Predictive Role of XRCC5/XRCC6 Genotypes in Digestive System Cancers

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Abstract

Cancers are a world-wide concern, while oral, esophageal and gastrointestinal cancers represent important caused of cancer-related mortality and contribute to a significant burden of human disease. The DNA repair systems are the genome caretaker, playing a critical role in carcinogenesis. However, the associations between the genomic variations of DNA repair genes and cancer risk are largely unknown. This review focuses on the polymorphic genotypes of non-homologous end-joining DNA repair system, highlighting the role two genes of this pathway, *XRCC5* and *XRCC6*, in susceptibility to digestive system cancers and discussing their contribution to personalized medicine.

Key Words: XRCC5, XRCC6, polymorphism, oral cancer, esophageal cancer, gastrointestinal cancer, colorectal cancer

1. Introduction

The human genome is maintained mainly by the DNA repair pathways which can sense the DNA damage and response to exo- or endogenous DNA damages. In the recent literature, six main DNA repair pathways are identified and studied via functional assays: (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, during the cell cycle arrest caused by DNA abnormality, if these repair pathways fail to repair the DNA damage, the cell itself can sense the defects as a "threaten" and trigger the cell to undergo programmed cell death. 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 step by step. 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/XRCC6 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.

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 [18, 19]. 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 [20, 21]. Currently, it is worldwide trend to approach the subtle variations among subjects by the single nucleotide polymorphism (SNP) technique, and investigate their association with human diseases.

The aim of this article is to summarize and evaluate associations between the SNPs of *XRCC5*/*XRCC6* genes with the susceptibility to digestive system cancers, including oral, esophageal, gastric and colorectal cancers. Among the digestive

cancers, gastric, liver, and esophageal cancers continued to stay among top five cancers during the past three decades. More interestingly, 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 none for the difficulty of sample collection. 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-approaching studies. Therefore, we hope this article can provide some useful markers for oncology early detection, prevention, and some candidates for anticancer intervention. To this aim, we have summarized the literature for oral (2.1), esophageal (2.2), gastric (2.3) and colorectal (2.4) cancers in the second section, and discussed the contribution of these findings to personalized medicine and therapy in the third section.

2. XRCC5/XRCC6 polymorphic studies in digestive cancers

2.1. 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. World Health Organization has estimated oral cancer to be the eighth most common cancer worldwide. As with other upper aerodigestive tract cancers, five-year survival rates for oral cavity cancers decrease with delayed diagnosis. Cancers of the oral cavity are thought to progress from premalignant/precancerous lesions, beginning as hyperplastic tissue and developing into invasive squamous cell carcinoma. The most important environmental risk factors for the development of oral cancer in the Western countries are the consumption of tobacco and alcohol [22, 23]. In Asia, the chewing of betel quid and/or betel nut, are responsible for a considerable percentage of oral cancer cases [24, 25]. So far, the genomic etiology of oral cancer is of great interest but largely unknown.

In Taiwan, where the oral cancer density is highest in the world, oral cancer is a fatal disease accounting for the fourth highest incidence of malignancy in males and the seventh in females [26]. 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 prevalent habits of smoking, alcohol drinking and betel quid chewing. In the literature, there were four papers investigated the associations of NHEJ genes with oral cancer in Taiwan. In 2008, our group had 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 [27]. In the next, we 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 [28]. In that study, it was reported that those people carried GT and TT genotype at *XRCC5* rs828907 had an 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 investigating the polymorphic genotypes of *XRCC4* and their association with oral cancer in Taiwan [29, 30]. These studies reported that the *XRCC4* rs3734091 and rs28360071 polymorphisms turned out to be associated with oral cancer risk. In 2008, a study investigating the Americans with oral premalignant lesions has found that there is no association between their *XRCC5* rs1051685 genotypes with the susceptibility [31]. The inconsistency can be explained by at least two directions, one is different populations from different ethnicities were investigated, and another is different SNPs were examined among these studies. The negative findings could not exclude the possibilities that other SNPs of the *XRCC5* may be found to be associated with oral cancer susceptibility, at the meanwhile, the positive findings should be verified in even larger sample size and checked of the functional differences caused by the polymorphic genotypes.

