmir;
Polymorphism;
RFLP;
TLR4

Abstract *Background:* Colorectal carcinogenesis has been found to be associated with the polymorphic status of Toll-like receptor 4 gene in various populations of the world.

Aim: The aim of the study was to determine the genetic association of *TLR4* gene polymorphisms (Asp299Gly and Thr399Ile) with disease susceptibility and risk development in colorectal cancer (CRC) patients of Kashmir, India.

Materials and methods: Genotype frequencies of *TLR4* polymorphisms (Asp299Gly and Thr399Ile) were compared between 120 CRC patients and 200 healthy controls using PCR-RFLP method.

Results: We did not find any significant association between the *TLR4* gene polymorphisms and the CRC cases ($p > 0.05$). However CT genotype (Thr399Ile) showed modest elevated risk of the development of CRC [OR = 1.78 95% CI (0.88–3.5)]. Also G allele (AG genotype) of *TLR-4* Asp299Gly polymorphism was found to be significantly associated with the male gender (p value = 0.006) and involvement of Nodes (p value = 0.01) whereas, *T* allele (CT genotype) of Thr399Ile polymorphism showed significant association with the smoking status (p value = 0.03).

Conclusion: Our results suggest that *TLR4* gene polymorphism is not a key modulator of the risk of developing colorectal cancer in Kashmiri population.

* Corresponding author at: Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdul Aziz University for Health Sciences, National Guard Health Affairs, P.O Box: 9515-Mail Code 6556, Jeddah 21423, Kingdom of Saudi Arabia. Tel.: +966 12 2246666x45768.
E-mail address: agasy@ngha.med.sa (A.S. Sameer).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2016.04.004

1. Introduction

Colorectal cancer (CRC) is one of the commonly diagnosed cancers in both men and women. It ranks third most common cancer in men and second most in women worldwide [1]. The incidence of this malignancy shows considerable variation among racially or ethnically defined populations in multiracial/ethnic countries. Kashmir (Indian Administered) has been reported as a high-incidence area of gastrointestinal cancers (GIT) [1–3]. In Kashmir valley CRC represents the third most common GIT cancer [3,4] after esophageal and gastric cancers; and among males CRC ranks as fourth common cancer and third among females [2,3].

Toll-like receptors (TLRs) play a vital role in immune responses, being involved in the regulation of inflammatory reactions and activation of the adaptive immune response to eliminate infectious pathogens and cancer debris [5–7].

The *TLR4* gene is mapped on chromosome 9 and consists of three exons. Two common non-synonymous SNPs (+896A/G rs4986790 and +1196C/T rs4986791) have been found in exon 3 of gene which induce substitution of amino acids Asp299Gly and Thr399Ile, respectively. The substitution of Asp299Gly disrupts the normal structure of the extracellular region of the *TLR4*, which may cause decreased ligand recognition or protein interaction and decreased responsiveness to lipopolysaccharide [8]. Consequently, such change can affect the transport of *TLR4* to the cell membrane and lead to an exaggerated inflammatory response with severe tissue destruction [9].

TLR4 Asp299Gly and Thr399Ile are known to co-segregate in 10% of the Caucasian populations and are reported to have positive correlation with susceptibility to several diseases. However, neither SNP is present in Asian populations [10].

CRC is one of the few cancers in which inflammation is the basic pathological incident which later on can transform into full blown malignancy [11–14]. Furthermore, a positive association between various innate immune system mediators and bacterial toxins with the development of CRC has also been found [15]; together with the fact that TLRs serve as the mediators of inflammation in the gut and are known to be crucial regulators in maintaining the balance between commensal bacteria in the gut and the mucosal immune system [16,17].

In addition, the potential association between *TLR4* genetic polymorphisms and the risk of bladder cancer, prostate cancer, breast cancer, melanoma [18–21], hepatocellular carcinoma [22], gastric cancer [23–25] and gastric mucosa associated lymphoid tissue lymphoma has been proposed [26]. Both *TLR4* polymorphisms are also involved in advancement of Head and neck squamous cell carcinoma (HNSCC) and may serve as biomarkers for HNSCC [27]. In fact, some studies have reported a significant role of *TLR4* polymorphisms in increasing the risk of colorectal cancer as well in their respective populations [28,29].

