Recent years have witnessed the incidence of cancer rise worldwide, with no end to the war against it in sight. It is believed that cancer emanates from a series of genetic alterations leading to the progressive disorder of the normal mechanisms that control cell proliferation, differentiation, death, and/or genomic stability. With our genome under constant exogenous and endogenous assault, cellular capacity to maintain genomic stability by means of various DNA repair mechanisms looms vital to preventing tumor initiation and progression. In the same vein, the relative role of DNA repair as biomarker for prognosis, predictor of drug and therapy response, or indeed as target for novel gene therapy, has been recently patented and is very promising. This paper summarizes studies probing association among nonhomologous end-joining genes XRCC4, XRCC5, and XRCC6 vis-à-vis oral cancer susceptibility, then discusses their role in carcinogenesis and personalized pharmacogenomics.

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1. Introduction

The human genome is maintained by repair pathways that sense DNA damage and respond to exogenous and/or endogenous DNA insult. Pathways identified include: (a) direct reversal repair, (b) nucleotide excision repair, (c) base excision repair, (d) homologous repair (HR), (e) nonhomologous end-joining (NHEJ), and (f) mismatch repair. Normally, if these repair pathways fail to repair the DNA damage, the same molecular machinery can sense defects as a “threat” and trigger apoptosis. Yet, when DNA damage is neither repaired nor turned to induction of cell apoptosis terminating unhealthy cells, DNA defects remain and are propagated to offspring cells. Under the later circumstances, carcinogenesis occurs. Decreased genomic integrity and stability in most cancer types and identification of cancer predisposition syndromes linked to defects in 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). It should be repaired in eukaryotes by two major pathways mentioned above: HR and NHEJ. The former is a template-guided, error-free pathway predominantly operating in S and G2 phases of the cell cycle and involves RAD51, its paralogs RAD51B/C/D, XRCC2/3, and p53, replication protein A (RPA), BRCA 1/2, BLM DNA helicase (BLM), and...
MUS81 [4]. The latter, by contrast, is a potentially less accurate form of DSB repair, with both termini of a broken DNA molecule processed to form compatible ends directly joined. In most cases, NHEJ results in loss of a few nucleotides at broken ends, making this pathway error-prone. This article focuses on XRCC4, XRCC5, and XRCC6, which play crucial roles in NHEJ, which is considered the major repair pathway of DSBs in eukaryotic cells during most phases of the cell cycle, particularly G0/G1 phases [5]. NHEJ involves the XRCC4, XRCC5/XRCC6 (also known as Ku80/Ku70), XRCC7 [DNA-dependent protein kinase catalytic subunit (DNA-PKcs)], Artemis, XRCC4-like factor (XLF), DNA ligase 4, Ataxia telangiectasia mutated kinase (ATM), p53, and MDM2 proteins [6,7]. NHEJ deficiencies can increase genomic instability [8,9] as well as tumorigenesis [10–13]. Still, the exact roles of these genes and their protein products, such as XRCC4, XRCC5, or XRCC6, in oral and/or other cancers are neither well investigated nor revealed.

Fig. 1 depicts the model for DSB repair via NHEJ, along with proteins involved.

XRCC4 is found to restore DNA DSB repair and the ability to support V(D)J recombination of transiently introduced substrates in the XR-1 CHO cell line [14]. The XRCC4 interacts directly with XRCC5/ XRCC6; it is hypothesized to serve as a flexible tether between XRCC5/XRCC6 and associated protein ligase 4 [15]. XRCC4 is required for precise end-joining of blunt DNA DSBs in mammalian fibroblasts [16]. A gene-targeting mutation mouse model shows, XRCC4 gene inactivation spawning late embryonic lethality accompanied by defective lymphogenesis and neurogenesis, manifested by extensive apoptotic death of newly generated postmitotic neuronal cells [12,17]. Findings demonstrate that differentiating lymphocytes and neurons strictly require XRCC4 proteins.

XRCC5 and XRCC6 usually form heterodimer Ku; they are probably among the first proteins bound to DNA ends at a DSB. The XRCC5/6–DNA complex recruits and activates XRCC7 [18,19]. XRCC5/6 dimer and XRCC7 are proposed to act in a synapsis process [18,19]. XRCC5 and XRCC6 knockout mice are growth retarded, radiosensitive and are severely immunodeficient [20,21]. B-cell development is arrested at an early stage due to profound deficiency in V(D)J recombination [20,21]. Although 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. These defects may also happen during human embryonic development. Human cells absorb hundreds of thousands exogenous and endogenous DNA insults daily. If cells fail to repair DSB, accumulated genomic instability will lead to apoptosis and cause embryonic lethality. Beyond a doubt, XRCC5 and XRCC6 are critical to both genomic stability and human ontogenesis.

