XRCC1 Gene Polymorphisms and miR-21 Expression in Patients with Colorectal Carcinoma

Kolorektal Karsinoması Olan Hastalarda XRCC1 Gen Polimorfizmleri ve miR-21 Ekspresyonu

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ABSTRACT

Objective: The objectives of this study were to evaluate the impact of two X-ray repair cross complementing 1 (XRCC1) gene polymorphisms (Arg194Trp and Arg399Gln) on the risk of development of colorectal cancer (CRC) and to assess the expression levels of microRNA-21 (miR-21) in CRC patients.

Materials and Methods: A case-control cross sectional study was conducted on 50 CRC patients and 50 cancer-free subjects. DNA and miR-21 were extracted from whole blood samples. The expression levels of the XRCC1 polymorphisms and miR-21 were assessed by real-time PCR in all subjects of the study.

Results: Genotype analysis revealed a significant association between CRC risk and both the Arg194Trp genotype (OR=11.407, 95% CI=4.039-32.221, p<0.001) and the Arg399Gln genotype (OR=3.778, 95% CI= 1.6-8.919, p=0.002). The expression levels of circulating miR-21 were able to detect CRC cases significantly (p=0.022) with a sensitivity of 82% and a specificity of 56% (Area under the curve (AUC)=0.633) but were unable to distinguish between early and late cases (AJCC classification) (p=0.194).

Conclusion: The XRCC1 Arg194Trp and Arg399Gln polymorphisms both confer high susceptibility for the development of CRC. Circulating miR-21 expression levels are a potentially diagnostic non-invasive genetic marker of CRC.

Keywords: Colorectal cancer, miRNA-21, XRCC1, DNA repair, single nucleotide polymorphisms

ÖZ

Amaç: Bu çalışmanın amacı iki XRCC1 gen polimorfizminin (Arg194Trp ve Arg399Gln) kolorektal kanser (KRK) gelişimi riski üzerindeki etkisini değerlendirmek ve KRK hastalarında microRNA-21 (miR-21) ekspresyonu düzeylerini incelemektir.

Gereç ve Yöntemler: 50 KRK hastası ve 50 kansersiz denekle vaka-kontrollü bir kesitsel çalışma gerçekleştirildi. Tüm kan numunelerinden DNA ve miR-21 alındı. XRCC1 polimorfizmlerinin ve miR-21'in ekspresyon seviyeleri, çalışmadaki tüm deneklerde gerçek zamanlı PCR ile değerlendirildi.

Bulgular: Genotip analizinde KRK riski ile Arg194Trp (OR=11.407, 95% CI=4.039-32.221, p<0,001) ve Arg399Gln (OR=3.778, 95% CI=1,6-8.919, p=0,002) genotipleri arasında önemli bir ilişki ortaya konuldu. Dolaşımdaki miR-21 ekspresyon düzeyleriyle KRK olguları %82 duyarlılık ve %56 özgüllük (AUC=0,633) ile anlamlı bir şekilde (p=0,022) saptanabildi, ancak erken ve geç vakalar ayırt edilemedi (AJCC sınıflandırması) (p=0,194).

Sonuç: XRCC1 Arg194Trp ve Arg399Gln polimorfizmlerinin her ikisi de KRK gelişimine yüksek düzeyde yatkınlık göstermektedir. Dolaşımdaki miR-21 ekspresyon seviyeleri potansiyel olarak KRK'nın tanısal non-invasiv genetik belirtecidir.

Anahtar Kelimeler: Kolorektal kanser, miRNA-21, XRCC1, DNA onarımı, SNPs

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Introduction

It has been reported in many previous studies that most nucleotide polymorphisms (SNPs) have no deleterious effects or negative health impacts because they are usually located in the non-coding regions of genes. SNPs are often used as genetic markers for the prediction of patient response to therapy and to predict susceptibility to the development or diagnosis of a particular disease [1, 2].