Therefore, the potential role of the *TLR4* polymorphisms in the development and progression of CRC becomes much more important to be researched upon, provided the limited data are available on colorectal cancer.

In the present study, we conducted a case–control study to find out the relevance of *TLR4* polymorphisms (Asp299Gly and Thr399Ile) in colorectal cancer patients of Kashmiri population. Furthermore we also investigated whether there is a link between the clinic-pathological variables and the *TLR4* Asp299Gly and/or Thr399Ilegenotypes and hence their role in modulating the risk of colorectal cancer.

2. Subjects and methods

2.1. Subjects

This study included 120 consecutive primary CRC patients recruited from Department of Surgery, Sher-I-Kashmir Institute of Medical Science, Kashmir, India. Tumor types and stages were determined by two experienced pathologists. The cases who had not received any chemo or radiotherapy were chosen for this study. Blood samples of 200 age and sex matched individuals with no signs of any malignancy were collected for controls. The mean age of both patient and control groups was 55 years.

Data on all CRC patients were obtained from personal interviews with patients and or guardians (for those who were illiterate), medical records and pathology reports. The data collected included sex, age, dwelling, tumor location, Dukes Stage, lymph node status. All patients and or guardians were informed about the study and their will to participate in this study was taken on predesigned questionnaire (Available on request). The collection and use of tumor and blood samples for this study were previously approved by the appropriate Institutional Ethics Committee. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. DNA extraction & genotype analysis

DNA extraction was performed using Ammonium Acetate Method. One μL (50 ng) of DNA was used as the template for each PCR cycle.

Analysis of *TLR-4* Asp299Gly and Thr399Ile Polymorphisms Genotyping was performed using PCR-RFLP method as described previously [30]. Primers (Genescript, USA, New Jersey) for *TLR4* Asp299Gly were forward (F5′ GATTAGCA TACTTAGACTACTACCTCCATG) and reverse (R5′ GATCAACTTC TGA AAAAGCATTCCCAC) and Nco-I enzyme (Fermentas, USA, Maryland) was used for digestion. Similarly, primers for *TLR4* Thr399Ile were forward (F5′ GGTTGC TGTTCTCAAAGTGATTTTGGGAGAA) and reverse (R5′ CCTGAAGACTGGAGAGTGAGTTAAATGCT) and Hinf-I (Fermentas) was used to carry out restriction digestion.

Briefly PCR was carried out in a final volume of 25 μL containing 50 ng genomic DNA templaten, 1× PCR buffer with 2 mM $MgCl_2$, 0.5 μM of each primer (Genescript), 50 μM dNTPs (Fermentas) and 0.5 U DNA polymerase (Fermentas). For PCR amplification, the standard program was used as follows: one initial denaturation step at 94 °C for 7 min, followed by 35 denaturation cycles of 30 sat 95 °C, 30 s of annealing at 55 °C, and 30 s of extension at 72 °C, followed by a final elongation cycle at 72 °C for 7 min.

PCR product of 249 bp and 406 bp for *TLR4* Asp299Gly and Thr399Ile respectively were resolved for confirmation of PCR through a 2–3 per cent agarose gel (Genie, India, Bangalore). Both amplicons were then restriction digested at 37 °C for genotypic of two SNPs.

For, *TLR4* Asp299Gly 249 bp was identified as homozygous wild type AA, while three bands of 249 bp, 226 bp and 23 bp were identified as heterozygous variant allele AG and the homozygous mutant type GG displayed two bands of 226 bp and 23 bp. For, *TLR4* Thr399Ile 406 bp was identified as wild type CC, while three bands of 406 bp, 377 bp and 29 bp were identified as heterozygous variant allele CT and the homozygous mutant type TT displayed two bands of 377 bp and 29 bp. The genotypes of >20 per cent of the samples were reassessed in a double-blind manner by two independent researchers, to confirm the results. A positive control for each polymorphism was used for 50 per cent of the samples.