2.2. Esophageal cancer

Esophageal squamous cell carcinoma (ESCC) is one of the common malignancies which with 5-year survival less than 10%. It is the seventh leading cause of cancer-related deaths in the world [32]. Epidemiologically, it is characterized by distinctly higher incidence in certain geographical locations, such as China [33]. Smoking tobacco and consuming alcohol are two environmental factors strongly associated with the risks of both ESCC and esophageal adenocarcinoma [34, 35]. ESCC shows a great variation in its geographic distribution and the incidence rates are remarkably higher in distinct high risk areas such as China, Singapore, Iran, France, South Africa, Puerto Rico, Chile, Brazil, Northern and Eastern Himalayan regions. In 1989, it is thought that the wide geographical variation in the incidence reflects a strong influence of environmental factors [36]. However, recent papers reporting that the high incidence of ESCC 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 [37-39].

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 subjects, while neither single SNP nor combined genotype has been found to be associated with esophageal cancer risk [40]. However, in those subjects with familial history of esophageal cancer, the C allele of *XRCC5* C74468A seemed to be a protective factor for the incidence [40]. Up to now, there was no report analyzing the association of *XRCC6* polymorphism with esophageal cancer risk.

2.3. Gastric cancer

Gastric cancer is the second most common malignancy and the second most frequent cause of cancer-related death in the world, responsible for approximately 934,000 new diagnoses annually (8.6% of new cancer cases) [41]. 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) [42]. 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) [43].

Now, gastric cancer is still a major health problem worldwide due to its

frequency, poor prognosis and limited treatment options. It is often diagnosed in advanced stages and consequently leads to poor prognosis. Although the mechanisms of gastric cancer were not yet elucidated, close relationship between gastric cancer and the provocation, maintenance and modulation of inflammation induced by *Helicobacter pylori* (*H. pylori*) was well accepted model for gastric carcinogenesis. In addition, 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. However, the genetic factors of gastric cancer are poorly understood.

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 [40]. 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 *XRCC5* 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.

2.4. Colorectal cancer

Colorectal cancer is the third most common malignant cancer worldwide. In 2010, an estimated 142,570 new cases of colorectal cancer (CRC) and 21,100 new cases of gastric adenocarcinoma (GA) will be diagnosed in the United States [44]. Noticeably, colorectal cancer remains a significant cause of morbidity and mortality in the United States, Taiwan and throughout the world [45]. Etiological studies have attributed more than 85% of colorectal cancer to environmental factors [46, 47], and in particular meat consumption, cigarette smoking, exposure to carcinogenic aromatic amines, such as arylamines and heterocyclic amines [48, 49]. These carcinogens are thought of as DNA damage inducers in responsible for DNA base damage, DNA single-strand breaks and DSBs [50].

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 [51]. 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* genotype) and environmental (smoking habit) 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. The contribution of XRCC5/XRCC6 biomarkers to personalized medicine

In this article, we have reviewed all the associations of *XRCC5* and *XRCC6* genotypes with the susceptibilities for digestive cancers in the literature, and summarized them concisely (Table 1). Generally speaking, individual cancer susceptibility is determined by three groups of factors, lifestyle/environmental factors, genetic/genomic factors, and age/gender factors. Among the three, the effects of lifestyle/environmental and age/gender factors may be influenced on somatic cells as genomic and epigenomic damage, which can be altered during the life span. However, the genomic/genetic factors confer a step-by-step but complicated and multi-pathway development of carcinogenesis. Clinical observation suggested that individuals may exhibit dramatic differences in their response to therapies and drugs, and that these variations could be inherited [52, 53]. SNPs could serve as not only the genomic markers,

but also the biomarkers in charge of personal cancer susceptibility. These SNPs 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 Table 1, we cancer molecular epidemiologists are devoted into the describing subtle differences among subjects in the distribution of genetic SNPs that affected DNA-repair enzymes, drug-metabolizing enzymes, cell-cycle controlling proteins, oncogenes, tumor suppression genes, and cellular transporters of cytotoxic chemotherapy, to reveal the overview of carcinogenesis. In this review, we can just focused on summarizing the state-of-arts studies on *XRCC5* and *XRCC6* genes, which are upstream and specifically critical in NHEJ, and their contribution to the digestive cancers. Although currently the hypothesis-free genome-wide association studies (GWAS) were largely applied to studies including cancer researches, the knowledge about the associations of specific genotypes with cancers is still limited and in urgent need. The contributions of the SNPs listed here in Table 1 to other human cancers and cancer-related diseases and their functional biological meanings to carcinogenesis need further investigations. At the meanwhile, they may serve as candidate targets pharmacogenomically for developing personalized anticancer drugs. The hypothesis

of how the *XRCC5*/*XRCC6* genotypes control the fate of cells after DSB insults is shown in Figure 2.

