The role of XRCC5/XRCC6 in carcinogenesis and pharmacogenomics

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Abstract

In the past decades, the incidence of cancer keeps its rapid increasing step all over the world and cancer is always an important threat to public health. It is believed that cancer is resulted from a series of genetic alterations leading to progressive disorder of the normal mechanisms controlling cell proliferation, differentiation, death, and/or genomic stability. The response of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are therefore essential in preventing tumor initiation and progression. From the same viewpoint, the relative role of DNA repair as a biomarker for prognosis, predicator of drug and therapy responses, or indeed as target for novel gene therapy is very promising. In this review, we have summarized the studies investigating the association between XRCC5/6 dimer and the susceptibility to multiple cancers, and discussed their role in carcinogenesis and application in anticancer drug discovery.

Keywords: XRCC5, XRCC6, polymorphism, cancer, carcinogenesis

1. Introduction

The human genome is maintained by interconnected checkpoints as well as the DNA

repair pathways that can sense the DNA damage and response to exogenous or endogenous DNA insults with a cascade signaling and removing of the DNA adducts. There are six main DNA repair pathways identified: (i) direct reversal repair; (ii) nucleotide excision repair; (iii) base excision repair; (iv) homologous repair (HR); (v) non-homologous end-joining (NHEJ); and (vi) mismatch repair. Normally, if these repair pathways fail to repair the DNA damage, the same molecular machinery can sense the defects as a “threaten” and trigger the apoptosis. However, when the DNA damage were neither repaired nor turned to the induction of cell apoptosis and terminating the unhealthy cell, the DNA defects will be left and propagated to its offspring cells. Under the later circumstances, carcinogenesis will occur. The decreasing of genetic/genomic integrity and stability in most cancer types and the identification of cancer predisposition syndromes linked to the defects of DNA repair pathways support the concept that DNA repair genes may play a critical role in opposing cancer initiation and progression.[1-3]

One of the most deleterious DNA damaging types is double strand break (DSB), which should be repaired in eukaryotes by two major pathways mentioned above: HR and NHEJ. HR is a template guided, error-free pathway predominantly operating in the S and G2 phases of the cell cycle and involves RAD51, its paralogs RAD51B/C/D, XRCC2/3, and p53, RPA, BRCA1/2, BLM and MUS81.[4] NHEJ, on the other hand, is

a potentially less accurate form of DSB repair, in which the two termini of the broken DNA molecule are processed to form compatible ends that are directly jointed. In most cases, NHEJ results in the loss of a few nucleotides at the broken ends, making this pathway error-prone. This article is focused on XRCC5/6 dimer which play crucial roles in the NHEJ pathway, as NHEJ is considered to be the major repair pathway of DSBs in eukaryotic cells during most phases of the cell cycle, particularly the G0/G1 phases.[5] NHEJ involves the XRCC5/XRCC6 (also known as Ku80/Ku70), XRCC7 (DNA-dependent protein kinase catalytic subunit; DNA-PKcs), Artemis, XLF, XRCC4, DNA ligase 4, ATM, p53 and MDM2 proteins.[6, 7] NHEJ deficiencies can lead to increased genomic instability[8, 9] and cause increased tumorigenesis.[10-13] However, the exact roles of these genes and their protein products, such as XRCC5 or XRCC6, in each type of cancers are not well investigated or revealed. The model for DSB repair via NHEJ and the proteins involved are shown in Figure 1.

XRCC5 and XRCC6 usually form the heterodimer Ku. They are probably among the first proteins that bind to the DNA ends at a DSB and the XRCC5/6–DNA complex recruits and activates XRCC7.[14, 15] XRCC5/6 dimer and XRCC7 are proposed to act in the synapsis process.[14, 15] Xrcc5 and xrcc6 knockout mice are growth retarded, radiosensitive and are severely immuno-deficient.[16, 17] B-cell development is arrested at an early stage due to a profound deficiency in V(D)J

recombination, which is commonly employed by vertebrates to generate diversity doe an adaptive immune response.[16, 17] Although the xrcc5- or xrcc6-deficient mice are visible, their cells have defects in DNA end joining, which manifest as irradiation sensitivity, growth defects, premature senescence, and inability to perform end-joining during V(D)J recombination. All these defects may also happen during human embryonic development. A human cell and statistically insulted by hundreds of thousands exogenous and endogenous DNA damage per day, and if the cell could not repair DSB well, the accumulated genomic instability would lead the cell to apoptosis and cause the embryonic lethality of the subject. There is no doubt that XRCC5 and XRCC6 are very critical in both genomic stability and human ontogenesis.

The 3D structure of the XRCC5 and XRCC6 heterodimer can help us understand their how certain domains interact with other DNA repair proteins for possible anticancer drug development.[18] XRCC5 and XRCC6 share a three-domain topology comprising (1) an N-terminal α/β domain, (2) a central β-barrel domain and (3) an α helical C-terminal arm. The C-terminal domains of XRCC5 contain of the XRCC7 recruitment element for the further formation of the XRCC5/6/7 holoenzyme to fulfill the synapsis of the broken DNA ends.[19, 20] In XRCC6, residues 536–560 and 561–609 of XRCC6 are both in charge of the DNA interaction.[21] The three-dimensional model of heterodimer structure of XRCC5/6 and associated proteins is shown in Figure 2.

Since each of the NHEJ genes plays a critical and specific role during the process of repairing the DSBs, any of them fails to finish its job correctly and immediately, the NHEJ capacity will become lower and the overall genomic instability will become higher. It is therefore tempting to speculate that defects in the NHEJ pathway may be associated with human cancers. Given this, it is puzzling that no direct genetic evidence has been found to link defective NHEJ genes with cancers. Among them, only mutations in two have been found to predispose carriers to a higher rate of genetic diseases, DNA ligase 4 and Artemis, which are associated with Nijmegen breakage syndrome-like syndrome and severe combined immunodeficiency, respectively.[22, 23] One explanation is that any severe defects (null mutants) in NHEJ-related genes would result in great genomic instability and might be incompatible with life, thus no cancer cases can be observed. The crucial and irreplaceable roles of these gene products may also increase the difficulty of approaching their physiological functions via single gene knockout mice models. For this reason, for these high-penetrance NHEJ genes, only subtle defects arising from low-penetrance alleles (e.g., hypomorphic mutant or polymorphic variant) would escape the cell cycle checkpoint surveillance and allow the cell to survive, and to accumulate enough unrepaired genomic alterations required for tumor formation.[24, 25] The studies applying single nucleotide polymorphism (SNP) technology, one of the

most subtle and powerful genetic analysis, to approach the associations of high-penetrance and low-penetrance genes with various cancers are of worldwide interest to approach the overall scene in cancer research.

The aim of this article is to summarize and evaluate associations between the SNPs of XRCC5/XRCC6 and the susceptibility to multiple cancers. Although the rapid development of genome-wide association studies and bioinformatics help a lot in revealing the secret of human genome in cancer, the knowledge of cancer genomics is still far from satisfying and in need of further multi-apporaching studies. For instance, The XRCC5/6 dimer plays an important role in NHEJ and maintaining of genomic stability, however, their role in carcinogenesis has not been worldwide studied. There are 1555 XRCC5 SNPs availably announced on the NCBI SNP website (http://www.ncbi.nlm.nih.gov/snp). Among them, 1432 are intronic, 32 are exonic, and others are located on locus regions, UTR regions, and alternative splicing sites. On the other hand, there are 683 CRCC6 SNPs, while 627 are intronic, 23 are exonic. Most of the SNPs have not been investigated about their functional influence in cell or animal models, not to mention their individual roles in cancers. It is very exciting that some of the SNPs of XRCC5 and XRCC6 have been found to be associated with the susceptibility to various cancers. More interesting, specific gene-environment interactions can be observed in these cancer patients who contact

with possible environmental carcinogens. Therefore, we infer the risk SNP genotypes of XRCC5/6 dimer and environmental carcinogens will have some joint effects and increase the susceptibility to cancers significantly. We hope this article can provide some novel useful markers for oncology early detection, prevention, and some candidates for anticancer intervention.

2. Literature survey

We conducted MEDLINE, Current Contents and Web of Science searches using "cancer" and "polymorphism", together with "XRCC5" or “XRCC6”, as keywords to search for papers published (from January 1, 1966 through September 21, 2010). Additional articles were identified through the references cited in the first series of articles selected. Articles included in the meta-analysis were in any language, with human subjects, published in the primary literature and had no obvious overlap of subjects with other studies. Case-control studies were eligible if they had determined the distribution of the relevant genotypes in cancer cases and in concurrent controls using a molecular method for genotyping.

3. The role of XRCC5/CRXX6 in cancer genomics

3.1. XRCC5/XRCC6 polymorphisms in glioma and meningioma

3.1.1. Glioma

Gliomas derived from glial cells surrounding neurons and accounted for 90% of brain tumors, and about 80% of the glioma patients could not survival for more than 12 months after their diagnosis. Studies have consistently shown that the risk of glioma is elevated 2-fold in first-degree relatives of patients with glioma and other primary brain tumors.[26] At present, most cases of glioma cannot be explained by endogenous or exogenous causes. High doses of ionizing radiation and rare genetic syndromes are the only generally accepted well-defined risk factors, and they explain a small percentage of all glioma cases. Ionizing radiation may cause DSBs, which should be repaired by HR or NHEJ. In 2007, Liu and his colleagues have reported that glioma risk was associated with XRCC5 and XRCC6.[27] Among the thirteen SNPs (eight of XRCC5 and five of XRCC6) they checked, three of XRCC5, rs828704, rs3770502 and rs9288516, and one of XRCC6, rs6519265, were significantly associated with glioma risk.[24] Their large sample size and haplotype analysis also revealed that one specific haplotype of XRCC5 (CAGTT) was associated with a 40% reduction in risk of developing glioma. Although their data did not directly prove the involvement of XRCC7, which is also a NHEJ protein interacting with XRCC5/6, they proposed a concept that the risk of glioma depends not only on individual NHEJ genes, but also on the interaction among SNPs of NHEJ genes. This team has investigated the

contribution of XRCC7 to glioma in 2004 with no positive association.[28]

3.1.2. Meningioma

Meningiomas are the most commonly reported type of brain tumor in the United States, which are often surgically curable. Among the risk environmental factors, ionizing radiation is the most consistent and powerful one for meningiomas.[29, 30] In 2005, a population in Israel was investigated of the sporadic and radiation-associated meningiomas with their genotypes of twelve genes, NF2, XRCC1, XRCC3, XRCC5, ERCC2, p16, Ki-ras, E-cadherin, PTEN, cyclin D1, TGFB1 and TGFBR2.[31] Only in the non-irradiated group, the genotypes of p16 (rs2811708) and PTEN (rs1234214) were found to be associated with meningiomas risk. As for the XRCC5 genotype of rs828699, there was no association with either sporadic or radiation-associated meningiomas,[31] which may be due to the small (less than 200 in each group) but ethics-diverse (including Asia, Africa and Europe) samples and their SNP selection, which was limited to one-gene one-SNP choosing strategy.