Since each NHEJ gene plays a critical and specific role in repairing DSBs, if any of them fails to finish its job correctly and immediately, NHEJ capacity is hindered and overall genomic instability raised. It is thus tempting to speculate that defects in the NHEJ pathway may be associated with human cancers. This makes it puzzling as to why no direct genetic linkage is found between defective NHEJ genes and cancers. Among them, mutations in two demonstrably predispose carriers to a higher rate of genetic disease, DNA ligase 4 and Artemis, both 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 result in great genomic instability and prove incompatible with life, hence no cancer observed. Crucial and irreplaceable roles of these gene products may raise difficulty of approaching their physiological function via knockout mice. Consequently, for these high-penetrance NHEJ genes, only subtle defects arising from low-penetration alleles like hypomorphic mutant or polymorphic variant would escape the cell cycle checkpoint surveillance and allow the cell to survive, as well as to accumulate enough unrepaired genomic alteration required for tumor formation [24,25]. Studies applying single nucleotide polymorphism (SNP) technology, among the most powerful and subtle genetic analyses, to approach associations of high- and low-penetration genes with various cancers are warranted to fulfill the overall scene in cancer research.

One aim of this article is to summarize the work in Terry Fox Cancer Research Lab of China Medical University and

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**Fig. 1** — Model for repair of double-strand breaks by nonhomologous end-joining.
Hospital, Taichung, Taiwan, R.O.C., and worldwide, assessing correlation between SNPs of XRCC4, XRCC5, and XRCC6 regarding susceptibility to oral cancer. While rapid development of genome-wide association studies and bioinformatics help reveal secrets of human genome in cancer, knowledge of cancer genomics is still far from satisfying and in urgent need of further multi-approach studies. In addition, most SNPs have not been investigated for functional influence in cell or animal models, not to mention individual roles in cancer. It is very exciting that some SNPs of XRCC4, XRCC5, and XRCC6 have been associated with the susceptibility to various cancers. More interesting, specific gene-environment interaction appear in cancer patients who contact possible environmental carcinogens. Therefore, we infer risky SNP genotypes of XRCC4, XRCC5, XRCC6, and environmental carcinogens have some joint effects and increase susceptibility to cancer significantly. We hope this article provides novel useful markers for oncology early detection, prevention, along with some candidates for anticancer intervention.

2. Literature survey

We conducted MEDLINE, Current Contents, and Web of Science searches using oral cancer, polymorphism, XRCC4, XRCC5, or XRCC6 as keywords to search for articles published (January 1, 1966, to December 31, 2010). Additional papers were identified by references cited in the first series of selected articles; those included in meta-analysis were in any language, with human patients, published in primary literature and had no obvious overlap with patients of other studies. Case-control studies were eligible if they determined distribution of relevant genotypes in cancer cases and in concurrent controls using a molecular method for genotyping.

3. Oral cancer in Taiwan and worldwide

Oral cancer specifies a subgroup of head and neck malignancies developing at the lips, tongue, salivary glands, gingiva, mouth floor, oropharynx, buccal surfaces and other intraoral locations. Oral cancer incidence has increased through 2007, estimated in the United States as 10.3 cases per 100,000 persons, with mortality of 2.5 per 100,000 [26]. An estimated 36,540 new cases will be diagnosed in America during 2010, with 7,880 deaths due to this disease [27]. Oral cancer is more common in men than in women and accounts for 3% of new cancer cases in American men [27]. The World Health Organization estimates oral cancer as the eighth-most common cancer worldwide. Most important environmental risk factors in Western countries are consumption of tobacco and alcohol [28,29]. In Asian countries like Taiwan and India, betel quid accounts for a considerable percentage of such cases [30,31]. To date, genomic etiology is of great interest but largely unknown. In Taiwan, whose oral cancer frequency ranks highest worldwide, this disease looms as fourth greatest malignancy among men and sixth in women [32]; its relatively high prevalence in Taiwan stems mainly from the high-risk group of 2.5 million people with tobacco, liquor, and/or betel nut habits.

4. Role of XRCC4 in oral cancer genomics

Few papers investigated association of NHEJ genes with oral cancer worldwide; the two correlating XRCC4 genotypes with oral cancer risk were performed in Terry Fox Cancer Research Lab [33,34]. These studies screened five genetic variants of the XRCC4 gene, two of which showed strong correlation with oral cancer susceptibility: XRCC4 codon 247 (rs3734091) and intron3 (rs28360071). The XRCC4 codon 247 (rs3734091) locates on the exon of XRCC4; this SNP is in charge of shifting amino acids of XRCC4 gene products, which may also alter its biologic function. For this age- and sex-matched case-control study, control and patient groups each recruited 318 patients, with A allele assessed as a risk factor. Persons with it show 2.04-fold higher susceptibility. Another study’s key variable was XRCC4 intron3 (rs28360071), deletion-insertion polymorphism (DIP) involved in 30 bp genetic variation. In all, 122 (38.4%) patients had D allele; the control group had only 91 (28.6%) such individuals. Statistically, people with D allele have a 1.55-fold higher risk than those with I allele.

