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Original Article

Association of NER pathway gene polymorphisms with susceptibility to laryngeal cancer in a Chinese population

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Abstract: We systematically analyzed the association of nine SNPs of seven key NER pathway genes with the development of laryngeal cancer patients, and investigated whether NER pathway polymorphisms could serve as potential biomarkers for laryngeal cancer risk. 271 patients with pathologically proven laryngeal cancer and 271 control subjects were included in our study. Genotyping of ERCC1 rs11615 and rs2298881, ERCC2 rs13181 and rs50871, ERCC3 rs4150441, ERCC4 rs6498486, ERCC5 rs2094258, XPA rs2808668 and XPC rs2228001 were analyzed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By conditional logistic regression analysis, individuals carrying the TT genotype of ERCC1 rs11615 were correlated with an increased risk of larynx cancer when compared with the CC genotype (OR=1.89, 95% CI=1.07-3.37; *P* value=0.02). Moreover, individuals with the GG genotype of ERCC2 rs50871 were associated with an elevated risk of larynx cancer when compare with the TT genotype (OR=2.03, 95% CI=1.15-3.63; *P* value=0.01). We found a significant interaction between ERCC2 rs50871 polymorphism and tobacco smoking in the risk of larynx cancer (*P* for interaction <0.05). In conclusion, our study showed that ERCC1 rs11615 and ERCC2 rs50871 polymorphisms could influence the risk of larynx cancer in Chinese population, particularly among smokers.

Keywords: NER pathway gene, single nucleotide polymorphism, laryngeal cancer

Introduction

Larynx cancer is an important entity of cancer, which accounts for 30%-40% of all malignant head and neck tumors [1, 2]. It is estimated that the there were 138,102 new cases with laryngeal cancer and 73,261 deaths from laryngeal cancer worldwide in 2012 [3]. It is well known that larynx cancer is a complex disease, which is caused by many environmental and genetic factors [4, 5]. Several environmental factors are reported to be associated with increased risk of laryngeal cancer, including smoking, alcohol consumption, exposure to carcinogens in the work environment, nutrition, and viral infections with human papilloma virus (HPV) and Eostein-Barr virus (EBV) [4, 5]. Many molecular factors are reported to be involved in the mechanisms of carcinogenesis in the larynx, such as RECQL5, nucleotide excision repair genes, NOD2 and GSTM1 genes [4-8].

DNA repair systems play a pivotal role in maintaining the stability and integrity of the genome, which include nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and double-strand break repair (DSBR) [9, 10]. Nucleotide excision repair (NER) is a versatile system that monitors and repairs DNA damage caused by both endogenous and exogenous factors. DNA repair gene polymorphisms may be responsible for modified function and/or proficiency of DNA repair, and may contribute to inter-individual variation of DNA repair capacity [9, 11, 12]. NER process include steps of damage recognition, damage demarcation and unwinding, damage incision, and new strand ligation, all of which require corresponding functional proteins [13]. Polymorphisms of core NER genes could change the NER ability by influencing the expression and function of important proteins, and thus altering individual susceptibility to cancers. Driven by such hypothesis,

Table 1. Baseline characteristics of larynx cancer patients and control subjects

Variables	Patients	%	Controls	%	χ^2-test	*P* value
Age, years						
<65	145	53.51	143	52.77		
≥65	126	46.49	128	47.23	0.03	0.86
Gender						
Female	183	67.53	183	67.53		
Male	89	32.84	89	32.84	0.00	1.00
Smoking status						
Non-smokers	126	46.49	152	56.09		
Smokers	145	53.51	119	43.91	4.99	0.03
Drinking status						
Non-drinkers	114	42.07	138	50.92		
Drinkers	157	57.93	133	49.08	4.27	0.04
Family history of cancer						
No	246	90.77	270	99.63		
Yes	25	9.23	1	0.37	23.27	0.00

polymorphisms of several NER genes have previously been studied in relation to the development of laryngeal cancer [4-6, 14], but the results are conflicting. In the present study, we systematically analyzed the association of nine SNPs of seven key NER pathway genes (ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, XPA and XPC) with the development of laryngeal cancer patients, and investigated whether NER pathway polymorphisms could serve as potential biomarkers for laryngeal cancer risk.