2.3. Statistical analysis

The observed frequencies of the above genotypes in patients with CRC were compared with the controls using chi-square or fisher exact tests when the expected frequencies were small. The chi-square test was used to verify whether the genotype distributions were in Hardy–Weinberg equilibrium (for allele and genotype frequencies in our population). Statistical significance was set at $p < 0.05$.Statistical analyses were performed using PASW version 18 software.

3. Results

A total of 120 cases and 200 control subjects were included in this study with prior consent. Out of 120 confirmed cases of CRC, 72 were males and 48 cases were females; 66 were rural 54 were urban; 52 cases had carcinoma in colon and 68 in rectum; 59 were smokers and 61 non- smokers. The mean age of patients having confirmed CRC was 55 years. Among control subjects, 149 consisted of males and 51 females. No significant gender- or age-related differences were observed between the groups ($p > 0.05$).

Among 120 CRC cases, we found the frequency of heterozygous state of *TLR-4* Asp299Gly polymorphism to be 10.8% (13/120) while the frequency in the general control population was 15.4% (31/200). Likewise the frequency of heterozygous state of *TLR-4* Thr399Ile polymorphism was found to be 15% (18/120) in cases and 9% (18/200) among controls. Variant homozygous genotype was not found in both the polymorphisms in either the cases or the controls. The overall association between the *TLR4* polymorphism and the CRC cases was found to be insignificant ($p > 0.05$) (Table 1). However CT genotype showed a modest elevated risk of the development of CRC [OR = 1.78 95% CI (0.88–3.5)]. Furthermore, analysis of the gene interactions of the two SNPs of *TLR4* gene also did not reveal any significant association between the genotypes ($p > 0.05$) (Table 2).

We also analyzed the correlation of *TLR*-4 Asp299Gly polymorphism with the clinicopathological characteristics and found the G allele carrier to be significantly associated with the males (p value = 0.006) and involvement of lymph nodes (p value = 0.01) (Table 3). The Thr399Ile polymorphism study showed the *T* allele carrier to be significantly associated with the smokers (p value = 0.03) (Table 4).

4. Discussion

Toll-like receptors (TLRs) belong to Type I Transmembrane Glycoproteins, Containing three important domains: (a) the 608 residue ecto-domain – found outside the cell or in cytoplasm and contains multiple leucine-rich repeats (LRR) participating in the recognition and binding of pathogens, (b) single trans membrane domain (STM), (c) 187 residue toll/interleukin 1 receptor (TIR) domain. LRRs harbor 24–29 residues that fold into parallel β-sheets [8,10,31].

It has been found by various studies that the low-functioning *TLR4* polymorphisms usually result in reduced inflammatory response correlating with the low-damaging infection but a persistent infection [32–34]. Genetic variants of *TLR4* polymorphisms Asp299Gly and Thr399Ile have been found to disrupt the structure of the extracellular region of *TLR4* protein. This in turn leads to the disturbance of normal TLR signaling creating a pro-inflammatory environment that favors tumor growth and chemo resistance and hence plays a pivotal role in carcinogenesis process [35], or immunosuppression induced by chronic inflammation [7,36,37].

TLR4 Asp299Gly gene variant has an impact on *TLR4* signaling and potentially on intestinal homeostasis due to impaired control signals at the epithelial cell level leading to chronic intestinal inflammation & interrupted intestinal homeostasis and hence may lead to CRC [38].

In this study we evaluated the distribution of *TLR4* Asp299Gly and *TLR4* Thr399Ile polymorphisms in CRC patients and controls, but we didn't find a significant statistical difference in the distribution among the two groups. Comparing all CRC patients to healthy controls, the adjusted OR for CRC risk was 1.5 (95% CI, 0.75–3.01) and 0.56 (95% CI, 0.27–1.12) for Asp299Gly and Thr399, respectively. We also did not find any homozygous variant genotype among any of the cases or controls of the study. The results are consistent with the already published studies [10].

Although none of the two polymorphisms was found to be significantly associated with CRC statistically, however G allele (AG genotype) of *TLR-4* Asp299Gly polymorphism was found to be significantly associated with the male gender (p value = 0.006) and involvement of Nodes (p value = 0.01) (Table 3) and *T* allele (CT genotype) of

Table 1 *TLR4* genotypes and allele frequencies between CRC cases and controls.