Expression of the X-ray cross-complementing group 1 (XRCC1) gene yields a scaffolding protein that binds to many repair enzymes, such as poly-ADP ribose polymerase, DNA ligase III, DNA polymerase β, polynucleotide kinase, AP endonuclease, proliferating cell nuclear antigen,

and 8-oxoguanine DNA glycosylase [3]. These DNA-repair enzymes have critical protective functions against all kinds of environmental carcinogens that damage human genome. The XRCC1 gene codes for a DNA-repair enzyme that is involved in base excision repair of oxidative DNA damage as well as single-strand break repair. Moreover, several studies have reported an association between XRCC1 Arg/Gln or Gln/Gln genotypes and the clinical response to chemotherapy in colorectal cancer (CRC) [4]. Huang et al. [3] found a strong relation between environmental risk factors such as smoking or drinking alcohol and the presence of XRCC1 gene polymorphisms and the risk of CRC. They reported that individuals who have the XRCC1 194Arg allele and are heavy smokers or subjects who have the XRCC1 399Arg/Gln genotype and have a drinking habit or are heavy smokers have an increased risk of CRC.

MicroRNAs (miRNAs) are small endogenous RNAs that modify either mRNA degradation or translational repression by binding to the 3′-untranslated region of mRNA [5]. Accumulating evidence suggests that microRNA-21 (miR-21) is involved in the development of malignant tumors through its effects on cell apoptosis, proliferation, invasion, and metastasis by targeting phosphatase, tensin homolog (PTEN), and tissue inhibitor of metalloproteinases 3 (TIMP3) or through its role in signaling pathways such as Wnt/β-catenin, PTEN/PI-3K/AKT, and RAS/MEK/ERK [6, 7]. Recent studies showed that miR-21 is frequently up-regulated in CRC and that elevated expression of miR-21 is associated with worse prognosis and inefficient response to chemotherapy [8, 9]. Moreover, Li et al. [10] found that knocking down miR-21 expression in the CRC cell line SW-480 suppressed the invasion, migration, and proliferation of the cells.

The objectives of our present study were to assess the impact of the Arg194Trp and Arg-399Gln XRCC1 gene polymorphisms on the risk of CRC and to measure the expression level of miR-21 in CRC patients.

Materials and Methods

Study population

Overall, 50 CRC patients and 50 cancer-free control subjects were included in the study. All subjects signed a written informed consent to participate in the study. This study got approval of Ethical committee of Faculty of Medicine Cairo University. The current study was conducted in compliance with Declaration of Helsinki ethical principles. Data for all subjects were obtained from medical records and personal interviews. CRC patients were classified according to the American Joint Committee on Cancer staging (AJCC).

Subjects of the study were divided into the following groups

Group 1

Fifty CRC patients (26 males and 24 females) with an average age of 49.54±15.37 [mean±standard deviation (SD)] years. Inclusion criteria were pathologically confirmed CRC cases by pathological examination of the colonoscopy findings from both sexes; ascending, transverse, or left-sided rectosigmoid cancer; and not having previously been treated. Exclusion criteria were non-eligible patients; recurrent tumor cases or a history of secondary tumors; and having received any intervention, including surgery, chemotherapy, or radiotherapy.

Group 2

Fifty cancer-free control subjects of both sexes (50 males and 50 females) with an average age of 49.18±16.17 years were included. They were chosen from symptomatic patients who were undergoing colonoscopy with confirmed non-cancerous lesions (with or without inflammatory bowel disease).

DNA extraction

Whole blood DNA was extracted with the Quick-gDNA™ MiniPrep kit (Zymoresearch, CA, USA, Catalogue no. D3024) according to the manufacturer's directions.

Real-time PCR

This step involved DNA amplification and detection of Arg194Trp (rs1799782) and Arg-399Gln (rs 25487) SNPs using Real MOD™ Real-time PCR Master Mix (Catalog no. 25341, Intron Biotechnology, Korea,)

DNA template concentration used for TaqMan SNP genotyping was 20 ng/µl in the quantitative (qRT-PCR) reaction mix. DNA concentration was determined by measuring the absorption at 260 nm using a Nanodrop ND-1000* spectrophotometer.