Some DNA repair genes in the same and other subpathways such as XRCC4 in NHEJ [54], *MGMT* in direct removal pathway [55, 56], *XRCC1* in base excision repair [57], *ERCC1* and *ERCC2* in NER [58, 59], *hMSH2* in mismatch repair [57], *hHR21* in HR [58], are all thought to be anticancer candidate targets. From now on, *XRCC5*/*XRCC6* may be added to 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 [60].

In this article, 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. Further incorporations among populations 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.

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Figure legends

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

Figure 2 The hypothesis of the XRCC5/XRCC6 genotypic control over the fate of cells.

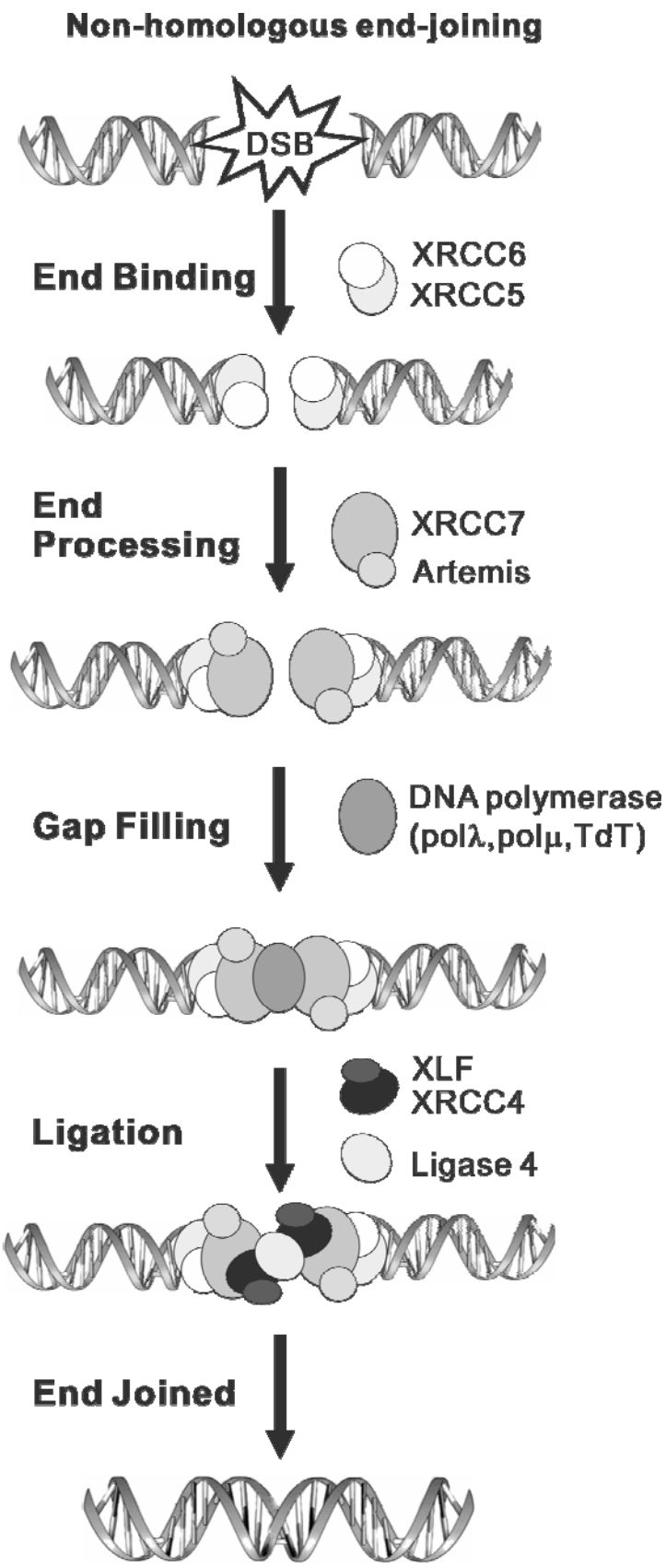
Non-homologous end-joining
DSB
End Binding
XRCC6
XRCC5
End
Processing
XRCC7
Artemis
Gap Filling
DNA polymerase
(polλ,polμ,TdT)
Ligation
XLF
XRCC4
Ligase 4
End Joined