3.2. XRCC5/XRCC6 polymorphisms in cancers of head and neck

3.2.1. Head and neck cancers

Head and neck cancer is one of the most common malignancies all over the world,

accounting for about 500,000 new cases each year. The head and neck cancer includes squamous cell carcinomas of the opal cavity, larynx and pharynx. The most well-known environmental factors for head and neck cancer are the consumption of tobacco and alcohol.[32, 33] As only a fraction of highly tobacco and alcohol consumers developed cancer during their life time, there may exist differences in individual susceptibility and complex gene-environment interaction. In 2004, Carles and his colleagues screened nine SNPs of seven DNA repair genes, including XPA, XPC, XPD/ERCC2, XPF, XPG, XRCC5 and XRCC1, for their associations with the cancer progress and survival rate after radiotherapy in head and neck patients. There was no association found between the outcomes and the genotypes of SNPs at rs1051677 or rs1051685 of XRCC5.[34] Instead, genetic polymorphisms in XPF/ERCC1 (rs735482), EPG/ERCC5 (rs1047768) and XPA (rs1800975) may significantly influence the outcomes of head and neck patients after radiotherapy.[34] In 2008, Werbrouck and his colleagues reported that there was no association among the head and neck cancer risk with genotypes of XRCC5 (rs3835), XRCC6 (rs2267437), or with the gene-smoking or gene-alcohol drinking interaction.[35] However, their small and non-representative sample may cause the decreasing of analyzing power. By the way, the 152 head and neck patients were further divided into oral cavity, pharynx and larynx groups for smoking and drinking analysis. Therefore, the borderline significant 2-fold decreased

risk (adjusted odds ratio=0.47 and *P*=0.08) for the carriers of GG variant XRCC6 genotype of rs2267437 should be rechecked in an expanded population and other ethics. In the next year, the same group has focused their investigation of the same head and neck patient population to the association of the side effects of intensity-modulated radiation therapy. Patients with CG or GG at rs2267437 of XRCC6 had a 4.08-fold significantly increased risk for severe acute dysphagia compared with patients with CC. Interestingly speaking, the differential response to radiation therapy of variant genotype of XRCC6 was not observed in mucositis or dermatitis, two common side effects of radiation. As for the XRCC5 genotype at rs3835, and ligase 4 genotypes at rs1805388 and rs1805386, there was not any association with the three side effects found in the same group of patients.[36] This is a pilot study in which the physical radiation therapy side effect evaluation parameters and genomic polymorphisms in the NHEJ genes were combined in the evaluation system that can be directly applied in clinical practice.

3.2.2. Oral cancer

Oral cancer specifically refers to a subgroup of head and neck malignancies that develop at the lips, tongue, salivary glands, gingiva, mouth floor, oropharynx, buccal surfaces and other intra-oral locations. Oral cancer incidence has keeping increased

till 2007, the estimated incidence of oral cancer in the United States was 10.3 cases per 100,000 persons, with a mortality rate of 2.5 per 100,000 persons.[37] It is estimated that there will be 36,540 new cases of oral cancer diagnosed in the United States in 2010 and 7,880 deaths due to this disease.[38] Oral cancer is more common in men than in women and accounts for 3% of new cancer cases in American male.[38] World Health Organization has estimated oral cancer to be the eighth most common cancer worldwide. The most important environmental risk factors for the development of oral cancer in the Western countries are the consumption of tobacco and alcohol.[39, 40] In Asian countries, such as Taiwan and India, the use of betel quid, is responsible for a considerable percentage of oral cancer cases.[41, 42] So far, the genomic etiology of oral cancer is of great interest but largely unknown.

In Taiwan, where the oral cancer density is highest worldwide, oral cancer is a fatal disease accounting for the fourth highest incidence of malignancy in males and the seventh in females.[43] The relatively high prevalence of oral cancer in Taiwan is mainly because there is a high-risk group of 2.5 million people with the habit of smoking, alcohol drinking and betel quid chewing. There were four papers investigated the associations of NHEJ genes with oral cancer in Taiwan. In 2008, Bau and his colleagues found that the C allele of XRCC6 rs5751129 was a risk marker for oral cancer susceptibility, while those of rs2267437, rs132770 and rs132774 were

not.[44] In the next, the team had enlarged the investigated population of control/case from 318/318 to 600/600, reporting that XRCC5 rs828907, but not rs11685387 or rs9288518, was associated with oral cancer susceptibility.[45] In addition, those people carried GT and TT genotype at rs828907 had a 1.6-fold enhanced risk when they had the habit of betel quid chewing. In addition to XRCC5 and XRCC6, there were two studies aiming at the SNPs of XRCC4 and their association with oral cancer in Taiwan.[46, 47] These studies reported that the XRCC4 rs3734091 and rs28360071 polymorphisms were associated. In 2008, a study investigating the subjects with oral premalignant lesions has found that there is no association between their XRCC5 rs1051685 genotypes with the susceptibility.[48]

3.3. XRCC5/XRCC6 polymorphisms in thyroid cancer

Thyroid cancer is the most frequent endocrine cancer and it appears mainly as sporadic form.[49] Thyroid cancer incidence have been keeping increased from 1973 to 2006 across USA, Europe, Asia, Oceania and South America with only few countries decreased such as Sweden, Norway, and Spain.[50] This change can be attributed primarily to an increase in papillary thyroid carcinoma, which increased 3.2-fold.[51] The most common histological varieties are non-familiar papillary and follicular thyroid carcinomas, the most frequent of all thyroid follicular-cell malignancies with

85-90% of incidence.[52] Thyroid cancer is more common in women than in men, while the mortality rates are significantly higher in men compared with women, largely due to late diagnosis and more advanced disease in men at the time of initial diagnosis.[52] The only verified cause of thyroid carcinogenesis is ionizing radiation (IR) exposure, though other risk factors have been pointed out as candidates, such as dietary iodine deficiency, hormonal factors, lymphocytic thyroiditis and familiar history.[52-54]

In 2010, Gomes and his colleagues have firstly investigated of the genetic role of XRCC4, ligase 4 and XRCC5 in thyroid cancer in a small Portuguese population with 109 cases and 217 controls.[55] Originally, they found that genotypes of four 3'UTR SNPs of XRCC5 including rs2440, rs6941, rs1051677 and rs1051685, were not associated with thyroid cancer risk. The authors turned to stratified the overall data by the histological types of cancer, and found that AA genotype of XRCC5 rs6941 (Ex21-238), compared with GG genotype, was associated with higher papillary tumor risk. This finding supported the hypothesis that different histological types can have different genetic basis. Also in the stratification analysis by gender, they found that XRCC5 rs1051677 (Ex21+338) and rs2440 (Ex21-352) were associated with an increased individual risk for thyroid cancer only in the males.[55] Their work may contribute to early detection and prediction of thyroid cancer susceptibility for the male population which usually are characterized by later diagnosis and higher

mortality rate.

3.4. XRCC5/XRCC6 polymorphisms in cancers of digestive system

Digestive tract cancers, such as gastric, liver, and esophageal cancers, continued to stay among top five cancers during the past three decades. The colorectal cancer is more and more serious in Asia, especially in China and Taiwan. However, the knowledge about the genomic effects on their incidence, prognosis, and responses to chemotherapy or radiotherapy is still very lacking. As for the pancreas cancer, the genomic studies were much fewer for the difficulty of sample collection. Followed are the previous studies reporting XRCC5/XRCC6 genotypes associated with these cancers in digestive system, and studies about liver and pancreas cancers are urgently in need.

3.4.1. Esophageal cancer

Esophageal squamous cell carcinoma, with 5-year survival less than 10%, has one of the highest malignant potentials of any tumor.[56] Epidemiologically, it is characterized by distinctly higher incidence in certain geographical locations, such as China.[57] Smoking tobacco and consuming alcohol are two factors strongly associated with a risk of developing esophageal squamous cell carcinoma and to a lesser degree

esophageal adenocarcinoma,[58, 59] and these risk factors have also been reported to interact in a multiplicative way in the etiology of this neoplasm.[60, 61] However, the recent papers reporting that the high incidence of esophageal cancer may result primarily from genetic rather than environmental factors for some patients, strengthens the importance of keeping on digging the genomic factors for esophageal cancer, which are still largely unknown.

In 2007, Dong and her colleagues have recruited 329 esophageal cancer patients and 631 cancer-free controls from China, where esophageal cancer is the fourth leading cause of the cancer death. The risk of esophageal cancer is highly associated with a family history, supporting the concept that genomic effects play an important role in its etiology. Two SNPs of XRCC5, C74468A and G74582A (Accession numbers: DQ787434 and DQ787434), were genotyped among the people, while neither single SNP nor combined genotype has been found to be associated with esophageal cancer risk.[62] However, in those subjects with familial history of esophageal cancer, the C allele of C74468A seemed to be a protective factor for the incidence.[62] There was no literature analyzing the association of XRCC6 polymorphism with esophageal cancer risk.

3.4.2. Gastric cancer

Gastric cancer causes a significant global health care burden, responsible for approximately 934,000 new diagnoses annually (8.6% of new cancer cases).[63] Almost two-thirds of cases occur in Eastern Europe, South America and Asia with 42% in China alone. In the United States, in 2009, an estimated 21,130 new cases (14th most common) of gastric cancer were diagnosed and was associated with 10,620 deaths (13th most common).[64] In Europe gastric cancer ranks 5th most prevalent with an estimated 159,900 new cases in 2006 and 118,200 deaths (4th most common cause of cancer-related death).[65]

Now, gastric cancer is still a major health problem worldwide due to its frequency, poor prognosis and limited treatment options. The most important etiological factors implicated in gastric carcinogenesis are diet and Helicobacter pylori infection. High intake of salted, pickled or smoked foods, as well as dried fish and meat and refined carbohydrates significantly increased the risk of developing gastric cancer while fibers, fresh vegetables and fruit were inversely associated with its risk. At present prevention is likely to be the most effective means to reduce its incidence and mortality, and the understanding of genomic effects on it and finding useful genomic markers are a powerful way for the dearly detection and prevention.

The group of Dong and her colleagues has found that in those subjects with familial history of gastric cancer, the C allele of XRCC5 C74468A seemed to be a

protective factor for the incidence.[62] A similar trend was found in the case of esophageal cancer. Also, in those subjects with familial history of gastric cancer, the A allele of G74582A seemed to be a protective factor for the incidence, which was not similar to the case of esophageal cancer. Interestingly, as for the esophageal and gastric cancer, there is both the similar (C allele of C74468A) and specific (A allele of G74582A) genomic influences from the same XRCC5 gene. There was no literature analyzing the association of XRCC6 polymorphism with esophageal cancer risk.

3.4.3. Colorectal cancer

Colorectal cancer remains a significant cause of morbidity and mortality in the United States, Taiwan and throughout the world.[66] Etiological studies have attributed more than 85% of colorectal cancer to environmental factors,[67, 68] and in particular meat consumption, cigarette smoking, exposure to carcinogenic aromatic amines, such as arylamines and heterocyclic amines.[69, 70] These carcinogens are thought of as DNA damage inducers in responsible for DNA base damage, DNA single-strand breaks and DSBs.[71]

In 2009, it has been reported in Taiwan, where the colorectal cancer is on the top on cancer incidence, that the XRCC5 rs828907 polymorphism was associated with increased colorectal cancer, while the XRCC5 rs11685387 and rs9288518 genotypes have no similar association. In the people with individual smoking habits, the

genomic effect of the XRCC5 rs828907 on colorectal cancer risk is even more significant with the T allele can obviously raise the colorectal risk by 2.54-fold. There was no significant joint effect between these genotypes and alcohol drinking on colorectal risk.[72] It is a pity that the diet habits, such as meat, vegetable/fruit and fish/shrimp consumption, can not be performed due to a lack of questionnaire information. But they have successfully established the relationship between genomic (XRCC5) and environmental (smoking) factors for colorectal cancer etiology. There was no literature analyzing the association of XRCC6 polymorphism with colorectal cancer risk, or the joint effects of genomic and environmental factors yet.