SNP	Controls (200)	Cases (120)	OR(95%CI)/Pearson p value	Fisher p value (One-tailed; Two-tailed)
Asp299Gly(A/G)				
AA	169 (84.5%)	107 (89.1%)	1.5 (0.75–3.01); 0.24	0.15, 0.31
AG	31 (15.4%)	13 (10.8%)		
Thr399Ile(C/T)				
CC	182 (90.9%)	102 (85.0%)	0.56 (0.27–1.12); 0.10	0.07; 0.14
CT	18 (9.0%)	18 (15.0%)		

Table 2 Comparative stats for the two polymorphisms for cumulative effect on CRC.

SNP		Asp299Gly (A/G)		OR(95%CI)	Fisher *p* value
		AA *n* = 107	AG *n* = 13		
Thr399Ile (C/T)	CC *n* = 102	93	9	0.33 (0.09–1.24)	0.105
	CT *n* = 18	14	4		

Table 3 Association of *TLR4* Thr299Ile polymorphism with various clinic-pathological characteristics in CRC cases.

Characteristics	*N* = 120	CC 102(85.0%)	CT 18(15.0%)	TT 0(0%)	OR (95%CI); *p* value
Age (years)					
>50	62(51.6%)	51	11	0	1.57 (0.56–4.37); 0.38
⩽50	58(48.3%)	51	7	0	
Gender					
Male	72(60.0%)	56	16	0	**6.57(1.43–30.07); 0.006**
Female	48 (40.0%)	46	2	0	
Dwelling					
Rural	66(55.0%)	55	11	0	1.34 (0.48–3.74); 0.57
Urban	54(45.0%)	47	7	0	
Smoking status					
Ever	59(49.1%)	48	11	0	1.76 (0.63–4.92); 0.27
Never	61(50.8%)	54	7	0	
Tumor location					
Colon	52(43.3%)	44	8	0	1.05 (0.38–2.89); 0.92
Rectum	68(56.6%)	58	10	0	
Tumor grading					
W.D	80(66.6%)	70	10	0	0.57 (0.20–1.58); 0.27
M.D and P.D	40(33.3%)	32	8	0	
Lymph node involved					
Yes	66(55%)	61	5	0	**0.25(0.08–0.78); 0.019**
No	54(45%)	41	13	0	

W.D = well differentiated; M.D = moderately differentiated; P.D = poorly differentiated.
Significance of the association is represented in bold font of the values ($p < 0.05$).

Thr399Ile polymorphism showed significant association with the smoking status (p value = 0.03).

A recent meta-analysis by Sheng et al. [39] found that the GG genotype carriers of *TLR4* gene had higher risk of developing CRC than AA + GA genotype carriers ($p = 0.05$). Furthermore, stratified analyses showed that the genetic models of the Asian group had higher risk of developing CRC than the Caucasian group. They concluded that the GG genotype of *TLR4* Asp299Gly and the TT genotype of *TLR4* Thr399Ile polymorphisms might be correlated with an increased risk of CRC, indicating that they may serve as biomarkers of disease progression

Another meta-analysis by Li et al. [40] found an empirical evidence of involvement of *TLR4* gene in driving CRC and this *TLR4* may serve as a potential biomarker for the early diagnosis of CRC. Zhang et al., [41] in their meta-analysis for *TLR4* gene polymorphisms found both *TLR4* (299/399) polymorphisms to be associated with increased cancer risk and one SNP (rs1927911) with decreased cancer risk.

Third meta-analysis by Jing et al. [7] has shown *TLR4* polymorphisms to be positively associated with the increased risk of digestive tract cancers. Their results suggested that Asp299Gly has a risk effect on gastrointestinal cancers but it tends to be a protective factor on prostate cancer. They also reported significant geographic and ethnic differences in the intercontinental incidence of Asp299Gly and Thr399Ile polymorphisms with both SNPs found to be present in 10% of Caucasian populations. Zou et al. [42] also reported similar results in their meta-analysis of *TLR4* gene polymorphisms and concluded that the association between *TLR4* Thr399Ile polymorphism and cancer risk was significant in both South Asians and East Asians, but not in Caucasians.

