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Decreased expression of DNA repair genes (*XRCC1* and *XPD/ERCC2*) in colorectal cancer in Iranian patients

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Abstract

The aim of this study was to investigate the role of the *XRCC1* and *XPD/ERCC2* in the susceptibility and in the progression of colorectal cancer (CRC) in Iranian patients. Epidemiological studies have shown a positive association between defective DNA repair capacity and CRC. The underlying mechanism of their involvement is not well understood. In the present study, we have analyzed the relationship between CRC and the expression of DNA repair genes namely X-ray repair cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (*XPD)* in 70 formalin fixed and paraffin embedded tumor tissues as well as normal adjacent tissues. The relative expression of XRCC1 and *XPD* in the mentioned tissues was performed for first time by quantitative real-time PCR (q-PCR). Results of this study demonstrated that difference of mean relative expression between tumor tissues and normal tissues is 64-fold *(XRCC1)* > 16-fold *(XPD)*. In multivariate logistic regression analysis, low expression of XRCC1 and *XPD* was associated with a statistically significant increased risk of CRC [crude odds ratios (ORs) (95%CI) OR 2.10; (1.06-4.17) and OR 5.24 (2.38-11.52), respectively]. In conclusion, our study demonstrated that reduced expression of XRCC1and *XPD* is associated with more than twofold increased risk in CRC.

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Introduction

Colorectal cancer (CRC) is one of the most common cancers in developed countries (Jemal *et al.*, 2006), and newly is becoming more prevalent in developing countries (Fengju *et al.*, 2000). The reasons of CRC can include high red meat intake (Chao *et al.*, 2005), high alcohol intake (Cho *et al.*, 2004) and smoking (Giovannucci, 2001). These factors cause DNA damages that it must repair by DNA repair systems. Epidemiological studies have shown a positive association between defective DNA repair capacity and CRC, and the underlying mechanism of their involvement is not well understood. We know many genes *(XRCC1, XPD/ERCC2,* and etc) involve in the DNA repair systems. Recently, more and more views support genetic and epigenetic predisposition as the basis of many diseases, especially cancers (Garinis *et al.*, 2002). These problems may disrupt role of DNA repair proteins and It means when DNA repair genes don't correctly act some diseases and cancers occur in human. Therefore, the aim of the present study was demonstration the role of the DNA repair genes (*XRCC1* and *XPD/ERCC2)* in the susceptibility and in the progression of colorectal cancer (CRC) in Iranian patients.

In the base excision repair (BER) pathway, X-ray repair cross-complementing group 1(XRCC1) acts as a scaffolding protein and lacks any enzymatic activity. XRCC1 also plays an important role in the repair of single-strand breaks. It is mentioned (Campalans *et al.*, 2005) that XRCC1 may be recruited to the site of the lesion involved in BER or single-strand break repair, which subsequently modulates the activities of many proteins. XRCC1 can also bind to DNA polymerase β as a scaffold protein and help in the replacement of oxidized base in BER. It forms a XRCC1–DNA ligase III heterodimer at the site of repair. Finally, DNA ligase III fills the nick region produced due to excision of oxidized base.

Another DNA repair gene XPD which encodes an ATP dependent DNA helicase is involved in the nucleotide excision repair (NER) and in basal transcription as a part of the transcription factor TFIIH (Lainé *et al.*,

2007). NER removes and corrects a variety of bulky lesions induced by ultraviolet rays, chemical adducts, and cross-links (Wood, 1999). During the NER pathway, XPD gene encodes a protein which possesses an ATP-dependent helicase activity. It is a subunit of TFIIH which helps in maintaining unwound DNA structure at damaged region (Lindahl and Wood, 1999).

Very few studies are available regarding the role of the expression levels of the two DNA repair genes (*XRCC1* and *XPD*) and their relationship with colorectal cancer. Therefore, we for first time have utilized quantitative real-time PCR for determination of relative expression levels of these two DNA repair genes. The involvement of candidate DNA repair genes (*XRCC1* and *XPD/ERCC2*) expression with CRC risk was determined in the formalin fixed and paraffin embedded tumor tissues (FFPE) and normal adjacent tissues of CRC patients in north east of Iran.