3.5. XRCC5/XRCC6 polymorphisms in lung cancer

Lung cancer is the leading cause of cancer-related death in the United States and in the world.[73, 74] Although tobacco smoking is the leading cause of lung cancer, only 10-15% of all smokers develop lung cancer, suggesting that there is a great variation among individuals in their susceptibility to lung carcinogenesis.[75, 76]

In Taiwan, lung cancer has high incidence, high mortality, a low 5-year survival rate, and famous world leading female adenocarcinoma cases. Tseng and his colleagues, provided evidence of high fractional allelic loss in lung cancer patients linking the defect in DSB repair with lung cancer etiology.[77, 78] In 2009, 152

non-small cell lung cancer patients and 162 gender- and age-matched controls were genotyped and analyzed by them. No association between lung cancer risk and polymorphisms in the XRCC5 (rs3835) or XRCC6 (rs2267437) was found, while there is positive finings in the other two genes in NHEJ systems, XRCC4 (rs18055377) and ligase 4 (rs1805388). The authors have put the upstream (XRCC5 and XRCC6) and downstream (XRCC4 and ligase 4) genes together for gene-gene interaction analysis, finding that the combinative of risk XRCC5 and XRCC6 genotypes, although not significant individually, increased the lung cancer risk to 4- to 65-fold compared with the wildtypes. Therefore, the small sample size is not a cause of the negative findings for XRCC5 and XRCC6, and indeed NHEJ plays an important role in lung carcinogenesis. In addition, they have linked the XRCC4 and ligase genotypes to the phenotype of the patients with high fractional allelic loss.[79] It is worthwhile to search for more SNPs in XRCC5 and XRCC6 to investigate their individual and gene-gene and gene-environment joint effects on lung cancer, together with genotype-phenotype correlation.

3.6. XRCC5/XRCC6 polymorphisms in breast cancer

Breast cancer is now the most common cancer affecting women throughout the world and the latest estimates suggest that more than 1,050,000 new breast cancer cases

occur worldwide annually.[80, 81] In western countries, one woman out of eight will develop breast cancer. Mortality of breast cancer among women has decreased a lot but incidence has doubled during the past 30 years probably because of new therapy, changes in the use of hormone replacement therapy in postmenopausal women and early diagnosis. However, breast cancer is still the first cause of death by cancer in woman under 65, and not ignorable.[81, 82]

In 2002, Goode and his colleagues has carried our a study recruiting up to 2473 breast cancer cases in USA and screening 2430 of their genotypes in 22 DNA repair, hormone metabolism, carcinogen metabolism and other genes.[83] None of XRCC5 and one of the XRCC6 (rs132788) was listed among the 21 SNPs they chosen for genotyping and analyzing, but with negative association results. In the same year, Kuschel and his colleagues have performed a case-control breast cancer study in Germany with 2205 cases and 1826 controls, finding that the same SNP of XRCC6 was not associated with breast cancer risk. In addition, they did not perform any XRCC5 genotyping for the SNP choosing technical was based on limited literature and knowledge at that period.[84] The genotypes of ligase 4 and XRCC2, were found to be marginally associated with breast cancer risk in their study, suggesting that DNA repair systems, including NHEJ, played an important role in breast carcinogenesis.[74] In 2009, Willems and his colleagues performed a study in Belgian at a much smaller scale with 206 cases and 171 controls, investigating the contribution of single SNP of XRCC6 genotype at

rs2267437 to breast cancer risk.[85] The results showed a significant odds ratio of 1.85 in sporadic, but not familial breast cancer patients, indicating that other factors besides genetic aptitude influence the association. They have further examined the influence of estrogen exposure. The hormone-related risk factors associated with breast cancer are an early age of first menarche, nulliparity or late first full-pregnant childbirth, and late menopause.[86] The G allele of rs2267437 was also associated with about 2-fold odds ratios in the subjects with early menarche age, and subjects with late (≥50 years old) menopause.[85] Their conclusion is that XRCC6 genotype was associated with breast cancer risk, which was stronger in female with a longer estrogen exposure.

Breast cancer in Asia is characterized by a lower incidence than in western populations, and by early tumor onset, both may be due to different genetic background, cultural habits and environmental exposures. In 2005, Lee and his colleagues have conducted a study investigating the Korean population, finding that XRCC6 G1796T (Accession: AY870329) was not associated with breast cancer risk.[87]

In Taiwan, breast cancer is the second leading cancer, important for its high incidence, high mortality, and early onset.[88, 89] In 2003, Fu and her colleagues screened thirty SNPs on NHEJ genes in Taiwanese. Among the NHEJ SNPs, only XRCC6 rs2267437 and XRCC4 rs2075685 were found to show significantly

differential distribution among 254 primary breast cancer patients and 379 healthy controls. Genotypes of five SNPs from the five NHEJ genes, the heterozygous and homozygous variants of XRCC5 rs38365, or XRCC7 rs2231178, and the homozygous wild-type of XRCC6 rs2267437, ligase 4 rs1805388, or XRCC4 rs2075685, were identified as putative high-susceptibility genotypes.[25] They proposed that the more high-susceptibility genotypes of SNPs, the higher the breast cancer risk became. These evidence supported the model of Pharoah indicating that familial breast cancers can not be explained by variations in BRCA1 and BRCA2, but by the joint effects of a large number of codominant alleles, each of which effect is associated with a small increase in risk.[90] In 2009, Wang and his colleagues has accessed the association of XRCC5 genotypes with Taiwanese breast cancer with a larger sample size, 1272 patients and 1272 age- and gender-matched controls.[91] A significantly different distribution was found in the frequency of the XRCC5 rs828907 genotype, but not the XRCC5 rs11685387 or rs9288518 genotypes, between the breast cancer and control groups. The T allele XRCC5 rs828907 conferred a significant increased risk of breast cancer, and the XRCC5 rs828907 GT and TT genotypes interacted with smoking habit conferring a 3.16-fold increased risk, while no joint effect with breastfeeding was found.[91] Bau and his colleagues have also performed genotype-phenotype studies measuring the individual NHEJ capacities,

which may contribute to personalized breast cancer risk prediction and evaluation.[24, 92]

3.7. XRCC5/XRCC6 polymorphisms in cancers of urinary system

Urinary cancers may include kidney cancer, ureter cancer, bladder cancer, and two male cancers, testis and prostate cancers. Epidemiologically, all of them seemed to be more common in male than female. The knowledge about the genomic effects on their incidence, prognosis, and responses to chemotherapy or radiotherapy is still very lacking. As for the ureter and testis cancers, there were almost no genomic studies for the difficulty of sample collection. Followed are the current SNP literatures about these cancers in urinary system, and the investigations about ureter and testis cancers are urgently warrant.

3.7.1. Renal cell carcinoma

Renal cell carcinoma is the third leading cause of death among genitourinary malignancies and the twelfth leading cause of cancer death overall. The incidence of renal cell carcinoma is about the figure of 2% worldwide. Due to a widespread use of abdominal imaging, localized tumors are frequently diagnosed nowadays. However, roughly one third of the patients will ultimately die from this disease even in USA,

where the healthy caring system is very high-qualified.[93]

In literature, gender, obesity, smoking, analgesic, diuretic abuse, and environmental factors are reported to be associated with renal cell carcinoma.[83] Cigarette smoking, for example, doubles the risk for renal cell carcinoma and contributes to as much as one third of all cases, yet only a fraction of smokers and a low number of nonsmokers develop renal cell carcinoma, which implies influence of host factors on individual susceptibility.[94] These individual differences in susceptibility to renal cell carcinoma may be attributed to genetic polymorphisms in DNA repair genes or others.[95] In 2008, Margulis and his colleagues have investigated thirteen SNPs in ten DSB repair genes, including XRCC2, XRCC3, NBS1, BRCA2, RAG1, ATM, and the four NHEJ genes, XRCC5, XRCC6, ligase 4 and XRCC4.[96] The original data showed that the SNPs of XRCC5 rs1805388 and XRCC6 rs132788 were not associated with renal cell carcinoma. However, the XRCC6 rs132788 genotype was considered one of the five critical genotypes determining the overall renal cell carcinoma risk in the classification and regression tree analysis, with the most effective NBS1 rs1805794. They concluded that individuals carrying more putative high-risk genotypes in the DSB repair pathway are at higher risks for renal cell carcinoma.[96]

3.7.2. Bladder cancer

Bladder cancer is the most common urinary tumor worldwide. In Europe, bladder cancer is the fourth most frequent cancer among men, accounting for 7% of total cancers.[97, 98] In USA, bladder cancer is the fifth highest cancer in men and seventh in women.[99, 100] Generally, bladder cancer is three times more common in men than women, and it is primarily a disease of the elderly, with 80% of the patients in the 50-79-year age group and a peak in the seventieth age. Environmental exposures to tobacco are the predominant risk factors for bladder cancer. The bladder cancer incidence is two to three folds higher among cigarette smokers as compared with non-smokers.[101] Occupational exposure to carcinogens, alcohol consumption, dietary factors and the use of hair dyes have also been suggested as risk factors for bladder cancer.[102-106]

Although loss of XRCC5 can result in the genome instability and in initialization of carcinogenesis, over-expression of XRCC5 is associated with the progression of bladder cancer.[107] The expression of XRCC6 is elevated in bladder tumor tissue[107] and XRCC6 may function as a caretaker gene for the development of T-cell lymphomas.[108] In 2008, Wang and his colleagues have published two papers investigating the roles of XRCC5[109] and XRCC6,[110] in bladder cancer in China. In the former study, a polymorphism with a variable number of tandem repeats (21-bp repeat

elements at the position 201 to 160 bp upstream to the initiation of transcription) in the XRCC5 was investigated of the association with bladder cancer risk. There are three different alleles, one includes the 42 nucleotide repeat elements (2R), another contains only one 21-nucleotide repeat in the rectangular box (1R), and still the other includes no repeat element (0R). The frequencies of the 2R/2R, 2R/1R, and 2R/0R genotypes among the cases were less than those for the controls, while the proportions of the 1R/1R, 1R/0R, and 0R/0R genotypes were greater. Overall the difference of the genotype distributions between the cases and the controls was significant, and individuals not carrying the 2R allele had a 1.75-fold increased risk of bladder cancer compared with those carrying the 2R allele.[109] The authors has also measured the promoter activities of the 2R, 1R and 0R alleles by transient transfection in HeLa, T24, and NIH3T3 cells, finding that fewer tandem repeats in the XRCC5 promoter increased the activity of the XRCC5 transcript.[109] The later paper investigating the same population has observed an association between XRCC7 rs7003908, but not XRCC6 rs2267437, genotype and the bladder cancer risk. Also, the risk is increased among the elder (>65 years old) smokers, suggesting that a gene-environment interaction may be involved in the development of bladder cancer.[110] It is reported that 2-naphthylamine and 4-aminobiphenyl, the compounds in tobacco smoke, can cause genetic damage in urothelium,[111, 112] which may enhance the cellular proliferation in

bladder carcinogenesis.[111, 113] In 2009, Michiels and his colleagues have analyzed the gene-environment joint effects on bladder cancer risk,[114] using the classification and regression tree method similar to that performed gene-gene interaction by Wu.[115] Smoking status and genotype data for up to 652 SNPs were incorporated in the analysis to explore gene-gene and gene-smoking interactions. The outcome is as expected, the smoking status is the most critical risk factor for bladder cancer. In ever smokers, a potential two-order interaction between the two SNPs, XRCC5 rs4674066 and ligase 1 rs2288878 was observed. The results suggested that smoking habits, XRCC5 CC and ligase 1 CT or TT, are sequentially three determinants for bladder cancer susceptibility in each subject. Very similar to this finding, Chang and his colleagues has found that there is a joint effects of XRCC5 genotype and personal smoking habits on bladder cancer risk in Taiwan.[116] In this study, a significant different distribution was found in the frequency of the XRCC5 rs828907, but not rs11685387 or rs9288518. In addition, those people carried GT and TT genotype at rs828907 had a 2.05-fold enhanced risk when they had the habit of tobacco smoking, but not alcohol consumption.[116]