Figure 1

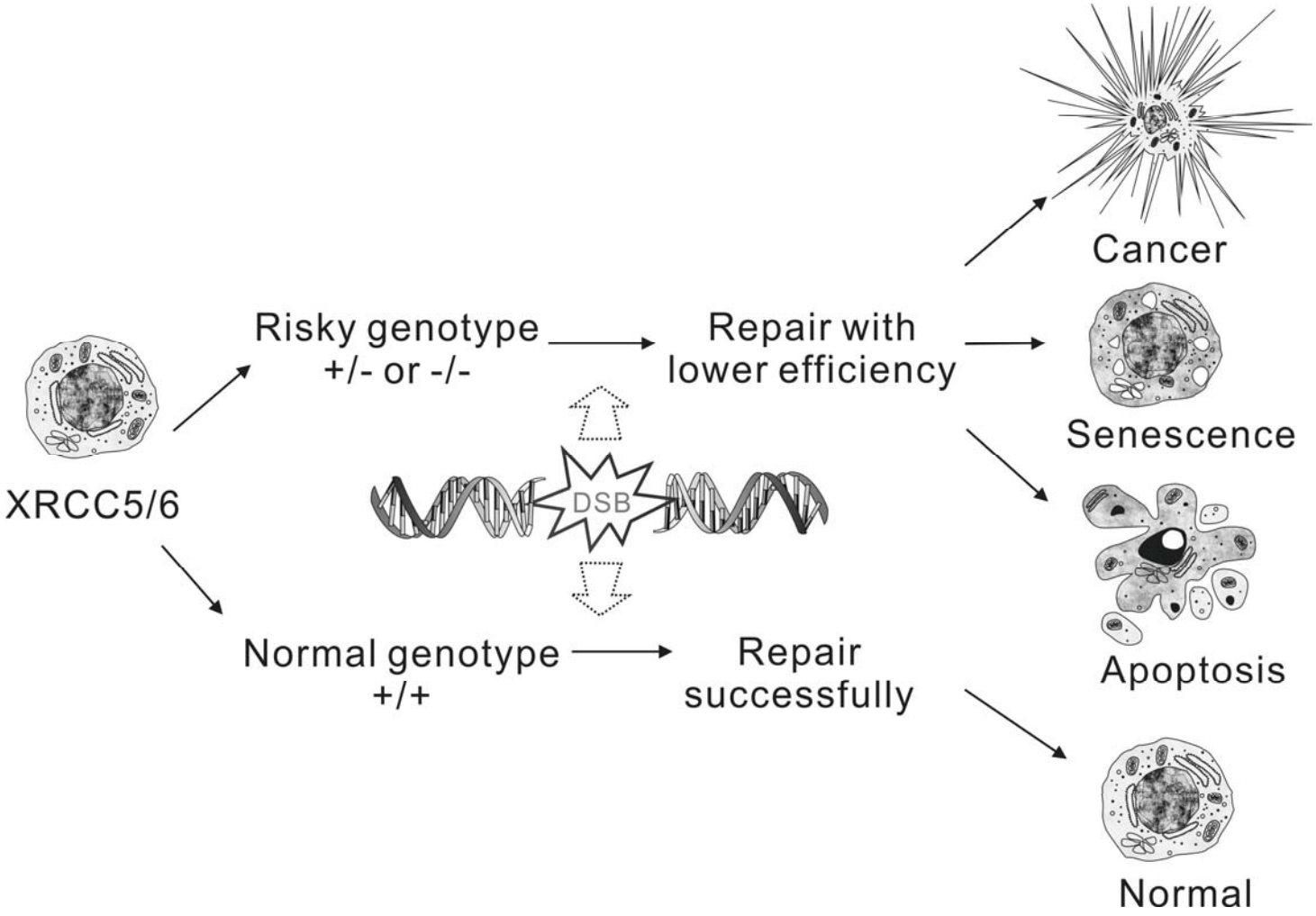
Cancer
Risky genotype
+/- or -/-
Repair with
lower efficiency
Senescence
XRCC5/6
DSB
Apoptosis
Normal genotype
+/+
Repair
successfully
Normal

Figure 2

Table 1. Summary of the associations for digestive cancers and the polymorphic genotype of XRCC5 and XRCC6 genes

Cancer	Author (ref number)	Gene	rs number	Location	Study subjects				
					Ethnic Country	Cases	Controls	Statistical Significance	Brief description
Oral Cancer	Hsu (28)	XRCC5	828907	Promoter	Taiwan	600	600	S	Allele C is of higher risk
			11685387	Promoter				NS	
			9288518	Intron 19				NS	
	Bau (27)	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	
Esophageal Cancer	Dong (40)	XRCC5	Accession number: DQ787434*	Intron16	China	329	631	S	Allele A is of higher risk
			Accession number: DQ787434*	Intron16				NS	
Gastric	Dong (40)	XRCC5	Accession	Intron16	China	255	631	S	Allele A is of higher risk

Cancer			number: DQ787434*						
			Accession number: DQ787434*	Intron16				S	Allele G is of higher risk
Colorectal Cancer	Yang (51)	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	

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