The qRT-PCR reaction mix per well consisted of 10 µL of 2x RealMOD™ Real-time PCR Master Mix solution, 7 µL nuclease-free water, 2 µL PCR primers, TaqMan® probe, and 1 µL template DNA. The Real-time PCR instrument (Applied Biosystems, SDS v 2.1 software, Step One System, RQ Manager 1.2) was programmed as follows: denaturation (95°C for 5 min) and then 45 cycles of denaturation (92°C for 15 sec) and annealing/extension (60 °C for 60 sec).

MiRNA extraction

MiRNAs were extracted from 300 µL whole blood with the miRNeasy extraction kit according to the manufacturer`s directions (Catalogue no. 217004, Qiagen, Valencia, CA, USA).

Quantification of miR-21 by reverse-transcriptase real-time PCR

a. Reverse transcriptase (RT)

The miRNAs were converted into cDNA with the TaqMan micro-RNA kit (Catalogue no. 4427975, ID 000397, Applied Biosystems) according to the manufacturer's directions.

b. Real-time PCR (qPCR):

The cDNA generated from the RT reaction was amplified using the TaqMan PCR Master Mix kit (Applied Biosystems, Cat No. 4440040), and a Step One System (RQ Manager 1.2, software v 2.1, Applied Biosystem) was used for the reactions. To determine the threshold cycle (CT) value from the amplification plot, RNU6B (Applied Biosystems) was used as the endogenous reference control gene for normalization control. Negative controls were used to exclude any contamination.

The cDNA was amplified using FAM/MGB probe (5' FAM-TCGGGCCTGGTTAGT MGB 3'), forward primer (5' CGTCTGATCTCG-GAAGCTAAGC 3'), and reverse primer (5' GGCGGTCTCCCATCCAA 3') (Gene Bank accession number NG043905.1)

The qRT-PCR reaction mix per well consisted of 2 µL nuclease-free water, 0.5 µL TaqMan microRNA Assay (20×), 6.5 µL TaqMan Universal Master Mix, and 3 µL cDNA. The RT-PCR instrument was programmed as follows: denaturation (95°C, 10 min) followed by 40 cycles of denaturation (95°C for 15 sec) and annealing/extension (60°C for 60 sec).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences version 22 (IBM Corp.; Armonk, NY, USA). Data were calculated as frequencies and relative frequencies for categorical variables and mean±SD for quantitative variables. Comparisons between groups were calculated with an unpaired *t*-test. For categorical data, the comparison was done using chi-square (c^2) test. When the expected frequency was less than 5, exact test was used. The receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was calculated to determine the best cutoff value for miRNA detection. Genotype frequencies were compared between groups using c^2 test. $p<0.05$ was considered statistically significant.

Results

Demographic results

Colorectal cancer patients were stratified as described by the AJCC staging system. Findings of the present study revealed that most of the CRC patients (78%) had an early-stage disease, in which there were no lymph node involvement, no metastases, and no local invasion beyond the colon. Most of the patients (82%) presented with a left-sided tumor, with or without extensions to the transverse or even the ascending colon. More than one-third of the CRC patients presented with low-grade adenocarcinoma.

Diagnostic results

XRCC1 gene polymorphisms

The homozygous wild types Arg/Arg for exon 399 and Arg/Arg for exon 194 were significantly more frequent in the control group compared to the CRC group. The homozygous 194Trp/Trp genotype was not found in control subjects but was detected in 8% of CRC patients, while the heterozygous 194Trp genotype was found in 12% of the controls and 56% of the cases. The frequency of the 194Trp allele was significantly higher in CRC cases (36%) in comparison to control subjects (6%). CRC cases showed significantly higher expression of the 399Gln allele (36%) compared to control subjects (18%). The homozygous 399Gln/Gln polymorphism was detected in 8% of CRC cases and 4% of the control subjects. The heterozygous 399Arg/399Gln genotype was found in 56% of CRC cases and 28% of the control subjects (Table 1).