3.7.3. Prostate cancer

Prostate cancer, a worldwide male disease, is the leading cause of illness and cancer

death in males.[117] In addition to age, race and a family history of prostate cancer, unbalanced diet, androgens, occupational chemicals, smoking, inflammation and obesity are considered to be additional secondary risk factors.[118] Recently, carbon ion radiotherapy with an established dose fractionation regimen has been shown to yield biochemically satisfactory relapse-free rates without local recurrence and with minimal morbidity.[119-121] In 2007 and 2008, Suga and his colleagues investigated the association between 450 SNPs in 118 candidate genes and radiation susceptibility in prostate cancer patients after carbon ion radiotherapy.[122, 123] The genotype of XRCC6 rs2267437, together with those for other four SNPs, SART1 rs2276015, ID3 rs2742946, EPDR1 rs1376264 and PAH rs1226758, were the determinants for the prediction of developing dysuria after carbon ion radiotherapy in prostate cancer patients. Despite the small population recruited, their work has set a very good example for the evaluation of side effects after clinical therapy, using the patients from a single hospital without the confounding effects of therapeutic protocols and differential scoring from various examiners and multiple institutions.[123]

3.8. Xrcc5/xrcc6 polymorphisms in myeloma

Multiple myeloma, also known as plasma cell myeloma, or as Kahler's disease, is a cancer of plasma cells, a type of white blood cell normally responsible for the

production of antibodies.[124] The disease develops in 1 to 4 per 100,000 people per year, more common in men, and is twice as common in blacks as it is in whites. Overall, it represents approximately 1% of all cancers and 2% of all cancer deaths.[124] The management of myeloma has benefited substantially from the introduction of three new drugs, the proteasome inhibitor bortezomib and the immunomodulators thalidomide and lenalidomide.[125] However, venous thromboembotic events with the subsequent risk of pulmonary embolism are major concerns in the treatment of patients with multiple myeloma with thalidomide. The susceptibility to developing venous thromboembotic events in response to thalidomide therapy is likely to be influenced by both genetic and environmental factors. In 2007, Hayden and his colleagues have screened 13 SNPs of XRCC5, including those located in intron regions, rs828704, rs2303400, rs207906, rs207908, rs207916, rs207922, rs6753002, rs207940, rs3770500, rs3770493, and three in 3'UTR, rs1051677, rs1051685 and rs2440, for the association of XRCC5 with myeloma risk. Only the genotype GG of rs1051685 was found to be significantly associated while others were all non-associated.[126] In 2008, Johnson and his colleagues have screened the effects of 3404 SNPs within 964 genes spanning 67 molecular pathways on the thalidomide-mediated venous thrombotic events in myeloma.[127] Overall, genes involved in drug transportation, drug metabolism, DNA repair, and cytokine balance

were found to be responsible for thalidomide-mediated venous thrombotic events in myeloma. The consistency finding of the XRCC5 rs2440 examined between these two studies was briefly discussed by the former group in a brief letter to the editor.[128] Their findings highlighted the significance of identifying genetic biomarkers, such as rs2440 in 3'UTR of XRCC5, that contributed to increased risk in myeloma patients and permit delineation of these biomarkers that may guide clinical strategy determination and therapeutic management for high-risk patients.

3.9. XRCC5/XRCC6 polymorphisms in cancer like disease

3.9.1 Pterygium

Pterygium is an invasive and fibrovascular overgrowth from the conjunctiva onto the cornea. The pathogenesis of this common ocular surface disorder is not well understood and the only treatment is surgical removal.[129] The pathology and etiology of pterygium were much alike to the cancer progression. The approaches and molecular knowledge investigating both of them may help the understanding of each other. In 2007, it is found that there were significant differences between pterygium and control groups in the distribution of genotype and allelic frequency in the XRCC6 promoter T-991C (rs5751129) polymorphism.[130] Individuals who carried at least one C allele (T/C and C/C) had a 2.83-fold increased risk of developing pterygium

compared to those who carried the T/T wild-type genotype. Moreover, individuals who carried at least one C allele (T/C and C/C) had a higher tendency to develop both sides of pterygium. In the XRCC6 rs2267437 polymorphism, there was no difference between both groups in the distribution of either genotype or allelic frequency.

4. XRCC5 and XRCC6 may contribute to individualized cancer pharmacogenomics

In this article, we have reviewed all the associations of XRCC5 and XRCC6 genotypes with the susceptibilities for various cancers in the literature, and summarized them concisely (Table 1). Among the SNPs investigated in different ethnicities and different cancers, some of the SNPs are very potential to serve as both cancer biomarkers in early detection and anticancer drug designing target sites, such as rs828704, rs828907, rs3770502 and rs9288516 of XRCC5, and rs2267437, rs5751129, rs6519285 of XRCC6. Clinical observation suggested that individuals exhibit differences in their response to drugs and that these variations could be inherited.[131, 132] Medical practice based on population responses did not reflect the best treatment for an individual.[133] Not until the Human Genome Project and the advances in genomic epidemiology together with systematic bioinformatics have the inter-individual and inter-ethic genetic variations come to light step by step. Although

the SNP and haplotype analyzing technology has become more and more mature and complex, the personalized cancer therapy and medicine depending much on the knowledge about cancer susceptibility, treatment outcome, responses to commonly used or gene-targeted anticancer drugs, and toxicities that are clustered among specific groups of patients in specific geographical regions, still necessarily need the help from the pharmacogenomics. The inter-individual variability in drug response can not satisfactorily be explained by ambiguous renal or liver functional differences, patients' age, morbidity, life style, or co-medication and compliance of patients, and the most possible explanation is falling on individual differences in their genomics. Polymorphisms in the human genome contribute to wide variations in how individuals respond to clinical medications, either by changing the pharmacokinetics (absorption, distribution, metabolism, and elimination) of anticancer drugs or by altering the cellular response to therapeutic agents such as radiotherapy.

As shown in the example of Table 1, cancer molecular epidemiologists are devoted themselves into the describing subtle differences among subjects in the distribution of genetic polymorphisms that affected DNA-repair enzymes, drug-metabolizing enzymes, cell-cycle controlling proteins, oncogenes, tumor suppression genes, and cellular transporters of cytotoxic chemotherapy. Because DNA repair enzymes are correctives for DNA damage induced by carcinogens and

even some anticancer drugs and radiotherapy, it is very likely that SNPs in DNA repair genes may play an important part in all the processes from cancer susceptibility to anticancer treatment outcomes. In this review, we focused on summarizing the SNPs of XRCC5 and XRCC6 genes, which are upstream and specifically critical in NHEJ, and their contribution to the common cancers around of living. It is necessary to discuss them together for they frequently form the heterodimer, as shown in Figure 2. However, the designing of anticancer drugs may directly aiming at binding with either or both of them, or indirectly modifying the interaction of them with other NHEJ proteins, such as XRCC7, or DNA itself. Among them, some SNPs, such as XRCC5 rs828907, are worth of paying more of our attention since they may serve as common SNPs for detecting and prediction for various cancers (so far for oral, breast and bladder cancers). The results of rs5751129 of XRCC6 were in similar case as those of rs828907 of XRCC5, showing that the polymorphic genotypes were associated with oral cancer and pterygium. The involvements of these SNPs in other human cancers and cancer-related diseases need further investigations and they may serve as candidate targets pharmacogenomically for developing personalized anticancer drugs. The hypothesis of how the XRCC5/6 genotypes control the fate of cells after DSB insults is shown in Figure 3.

Some DNA repair genes in the same and other subpathways such as XRCC4 in

NHEJ,[134] MGMT in direct removal pathway,[135, 136] XRCC1 in base excision repair,[137] ERCC 1 and 2 in NER,[138, 139] hMSH2 in mismatch repair,[137] hHR21 in HR,[138] are all thought to be anticancer candidate targets. From now on, XRCC5/6 may be added into the list above. It should be also paid attention that anticancer drugs may induce DSBs itself in the feasibility of chemotherapy. In the other way, co-treatments of DNA-damaging agents and radiation have a central role besides other cancer treatment modalities. The balance between DNA damage and capacity of DNA repair mechanisms determines the final therapeutic outcome. The capacity of cancer cells to complete DNA repair mechanisms is important for therapeutic resistance and has a negative impact upon therapeutic efficacy. Pharmacological inhibition of recently detected targets of DNA repair with several small-molecule compounds, therefore, has the potential to enhance the cytotoxicity of anticancer agents. Futami and his colleagues also discovered that inhibition of the expression of various genes associated with chromosome stabilization induces cancer cell-specific apoptosis and inhibits cell proliferation.[140]

5. Current and future developments

The story of “one size fits all” should be never spread, and pharmacogenomics is the most basic and essential part for individualized therapy and medicine. It is promising

to know that the potential of translational medical science has become reality in the field of pharmacogenomics, with the classical examples of UGT1A1 and irinotecan, TMPT and thiopurine, and CYP2D6 and tamoxifen. However, the fight with cancers is just at the beginning.

In this summary, most of the studies are case-control investigations for one or two ethnics. The inconsistency of choosing the SNPs and insufficiency sample size limited the multiple comparisons of the human populations around the world. The good examples of classification and regression tree analysis by some groups[95, 113] are straightforward to the goal of personalized medicine. Further incorporations and integrations of genotype-phenotype relationship analysis, population-based tissue and blood functional measurements, clinical outcome records, especially those in chemo- and radiotherapy responses, are **in urgent need** for international studies on inter-ethnic variations, using these pharmacogenomic biomarkers. The integration of pharmacogenomic biomarkers, phenotypic biomarkers, pathological biomarkers, is necessary in the systems for cancer risk prediction, and personalized medicine and therapy evaluation.

The knowledge about these pharmacogenomic biomarkers may provide new directions and practical tools for personalized medicine. After the knowing of specific critical SNPs, especially those located in exons and the genetic polymorphisms may

lead to alterations in the protein structures (so-called nonsynonmous SNPs), the scientists in bioinformatics may perform the molecular dynamic simulation among the docking sites between the target proteins, gaining the insight into the impact of these SNPs on structural changes. Quantitative structure-activity relationship analysis can be used to quantitatively analyze the impact of those non-synonymous polymorphisms on the function of the target protein. These methods would provide powerful and practical tools for high-speed screening of synthetic and natural compounds, and the deduced data can be applied to the molecular design for new anticancer drugs.

Conflict of interests

All the authors declare no competing financial interests.

Executive summary

XRCC5/XRCC6 played critical roles in genomic instability and carcinogenesis

- Defects in DNA repair systems were closely related to genome instability and carcinogenesis.
- Homologous recombination and non-homologous end-joining were two major DNA repair systems for DNA double strand breaks, one of the most deleterious DNA damaging types.

- XRCC5/XRCC6 was the upstream heterodimer in detecting double strand breaks for the non-homologous end-joining repair system.