Reports also checked joint effect of risky genotype and environmental exposure in oral cancer susceptibility. Briefly, risky genotype XRCC4 codon 247 (rs3734091) has synergistic effect with smoking on oral cancer risk, as does risky genotype intron3 (rs28360071) with both smoking and betel quid chewing. Joint effects indicate not only the XRCC4 gene but also the NHEJ DSB repair system as involved. Genetic factors indeed interact with environmental factors in overall cancer susceptibility.

5. Role of XRCC5 and XRXX6 in cancer genomics

In 2008, Terry Fox Cancer Research Lab found C allele of XRCC6 rs5751129 a marker for oral cancer risk; those of rs2267437, rs132770 and rs132774 were not [35]. Our team enlarged their population control/case from 318/318 to 600/600, reporting XRCC5 rs828907, but not rs11685387 or rs9288518, as associated with susceptibility [36]. Patients carrying GT and TT genotype at rs828907 showed 1.6-fold risk if they habitually chewed betel quid. A 2008 study of oral premalignant lesions found no correlation between XRCC5 rs1051685 genotypes and susceptibility [37].

6. XRCC4, XRCC5, and XRCC6 for individualized cancer pharmacogenomics

This article has reviewed association of XRCC4, XRCC5, and XRCC6 genotypes with susceptibility described oral cancer in literature, as summarized in Table 1. Clinical observation hints individual differences in response to drugs, variations that could be genetic [38,39]. Medical practice based on population response did not reflect ideal treatment for individuals [40]. Not until the Human Genome Project and advances in genomic epidemiology, along with systematic bioinformatics, have individual and/or ethnic genetic variations come to light step by step. Although the SNP and haplotype analyzing
technology has grown ever more mature and complex, personalized cancer therapy and medicine heavily dependent on knowledge of susceptibility, treatment outcome, response to commonly used or gene-targeted anticancer drugs, and toxicities clustered among specific groups of patients in specific geographical regions, still need help from pharmacogenomics. Inter-individual variability in drug response cannot be satisfactorily explained by ambiguous renal or liver functional differences, patient age, morbidity, lifestyle, or comedication and patient compliance. Any solution is doomed to fail via individual genomics; polymorphisms lead to wide variation in how individuals respond to medication, either by changing pharmacokinetics (absorption, distribution, metabolism, and/or elimination) of anticancer drugs or by altering cellular response to therapeutic agents like radiotherapy.

As shown in Table 1, cancer molecular epidemiologists devote themselves to the description of subtle differences among patients in genetic polymorphism distribution that affected DNA-repair enzymes, drug-metabolizing enzymes, cell-cycle controlling proteins, oncogenes, tumor suppression genes, and cellular transporters of cytotoxic chemotherapy. With DNA repair enzymes as correctives for DNA damage induced by carcinogens and even some anticancer drugs and radiotherapy, SNPs in DNA repair genes may very likely play important roles in all processes from cancer susceptibility to anticancer treatment outcome. This review summarizes SNPs of XRCC4, XRCC5, and XRCC6 genes specifically critical in NHEJ, plus their contribution to common cancers around of living. Among them, SNPs like XRCC5 rs828907, may merit our attention since they serve as biomarkers for early detection and prediction of various cancers (to date, oral, breast, and bladder cancers) [30,41,42]. The rs5751129 of XRCC6 was similar to such cases as rs828907 of XRCC5, associated with oral cancer and pterygium[35,43]. Involvement of these SNPs in other human cancers and related diseases warrant further investigation and may serve as pharmacogenomic targets for concocting personalized drugs.

Some DNA repair genes in the same and other subpathways, such as O(6)-methylguanine-DNA methyltransferase (MGMT) in direct removal pathway [44,45], XRCC1 in base excision repair [46], ERCC 1 and 2 in nucleotide excision repair (NER) [47,48], hMSH2 in mismatch repair [46], and hHR21 in HR [47] are all seen as anticancer candidate targets. Henceforth, XRCC4, XRCC5 and XRCC6 may be added to the list above. Association studies of XRCC4, XRCC5, and XRCC6 genotypes in other cancers and cancer-related diseases all supported the concept of NHEJ and DNA repair system playing a key role in