Omrane et al. [28] found a strong association between both of the *TLR4* polymorphisms and CRC. They reported a significant

Table 4 Association of *TLR4* Asp299Gly polymorphism with various clinic-pathological characteristics in CRC cases.

Characteristics	$N = 120$	AA 107 (89.1%)	AG 13 (10.8%)	GG 0(0%)	OR (95%CI); p value
Age (years)					
⩽50	62(51.6%)	54	8	0	1.57 (0.48–5.11); 0.45
>50	58(48.3%)	53	5	0	
Gender					
Male	72(60.0%)	63	9	0	1.57 (0.45–.5.42); 0.47
Female	48(40.0%)	44	4	0	
Dwelling					
Rural	66(55.0%)	62	4	0	0.32 (0.09–1.11); 0.06
Urban	54(45.0%)	45	9	0	
Tumor location					
Colon	52(43.3%)	48	4	0	0.54 (0.15–1.88); 0.33
Rectum	68(56.6%)	59	9	0	
Smoking status					
Ever	59(49.1%)	49	10	0	**3.94(1.02–15.14); 0.03**
Never	61(50.8%)	58	3	0	
Tumor grading					
W.D	80(66.6%)	71	9	0	1.14 (0.32–3.95)
M.D and P.D	40(33.3%)	36	4	0	
Lymph node involved					
Yes	66(55.0%)	57	9	0	1.97 (0.57–6.80); 0.27
No	54(45.0%)	50	4	0	

W.D = well differentiated; M.D = moderately differentiated; P.D = poorly differentiated.
Significance of the association is represented in bold font of the values ($p < 0.05$).

association of TL4 polymorphisms with numerous clinic-pathological parameters (tumor differentiation ($p = 0.027$) and tumor architecture ($p = 0.02$), advanced stage ($p = 0.03$) and concluded that *TLR4* polymorphisms may serve as potential biomarker of CRC progression.

Pimentel-Nunes et al. [29] in their study on European CRC patients reported that the functional polymorphisms in *TLR2* and *TLR4* significantly alter the risk of developing CRC. Furthermore they also reported that the risk of cancer gets exemplified in the overweight individuals.

However, there are a number of studies on various populations which have observed a non-association of *TLR4* gene polymorphisms with the risk of modulating CRC [43,44]. A number of studies have shown overexpression of *TLR4* to be associated with the metastatic colorectal cancer [45–47]; while as a few have shown that loss of expression or strong downregulation of *TLR4* is associated with the metastatic colorectal cancer [48], while some reported no change in expression by variant status of *TLR4* [11].

Furthermore, a number of studies reported from India on various cancers have implicated *TLR4* polymorphism in modulating the risk of cancers [49–52]. So, our study is also an extension of such efforts to elucidate the role played by various genetic polymorphisms in modulating the risk of CRC.

5. Conclusion

In conclusion, our results suggest that *TLR4* gene polymorphism is not a key modulator of the risk of developing colorectal cancer in Kashmiri population.

Conflict of interest

None exist between any parties.

Acknowledgments

The authors wish to thank each and every CRC patient who took part in this study and cooperated during the interview and sample collection. The authors gratefully acknowledge the financial support provided by the Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, Kashmir, for this work. We also thank the Department of Immunology & Molecular Medicine, SKIMS, Soura, Kashmir for providing all the technical support. We also thank the head and technical staff of the operating theater in the Department of General Surgery, SKIMS, Soura, Kashmir who helped us with tissue procurement, and the anonymous pathologists at the Department of Pathology, SKIMS, Soura, Kashmir for the histopathological assessment of the tumor tissues.

References

[1] Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality Rates and trends-an update. Cancer Epidemiol Biomarkers Prev 2015.

[2] Wani MA, Jan FA, Khan NA, Pandita KK, Khurshid R, Khan SH. Cancer trends in Kashmir; common types, site incidence and demographic profiles: National Cancer Registry 2000–2012. Indian J Cancer 2014;51(2):133–7. http://dx.doi.org/10.4103/0019-509X.138188.