Some SNPs on XRCC5/XRCC6 were investigated of their associations with specific cancer risks

- Molecular epidemiologists can investigate the association of XRCC5/XRCC6 genotypes with cancer risks via genome-wide, pathway-based and candidate-gene approaches.
- The XRCC5/XRCC6 polymorphic genotypes have been investigated of their associations with glioma, meningiomas, head and neck, thyroid, digestive system, lung, breast, urinary system cancers, and cancer like disease pterygium.
- The haplotypes, gene-gene and gene-environment interactions for carcinogenesis, and their associations with prognosis and anticancer treatment response may also be evaluated in population study.

XRCC/XRCC6 may contribute to individualized cancer pharmacogenomics

- The XRCC5/XRCC6 may be potential targets for anticancer drug development.
- The genotypes of XRCC5/XRCC6 SNPs may be potential bio-predictors of personal cancer risk and cancer prognosis outcome.
- The genotyping of XRCC5/XRCC6 SNPs, together with individual clinical data, may also be helpful in individualized cancer therapy strategy determination.

Future prospective on the road ahead

- The worldwide cancer epidemiologists should make progress in international integration for studies in cancer genomics and pharmacogenomics to make cancer eliminated.
- Larger sample sizes, more validations in different ethnicities and more detail clinical information should be incoperated into the worldwide cancer prediction system of individualized cancer pharmacogenomics.

Bibliography

Papers of special note have been highlighted as:

* = of interest,

** = of considerable interest

1 Hakem R. DNA-damage repair; the good, the bad, and the ugly. *EMBO J* 2008; **27**: 589–605.

2. Spry M, Scott T, Pierce H, D'Orazio JA. DNA repair pathways and hereditary cancer susceptibility syndromes. *Front Biosci* 2007; **12**: 4191–4207.

3. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J Natl Cancer Inst* 2000; **92**: 874–897.

4 Valerie K, Povirk LF. Regulation and mechanisms of mammalian

double-strand break repair. *Oncogene* 2003; **22:** 5792-5812.

5 Pfeiffer P, Goedecke W, Kuhfittig-Kulle S, Obe G. Pathways of DNA double-strand break repair and their impact on the prevention and formation of chromosomal aberrations. *Cytogenet Genome Res* 2004; **104:** 7-13.

6 Bassing CH, Chua KF, Sekiguchi J, Suh H, Whitlow SR, Fleming JC *et al*. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc Natl Acad Sci U S A* 2002; **99:** 8173-8178.

7 Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. *Cell Res* 2008; **18:** 134-147.

8 Gilley D, Tanaka H, Hande MP, Kurimasa A, Li GC, Oshimura M *et al*. DNA-PKcs is critical for telomere capping. *Proc Natl Acad Sci U S A* 2001; **98:** 15084-15088.

9 Goytisolo FA, Samper E, Edmonson S, Taccioli GE, Blasco MA. The absence of the dna-dependent protein kinase catalytic subunit in mice results in anaphase bridges and in increased telomeric fusions with normal telomere length and G-strand overhang. *Mol Cell Biol* 2001; **21:** 3642-3651.

10 Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE *et al*. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 2000; **404:** 510-514.

11 Ferguson DO, Alt FW. DNA double strand break repair and chromosomal translocation: lessons from animal models. *Oncogene* 2001; **20:** 5572-5579.

12 Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM *et al*. Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature* 2000; **404:** 897-900.

13 Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, Fleming J *et al*. Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. *Cell* 2002; **109:** 811-821.

14 Lieber MR. The mechanism of human nonhomologous DNA end joining. *J Biol Chem* 2008; **283:** 1-5.

* The article updated and summarized the genes/molecules involved in non-homologous end-joining system, together with the detail mechanism and regulation among them.

15 Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol* 2003; **4:** 712-720.

16 Gu Y, Seidl KJ, Rathbun GA, Zhu C, Manis JP, van der Stoep N *et al*. Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity* 1997; **7:** 653-665.

17 Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, Nussenzweig MC *et al*. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* 1996; **382:** 551-555.

18 Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 2001; **412:** 607-614.

19 Spagnolo L, Rivera-Calzada A, Pearl LH, Llorca O. Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. *Mol Cell* 2006; **22:** 511-519.

* The article reported the most updated structure of DNA-PKcs/XRCC5/XRCC6 complex and the role of them in non-homologous repair system.

20 Sibanda BL, Chirgadze DY, Blundell TL. Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. *Nature* 2010; **463:** 118-121.

21 Zhang Z, Zhu L, Lin D, Chen F, Chen DJ, Chen Y. The three-dimensional structure of the C-terminal DNA-binding domain of human Ku70. *J Biol Chem* 2001; **276:** 38231-38236.

22 Li L, Moshous D, Zhou Y, Wang J, Xie G, Salido E *et al*. A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabascan-speaking

Native Americans. *J Immunol* 2002; **168:** 6323-6329.

23 O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B *et al*. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell* 2001; **8:** 1175-1185.

24 Bau DT, Mau YC, Ding SL, Wu PE, Shen CY. DNA double-strand break repair capacity and risk of breast cancer. *Carcinogenesis* 2007; **28:** 1726-1730.

** The article convincingly provided a phenotypic evidence for the association between lower DNA double strand end-joining capacity and higher risk of breast cancer.

25 Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY *et al*. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. *Cancer Res* 2003; **63:** 2440-2446.

* The first article showed that XRCC6 genotype was positively associated with cancer risk.

26 Hemminki K, Li X. Familial risks in nervous system tumors. *Cancer Epidemiol Biomarkers Prev* 2003; **12:** 1137-1142.

27 Liu Y, Zhang H, Zhou K, Chen L, Xu Z, Zhong Y *et al*. Tagging SNPs in non-homologous end-joining pathway genes and risk of glioma.

Carcinogenesis 2007; **28:** 1906-1913.

* The first article showed that XRCC5 genotype was positively associated with cancer risk.

28 Wang LE, Bondy ML, Shen H, El-Zein R, Aldape K, Cao Y *et al*. Polymorphisms of DNA repair genes and risk of glioma. *Cancer Res* 2004; **64:** 5560-5563.

29 Karlsson P, Holmberg E, Lundell M, Mattsson A, Holm LE, Wallgren A. Intracranial tumors after exposure to ionizing radiation during infancy: a pooled analysis of two Swedish cohorts of 28,008 infants with skin hemangioma. *Radiat Res* 1998; **150:** 357-364.

30 Preston DL, Ron E, Yonehara S, Kobuke T, Fujii H, Kishikawa M *et al*. Tumors of the nervous system and pituitary gland associated with atomic bomb radiation exposure. *J Natl Cancer Inst* 2002; **94:** 1555-1563.

31 Sadetzki S, Flint-Richter P, Starinsky S, Novikov I, Lerman Y, Goldman B *et al*. Genotyping of patients with sporadic and radiation-associated meningiomas. *Cancer Epidemiol Biomarkers Prev* 2005; **14:** 969-976.

32 De Stefani E, Boffetta P, Deneo-Pellegrini H, Ronco AL, Acosta G, Ferro G *et al*. The effect of smoking and drinking in oral and pharyngeal cancers: a case-control study in Uruguay. *Cancer Lett* 2007; **246:** 282-289.

33 Sturgis EM, Wei Q, Spitz MR. Descriptive epidemiology and risk factors for head and neck cancer. *Semin Oncol* 2004; **31:** 726-733.

34 Carles J, Monzo M, Amat M, Jansa S, Artells R, Navarro A *et al*. Single-nucleotide polymorphisms in base excision repair, nucleotide excision repair, and double strand break genes as markers for response to radiotherapy in patients with Stage I to II head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 2006; **66:** 1022-1030.

35 Werbrouck J, De Ruyck K, Duprez F, Van Eijkeren M, Rietzschel E, Bekaert S *et al*. Single-nucleotide polymorphisms in DNA double-strand break repair genes: association with head and neck cancer and interaction with tobacco use and alcohol consumption. *Mutat Res* 2008; **656:** 74-81.

36 Werbrouck J, De Ruyck K, Duprez F, Veldeman L, Claes K, Van Eijkeren M *et al*. Acute normal tissue reactions in head-and-neck cancer patients treated with IMRT: influence of dose and association with genetic polymorphisms in DNA DSB repair genes. *Int J Radiat Oncol Biol Phys* 2009; **73:** 1187-1195.

37 Altekruse SF KC, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J *et al*. SEER Cancer Statistics Review 1975-2007. *National Cancer Institute* (in press).

38 American Cancer Society. Cancer Facts and Figures 2010. *Atlanta, Ga*.

American Cancer Society 2010.

* The article illustrated the most updated database of cancers in USA for the epidemiological reference and comparison.

39 Ogden GR. Alcohol and oral cancer. *Alcohol* 2005; **35:** 169-173.

40 Warnakulasuriya S, Sutherland G, Scully C. Tobacco, oral cancer, and treatment of dependence. *Oral Oncol* 2005; **41:** 244-260.

41 Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. *Respirology* 2003; **8:** 419-431.

42 Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. *Mutagenesis* 2004; **19:** 251-262.

43 Department of Health, Taiwan. *Cancer registration system annual report*. Department of Health: Taiwan, 2009.

44 Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN *et al*. Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. *Oral Oncol* 2008; **44:** 1047-1051.

45 Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH *et al*. Association between DNA double strand break gene Ku80 polymorphisms and oral cancer susceptibility. *Oral Oncol* 2009; **45:** 789-793.

46 Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN *et al*. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. *Oral Oncol* 2008; **44:** 898-902.

47 Tseng HC, Tsai MH, Chiu CF, Wang CH, Chang NW, Huang CY *et al*. Association of XRCC4 codon 247 polymorphism with oral cancer susceptibility in Taiwan. *Anticancer Res* 2008; **28:** 1687-1691.

48 Yang H, Lippman SM, Huang M, Jack-Lee J, Wang W, Spitz MR, Wu X. Genetic polymorphisms in double-strand break DNA repair genes associated with risk of oral premalignant lesions. *Eur J Cancer* 2008; **44**: 1603-1611.

49 Sherman SI. Thyroid carcinoma. *Lancet* 2003; **361**: 501–511.

50 Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A *et al*. International patterns and trends in thyroid cancer incidence, 1973–2002. *Cancer Causes Control* 2009; **20**: 525–531.

51 Cramer JD, Fu P, Harth KC, Margevicius S, Wilhelm SM. Analysis of the rising incidence of thyroid cancer using the Surveillance, Epidemiology and End Results national cancer data registry. *Surgery* 2010; **148**: 1147-1153.

52 Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer* 2006; **6**: 292-306.

53 Silva SN, Gil OM, Oliveira VC, Cabral MN, Azevedo AP, Faber A *et al*.

Association of polymorphisms in ERCC2 gene with non-familial thyroid cancer risk. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 2407-2412.

54 Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis* 2000; **21**: 427-433.

55 Gomes BC, Silva SN, Azevedo AP, Manita I, Gil OM, Ferreira TC *et al*. The role of common variants of non-homologous end-joining repair genes XRCC4, LIG4 and Ku80 in thyroid cancer risk. *Oncol Rep* 2010; **24**:1079-1085.

56 Ando N, Ozawa S, Kitagawa Y, Shinozawa Y, Kitajima M. Improvement in the results of surgical treatment of advanced squamous esophageal carcinoma during 15 consecutive years. *Ann Surg* 2000; **232:** 225-232.

57 Zhang H, Chen Z, Cheng J, Zhu X, Guo W, Hu A *et al*. The high incidence of esophageal cancer in parts of China may result primarily from genetic rather than environmental factors. *Dis Esophagus* 2010; **23:** 392-397.

58 International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: In Alcohol drinking*. France IARC Press: Lyon, 1988.