Material and methods

Patient population and tissue samples

Seventy patients with histologically confirmed colorectal cancer (primary sporadic CRC) were examined in the present study. All patients underwent colorectal resection primarily at Neyshabur Medical Science Faculty Hospital (Neyshabur, Iran) from January 2008 to February 2013. None of the patients were treated previously (radiotherapy or chemotherapy). Patients who had other malignant disease except CRC were excluded. This study was approved by the IAU- Neyshabur Branch and Neyshabur University of Medical Sciences. All tissue samples were collected within 10 minutes of resection. Tumor samples were divided into two parts; one part was sent for histopathological diagnosis and staging and the other half was stored as formalin fixed paraffin embedded (FFPE) as well as adjacent normal samples (normal non tumorous healthy tissue at >2 cm distance from the tumor) (Li *et al.*, 2012). Histopathological grades and clinical staging were evaluated according to the standard criteria (Edge *et al.*, 2010). Only histopathologically confirmed cases were included for further molecular

analysis.

RNA extraction from tissue samples and cDNA preparation

RNA isolated 5 times with the Jena Bioscience kit (Cat.-No. PP-210xs, Germany). Before all extractions FFPE tissue specimens was deparaffinized by xylem. Quantity/quality was checked spectrophotometrically /1% agarose gel electrophoresis and stored for further use at -20°C. cDNA was prepared according to manufacturer's instructions of kit (AccuPower RoketScript RT PerMix) from BioNEER company of korea.

Real-time quantitative PCR (qPCR)

Real Time PCR was done using gene specific primers (Table 1). The PCR was carried out in a volume of 20 μl in Applied Biosystems step one Thermocycler using SYBR® Green PCR Master Mix (Part number 4309155) under the following conditions:

denaturation at 94 °C for 10 s; annealing (as per primer) for 20 s; extension at 72 °C for 5 s. The DNA repair genes mRNA expression was normalized to the housekeeping gene *GAPDH*. This reference gene previously has been shown to be appropriate for normalization in human tumor and normal colorectal tissue (Caradec *et al.*, 2010). The comparative CT method was used to evaluate the differential DNA repair genes expression in each patient sample (includes tumor and normal adjacent samples). The

relative amount of each target gene to *GAPDH* was determined using the following equation:

$\Delta\Delta Ct^{-2}$; where $\Delta\Delta Ct$ gene = [Ct $_{gene}$ - Ct $_{reference}$] tumor tissue - [Ct $_{gene}$ −Ct $_{reference}$] normal tissue.

For each target gene we performed three replicates of q-PCR.

Statistical analysis

Statistical analyses were performed with SPSS statistical software, version 20.0 for Windows (SPSS IBM, IL, USA). Statistical significance was defined as $P < 0.05$.

Results

Patients and tumor characteristics

A total of 140 consecutive surgically resected FFPE tissue specimens comprising of 70 tumor tissues and 70 adjacent normal control regions of 70 primary sporadic CRC were collected for analysis in the present study. The age range of patients was from 35 to 87 years (median, 66 years). Patients included 47 men (67.1%) and 23 women (32.9%). Of the 70 cases analyzed, tumor was located in the colon, rectum and rectosigmoid regions in 55.7%, 31.4% and 12.9% cases, respectively. Histological examination was done in all of the cases to evaluate tumor histotype. Details of each patient related to their demographic profile, clinical characteristics and average of relative expression repair genes in 70 CRC tissues have been shown in table 2.

Table 1. Primer sequences used in q-PCR (Kumar *et al.*, 2012; Ivanova *et al.*, 2007).

Gene	Primer	Sequences	Amplicon size (bp)
XRCC1	Forward	5′-CTGGGACCGGGTCAAAAT-3′	60
	Reverse	5′-CAAGCCAAAGGGGGAGTC-3′	
XPD/ERCC2	Forward	5′-GGCCTCCTGGTATGAGCA-3′	70
	Reverse	5′-CCTGGGTCTCAATAAAGAGCA-3′	
GAPDH	Forward	5′-GCTCTCTCCTCCTGTTCG-3′	115
	Reverse	5′-ACGACCAAATCCGTTGACTC-3′	

Repair genes expression

We performed a quantitative -PCR assay to analyze the mRNA expression of *XRCC1 and XPD/ERCC2* in FFPE tissue samples of colorectal cancer (tumor and

normal adjacent tissues). We could detect *XRCC1* and *XPD/ERCC2* mRNA expression in tumor tissue than normal is 0.0156- fold and 0.0625-fold, respectively, or we can say that *XRCC1* and *XPD/ERCC2* mRNA

expression in normal tissue than tumor is about 64-fold and 16-fold, respectively (Table 2 and Table3). Therefore, their expression was significantly reduced in colorectal cancer compared to normal adjacent tissue.