Colorectal cancer risk was significantly linked with the homozygous 194Trp/Trp genotype (p=0.0), and there was a strong significant association with the heterozygous 194 genotype (OR=11.407, 95%CI=4.039-32.221). This significance became more pronounced if the homozygous 194Trp was combined with heterozygous 194 genotype (OR=13.037, 95%CL=4.654-36.52). In contrast, CRC risk showed no significant association with the homozygous 399Gln genotype (p=0.187). However, a strongly significant association could be shown when combined with the heterozygous 399 genotype (OR=3.778, 95%CI=1.65–8.651, p=0.001) (Table 2).

MiR-21

Colorectal cancer cases showed significantly higher expression levels of miR-21 (31.65±4.89) in comparison to control subjects (27.77±8.55) (p<0.022).

Table 1. XRCC1 genotype in CRC cases and matched controls

	Exon 194				Exon 399			
Genotype Group	Arg/Arg No. (%)	Arg/Trp No. (%)	Trp/Trp No. (%)	Trp-allele Frequency	Arg/Arg No. (%)	Arg/Gln No. (%)	Gln/Gln No. (%)	Gln-allele frequency
Patients (n=50)	18 (36%)	28 (56%)	4 (8%)	36 (36%)	18 (36)	28 (56)	4 (8%)	36 (36%)
Control (n=50)	44 (88%)	6 (12%)	0 (0%)	6 (6%)	34 (68%)	14 (28)	2 (4%)	18 (18%)

CRC: colorectal cancer

Table 2. Estimates of the relative risk for CRC associated with the 399Gln and 194Trp genotypes

Genotype	CRC (n=50)	Controls (n=50)	Odds ratio (OR)	95% confidence interval (CI)	p
Exon 194 polymorphism					
Arg/Arg	18	44	1		
Arg/Trp	28	6	11.407	4.039-32.221	<0.001
Trp/Trp	4	0	----	----	0.01
Arg/Trp + Trp/Trp	32	6	13.037	4.654-36.52	<0.001
Exon 399 polymorphism					
Arg/Arg	18	34	1		
Arg/Gln	28	14	3.778	1.6-8.919	0.002
Gln/Gln	4	2	3.778	0.630-22.648	0.187
Arg/Gln + Gln/Gln	32	16	3.778	1.65-8.651	0.001

CRC: colorectal cancer

The homozygous 194Arg/Arg and the 399Arg/Arg genotypes served as reference categories.

Table 3. Analysis of the ROC curve of miR-21 for the detection of CRC cases

		95% Confidence Interval					
AUC	p	Lower Bound	Upper Bound	Cutoff value	Youden index	Sensitivity (%)	Specificity (%)
0.633	0.022	0.519	0.746	30.795	0.38	82	56

CRC: colorectal cancer

ROC curve analysis (Figure 1) showed that the miR-21 levels had a significant impact on discriminating CRC cases from control subjects. The AUC was 0.633 (95%CI=0.519-0.746), and the specificity and sensitivity were 56% and 82%, respectively (Table 3) using a cutoff value of 30.795 (Youden index=0.38). We found no statistically significant diagnostic value of miR-21 expression to discriminate between early and late stages of CRC (p=0.194).

The ROC analysis for detection of the late CRC cases revealed that the whole blood miR-21 levels were unable to differentiate between the early CRC cases and late cases. The AUC value was 0.629 (95% CI=0.458-0.801) (Figure 2).

Discussion

A case-control study was conducted in 50 CRC cases and 50 control subjects to assess the impact of two XRCC1 gene polymorphisms (194Arg/Trp and 399Arg/Gln) and to assess miR-21 expression as a risk factor for the development of CRC. We found significantly higher expression of the heterozygous Arg194Trp genotype in CRC cases, and the 194Trp allele was significantly more frequent in CRC cases than in control subjects. Moreover, expression of the heterozygous 399Arg/Gln genotype was significantly elevated in CRC cases compared to control subjects. The 399Gln allele frequency was highly significant in CRC cases. Our findings are comparable to those reported by Karam et al. [11] who found a significant association between both XRCC1 A399G polymorphisms and histological grading of CRC.