59 International Agency for Research on Cancer. *International Agency for Research on Cancer monograph 83: Tobacco smoke and involuntary smoking*. France IARC Press: Lyon, 2004.

60 Lee CH, Lee JM, Wu DC, Hsu HK, Kao EL, Huang HL *et al*. Independent and combined effects of alcohol intake, tobacco smoking and betel quid chewing on the risk of esophageal cancer in Taiwan. *Int J Cancer* 2005; **113:** 475-482.

61 International Agency for Research on Cancer. *International Agency for Research on Cancer monographs on the evaluation of carcinogenic risks to human: Tobacco smoking*. France IARC Press: Lyon, 1986.

62 Dong XJ, Wang N, Guo W, Zhou RM, Zhang XJ, Li Y. Correlations of XRCC5 polymorphisms to genetic susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a high incidence region. *Ai Zheng* 2007; **26:** 280-284.

63 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55:** 74-108.

64 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59:** 225-249.

65 Jackson C, Cunningham D, Oliveira J. Gastric cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2009; **20 Suppl 4:** 34-36.

66 Gellad ZF, Provenzale D. Colorectal cancer: national and international perspective on the burden of disease and public health impact.

Gastroenterology 2010; **138:** 2177-2190.

67 Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J Natl Cancer Inst* 1981; **66:** 1191-1308.

68 Thomas HJ. Familial colorectal cancer. *Bmj* 1993; **307:** 277-278.

69 Giovannucci E, Colditz GA, Stampfer MJ, Hunter D, Rosner BA, Willett WC *et al*. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. *J Natl Cancer Inst* 1994; **86:** 192-199.

70 Heineman EF, Zahm SH, McLaughlin JK, Vaught JB. Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of US veterans and a review. *Int J Cancer* 1994; **59:** 728-738.

71 Pryor WA, Hales BJ, Premovic PI, Church DF. The radicals in cigarette tar: their nature and suggested physiological implications. *Science* 1983; **220:** 425-427.

72 Yang MD, Hsu YM, Kuo YS, Chen HS, Chang CL, Wu CN *et al*. Significant association of Ku80 single nucleotide polymorphisms with colorectal cancer susceptibility in Central Taiwan. *Anticancer Res* 2009; **29:** 2239-2242.

73 Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007; **57:** 43-66.

74 Sanchez-Cespedes M. Lung cancer biology: a genetic and genomic

perspective. *Clin Transl Oncol* 2009; **11:** 263-269.

75 Shields PG. Molecular epidemiology of smoking and lung cancer. *Oncogene* 2002; **21:** 6870-6876.

76 Shields PG, Harris CC. Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. *J Clin Oncol* 2000; **18:** 2309-2315.

77 Tseng RC, Chang JW, Hsien FJ, Chang YH, Hsiao CF, Chen JT *et al*. Genomewide loss of heterozygosity and its clinical associations in non small cell lung cancer. *Int J Cancer* 2005; **117:** 241-247.

78 Tseng RC, Hsieh FJ, Hsu HS, Wang YC. Minimal deletion regions in lung squamous cell carcinoma: association with abnormality of the DNA double-strand break repair genes and their applications on gene identification and prognostic biomarkers. *Lung Cancer* 2008; **59:** 332-339.

79 Tseng RC, Hsieh FJ, Shih CM, Hsu HS, Chen CY, Wang YC. Lung cancer susceptibility and prognosis associated with polymorphisms in the nonhomologous end-joining pathway genes: a multiple genotype-phenotype study. *Cancer* 2009; **115:** 2939-2948.

80 Agurs-Collins T, Dunn BK, Browne D, Johnson KA, Lubet R. Epidemiology of health disparities in relation to the biology of estrogen receptor-negative breast cancer. *Semin Oncol* 2010; **37:** 384-401.

81 Coughlin SS, Ekwueme DU. Breast cancer as a global health concern. *Cancer Epidemiol* 2009; **33:** 315-318.

82 Freres P, Collignon J, Gennigens C, Scagnol I, Rorive A, Barbeaux A *et al*. "Triple negative" breast cancer. *Rev Med Liege* 2010; **65:** 120-126.

83 Goode EL, Dunning AM, Kuschel B, Healey CS, Day NE, Ponder BA *et al*. Effect of germ-line genetic variation on breast cancer survival in a population-based study. *Cancer Res* 2002; **62:** 3052-3057.

84 Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A, Lipscombe JM *et al*. Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Genet* 2002; **11:** 1399-1407.

85 Willems P, De Ruyck K, Van den Broecke R, Makar A, Perletti G, Thierens H *et al*. A polymorphism in the promoter region of Ku70/XRCC6, associated with breast cancer risk and oestrogen exposure. *J Cancer Res Clin Oncol* 2009; **135:** 1159-1168.

86 McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *Bmj* 2000; **321:** 624-628.

87 Lee KM, Choi JY, Kang C, Kang CP, Park SK, Cho H *et al*. Genetic polymorphisms of selected DNA repair genes, estrogen and progesterone receptor status, and breast cancer risk. *Clin Cancer Res* 2005; **11:** 4620-4626.

88 Cheng SH, Tsou MH, Liu MC, Jian JJ, Cheng JC, Leu SY *et al*. Unique features of breast cancer in Taiwan. *Breast Cancer Res Treat* 2000; **63:** 213-223.

89 Kuo WH, Yen AM, Lee PH, Chen KM, Wang J, Chang KJ *et al*. Cumulative survival in early-onset unilateral and bilateral breast cancer: an analysis of 1907 Taiwanese women. *Br J Cancer* 2009; **100:** 563-570.

90 Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet* 2002; **31:** 33-36.

91 Wang HC, Liu CS, Chiu CF, Chiang SY, Wang CH, Wang RF *et al*. Significant association of DNA repair gene Ku80 genotypes with breast cancer susceptibility in Taiwan. *Anticancer Res* 2009; **29:** 5251-5254.

92 Bau DT, Fu YP, Chen ST, Cheng TC, Yu JC, Wu PE *et al*. Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by BRCA1. *Cancer Res* 2004; **64:** 5013-5019.

93 Lipworth L, Tarone RE, McLaughlin JK. The epidemiology of renal cell carcinoma. *J Urol* 2006; **176:** 2353-2358.

94 Hunt JD, van der Hel OL, McMillan GP, Boffetta P, Brennan P. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. *Int J*

Cancer 2005; **114:** 101-108.

95 Hirata H, Hinoda Y, Matsuyama H, Tanaka Y, Okayama N, Suehiro Y *et al*. Polymorphisms of DNA repair genes are associated with renal cell carcinoma. *Biochem Biophys Res Commun* 2006; **342:** 1058-1062.

96 Margulis V, Lin J, Yang H, Wang W, Wood CG, Wu X. Genetic susceptibility to renal cell carcinoma: the role of DNA double-strand break repair pathway. *Cancer Epidemiol Biomarkers Prev* 2008; **17:** 2366-2373.

97 Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94:** 153-156.

98 Cancer Incidence. *IARC cancer Base No. 5: Mortality and Prevalence Worldwide. Version 1,0*. France IARC Press: Lyon, 2001.

99 Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A *et al*. Cancer statistics, 2005. *CA Cancer J Clin* 2005; **55:** 10-30.

100 Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970-2002. *Jama* 2005; **294:** 1255-1259.

101 International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Smoking*. France IARC Press: Lyon, 1985.

102 Bruemmer B, White E, Vaughan TL, Cheney CL. Nutrient intake in relation to

bladder cancer among middle-aged men and women. *Am J Epidemiol* 1996; **144:** 485-495.

103 Bruemmer B, White E, Vaughan TL, Cheney CL. Fluid intake and the incidence of bladder cancer among middle-aged men and women in a three-county area of western Washington. *Nutr Cancer* 1997; **29:** 163-168.

104 Gago-Dominguez M, Castelao JE, Yuan JM, Yu MC, Ross RK. Use of permanent hair dyes and bladder-cancer risk. *Int J Cancer* 2001; **91:** 575-579.

105 Sun CL, Yuan JM, Arakawa K, Low SH, Lee HP, Yu MC. Dietary soy and increased risk of bladder cancer: the Singapore Chinese Health Study. *Cancer Epidemiol Biomarkers Prev* 2002; **11:** 1674-1677.

106 Zeegers MP, Tan FE, Verhagen AP, Weijenberg MP, van den Brandt PA. Elevated risk of cancer of the urinary tract for alcohol drinkers: a meta-analysis. *Cancer Causes Control* 1999; **10:** 445-451.

107 Stronati L, Gensabella G, Lamberti C, Barattini P, Frasca D, Tanzarella C *et al*. Expression and DNA binding activity of the Ku heterodimer in bladder carcinoma. *Cancer* 2001; **92:** 2484-2492.

108 Li GC, Ouyang H, Li X, Nagasawa H, Little JB, Chen DJ *et al*. Ku70: a candidate tumor suppressor gene for murine T cell lymphoma. *Mol Cell* 1998; **2:** 1-8.

109 Wang S, Wang M, Yin S, Fu G, Li C, Chen R *et al*. A novel variable number of tandem repeats (VNTR) polymorphism containing Sp1 binding elements in the promoter of XRCC5 is a risk factor for human bladder cancer. *Mutat Res* 2008; **638:** 26-36.

110 Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD *et al*. Genetic variants of the XRCC7 gene involved in DNA repair and risk of human bladder cancer. *Int J Urol* 2008; **15:** 534-539.

111 Johansson SL, Cohen SM. Epidemiology and etiology of bladder cancer. *Semin Surg Oncol* 1997; **13:** 291-298.

112 Dolin PJ. An epidemiological review of tobacco use and bladder cancer. *J Smoking Related Dis* 1991; **2:** 129-143.

113 Lijinsky W, Thomas BJ, Kovatch RM. Systemic and local carcinogenesis by directly acting N-nitroso compounds given to rats by intravesicular administration. *Carcinogenesis* 1992; **13:** 1101-1105.

114 Michiels S, Laplanche A, Boulet T, Dessen P, Guillonneau B, Mejean A, *et al*. Genetic polymorphisms in 85 DNA repair genes and bladder cancer risk. *Carcinogenesis* 2009; **30:** 763-768.

* The article further evaluated the susceptibility of bladder cancer to 85 genes (652 SNPs) and smoking habits using the classification and regression tree (CART)

same as below, open the access for gene-environment joint effect evaluation.

115 Wu X, Gu J, Grossman HB, Amos CI, Etzel C, Huang M *et al*. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. *Am J Hum Genet* 2006; **78:** 464-479.

* The article very successfully evaluated the susceptibility of bladder cancer to 44 SNPs using the classification and regression tree (CART), establishing a very good example for gene-gene joint effect evaluation.

116 Chang CH, Chiu CF, Liang SY, Wu HC, Chang CL, Tsai CW *et al*. Significant association of Ku80 single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. *Anticancer Res* 2009; **29:** 1275-1279.

117 Gronberg H. Prostate cancer epidemiology. *Lancet* 2003; **361:** 859-864.

118 Hsing AW, Chokkalingam AP. Prostate cancer epidemiology. *Front Biosci* 2006; **11:** 1388-1413.

119 Akakura K, Tsujii H, Morita S, Tsuji H, Yagishita T, Isaka S *et al*. Phase I/II clinical trials of carbon ion therapy for prostate cancer. *Prostate* 2004; **58:** 252-258.

120 Ishikawa H, Tsuji H, Kamada T, Hirasawa N, Yanagi T, Mizoe JE *et al*. Risk factors of late rectal bleeding after carbon ion therapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2006; **66:** 1084-1091.

