行政院國家科學委員會專題研究計畫　成果報告

基因修補酵素 XRCC1 與 hOGG1 與食道癌關係之研究(2/2)

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計畫主持人：李章銘

計畫參與人員：郭小翠

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附件一

行政院國家科學委員會補助專題研究計畫

■成果報告

□期中進度報告

基因修補酵素 hOGG1 與食道癌關係之研究

計畫類別：■ 個別型計畫　　□ 整合型計畫

計畫編號：NSC 92-2314-B-002-225

執行期間：　九十一 年 八 月 一 日至 九十三 年 七 月 三十一 日

計畫主持人：李章銘醫師

共同主持人：

計畫參與人員： 郭小翠小姐

成果報告類型(依經費核定清單規定繳交)：□精簡報告　■完整報告

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢

□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：臺大醫院外科

中　華　民　國　九十三　年　十一　月　八　日

中文摘要：

關鍵詞：食道癌,基因多型性, LOH, hOGG1

食道癌的發生與環境和遺傳因子有密切的關係。在我們過去的研究中發現，吸煙、喝酒、嚼檳榔在台灣可增加食道癌的致癌危險性，這三種環境因子，彼此間有明顯的加成作用。另一方面，個體在先天的遺傳變異，也可左右食道癌發生的風險，各種解毒酵素，如 GSTP1、P53 腫瘤抑制基因，基因修輔酵素如 XRCC1、hOGG1 等遺傳因子之基因多型式，亦可決定個體對食道癌的易感受性。由於各種環境毒物，大部份都是透過對基因的損傷而促成癌化的作用，因此基因修補酵素的正常功能可能在癌化的預防的過程中，扮演著重要的角色，因此本研究將進一步探討 hOGG1 在食道癌癌化與腫瘤行為的角色。我們假設 hOGG1 的功能變異，包括遺傳子喪失、基因多型式、蛋白質表現可影響食道組織基因修補能力，而導致食道癌癌化、機轉之啟動。

在過去兩年中，我們完成了 hOGG1 基因多型性的基因檢定，我們發現 hOGG1 具有 cys 遺傳子者，分別在沒有嚼食檳榔、沒有飲酒及沒有抽煙習慣的個體中，可增加其罹患食道癌的危險性。其 OR（95% 信賴區間）分別為：1.86（1.08-5.31）及 3.09（1.22-7.81）。我們也在病患腫瘤檢體中，測定了 hOGG1 於 Exon 7 及 intron 4 等遺傳位置之遺傳子喪失的情形，我們發現食道癌在這些區域，有極高的比例有遺傳子喪失的情形，其比例分別為 55.66% （Exon 7）及 62.22% （intron 4）。在免疫螢光染色中，我們也發現了將近 82% 的腫瘤不表現 hOGG1 腫瘤蛋白。我們的研究顯示 hOGG1 基因功能改變或是喪失在食道癌發展的過程中扮演著重要角色。而 hOGG1 遺傳位置基因體的喪失，可影響食道癌病患治療的療效，在 hOGG1 Exon 有基因體喪失(LOH)病患在治療後，有較好的療效，另一方面在 Intron 4 產生 deletion，則病患治療的癒後則較不理想。在 exon 7 hOGG1 LOH 的作用，以抽煙、喝酒族群的病患，作用較明顯，而 Intron 4 作用則是於非抽煙、喝酒、嚼檳榔的病患較明顯。本研究顯示 hOGG1 的功能不只影響健康者，產生食道癌的危險性，且進一步能影響腫瘤的細胞行為，而決定病患的癒後。

爲了探討 hOGG1 的細胞生理學意義，以便解釋其對食道癌治療後之存活率的影響，我們使用了 CE81T/VGH 及 CE48T/VGH 兩株食道癌細胞，檢測抑制 hOGG1 的表達之後，對細胞存活與增生的影響。我們先確定 hOGG1 基因在此二細胞株的表達之後，再以 siRNA 抑制 hOGG1 於此二細胞株的表達，我們發現經 siRNA 抑制 hOGG1 表達之後，可增加 H_2O_2 在食道癌細胞株的細胞毒殺作用，另一方面則可以抑制 EGF 所引起的細胞增生反應。我們的體外實驗顯示，hOGG1 的功能與食道癌的細胞存活與抑制細胞增生有關，藉由這些機轉可能進一步影響患者治療的存活。