Materials and methods

Patients

Patients with pathologically proven laryngeal cancer were recruited in the period between January 2012 and December 2014. All the cases were newly diagnosed and previously untreated. A total of 292 patients with laryngeal cancer were collected into our study, and finally 271 patients agreed to participate into our study, with a participation rate of 92.81%.

Clinical characteristics including basic medical data were obtained from medical records. The control group consisted of 271 subjects without malignant pathologies consulting between January 2012 and December 2014 in the same hospital. The controls were recruited simultaneously from similar geographic areas and matched with patients in terms of age, gender and social conditions.

Cases and controls were interviewed using a standardized questionnaire including socio-demographic characteristics, such as sex, age, occupation, residence and lifestyle habits. Lifetime consumption of tobacco smoking and alcohol drinking were also collected. Subjects who had smoked cigarettes at least one cigarette a week of more than one year previously were defined as smokers. Similarly, subjects who had drunk alcoholic beverages at least once a week for more than one year previously were defined as drinkers. All the laryngeal cancer patients and control subjects gave their informed consent.

Blood samples and genotyping

Each patient was asked to provide 5 ml peripheral blood and kept in -70°C until use. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Beijing Bioteke Co. Ltd. Beijing, China). Genotyping of ERCC1 rs11615 and rs2298881, ERCC2 rs13181 and rs50871, ERCC3 rs4150441, ERCC4 rs6498486, ERCC5 rs2094258, XPA rs2808668 and XPC rs2228001 were analyzed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The 25 μL PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/L of dNTPs, 2 mmol/L of $MgCl_2$ and 1 U of Taq DNA polymerase. The PCR condition was conducted using the following steps: an initial denaturation at 95°C for 5 min, 35 cycles of amplification with denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 30 sec, followed by a final extension step of 7 min at 72°C. Digestion products were separated by electrophoresis on ethidium bromide stained agarose gel and visualized under UV light. Two researchers without the knowledge of case or control status blindly conducted all assays. Additionally, approximately 10% of the samples were randomly selected and retested, and the results were 100% concordant.

Table 2. Detailed information of nine SNPs in NER pathway

Genes	SNP	Base change	SNP location	HWE in controls	MAF	
					In database	In controls
ERCC1	rs11615	C>T	Exon	0.57	0.311	0.310
	rs2298881	C>A	Promoter	0.72	0.211	0.264
ERCC2	rs13181	T>G	Exon	0.80	0.237	0.286
	rs50871	T>G	Intron	0.52	0.289	0.303
ERCC3	rs4150441	G>A	Intron	0.008	0.399	0.378
ERCC4	rs6498486	A>C	5' Upstream	0.99	0.282	0.292
ERCC5	rs2094258	G>A	Promoter	0.83	0.214	0.253
XPA	rs2808668	T>C	Intron	0.11	0.367	0.349
XPC	rs2228001	A>C	Exon	0.84	0.315	0.323

Statistical analysis

Means of quantitative variables were compared between groups using Student *t*-test after log transformation to obtain normal distribution, while distributions of categorical variables were compared by Pearson χ^2-test. Hardy-Weinberg equilibrium (HWE) was examined using a Chi-square (χ^2)-test with one degree of freedom. Multiple logistic regression models were established to estimate relative risks of tobacco and alcohol consumption as well as risks related to each SNP after adjustment for age, gender, smoking and alcohol drinking. Additional regression models were designed where subjects were stratified on smokers and non smoker subgroups and genetic common type and polymorphism carriers of the studied variations. Risks attributable to combined genotypes were also assessed using recoding of genotypic classes for pairs of markers. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. All tests were two-sided with a significant level of *P*-value <0.05. The SPSS software (SPSS, Chicago, IL) was used for statistical analyses.