121 Tsuji H, Yanagi T, Ishikawa H, Kamada T, Mizoe JE, Kanai T *et al*. Hypofractionated radiotherapy with carbon ion beams for prostate cancer. *Int J Radiat Oncol Biol Phys* 2005; **63:** 1153-1160.

122 Suga T, Ishikawa A, Kohda M, Otsuka Y, Yamada S, Yamamoto N *et al*. Haplotype-based analysis of genes associated with risk of adverse skin reactions after radiotherapy in breast cancer patients. *Int J Radiat Oncol Biol Phys* 2007; **69:** 685-693.

123 Suga T, Iwakawa M, Tsuji H, Ishikawa H, Oda E, Noda S *et al*. Influence of multiple genetic polymorphisms on genitourinary morbidity after carbon ion radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2008; **72:** 808-813.

124 Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. *Lancet* 2009; **374:** 324-339.

125 Azais I, Brault R, Debiais F. New treatments for myeloma. *Joint Bone Spin*e 2010; **77:** 20-26.

126 Hayden PJ, Tewari P, Morris DW, Staines A, Crowley D, Nieters A *et al*. Variation in DNA repair genes XRCC3, XRCC4, XRCC5 and susceptibility to myeloma. *Hum Mol Genet* 2007; **16:** 3117-3127.

127 Johnson DC, Corthals S, Ramos C, Hoering A, Cocks K, Dickens NJ *et al*.

Genetic associations with thalidomide mediated venous thrombotic events in myeloma identified using targeted genotyping. *Blood* 2008; **112:** 4924-4934.

128 Tewari P, Kenny E, Staines A, Chanock S, Browne P, Lawler M. Genetic variants in XRRC5 may predict development of venous thrombotic events in myeloma patients on thalidomide. *Blood* 2009; **113:** 5691-5692.

129 Detorakis ET, Drakonaki EE, Spandidos DA. Molecular genetic alterations and viral presence in ophthalmic pterygium. *Int J Mol Med* 2000; **6:** 35-41.

130 Tsai YY, Bau DT, Chiang CC, Cheng YW, Tseng SH, Tsai FJ. Pterygium and genetic polymorphism of DNA double strand break repair gene Ku70. *Mol Vis* 2007; **13**: 1436-1440.

131 Evans WE, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med* 2003; **348:** 538-549.

132 Kawai Y, Kikuchi T, Mitani Y, Kogo Y, Itoh M, Usui K *et al*. Sensitive detection of EGFR mutations using a competitive probe to suppress background in the SMart Amplification Process. *Biologicals* 2008; **36:** 234-238.

133 Haselden JN, Nicholls AW. Personalized medicine progresses. *Nat Med* 2006; **12:** 510-511.

* The article highlighted the concepts and promising progresses of personalized

medicine.

134 Wu CN, Liang SY, Tsai CW, Bau DT. The role of XRCC4 in carcinogenesis and anticancer drug discovery. *Recent Pat Anticancer Drug Discov* 2008; **3:** 209-219.

* The article reviewed all the association studies in XRCC4 in carcinogenesis. XRCC4 also played an important role in non-homologous end-joining system.

135 Dolan ME, Moschel RC, Pegg AE. Depletion of mammalian O6-alkylguanine-DNA alkyltransferase activity by O6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. *Proc Natl Acad Sci U S A* 1990; **87:** 5368-5372.

136 Kaina B, Muhlhausen U, Piee-Staffa A, Christmann M, Garcia Boy R, Rosch F *et al*. Inhibition of O6-methylguanine-DNA methyltransferase by glucose-conjugated inhibitors: comparison with nonconjugated inhibitors and effect on fotemustine and temozolomide-induced cell death. *J Pharmacol Exp Ther* 2004; **311:** 585-593.

137 Andreassen CN, Alsner J, Overgaard M, Overgaard J. Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. *Radiother Oncol* 2003; **69:** 127-135.

138 Isla D, Sarries C, Rosell R, Alonso G, Domine M, Taron M *et al*. Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann Oncol* 2004; **15:** 1194-1203.

139 Ryu JS, Hong YC, Han HS, Lee JE, Kim S, Park YM *et al*. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004; **44:** 311-316.

140 Futami K, Takagi M, Shimamoto A, Sugimoto M, Furuichi Y. Increased chemotherapeutic activity of camptothecin in cancer cells by siRNA-induced silencing of WRN helicase. *Biol Pharm Bull* 2007; **30:** 1958-1961.

Figure legends

Figure 1 A model for repair of double-strand breaks by non-homologus end-joining.

Figure 2 Overview of XRCC5/XRCC6 heterodimer structure and associated protein. (A)The crystal structure of XRCC5/6 molecules. XRCC5 is colord black and XRCC6 grey. The C terminus of XRCC6 from the C-terminal arm through to the terminus is labeled and the disordered linker region between residues 539-558 are not shown. (B) Molecular surface of the XRCC7 molecules, the major functional domains are labeld. (C) The structure of C terminus of XRCC5.

Figure 3 The hypothesis of the XRCC5/XRCC6 genotypic control over the fate of cells.

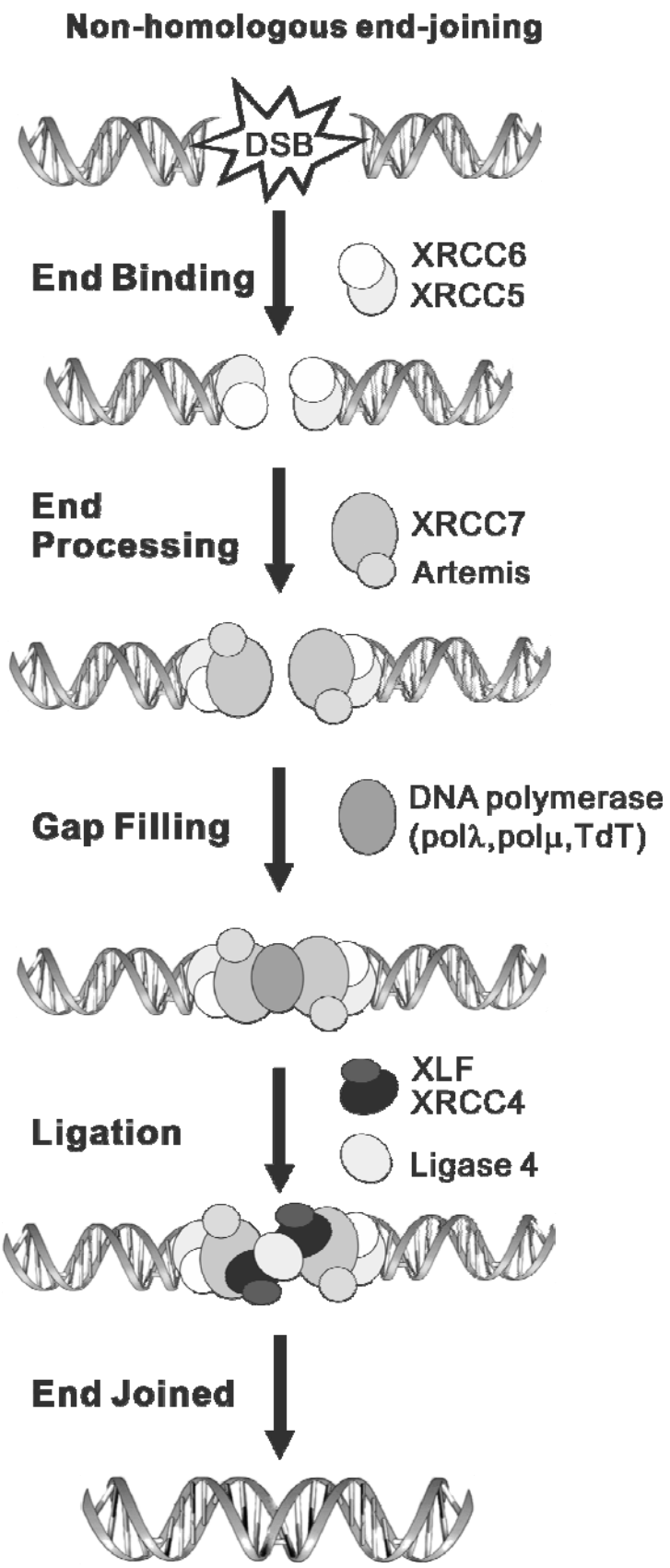
Non-homologous end-joining
DSB
End Binding
XRCC6
XRCC5
End
Processing
XRCC7
Artemis
Gap Filling
DNA polymerase
(polλ,polμ,TdT)
Ligation
XLF
XRCC4
Ligase 4
End Joined

Figure 1

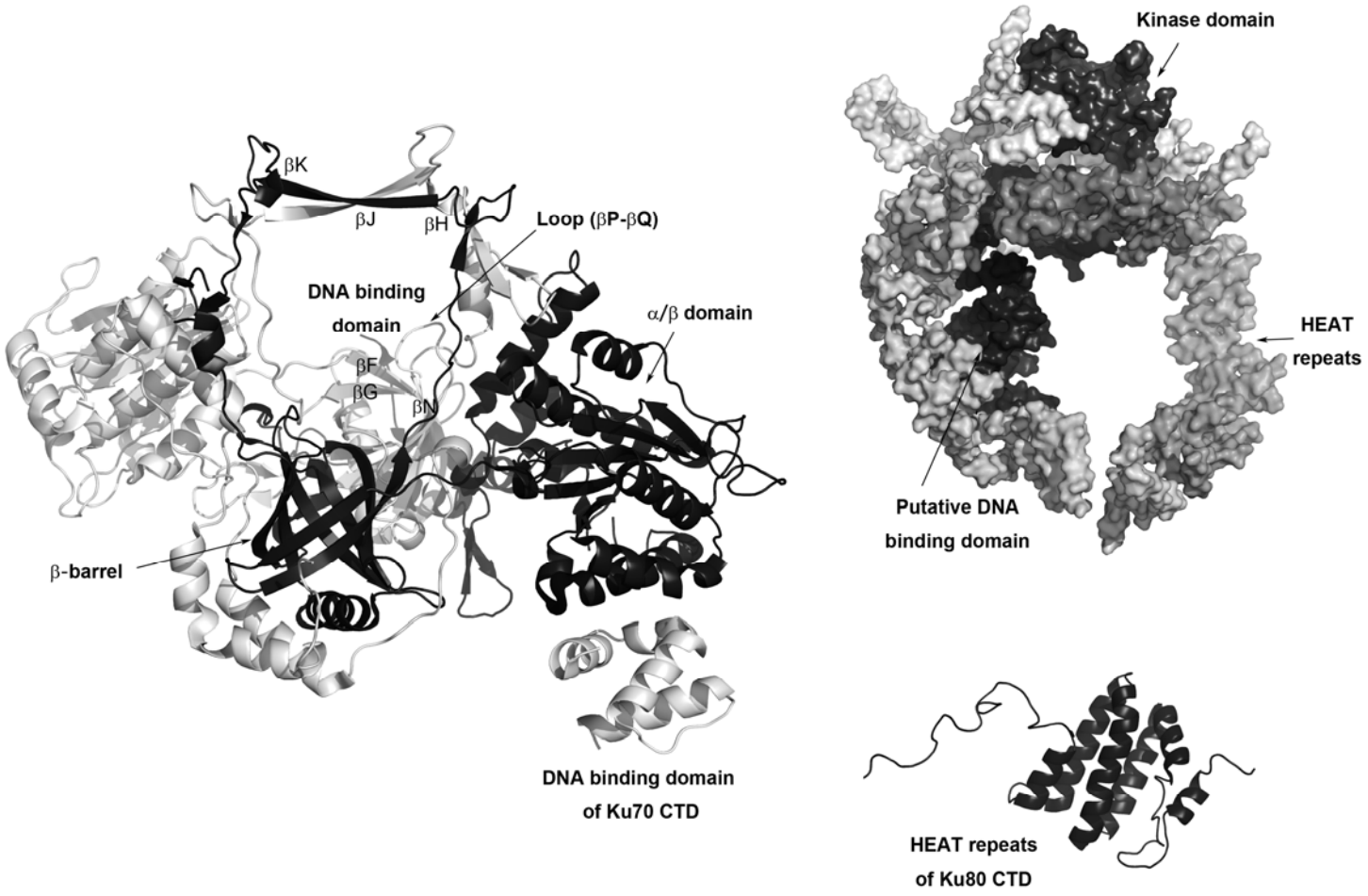
βK
βJ
βH
Loop (βP-βQ)
DNA binding
domain
α/β domain
βF
βG
βN
β-barrel
DNA binding domain
of Ku70 CTD
Kinase domain
HEAT
repeats
Putative DNA
binding domain
HEAT repeats
of Ku80 CTD