[3] Sameer AS. Colorectal cancer: a researcher's perspective of the molecular angel's gone eccentric in the Vale of Kashmir. Tumor Biol 2013;34:1301–15.
[4] Sameer AS. Colorectal cancer: molecular mutations and polymorphisms. Front Oncol 2013;3:114. http://dx.doi.org/10.3389/fonc.2013.00114.
[5] Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001;2(8):675–80.
[6] Wang RF, Miyahara Y, Wang HY. Toll-like receptors and immune regulation: implications for cancer therapy. Oncogene 2008;27(2):181–9. http://dx.doi.org/10.1038/sj.onc.1210906.
[7] Jing JJ, Li M, Yuan Y. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in cancer: a meta-analysis. Gene 2012;499(2):237–42. http://dx.doi.org/10.1016/j.gene.2012.03.045, Epub 2012 Mar 13.
[8] Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. *TLR4* mutations are associated with endotoxin hypo responsiveness in humans. Nat Genet 2000;25(2):187–91.
[9] Zhu L, Yuan H, Jiang T, Wang R, Ma H, et al. Association of *TLR2* and *TLR4* polymorphisms with risk of cancer: a meta-analysis. PLoS One 2013;8(12):e82858.
[10] El-Omar EM, Ng MT, Hold GL. Polymorphisms in Toll-like receptor genes and risk of cancer. Oncogene 2008;27(2):244–52. http://dx.doi.org/10.1038/sj.onc.1210912.
[11] Proença MA, de Oliveira JG, Cadamuro AC, Succi M, Netinho JG, Goloni-Bertolo EM, et al. *TLR2* and *TLR4* polymorphisms influence mRNA and protein expression in colorectal cancer. World J Gastroenterol 2015;21(25):7730–41. http://dx.doi.org/10.3748/wjg.v21.i25.7730.
[12] Slattery ML, Fitzpatrick FA. Convergence of hormones, inflammation, and energy-related factors: a novel pathway of cancer etiology. Cancer Prev Res (Phila) 2009;2(11):922–30. http://dx.doi.org/10.1158/1940-6207.CAPR-08-0191.
[13] Mantovani A. Cancer: inflammation by remote control. Nature 2005;435(7043):752–3.
[14] Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J PhysiolGastrointest Liver Physiol 2004;287(1):G7–G17.
[15] Wogan GN, Dedon PC, Tannenbaum SR, Fox JG. Infection, inflammation and colon carcinogenesis. Oncotarget 2012;3(8):737–8.
[16] Slattery ML, Herrick JS, Bondurant KL, Wolff RK. Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. Int J Cancer 2012;130(12):2974–80. http://dx.doi.org/10.1002/ijc.26314.
[17] Fukata M, Abreu MT. Pathogen recognition receptors, cancer and inflammation in the gut. Curr Opin Pharmacol 2009;9:680–7.
[18] Shen Y, Liu Y, Liu S, Zhang A. Toll-like receptor 4 gene polymorphisms and susceptibility to bladder cancer. Pathol Oncol Res 2013;19:275–80.
[19] Zheng SL, Augustsson-Balter K, Chang B, Hedelin M, Li L, Adami HO, et al. Sequence variants of toll-like receptor 4 are associated with prostate cancer risk: results from the Cancer Prostate in Sweden Study. Cancer Res 2004;64(8):2918–22.
[20] Theodoropoulos GE, Saridakis V, Karantanos T, Michalopoulos NV, Zagouri F, Kontogianni P, et al. Toll-like receptors gene polymorphisms may confer increased susceptibility to breast cancer development. Breast 2012;21(4):534–8.
[21] Gast A, Bermejo JL, Claus R, Brandt A, Weires M, Weber A, et al. Association of inherited variation in Toll-like receptor genes with malignant melanoma susceptibility and survival. PloS One 2011;6(9):e24370.
[22] Minmin S, Xiaoqian X, Hao C, Baiyong S, Xiaxing D, Junjie X, et al. Single nucleotide polymorphisms of Toll-like receptor 4 decrease the risk of development of hepatocellular carcinoma. PLoS One 2011;6(4):e19466.