Results

The characteristics of the patients and controls were presented in **Table 1**, including age, gender, smoking and drinking status, and family history of cancer. The mean ages of patients and controls were 63.70±10.50 and 64.50±9.60 years, respectively. There were no significant differences between larynx cancer patients and controls in terms of age and gender. By comparing baseline characteristics between larynx cancer patients and controls, larynx cancer patients were more likely to be a smokers and drinkers, and have a family history of cancer in first relatives.

The genotype distributions of ERCC1 rs11615 and rs2298881, ERCC2 rs13181 and rs50871, ERCC4 rs6498486, ERCC5 rs2094258, XPA rs2808668 and XPC rs2228001 confirmed with Hardy-Weinberg equilibrium in the controls, while ERCC3 rs4150441 was not (**Table 2**). The minor allele frequencies (MAF) of the nine in control subjects were similar with them in dbSNP database.

The association between the nine SNPs in NER pathway genes and risk of larynx cancer was shown in **Table 3**. By conditional logistic regression analysis, individuals carrying the TT genotype of ERCC1 rs11615 were correlated with an increased risk of larynx cancer when compared with the CC genotype (OR=1.89, 95% CI=1.07-3.37; *P* value=0.02). Moreover, individuals with the GG genotype of ERCC2 rs50871 were associated with an elevated risk of larynx cancer when compare with the TT genotype (OR=2.03, 95% CI=1.15-3.63; *P* value=0.01). However, ERCC1 rs2298881, ERCC2 rs13181, ERCC3 rs4150441, ERCC4 rs6498486, ERCC5 rs2094258, XPA rs2808668 and XPC rs2228001 polymorphisms were not associated with risk of larynx cancer.

We further analyzed the association between ERCC1 rs11615 and ERCC2 rs50871 and risk of larynx cancer stratified by smoking status, drinking status and family history of cancer in first relatives (**Table 4**). Individuals carrying the TG+GG genotype of ERCC2 rs50871 were associated with risk of larynx cancer in smokers (OR=2.87, 95% CI=1.68-4.90; *P* value <0.001), non-drinkers (OR=2.14, 95% CI=1.25-3.66; *P* value=0.003), drinkers (OR=2.11, 95% CI=1.28-3.47; *P* value=0.002) and those with family history of cancer (OR=1.92, 95% CI=1.33-2.77; *P* value <0.001). Moreover, we found a significant interaction between ERCC2 rs50871 polymorphism and smoking status in the risk of larynx cancer (*P* for interaction <0.05).

Table 3. Association between NER pathway genes and risk of larynx cancer

Genes	SNP	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
ERCC1	rs11615						
	CC	109	40.22	131	48.34	1.0 (Ref.)	-
	CT	118	43.54	112	41.33	1.27 (0.87-1.85)	0.26
	TT	44	16.24	28	10.33	1.89 (1.07-3.37)	0.02
	rs2298881						
	CC	136	50.18	148	54.61	1.0 (Ref.)	-
	CA	107	39.48	103	38.01	1.13 (0.78-1.64)	0.50
	AA	28	10.33	20	7.38	1.52 (0.79-2.99)	0.18
ERCC2	rs13181						
	TT	124	45.76	139	51.29	1.0 (Ref.)	-
	TG	115	42.44	109	40.22	1.18 (0.81-1.72)	0.36
	GG	32	11.81	23	8.49	1.55 (0.83-2.95)	0.14
	rs50871						
	TT	110	40.59	134	49.45	1.0 (Ref.)	-
	TG	116	42.80	110	40.59	1.28 (0.88-1.88)	0.18
	GG	45	16.61	27	9.96	2.03 (1.15-3.63)	0.01
ERCC3	rs4150441						
	GG	105	38.75	115	42.44	1.0 (Ref.)	-
	GA	111	40.96	107	39.48	1.14 (0.77-1.68)	0.50
	AA	55	20.30	49	18.08	1.23 (0.75-2.02)	0.39
ERCC4	rs6498486						
	AA	126	46.49	136	50.18	1.0 (Ref.)	-
	AC	116	42.80	112	41.33	1.18 (0.77-1.62)	0.54
	CC	29	10.70	23	8.49	1.36 (0.72-2.60)	0.31
ERCC5	rs2094258						
	GG	140	51.66	152	56.09	1.0 (Ref.)	-
	GA	106	39.11	101	37.27	1.14 (0.79-1.65)	0.47
	AA	25	9.23	18	6.64	1.51 (0.75-3.07)	0.21
XPA	rs2808668						
	TT	109	40.22	121	44.65	1.0 (Ref.)	-
	TC	118	43.54	111	40.96	1.18 (0.80-1.73)	0.38
	CC	44	16.24	39	14.39	1.25 (0.73-2.14)	0.38
XPC	rs1870134						
	GG	111	40.96	125	46.13	1.0 (Ref.)	-
	GC	123	45.39	117	43.17	1.18 (0.81-1.72)	0.36
	CC	37	13.65	29	10.70	1.44 (0.80-2.59)	0.19