Dimberg et al. [12] reported that the XRCC1 gene has three main SNPs that are located in codon 399 (exon 10), codon 194 (exon 6), and codon 280 (exon 9). The three common XRCC1 gene polymorphisms usually oc-

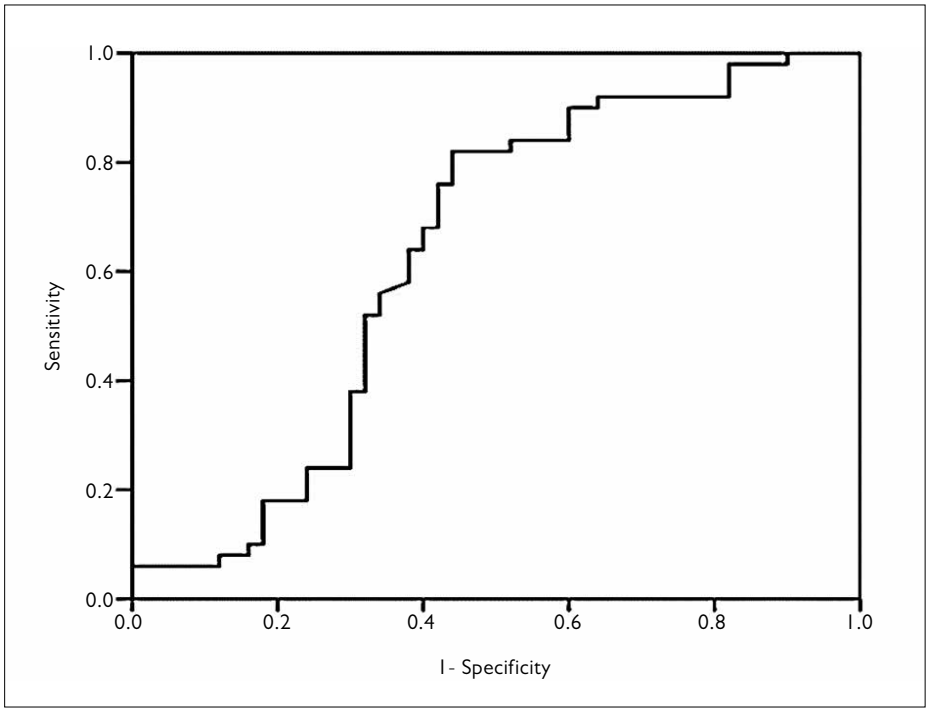


Figure 1. ROC curve for miR-21 in all CRC cases
CRC: colorectal cancer

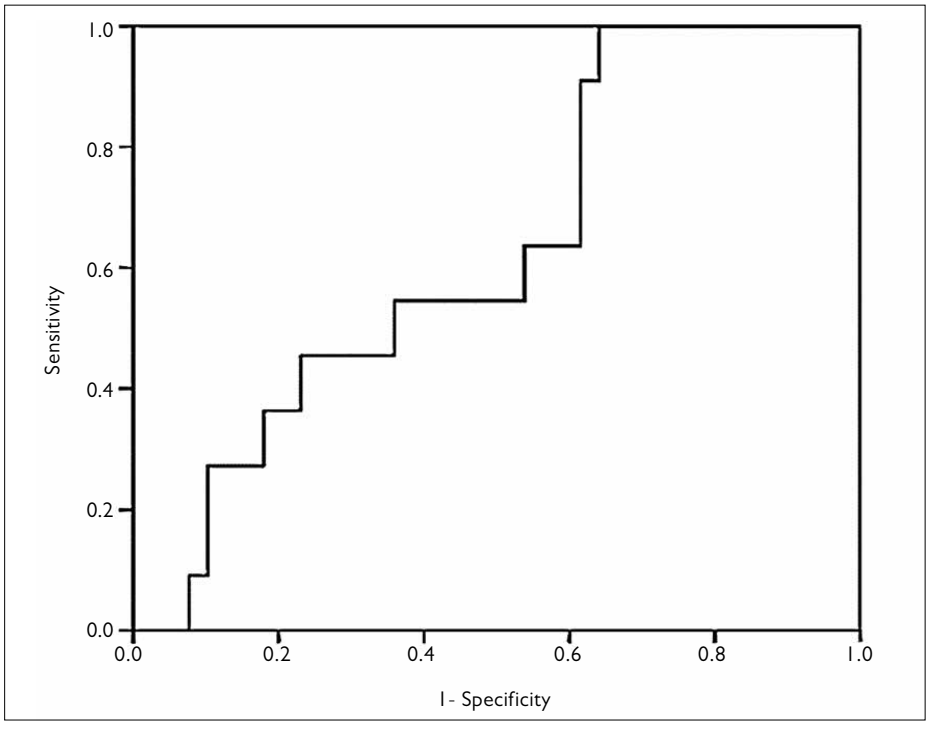


Figure 2. ROC curve for miR-21 in late CRC cases
CRC: colorectal cancer

cur in conserved sequences, which influence the gene-coding regions. The XRCC1 gene is a DNA-repair gene that encodes a protein that plays a major role in single-strand break repair and base excision repair systems. A recent large-scale meta-analysis confirmed the presence of a significant link between XRCC1 399Arg/Gln polymorphism and the development of CRC [13].

In agreement with our results, Halim et al. [14] reported that the 194Trp allele is significantly associated with high risk for the development of CRC. The frequency of the 194Trp allele in our study was similar to that previously reported in studies from Egypt and Mexico [15, 16].

Moreover, Dai et al. [17] showed that the XRCC1 194Arg/Trp gene polymorphism is associated with increased risk for CRC, reduced chemotherapy response, and reduced survival. Another study conducted by Nissar et al. [18] among a Kashmiri population found a significant impact of the Trp/Trp and Arg/Trp genotypes on the development of CRC.

Some studies have reported contradictory results regarding the association between the risk of CRC development and the presence of XRCC1 polymorphisms [19, 20]. These findings might be explained by the fact that the development of CRC is influenced by interactions between environmental factors and genetic factors. Zhang et al. [21] found that smoking significantly affects the association between XRCC1 399Arg/Gln and CRC risk in certain Chinese populations. Moreover, Brevik et al. [22] showed that free radical damage generated by a diet rich in red meat plays a significant contribution to the development of CRC.