<table>
<thead>
<tr>
<th>Disease</th>
<th>Author, year (ref number)</th>
<th>Gene</th>
<th>rs number</th>
<th>Location</th>
<th>Study subjects</th>
<th>Statistical Significance</th>
<th>Brief description</th>
</tr>
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<tbody>
<tr>
<td>Oral Premalignant Lesion</td>
<td>Yang, 2008 (37)</td>
<td>XRCC5</td>
<td>1051685</td>
<td>3'UTR America</td>
<td>147</td>
<td>147</td>
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<td>Oral Cancer</td>
<td>Chiu, 2008 (33)</td>
<td>XRCC4</td>
<td>6869366</td>
<td>Promoter Taiwan</td>
<td>318</td>
<td>318</td>
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<td></td>
<td>28360071</td>
<td>Intron 3</td>
<td></td>
<td>NS</td>
<td>Allele deletion is risky (vs insertion), and had joint effects with smoking and betel quid chewing habits</td>
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<td>28360317</td>
<td>Intron 7</td>
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<td>NS</td>
<td>Allele A is risky, and had joint effects with smoking habit</td>
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<td>1805377</td>
<td>Intron 7</td>
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<td>Codon 247</td>
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<td>Hsu, 2009 (36)</td>
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<td>11685387</td>
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<td>9288518</td>
<td>Intron 19</td>
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<td></td>
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<td>XRCC6</td>
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<td>2267437</td>
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<td>Allele T is risky, and had joint effects with betel quid chewing habits</td>
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<td>132770</td>
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<td>132774</td>
<td>Intron 3</td>
<td></td>
<td>NS</td>
<td>Allele T is risky, and had joint effects with betel quid chewing habits</td>
</tr>
</tbody>
</table>

S: statistically significant; NS: not statistically significant.
human carcinogenesis, such as in glioma [49,50], skin cancer [51], head and neck cancers [33–37,52,53], colorectal cancer [54], lung cancer [55,56], breast cancer [41,47–49,57–59], renal cell carcinoma [60,61], bladder cancer [42,62–64], and myeloma [65]. It may also be of importance for anticancer drugs inducing DSBs in feasibility of cotherapy. Alternatively, cotreatments of DNA-damaging agents and radiation play a central role besides other cancer treatment modalities. Balance between damage and capacity of repair mechanisms determines the final therapeutic outcome. The capacity of cells to complete DNA repair mechanisms is vital to therapeutically resistant and impacts therapeutic efficacy. Thus, pharmacologic inhibition of recently detected DNA repair targets with small-molecule compounds has the potential to enhance cytotoxicity of anticancer agents. Futami and colleagues [66] found inhibited gene expression associated with chromosome stabilization inducing cancer cell-specific apoptosis and inhibit cell proliferation.

7. Current and future works in the war against cancer

The story of “one size fits all” should be never spread; pharmacogenomics is the most fundamental element 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 classical examples UGT1A1 and irinotecan, thiopurine methyltransferase (TMPT) and thiopurine, CYP2D6 and tamoxifen. Yet the fight against cancer is barely underway. Terry Fox Cancer Research Lab at the China Medical University Hospital teamed with many outstanding scientists and surgeons in establishing and perfecting an oral cancer mouse model by Dr. N.W. Chang, unique pairs of oral cancer patient primary cultured cells from both tumor and portal sites (as far apart as possible) of experimental animal and human patients by ourselves. We have deeply devoted ourselves at all times to the phenotype and genotype correlation study, e.g., individual phenotypic NHEJ capacity and XRCC4, XRCC5, or XRCC6 haplotype analysis. A promising genotype-phenotype correlation platform could reveal the network of all biosignaling from genomics, transcriptomics, and proteomics to functional levels. We visited the M. D. Anderson Cancer Center in Dallas, Texas, to gain experience in genome-wide, high-level gene-gene and environment analyzing system, contributing all of our efforts to make cancer history. We believe that further incorporation and integration of genotype-phenotype analysis, population-based tissue and blood functional measurements, clinical outcome records, and especially in chemo- and radiotherapy response, are highly respected for international studies of ethnic variation using these pharmacogenomic biomarkers. The integration of pharmacogenomic, phenotypic, and pathologic biomarkers is pivotal in cancer risk prediction, along with personalized medicine and therapy evaluation.

The war against cancer is far from won; all oncologists from bench to bed face colossal tasks. All the above knowledge of these pharmacogenomic biomarkers may provide new directions and practical tools for personalized medicine. Learning about specific/critical SNPs, especially those in exons and genetic polymorphisms, may lead to alterations in protein structures (the so-called nonsynonymous SNPs). Scientists in bioinformatics may perform molecular dynamic simulation of docking sites between target proteins, gaining insight into the impact of these SNPs on structural changes. Quantitative structure-activity relationships can aid in quantitative analyzing impact of non-synonymous polymorphisms on functions of target protein. These methods could supply powerful and practical tools for high-speed screening of synthetic and natural compounds; deduced data can be applied to molecular design of new drugs.

References