[1]Adjusted for age, gender, smoking and drinking status, and family history of cancer in the first relatives.

Discussion

Polymorphisms have an important role in the regulation of gene expression, and can contribute to the differences between individuals in the susceptibility to a disease and its severity. The regulation of DNA repair is a key factor in the multistep process of carcinogenesis, and NER pathway genes are important parts of the DNA repair machinery. In our study, we suggest that the ERCC1 rs11615 and ERCC2 rs50871 polymorphisms are correlated with risk of larynx cancer.

Nucleotide excision repair (NER) is an important mechanism of the DNA repair pathway, and it maintains genomic integrity by removing DNA interstrand crosslinks [15, 16]. Both products of ERCC1 and ERCC2 genes are two important rate-limiting enzymes in the NER pathway. ERCC1 is a subunit of the NER complex and interacts with XPA, XPF and/or RPA, and this gene guides the 5' cleavage activity in the NER pathway [17, 18]. Cells from ERCC1-deficient mice usually present a high mutation frequency, an elevated level of genomic instability and a reduced frequency of S-phase-dependent illegitimate chromosome exchange, a response adopted by rodent cells to prevent the accumulation of DNA double strand breaks [19]. The ERCC2 protein is encoded by the gene located at chromosome 19q-13.3, and it possesses both single strand DNA-dependent ATPase and 5'-3' DNA helicase activities and participates in DNA unwinding during NER [20, 21]. Therefore, the polymorphisms in functional SNPs of ERCC1 and ERCC2 could influence the DNA repair capacity and the risk of larynx cancer.

Recently, several previous molecular studies have indicated that SNPs in DNA repair genes may contribute to the development of larynx cancer [4, 5, 14, 22], but the results were inconclusive. Li et al. conducted a 1:1 matched case-control study in a Chinese population, and

Table 4. Association between ERCC1 rs11615 and ERCC2 rs50871 polymorphisms and risk of larynx cancer stratified by demographic characteristics

Variables	rs11615 (case/control)		OR (95% CI)	*P* value	rs50871 (case/control)		OR (95% CI)	*P* value
	CC	CT+TT			TT	TG+GG		
Smoking status								
Non-smokers	48/78	72/80	1.46 (0.88-2.43)	0.12	64/62	93/59	1.53 (0.92-2.53)	0.08
Smokers	61/84	59/60	1.35 (0.81-2.27)	0.22	46/99	68/51	2.87 (1.68-4.90)	<0.001
Drinking status								
Non-drinkers	46/68	67/71	1.39 (0.82-2.38)	0.19	48/66	84/54	2.14 (1.25-3.66)	0.003
Drinkers	63/94	64/69	1.38 (0.85-2.27)	0.17	62/95	77/56	2.11 (1.28-3.47)	0.002
Family history of cancer								
No	99/147	130/140	1.38 (0.96-1.99)	0.07	106/140	160/110	1.92 (1.33-2.77)	<0.001
Yes	10/15	1/0	-	-	4/21	1/0	-	-