Additionally, to explore gene–gene interaction, we observed no significant Spearmen correlation between XRCC1 vs. XPD (p>0.05).

Table 2. Demographic, clinical characteristics and average of relative expression repair genes in 70 CRC tissues.

Patient characteristic	No. of samples (%)	Fold difference in Target relative to normal tissue ($2^{-\Delta\Delta Ct}$)			
		XRCC1		XPD	
		Average	P Value	Average	P Value
Total	70 (100)	0.0153 (3-6)		0.0613 (2-4)	
Gender					
Male	47 (67.1)	0.0154	0.222	0.0597	0.232
Female	23 (32.9)	0.0152		0.0620	
Age					
≤50	28 (40)	0.0175	0.052*	0.0633	0.035*
50 <	42 (60)	0.0132		0.0589	
Tumor location					
Colon	39 (55.7)	0.0150	0.855	0.0617	0.584
Rectum	22 (31.4)	0.0155		0.0610	
Rectosigmoid	9 (12.9)	0.0161		0.0598	
Tumor stage					
I	15 (21.4)	0.0170	0.050*	0.0641	0.053*
II	33 (47.1)	0.0152		0.0621	
III	17 (24.3)	0.0145		0.0610	
IV	5 (7.1)	0.0142		0.0600	
Tumor grade#					
WD	20 (28.6)	0.0154	0.323	0.0616	0.398
MD	33 (47.1)	0.0153		0.0592	
PD	12 (17.1)	0.0146		0.638	
U	5 (7.1)	0.0163		0.0676	

#WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; U, undifferentiated.

*p value <0.05 was taken as significant.

Discussion

Here, we discuss regarding the data on gene expression level of tow well-studied DNA repair genes, namely *XRCC1* and *XPD/ERCC2* and their association with CRC in Iranian patients. Our results exhibit a significant reduction in the mRNA levels of XRCC1 > XPD in tumor tissues as compared to the normal adjacent tissues in CRC patients. Multiple molecular pathways have been considered to be responsible for alterations in gene expression during the progression of CRC. Several DNA repair pathways are involved in the maintenance of genetic stability. Base excision repair (BER) and nucleotide excision repair (NER) are the major pathway involved in removal of endogenous and mutagen induced DNA damage. XRCC1 deficiency in BER and XPD

deficiency in NER may dangerous for organism. The genomic fidelity and genetic stability depends on the efficiency of DNA repair capacity (DRC) when an organism is exposed to environmental and endogenous carcinogens. As we demonstrated in this study the positive correlation of DRC with age and low expression of DNA repair genes may reflect greater need to repair DNA damage (Takahashi *et al.*, 2005; Chen *et al.*, 2003). Reduced expression of P[16] (Attri *et al*, 2005), MGMT in esophageal squamous cell carcinoma (Liu *et al.*, 2007), *XPG/ERCC5*, *CSB/ERCC6* in lung cancer (Cheng *et al.*, 2002) and CDH1 in esophageal cancer (Ling *et al*, 2011) has been reported. The activity of DNA repair protein and enzyme may be affected by the level of transcription, splicing, stability of mRNA, translation, protein stability, posttranslational modification, and by the action of inhibitors or stimulators. In addition, there may be large number of factors such as epigenetic factors (e.g., DNA methylation) or stochastic factors that affect enzyme activity. Some studies demonstrated that genetic polymorphisms of the *XRCC1 and XPD* genes are associated with colorectal cancer, lung cancer and cervical cancer (Skjelbred *et al.*, 2006; Zhang *et al.*, 2012; Dai *et al.*, 2012) and response to platinum-based chemotherapy in non-small-cell lung cancer, colorectal cancer, and breast cancer (Gurubhagavatula *et al.*, 2004).Positive association of XRCC1 Arg399Gln variants with mesothelioma and in head and neck cancer (Betti *et al.*, 2011; Kowalski *et al.*, 2009] suggest that Gln/Gln homozygote of this polymorphism may be linked with decreased effectiveness of BER by an alteration in BRCT (BRCA1 carboxyl-terminal domain) domain of the XRCC1 (Chang *et al.*, 2009). This results in reduced binding of XRCC1 by PARP, leading to the accumulation of DNA damage (Rodriguez *et al.*, 2003). Also, by computational study it was demonstrated that Arg560Trp and Arg107His alter stability and function of XRCC1 and then this protein do not act normally and may cause cancer (Mehrzad *et al.*, 2014).