We found significantly higher expression levels of circulating miR-21 in CRC patients compared to controls, and the ROC curve showed an 82% sensitivity and 56% specificity. However, the ROC curve was unable to discriminate between early and late CRC cases. Bastaminejad et al. [23] reported that miR-21 expression levels in serum were significantly elevated in CRC cases with an 86.05% sensitivity and 72.97% specificity. A meta-analysis that included articles published from 2009 to 2014 where the participants include Caucasians, Asians, and a few Africans showed that circulating miR-21 is useful as an early diagnostic biomarker for several malignant tumors, including CRC [24, 25]. Moreover, Sheng et al. [26] found that the protein kinase/phosphatase/phosphatidyl-inositol kinase (AKT/PTEN/PI3K) signaling pathway is intimately linked with many malignant tumors and their progression, drug resistance, immunity, angiogenesis, and metastasis [27, 28]. Furthermore, there have been several studies showing that miR-21 has a significant role in regulating the AKT/ PTEN/ PI3K signaling pathway and contributes to the initiation of carcinogenesis, tumor progression, and metastasis [29, 30]. Sheng et al. [26] reported that the PTEN gene is a direct target gene of miR-21. Thus, gene expression levels of phosphatase and its downstream AKT and PI3K genes might be controlled by regulating miR-21 expression, which in turn regulates the development of CRC.

Limitations of the present study include the small number of CRC cases as well as the presence of small number in each CRC patient subgroup that was categorized according to the tumor grade or stage.

To the best of our knowledge there are no relations between mi-RNA21 and XRCC1 gene polymorphisms.