Abstract：

Keywords:Esophageal cancer, hOGG1,Polymorphism,LOH

The risk to develop esophageal cancer is associated with a variety of environmental and genetic factors. Previously, we have found that individual susceptibility to esophageal cancer in Taiwan was closely related to the consumption of tobacco, alcohol and areca. These factors exerted a synergistic effect on the risk for esophageal cancer. On the other hand, the genetic variation was also found important in determining the individual susceptibility to esophageal cancer. Genetic polymorphisms of the xenobiotic metabolizing enzymes, GSTP1, GSTT1, and CYP1A1, tumor suppressor gene, p53, or DNA repair enzymes, XRCC1 or hOGG1, can significantly influence the risk of esophageal cancer. Given that the DNA damage is the common form induced by the

environmental carcinogens and the main contributor to esophageal carcinogenesis, the individual variation in the repairing capacity for damaged DNA would play a important role in determine the tendency for individual carcinogenesis of the esophagus. Therefore, the aim of this study was to investigate whether the genetic alteration of the DNA repair genes, hOGG1, and XRCC1 can modify the individual risk to esophageal cancer. Previously, we have developed the technique of laser capture microdissection (LCM) to obtain pure tumor DNA for analysis. Using this technique, we have alleotyping the hOGG1 on intron 4 and exon 7, where the genetic polymorphisms locates. We also genotyped the patients of esophageal cancer for the hOGG1 Ser 326 Cys genetic polymorphism on intron 7. The expression of the hOGG1 in tumor was also examined by immunohistochemical staining. Totally, we evaluated the status of LOH in tumor from 90 patients. Sixty-seven of them further received immunohistochemical staining for the hOGG1 expression. 204 patients and 266 normal control received genotyping for the hOGG1 polymorphisms. We found that, the risk for the esophageal cancer was enhanced by the presence of the hOGG1 326 Cys allele in the males who did not regularly consume tobacco, alcohol, or areca nut, with ORs(95% CI) being 3.09 (1.22-7.81) for the non-smokers, 2.40 (1.08-5.31) for the non-alcohol drinkers, and 1.86 (0.99-3.49) for the non-areca chewers. Using the technique of Laser Capture Microdissection, we have alleotyping the hOGG1 on intron 4 and exon 7, where the genetic polymorphisms locates. We also found the allelic loss in genetic locus of hOGG1 is a very common episode with 62.2% of cases on the intron 4 and 56.7% of cases on the exon 7. The expression of the hOGG1 in the tumor tissue were also reduced, with 82% of the patients having tumor without expressing or expressing only a very low level of hOGG1 (less than 10% of the tumor cells).The pattern of protein expression and allelic deletion of hOGG1 was not significantly affected by the individual exposure to tobacco, alcohol, or areca nut. However, the deletion status intron 4 and exon 7 of hOGG1 can influence the survival of esophageal cancer after treatment. Those patients who had LOH in the exon 7 of hogg1 have better prognosis than the patients without LOH ($p=0.06$). This effect looked to be more evident in the cigarette smokers ($p<0.05$), and alcohol drinkers ($p<0.05$). The status of lymphnode metastasis was also associated with the deletion of hOGG1 in exon 7 (OR: **2.76**; 95% CI: **1.03-7.38**). On the contrast, those patient without LOH in the intron 4 of hogg1 have better prognosis after surgery for esophageal cancer ($p=0.08$). This effect was more prominent in the patients did not smoke cigarette ($p<0.05$) or drink alcohol ($p<0.05$). This implies that repair of the oxidative damaged DNA, 8-oxoG, is not only an important mechanism for prevention of neoplasm in the esophagus but also a significant factor affecting the tumor behaviour of esophageal cancer which might act with the factor of environmental exposure.

研究目的

To investigate the role of the genetic alteration and expression of XRCC1 and hOGG1 in the esophageal carcinogenesis, we focused the genetic alteration of hOGG1 and XRCC1 including allelic loss and genetic polymorphism in esophageal cancer. We also studied the expression of XRCC1 and hOGG1 in the tumor tissue of the esophageal cancer.