Figure 2

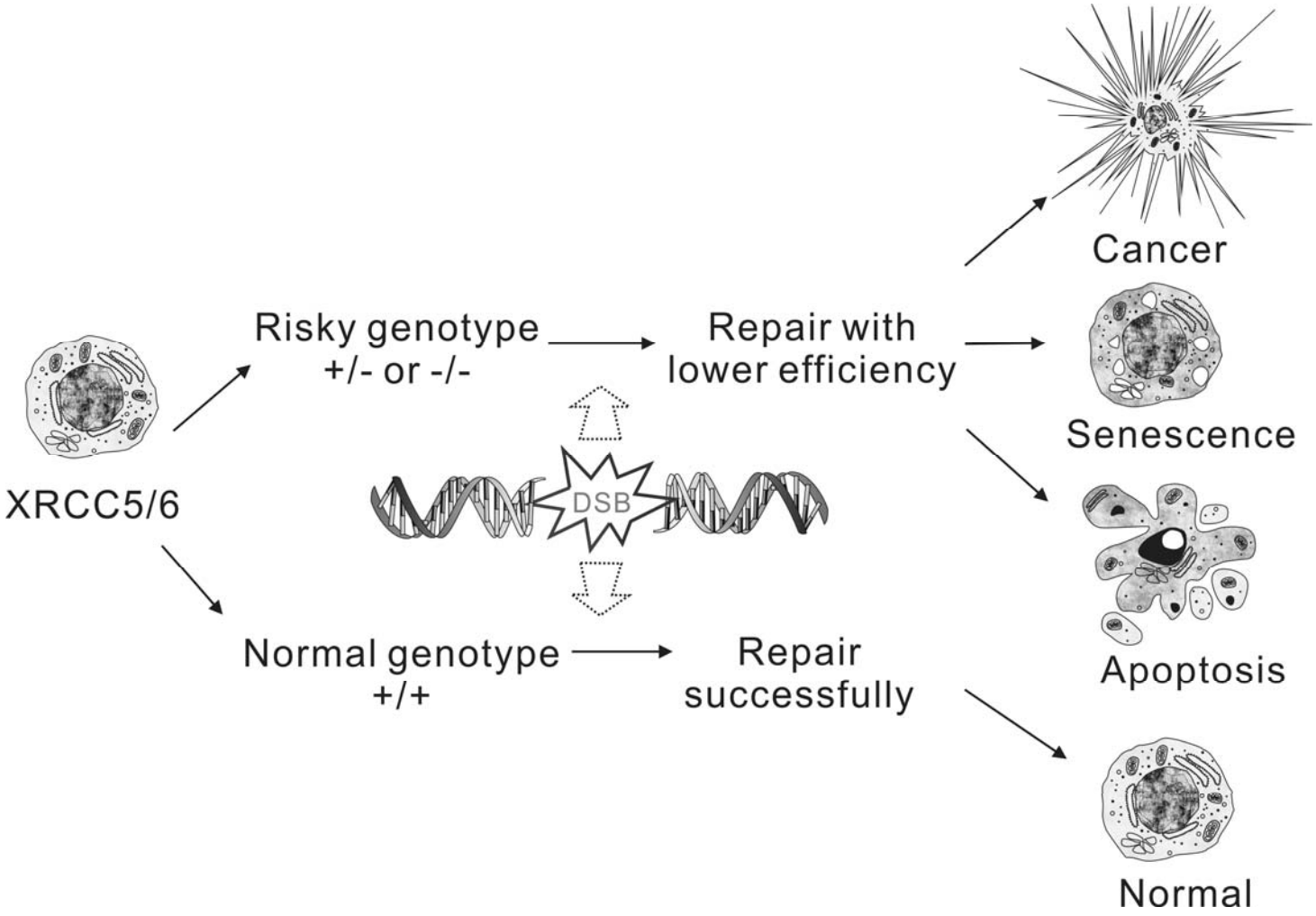
Cancer
Risky genotype
+/- or -/-
Repair with
lower efficiency
Senescence
XRCC5/6
DSB
Apoptosis
Normal genotype
+/+
Repair
successfully
Normal

Figure 3

Table 1. Summary of the results of the investigations for various cancers and the polymorphic genotype of XRCC5 and XRCC6 genes

Disease	Author, year (ref number)	Gene	rs number	Location	Study subjects				
					Ethnic Country	Cases	Controls	Statistical Significance	Brief description
Glioma	Liu, 2007 (27)	XRCC5	828704	Intron 8	China	771	752	S	Allele A is of higher risk
			16855458	Intron 13				NS	
			16855489	Intron 14				NS	
			668844	Intron 14				NS	
			207916	Intron 16				NS	
			3770502	Intron 16				S	Allele A is of higher risk
			9288516	Intron 16				S	Allele A is of higher risk
			1051677	3'UTR				NS	
		XRCC6	132770	5'UTR				NS	
			2267437	Intron 1				NS	
			12163239	Intron 3				NS	
			6519265	Intron 3				S	Allele A is of higher risk
			132793	3' flanking				NS	
Meningioma	Sadetzki, 2005 (31)	XRCC5	828699	Intron 6	Israel	219	221	NS	
Head and	Carles, 2006 (34)	XRCC5	1051677	3'UTR	Spain	108		NS	

Neck Cancer									
			1051685	3’UTR				NS	
	Werbrouck, 2008 (35)	XRCC5	3835	Intron 19	Belgium	152	157	NS	
		XRCC6	2267437	Promoter				NS	
	Werbrouck, 2009 (36)	XRCC5	3835	Intron 19	Belgium	152	157	NS	
		XRCC6	2267437	Promoter				S	Allele G is of higher risk for dysphasia after radiotherapy
Oral Premalignant Lesion	Yang, 2008 (48)	XRCC5	1051685	3’UTR	America	147	147	NS	
Oral Cancer	Hsu, 2009 (45)	XRCC5	828907	Promoter	Taiwan	600	600	S	Allele C is of higher risk
			11685387	Promoter				NS	
			9288518	Intron 19				NS	
	Bau, 2008 (44)	XRCC6	5751129	Promoter	Taiwan	318	318	S	Allele T is of higher risk, and interacted with betel quid chewing habits
			2267437	Promoter				NS	
			132770	Promoter				NS	
			132774	Intron 3				NS	

Thyroid Cancer	Gemes, (55)	2010	XRCC5	2440	3'UTR	Portugal	109	217	S	Allele A is of higher risk for male but not for female
				6941	3'UTR				S	Allele A is of higher risk in papillary tumor subgroup but not follicular tumor subgroup
				1051677	3'UTR				S	Allele C is of higher risk for male but not for female
				1051685	3'UTR				NS	
Esophageal Cancer	Dong, 2007 (62)		XRCC5	Accession number: DQ787434*	Intron16	China	329	631	S	Allele A is of higher risk
				Accession number: DQ787434*	Intron16				NS	
Gastric Cancer	Dong, 2007 (62)		XRCC5	Accession number: DQ787434*	Intron16	China	255	631	S	Allele A is of higher risk
				Accession number: DQ787434*	Intron16				S	Allele G is of higher risk

Colorectal Cancer	Yang, 2009 (72)	XRCC5	828907	Promoter	Taiwan	362	362	S	Allele T is of higher risk, and interacted with smoking habits
			11685387	Promoter				NS	
			9288518	Intron 19				NS	
Lung Cancer	Tseng, 2009 (79)	XRCC5	3835	Intron19	Taiwan	152	162	NS	Gene-gene joint effect with XRCC6
		XRCC6	2267437	Promoter				NS	Gene-gene joint effect with XRCC5
Breast Cancer	Goode, 2002 (83)	XRCC6	132788	Exon 4	British	2430	1370	NS	No effects on survival rate
	Kuschel, 2002 (84)	XRCC6	132788	Exon 4	Germany	2205	1826	NS	
	Willems, 2009 (85)	XRCC6	2267437	Promoter	Belgium	206	171	S	Allele G is of higher risk in sporadic but not familial breast cancer
	Lee, 2005 (87)	XRCC6	Accession number: AY870329*	Intron 2	Korea	872	671	NS	
	Fu, 2003 (25)	XRCC5	3835	Intron 19	Taiwan	254	379	NS	
			3834	Intron 19				NS	
		XRCC6	2267437	Promoter				S	Allele C is of higher risk
			132788	Exon 4				NS	
			132793	3’ flanking				NS	
	Wang, 2009 (91)	XRCC5	828907	Promoter	Taiwan	1272	1272	S	Allele T is of higher risk

			11685387	Promoter				NS	
			9288518	Intron 19				NS	
Renal Cell Carcimoma	Margulis, 2008 (96)	XRCC5	1051685	3'UTR	America	326	335	NS	
		XRCC6	132788	Exon 13				NS	
Bladder Cancer	Wang, 2008 (109)	XRCC5		-210~-160 repeats	China	213	235	S	Allele with fewer tandem repeats is of higher risk
	Wang, 2008 (110)	XRCC6	2267437	Promoter	China	213	235	NS	
	Michiels, 2009 (114)	XRCC5	4674066	Intron 19	France	210	326	NS	Gene-gene and gene-environment joint effects with smoking habits and ligase 1 genotype
	Chang, 2009 (116)	XRCC5	828907	Promoter	Taiwan	288	288	S	Allele T is of higher risk
			11685387	Promoter				NS	
			9288518	Intron 19				NS	
Prostate Cancer	Suga, 2008 (123)	XRCC6	2267437	Promoter	Japan	197	227	S	Allele G is of higher risk
Myeloma	Hayden, 2007 (126)	XRCC5	828704	Intron 8	Countries in Europe:	306	263	S	Allele G is of higher risk

			2303400	Intron 12	Germany,			NS	
			207906	exon 14	Italy, Spain,			NS	
			207908	Intron 14	Ireland,			NS	
			207916	Intron 16	France and			NS	
			207922	Intron 16	Czech			NS	
			6753002	Intron 16	Republic			NS	
			207940	Intron 16				NS	
			3770500	Intron 16				NS	
			3770493	Intron 16				NS	
			1051677	3'UTR				NS	
			1051685	3'UTR				NS	
			2440	3'UTR				NS	
Pterygium	Tsai, 2007 (130)	XRCC6	5751129	Promoter	Taiwan	128	114	S	Allele C is of higher risk
			2267437	Promoter				NS	

S: statistically significant; NS: not statistically significant; * Accession number was provided instead for the rs number is not available.