The presence of either the 194Trp allele or 399Gln allele is associated with greater risk for CRC. Circulating miR-21 expression levels can be used as an early diagnostic marker for CRC cases but cannot discriminate early from late cases of CRC.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Cairo University Faculty of Medicine (2015).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - H.F., D.S., H.S.; Design - D.S., H.S., H.M.; Supervision - H.F., D.S., H.S.; Resource - H.M., H.S., N.A.; Materials - H.M., N.A.; Data Collection and/or Processing - H.F., D.S.; Analysis and /or Interpretation - H.M., N.A.; Literature Search - H.M., H.S., N.A.; Writing - H.F., H.M., N.A.; Critical Reviews - H.F., D.S., H.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Mazzuca F, Botticelli A, Mazzotti E, et al. CYP19A1 genetic polymorphisms rs4646 and osteoporosis in patients treated with aromatase inhibitor-based adjuvant therapy. Eurasian J Med 2016; 48: 10-4.
2. Atis O, Sahin S, Ceyhan K, Ozyurt H, Akbas A, Benli I. the distribution of apolipoprotein E gene polymorphism and apolipoprotein E levels among coronary artery patients compared to controls. Eurasian J Med 2016; 48: 90-4.
3. Huang Y, Li X, He J, et al. Genetic polymorphisms in XRCC1 genes and colorectal cancer susceptibility. World J Surg Oncol 2015; 13: 244.
4. Khan NP, Pandith AA, Yousuf A, et al. The XRCC1Arg399Gln gene polymorphism and risk of colorectal cancer: a study in Kashmir. Asian Pac J Cancer Prev 2013; 14: 6779-82.
5. Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer--a brief overview. Adv Biol Regul 2015; 57: 1-9.
6. Xu LF, Wu ZP, Chen Y, Zhu QS, Hamidi S, Navab R. MicroRNA-21 (miR-21) regulates cellular proliferation, invasion, migration, and apoptosis by targeting PTEN, RECK and Bcl-2 in lung squamous carcinoma, Gejiu City, China. PLoS One 2014; 9: e103698.
7. Yu Y, Kanwar SS, Patel BB, et al. MicroRNA-21 induces stemness by downregulating transforming growth factor beta receptor 2 (TGF-betaR2) in colon cancer cells. Carcinogenesis 2012; 33: 68-76.
8. Pan X, Wang ZX, Wang R. MicroRNA-21: A novel therapeutic target in human cancer. Cancer Biol Ther 2010; 10: 1224-32.
9. Lin PL, Wu DW, Huang CC, et al. MicroRNA-21 promotes tumour malignancy via increased nuclear translocation of beta-catenin and predicts poor outcome in APC-mutated but not in APC-wild-type colorectal cancer. Carcinogenesis 2014; 35: 2175-82.
10. Li C, Zhao L, Chen Y, et al. MicroRNA-21 promotes proliferation, migration, and invasion of colorectal cancer, and tumor growth associated with down-regulation of sec23a expression. BMC Cancer 2016; 16: 605.
11. Karam RA, Al Jiffry BO, Al Saeed M, Abd El Rahman TM, Hatem M, Amer MG. DNA repair genes polymorphisms and risk of colorectal cancer in Saudi patients. Arab J Gastroenterol 2016; 17: 117-20.
12. Dimberg J, Skarstedt M, Slind Olsen R, Andersson RE, Matussek A. Gene polymorphism in DNA repair genes XRCC1 and XRCC6 and association with colorectal cancer in Swedish patients. APMIS 2016; 124: 736-40.
13. Forat-Yazdi M, Gholi-Nataj M, Neamatzadeh H, Nourbakhsh P, Shaker-Ardakani H. Association of XRCC1 Arg399Gln polymorphism with colorectal cancer risk: A HuGE meta analysis of 35 studies. Asian Pac J Cancer Prev 2015; 16: 3285-91.