研究背景

Esophageal squamous cell carcinoma (EPC) is still an important disease threatening public health throughout the world. Geographic variation in the incidence of this cancer strongly suggests that environmental factors are involved in its etiology and carcinogenesis. The annual incidence of EPC in Taiwan is 6.93 per 100,000 of the population, very similar to that in most Western countries, and in sharp contrast to certain high-risk areas in Northern China, such as Linxian in Henan Province, where the annual incidence of EPC exceeds 100 per 100,000 of the population (Yang 1980; Blot and McLaughlin, 1999). To explore possible differences in risk factors, we and others have conducted epidemiological studies, the results of which indicated that the risk of EPC in our population is very similar to that reported in the Western countries, China and Indian and that the important risk factors are cigarette smoking, alcohol consumption, and areca chewing (Wu et al., 2001; Lee et al., 2000; Brown et al., 1994; Cheng et al., 1995; Franceschi et al., 1990; Gao et al., 1994; Garidou et al., 1996; Launoy et al., 1997; Sankaranarayanan et al., 1991; Tavani et al., 1993). Furthermore, the risk is positively correlated with the dosage of exposure and is enhanced synergistically by combinations of these factors (Wu et al., 2000; Lee et al., 2000).

Individuals exposed to the products of cigarette smoke have a higher susceptibility of DNA damage as evidenced by the increased frequency of PAH-DNA adduct formation and DNA strand breaks in the leukocytes of smokers (Van Schooten et al ,1997). Excessive alcohol consumption can also lead to DNA damage, mainly through the production of reactive oxygen species, lipid peroxidation products, or acetaldehyde (Brooks et al,1997). The frequency of single-strand breaks in DNA also increases after chronic alcohol exposure (Daiker et al,2000). Interestingly, this mutagenic effect is synergetic with that of tobacco smoking and can be reversed by abstinence (Castelli et al,1999), suggesting a causal link between alcohol consumption and DNA damage. Areca chewing is popular in Southeastern Asia, and is closely related to the incidence of oral cancer in Taiwan (Ko et al,1995). Taiwanese areca consumers usually sandwich the betel nut between Piper betel inflorescence (sometimes substituted by betel leaf) and lime paste, and do not use tobacco. Chewing areca nut also provide the individual a higher genotoxic propensity. The high safrole content (15 mg/gm) of the Piper betel of areca nut can lead to the formation of a safrole-DNA adduct (Wang et al,1993; Miller et al,1983). Hydrogen peroxide in the areca extract is also able to induce oxidative DNA damage, resulting in DNA base alteration and strand breaks (Liu et al,1996) Given the fact that DNA damage induced by these carcinogens is the most important etiology in EPC tumorigenesis, the capacity for DNA repair might play a vital role in preventing neoplastic transformation of the esophagus and variation in the DNA repairing capacity induced by the genetic polymorphism, deletion or mutation might contribute to the individual carcinogenic susceptibility.

The hOGG1 gene, localized on chromosome 3p25, encodes a glycosylase that repair the DNA carrying the 8-oxoG base pair, an important product in oxdative DNA damage and leading to the formation of GC to TA transversion during its replication (Radicella JP et al, 1997) Chinese hamster ovary cell lines (AA8 and AS52) , transfected to overexpress hOgg1 protein, would have up to three-fold of increase in repairing capacity for 8-oxoG, induced by either potassium bromate or photosensitizer plus light (Hollenbach S et al., 1999) The OGG1 deficient cell generated from the ogg1 -/- animals also demonstrated a complete abolishment of repairing the 8-OH-G residues in the

non-transcribed DNA. However, the transcription coupled repaire for 8-OH-G is largely spared, indicating that 8-OH-G in the actively transcribed gene is repaired through a OGG1 independent pathway (Dhenaut A et al., 2001). Due to a genetic polymorphism at codon 326, hOGG1-Ser326 and hOGG1-Cys326 proteins were produced in human cells. Activity in the repair of oh8Gua was greater in hOGG1-Ser326 protein than in hOGG1-Cys326 protein in the complementation assay of an E. coli mutant defective in the repair of oh8Gua. (Kohno T et al., 1998). Individual genetic variation in codon 326 (Ser/Cys) of hOGG1 was also found significant in affecting individual suspetibility to lung cancer or esophageal cancer in Asian (Xing DY et al., 2001; Sugimura H et al., 1999). Loss of function due to allelic imbalance were also found in cancers of the lung or head and neck (Fan CY et al., 2001; Wikman H et al., 2000). These data suggested that the hOGG1 might play an important role for the carcinogenesis in cancers of aerodigestive tract.