[23] Zhou Q, Wang C, Wang X, Wu X, Zhu Z, et al. Association between *TLR4* (+896A/G and +1196C/T) polymorphisms and gastric cancer risk: an updated meta-analysis. PLoS One 2014;9(10):e109605. http://dx.doi.org/10.1371/journal.pone.0109605.
[24] Chen J, Hu S, Liang S, Chen Q, Yang Q, Zheng W, et al. Associations between the four toll-like receptor polymorphisms and the risk of gastric cancer: a meta-analysis. Cancer Biother Radiopharm 2013;28(9):674–81. http://dx.doi.org/10.1089/cbr.2012.1395.
[25] Hold GL, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, et al. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. Gastroenterology 2007;132(3):905–12.
[26] Türe-Ozdemir F, Gazouli M, Tzivras M, Panagos C, Bovaretos N, Petraki K, et al. Association of polymorphisms of NOD2, *TLR4* and CD14 genes with susceptibility to gastric mucosa-associated lymphoid tissue lymphoma. Anticancer Res 2008;28(6A):3697–700.
[27] Bergmann C, Bachmann HS, Bankfalvi A, Lotfi R, Pütter C, Wild CA, et al. Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in head and neck squamous cell carcinomas. J Transl Med 2011;9:139. http://dx.doi.org/10.1186/1479-5876-9-139.
[28] Omrane I, Baroudi O, Kourda N, Bignon YJ, Uhrhammer N, Desrichard A, et al. Positive link between variant Toll-like receptor 4 (Asp299Gly and Thr399Ile) and colorectal cancer patients with advanced stage and lymph node metastasis. Tumour Biol 2014;35(1):545–51. http://dx.doi.org/10.1007/s13277-013-1075-6.
[29] Pimentel-Nunes P, Teixeira AL, Pereira C, Gomes M, Brandão C, Rodrigues C, et al. Functional polymorphisms of Toll-like receptors 2 and 4 alter the risk for colorectal carcinoma in Europeans. Dig Liver Dis 2013;45(1):63–9. http://dx.doi.org/10.1016/j.dld.2012.08.006, Epub 2012 Sep 19.
[30] Lorenz E, Frees KL, Schwartz DA. Determination of the *TLR4* genotype using allele-specific PCR. Biotechniques 2001;31(1):22–4.
[31] Kutikhin AG. Impact of Toll-like receptor 4 polymorphisms on risk of cancer. Hum Immunol 2011;72(2):193–206. http://dx.doi.org/10.1016/j.humimm.2010.11.003.
[32] Agnese DM, Calvano JE, Hahm SJ, Coyle SM, Corbett SA, Calvano SE, et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk gram-negative infections. J Infect Dis 2002;186(10):1522–5.
[33] Child NJ, Yang IA, Pulletz MC, de Courcy-Golder K, Andrews AL, Pappachan VJ, et al. Polymorphisms in Toll-like receptor 4 and the systemic inflammatory response syndrome. Biochem Soc Trans 2003;31(Pt 3):652–3.
[34] Faber J, Meyer CU, Gemmer C, Russo A, Finn A, Murdoch C, et al. Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy. Pediatr Infect Dis J 2006;25(1):80–1.
[35] Chen R, Alvero AB, Silasi DA, Mor G. Inflammation, cancer and chemoresistance: taking advantage of the toll-like receptor signaling pathway. Am J Reprod Immunol 2007;57(2):93–107.
[36] Noreen M, Arshad M. Association of *TLR1*, *TLR2*, *TLR4*, *TLR6*, and TIRAP polymorphisms with disease susceptibility. Immunol Res 2015;62(2):234–52. http://dx.doi.org/10.1007/s12026-015-8640-6.
[37] Noreen M, Shah MA, Mall SM, Choudhary S, Hussain T, Ahmed I, et al. *TLR4* polymorphisms and disease susceptibility. Inflamm Res 2012;61(3):177–88. http://dx.doi.org/10.1007/s00011-011-0427-1.
[38] Davoodi H, Hashemi SR, Seow HF. Increased NFk-B activity in HCT116 colorectal cancer cell line harboring *TLR4* Asp299Gly variant. Iran J Allergy Asthma Immunol 2012;11(2):121–32, 011.02/ijaai.121132.
[39] Sheng WY, Yong Z, Yun Z, Hong H, Hai LL. Toll-like receptor 4 gene polymorphisms and susceptibility to colorectal cancer: a meta-analysis and review. Arch Med Sci 2015;11(4):699–707.