they reported that ERCC1 rs11615 and ERCC5 rs17655 were correlated with development of laryngeal cancer in a Chinese population, especially in smokers and drinkers [4]. Lu et al. also conducted a case-control study in a Chinese population, and they also suggested that ERCC1 rs11615 and ERCC5 rs17655 polymorphisms are correlated with an elevated risk of laryngeal cancer [5]. Abbasi et al. reported the association between 14 SNPs in eight NER genes and laryngeal cancer risk, and they found that ERCC5 Asp1104His, RAD23B Ala249Val and ERCC6 Arg1230Pro were associated with the development of larynx cancer [14]. Cui et al. investigated the effects of XPG His1104Asp polymorphism on the risk of larynx risk, and they found that this gene polymorphism may influence the susceptibility to the risk of larynx cancer [22]. The discrepancies of these results may be caused by different in sample size, cancer patients and controls selection, and study design. In our study, we found that ERCC1 rs11615 and ERCC2 rs50871 polymorphisms could influence the development of larynx cancer. Therefore, further large sample studies are greatly needed to confirm our results.

It is well known that genetic factors may interact with environmental factors, such as tobacco smoking and alcohol drinking, in the development of cancers. It is reported that NER is a critical pathway in regulating the susceptibility to larynx cancer, because NER pathway is an important mechanism for repairing bulky and helical and distorting DN adducts caused by cigarette smoke [23-25]. Proteins in the NER pathway could have a key role in repairing different types of oxidative damage [26-28]. In our study, we reported the ERCC2 rs50871 polymorphism interacted with tobacco smoking in the risk of larynx cancer, which proved the above-mentioned hypothesis.

Several limitations should be considered in our study. First, ERCC3 rs4150441 did not confirm with Hardy-Weinberg equilibrium in the controls, since the included controls may have various non-malignant diseases. However, this bias could be corrected by matching of controls to patients. Second, the sample size relative small, which might suffer from lack of power to find association of DNA repair genes with the risk of larynx cancer.

In summary, our study showed that ERCC1 rs11615 and ERCC2 rs50871 polymorphisms could influence the risk of larynx cancer in Chinese population, particularly among smokers. Future studies using larger patient sample and employing either similar or different analytic strategies may help to elucidate the impact of these polymorphisms on the development of larynx cancer.

Disclosure of conflict of interest

None.

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References

[1] Markou K, Christoforidou A, Karasmanis I, Tsiropoulos G, Triaridis S, Constantinidis I, Vital V, Nikolaou A. Laryngeal cancer: epidemiological data from Northern Greece and review of the literature. Hippokratia 2013; 17: 313-8.

[2] Takiar R, Krishnan SK, Shah VP. A Model Approach to Calculate Cancer prevalence from 5 Years Survival Data for Selected Cancer Sites in India -Part II. Asian Pac J Cancer Prev 2014; 15: 5681-4.

[3] GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. accessed in 2015-05-05.

[4] Li X, Xu J, Yang X, Wu Y, Cheng B, Chen D, Bai B. Association of single nucleotide polymorphisms of nucleotide excision repair genes with laryngeal cancer risk and interaction with cigarette smoking and alcohol drinking. Tumour Biol 2014; 35: 4659-65.

[5] Lu B, Li J, Gao Q, Yu W, Yang Q, Li X. Laryngeal cancer risk and common single nucleotide polymorphisms in nucleotide excision repair pathway genes ERCC1, ERCC2, ERCC3, ERCC4, ERCC5 and XPA. Gene 2014; 542: 64-8.

[6] Qi Y, Zhou X. Haplotype analysis of RECQL5 gene and laryngeal cancer. Tumour Biol 2014; 35: 2669-73.

[7] Liu J, He C, Xu Q, Xing C, Yuan Y. NOD2 polymorphisms associated with cancer risk: a meta-analysis. PLoS One 2014; 9: e89340.