Table 3. Relative expression of DNA repair genes in colorectal cancer (n = 70).

Gene	Tissue	Average Ct ±*SD	CtΔ (Ct Target − Ct Reference)	Ct (ΔΔ ΔCt Tumor - ΔCt Normal)	Fold difference in XRCC1 relative to Normal ($2^{-\Delta\Delta Ct}$)	Fold difference in XPD /ERCC2 relative to Normal ($2^{-\Delta\Delta Ct}$)
XRCC1 (Target)	Normal	15.50± 0.08	-6.53± 0.04	6.08± 0.07	0.0153	0.0613
	Tumor	0.07±21.95	-0.45± 0.07			
XPD /ERCC2 (Target)	Normal	27.99 ±0.03	5.96± 0.05	4.30± 0.08		
	Tumor	32.66±0.09	10.26± 0.08			
GAPDH (Referenc)	Normal	22.03 ± 0.03	*SD: Standard Deviation			
	Tumor	22.40 ± 0.04				

The difference between the expression pattern of tumor tissues and normal tissues suggest effects of different genetic mutations, single nucleotide polymorphism (SNP), epigenetic characteristics, or some environmental factors. The reduced expression of these DNA repair genes may likely to exhibit a reduced DNA repair activity, consequently leading to the accumulation of more damages in CRC.

According to data of present study, approximately 64-fold lowered expression in the case of XRCC1 in tumor tissues than normal tissues agrees well with the risk data available for other cancers (Bajpai *et al.*, 2013; Mahjabeen *et al.*, 2012). In the case of XPD, the reduction in expression level was about 16-fold. The results suggest an association between these tow genes with increased risk of CRC. Similarly, Wei *et al.* (2009) reported 1.6–2.5-fold reduced expression of these DNA repair genes in the tissue of colorectal patients. However, some studies reported an increase in the expression of DNA repair genes in colorectal adenomas and carcinomas and in lung cancer (Hatt *et al.*, 2008). One study (Polosak *et al.*, 2010) determined that the reduced expression of XPD may be associated with age. The relation of XPD Asp312Asn and Lys751Gln polymorphism has been a

subject of many phenotypic studies relevant to DNA repair and carcinogenesis (Sliwinski *et al.*, 2010). Some studies demonstrated relation of reduced repair capacity with the mutant allele of XPD Lys751Gln. Le Morvan *et al.* (2006) showed that ERCC2 expression in the NCI-60 tumor cell line panel was associated with reduced DNA nucleotide excision repair activity and enhanced drug cytotoxicity. Rusin *et al.* (2009) showed that the impaired DNA repair activity may play an important role in genotoxic susceptibility of patients with head and cancer and further suggest that treatment with DNA-reactive drugs and γ-radiation could be considered an effective therapy against SCCHN. Recently, It was reported (Lai *et al.*, 2009) no difference in protein expression of patients with/without Lys751Gln polymorphism. However, Wolfe *et al.* (2007) suggested that Lys751Gln polymorphisms could alter mRNA secondary structure, indicating that these SNPs potentially affect local folding and mRNA stability.

In conclusion, our study demonstrated that reduced expression levels of XRCC1 and XPD involved in DNA damage and repair process are of high risk in CRC. Maximum reduction in the expression levels of XRCC1 indicates an essential role of this gene in the etiology of CRC in North East Iranian population. The expression characteristics of these genes with protein quantity and activity could be further validated in a larger section of society for screening of high-risk populations.

Acknowledgements

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