[40] Li X-X, Sun G-P, Meng J, Li X, Tang Y-X, et al. Role of Toll-like receptor 4 in colorectal carcinogenesis: a meta-analysis. PLoS One 2014;9(4):e93904. http://dx.doi.org/10.1371/journal.pone.0093904.

[41] Zhang K, Zhou B, Wang Y, Rao L, Zhang L. The *TLR4* gene polymorphisms and susceptibility to cancer: a systematic review and meta-analysis. Eur J Cancer 2013;49(4):946–54. http://dx.doi.org/10.1016/j.ejca.2012.09.022, Epub 2012 Oct 17.

[42] Zou TH, Wang ZH, Fang JY. Positive association between Toll-like receptor 4 gene +896A/G polymorphism and susceptibility to gastric carcinogenesis: a meta-analysis. Tumour Biol 2013;34 (4):2441–50. http://dx.doi.org/10.1007/s13277-013-0795-y, Epub 2013 Apr 17.

[43] Davoodi H, Seow HF. Variant Toll-like receptor4 (Asp299Gly and Thr399Ile alleles) and Toll-like receptor2 (Arg753Gln and Arg677Trp alleles) in colorectal cancer. Iran J Allergy Asthma Immunol 2011;10(2):91–9, 010.02/ijaai.9199.

[44] Guo Q, Zhu J, Xia B. Polymorphism of CD14 gene but not the mutation of *TLR4* gene is associated with colorectal cancer in Chinese patients. J Gastroenterol Hepatol 2006;21(1 Pt 1):92–7.

[45] Cammarota R, Bertolini V, Pennesi G, Bucci EO, Gottardi O, Garlanda C, et al. The tumor microenvironment of colorectal cancer: stromal *TLR-4* expression as a potential prognostic marker. J Transl Med 2010;8:112. http://dx.doi.org/10.1186/1479-5876-8-112.

[46] Doan HQ, Bowen KA, Jackson LA, Evers BM. Toll-like receptor 4 activation increases Akt phosphorylation in colon cancer cells. Anticancer Res 2009;29(7):2473–8.

[47] Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology 2007;133(6):1869–81, Epub 2007 Sep 14.

[48] Simiantonaki N, Kurzik-Dumke U, Karyofylli G, Jayasinghe C, Michel-Schmidt R, Kirkpatrick CJ. Reduced expression of *TLR4* is associated with the metastatic status of human colorectal cancer. Int J Mol Med 2007;20(1):21–9.

[49] Qadri Q, Rasool R, Afroze D, Naqash S, Gulzar GM, Yousuf A, et al. Study of *TLR4* and IL-8 gene polymorphisms in H.pylori-induced inflammation in gastric cancer in an ethnic Kashmiri population. Immunol Invest 2014;43(4):324–36. http://dx.doi.org/10.3109/08820139.2013.854378.

[50] Priyadarshini A, Chakraborti A, Mandal AK, Singh SK. Asp299Gly and Thr399Ile polymorphism of *TLR-4* gene in patients with prostate cancer from North India. Indian J Urol 2013;29:37–41.

[51] Srivastava K, Srivastava A, Kumar A, Mittal B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. Liver Int 2010;30(7):1067–72. http://dx.doi.org/10.1111/j.1478-3231.2010.02268.x.

[52] Pandey S, Mittal RD, Srivastava M, Srivastava K, Singh S, Srivastava S, et al. Impact of Toll-like receptors *TLR 2* (-196 to -174 del) and *TLR 4* (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. Gynecol Oncol 2009;114 (3):501–5. http://dx.doi.org/10.1016/j.ygyno.2009.05.032.