14. Halim NH, Chong ET, Goh LP, et al. Variant alleles in XRCC1 Arg194Trp and Arg399Gln polymorphisms increase risk of gastrointestinal cancer in Sabah, North Borneo. Asian Pac J Cancer Prev 2016; 17: 1925-31.
15. Abdel-Rahman SZ, Soliman AS, Bondy ML, et al. Inheritance of the 194Trp and the 399Gln variant alleles of the DNA repair gene XRCC1 are associated with increased risk of early-onset colorectal carcinoma in Egypt. Cancer Lett 2000; 159: 79-86.
16. Muñiz-Mendoza R, Ayala-Madrigal ML, Partida-Pérez M, et al. MLH1 and XRCC1 polymorphisms in Mexican patients with colorectal cancer. Genet Mol Res 2012; 11: 2315-20.
17. Dai Q, Luo H, Li XP, Huang J, Zhou TJ, Yang ZH. XRCC1 and ERCC1 polymorphisms are related to susceptibility and survival of colorectal cancer in the Chinese population. Mutagenesis 2015; 30: 441-9.
18. Nissar S, Lone TA, Banday MZ, et al. Arg-399Gln polymorphism of XRCC1 gene and risk of colorectal cancer in Kashmir: A case control study. Oncol Lett 2013; 5: 959-63.
19. Liu L, Miao L, Ji G, Qiang F, Liu Z, Fan Z. Association between XRCC1 and XRCC3 polymorphisms and colorectal cancer risk: a meta-analysis of 23 case-control studies. Mol Biol Rep 2013; 40: 3943-52.
20. Mao D, Zhang Y, Lu H, Fu X. Association between X-ray repair cross-complementing group 1 Arg194Trp polymorphism and colorectal cancer risk. Tumour Biol 2013; 34: 2529-38.
21. Zhang SH, Wang LA, Li Z, et al. APE1 polymorphisms are associated with colorectal cancer susceptibility in Chinese Hans. World J Gastroenterol 2014; 20: 8700-8.
22. Brevik A, Joshi AD, Corral R, et al. Polymorphisms in base excision repair genes as colorectal cancer risk factors and modifiers of the effect of diets high in red meat. Cancer Epidemiol Biomarkers Prev 2010; 19: 3167-73.
23. Bastaminejad S, Taherikalani M, Ghanbari R, Akbari A, Shabab N, Saidijam M. Investigation of microRNA-21 expression levels in serum and stool as a potential non-invasive biomarker for diagnosis of colorectal cancer. Iran Biomed J 2016; 21: 106-13.
24. Wu K, Li L, Li S. Circulating microRNA-21 as a biomarker for the detection of various carcinomas: an updated meta-analysis based on 36 studies. Tumour Biol 2015; 36: 1973-81.
25. Zanutto S, Pizzamiglio S, Ghilotti M, et al. Circulating miR-378 in plasma: a reliable, haemolysis-independent biomarker for colorectal cancer. Br J Cancer 2014; 110: 1001-7.
26. Sheng WZ, Chen YS, Tu CT, He J, Zhang B, Gao WD. MicroRNA-21 promotes phosphatase gene and protein kinase B/phosphatidylinositol 3-kinase expression in colorectal cancer. World J Gastroenterol 2016; 22: 5532-9.
27. McCubrey JA, Steelman LS, Chappell WH, et al. Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascade inhibitors: how mutations can result in therapy resistance and how to overcome resistance. Oncotarget 2012; 3: 1068-111.
28. Hafsi S, Pezzino FM, Candido S, et al. Gene alterations in the PI3K/PTEN/AKT pathway as a mechanism of drug-resistance (review). Int J Oncol 2012; 40: 639-44.
29. Xiong B, Cheng Y, Ma L, Zhang C. MiR-21 regulates biological behavior through the PTEN/PI-3 K/Akt signaling pathway in human colorectal cancer cells. Int J Oncol 2013; 42: 219-28.
30. Han M, Liu M, Wang Y, et al. Antagonism of miR-21 reverses epithelial-mesenchymal transition and cancer stem cell phenotype through AKT/ERK1/2 inactivation by targeting PTEN. PLoS One 2012; 7: e39520.