[8] Zhang Y, Chen W, Ji JF, Wang ZY, Wu MH, Zhang K, Wang QP. GSTM1 null polymorphisms is associated with laryngeal cancer risk: a meta-analysis. Tumour Biol 2014; 35: 6303-9.

[9] Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11: 1513-30.

[10] Lindahl T, Wood RD. Quality control by DNA repair. Science 1999; 286: 1897-905.

[11] Monaco R, Rosal R, Dolan MA, Pincus MR, Brandt-Rauf PW. Conformational effects of a common codon 399 polymorphism on the BRCT1 domain of the XRCC1 protein. Protein J 2007; 26: 541-546.

[12] Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000; 21: 551-5.

[13] Nouspikel T. DNA repair in mammalian cells: Nucleotide excision repair: variations on versatility. Cell Mol Life Sci 2009; 66: 994-1009.

[14] Abbasi R, Ramroth H, Becher H, Dietz A, Schmezer P, Popanda O. Laryngeal cancer risk associated with smoking and alcohol consumption is modified by genetic polymorphisms in ERCC5, ERCC6 and RAD23B but not by polymorphisms in five other nucleotide excision repair genes. Int J Cancer 2009; 125: 1431-9.

[15] Neumann AS, Sturgis EM, Wei Q. Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. Mol carcinog 2005; 42: 65-92.

[16] Wu Q, Christensen LA, Legerski RJ, Vasquez KM. Mismatch repair participates in error-free processing of DNA interstrand crosslinks in human cells. EMBO Rep 2005; 6: 551-7.

[17] Volker M, Moné MJ, Karmakar P, van Hoffen A, Schul W, Vermeulen W, Hoeijmakers JH, van Driel R, van Zeeland AA, Mullenders LH. Sequential assembly of the nucleotide excision repair factors in vivo. Mol Cell 2001; 8: 213-24.

[18] Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei YF, Moggs JG, Carter KC, Shell BK, Evans E, de Jong MC, Rademakers S, de Rooij J, Jaspers NG, Hoeijmakers JH, Wood RD. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell 1996; 86: 811-22.

[19] Melton DW, Ketchen AM, Núñez F, Bonatti-Abbondandolo S, Abbondandolo A, Squires S, Johnson RT. Cells from ERCC1-deficient mice show increased genome instability and a reduced frequency of S-phase-dependent illegitimate chromosome exchange but a normal frequency of homologous recombination. J Cell Sci 1998; 11: 395-404.

[20] Sung P, Bailly V, Weber C, Thompson LH, Prakash L, Prakash S. Human xeroderma pigmentosum group D gene encodes a DNA helicase. Nature 1993; 365: 852-855.

[21] de Boer J, Hoeijmakers JH. Nucleotide excision repair and human syndromes. Carcinogenesis 2000; 21: 453-460.

[22] Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, Cozen W, Mack TM, Zhang ZF. Polymorphism of Xeroderma Pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer 2006; 118: 714-20.

[23] Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004; 73: 39-85.

[24] DeMarini DM, Shelton ML, Levine JG. Mutation spectrum of cigarette smoke condensate in Salmonella: comparison to mutations in smoking-associated tumors. Carcinogenesis 1995; 16: 2535-42.

[25] Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, Yamaguchi R, Itoh H, Kasai H. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 1997; 18: 1763-6.

[26] Reardon JT, Bessho T, Kung HC, Bolton PH, Sancar A. In vitro repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. Proc Natl Acad Sci U S A 1997; 94: 9463-8.

[27] Torres-Ramos CA, Johnson RE, Prakash L, Prakash S. Evidence for the involvement of nucleotide excision repair in the removal of abasic sites in yeast. Mol Cell Biol 2000; 20: 3522-8.

[28] Fleck O, Lehmann E, Schär P, Kohli J. Involvement of nucleotide-excision repair in msh2 pms1-independent mismatch repair. Nat Genet 1999; 21: 314-7.