Loss-of-function mutation in tumor suppressor genes (TSGs) is important in the development of hereditary and sporadic cancers. In the model proposed by Knudson, loss of TGS function usually results from two independent mutagenic “hits” on the TGS alleles(Knudson et al,1985). The first mutation is commonly a subtle change to the TSG itself, whereas the second is more likely to involve major loss of the remaining normal TSG allele by non-disjunction (leading to the loss of a whole chromosome), mitotic recombination, or interstitial deletion. Under such circumstances, the loss of genetic markers near putative TGS detected by loss of heterozygosity (LOH), would provide important clues about the sites of TSGs and their involvement in tumorigenesis (Lasko et al,1991).

There is one methodological limitation , however, that always affects valid assessment of LOH results. Since detection of LOH is based on the comparison of tumor cells and corresponding normal tissues in order to identify genetic deletion that occurs specifically in tumor tissues, it is essential to be able to obtain pure tumor cells to provide the homogenous materials required for reliable analysis. However, the infiltrating nature of most invasive solid tumors, including esophageal cancer, leads to contamination of the surrounding normal tissue and creates technical problems in LOH analysis. To overcome this problem, the labor-intensive, inaccurate, and time-consuming process of tissue microdissection must be used, which originally involved manual or micromanipulator-guided scraping of the area of interest of a thin tissue section. The recently developed technique of laser capture microdissection (LCM) has greatly facilitated this procedure, and now provides and efficient and reliable one-step method for obtaining pure populations of cells from specific microscopic regions of tissue sections under direct visualization. LCM was used in this study to obtain pure tumor cells. From the perspective of etiology, more importantly, many esophageal cancers display a high degree of intratumor heterogeneity in terms of histologic appearance and phenotypic character, and do not exhibit easily observable morphological manifestations to define sequential evolution. By using LCM in the present study, the effects of heterogeneity within a tumor was minimized by ensuring that genetic and phenotypic examinations are carried out on the same tumor cells, which allowed a more precise evaluation of specific association between genetic alteration and pathological manifestation.

材料與方法

Study Population

This project was part of an ongoing cooperative study aimed at understanding the causes esophageal cancer in Taiwan. It included incident cases of esophageal squamous cell carcinoma diagnosed at National Taiwan University Hospital from 1996 to 2003. The patients was randomly selected from this esophageal cancer cohort. Institutional review board-approved informed consent was obtained from each patient prior to tissue acquisition. Their background of environmental exposure including smoking, drinking and other possible risk factors for esophageal cancer was collected by structured questionnaire. Normal control mastched with sex and age was selected from the health examination ward. Peripheral blood of the patients was drawn before surgery or chemoirradiation. All specimens collected from the patients was maintained at -80^{o}C until subsequent analysis. Blood also was drawn from a control group of normal healthy persons matched for age (with a difference of less than 5 years) and sex, who came to National Taiwan University Hospital for routine medical examination.

DNA Extraction from the Blood

Genomic DNA was prepared from the leukocytes with serial extraction by phenol and chloroform, and then ethanol precipitation. The content of DNA of each sample was analyzed by spectrophotometry, and about 1 μg of DNA was taken from each sample for PCR amplification.

Genotyping of XRCC1 and hOGG1

The genotype of *XRCC1 and hOGG1* was determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) for XRCC1 and PCR-SSCP for hOGG1 with slight modification (Lunn et al., 1999, Sugimura et al., 1999). The genomes of codons 194, 280 and 399 of XRCC1 was amplified in a PCR using the primers as follows: 26240F: GTT CCG TGT GAA GGA GGA GGA and 26377R: CGA GTC TAG GTC TCA ACC CTA CTC ACT for codon 194; and 27405F:TTG ACC CCC AGT GGT GCT AA and 28265R GGC TGG GAC CAC CTG TGT T for codons 280 and 399. The PCR reaction was started with a combination of the reagents: 100 ng of genomic DNA, 5 mM dNTPs, 5 pmol each primer, and 1unit (in 50 μl) of Taq DNA polymerase (Amersham Pharmacia Biotech), which was added into the PCR buffer containing 10mM Tris-HCl [pH 9.0], 1.5 mM $MgCl_2$, and 50 mM KCl. The reactions was carried out in the following thermocycle conditions: denaturation at 94^{o}C for 4 min., 35 cycles of 40 sec. at 94^{o}C, 30 sec. at 55^{o}C, and 40 sec. at 72 ^{o}C subsequently, followed by a 10-min. extension period at 72 ^{o}C. Following PCR, 10 μl aliquots was removed and subjected to restriction digestion separately with 2 units of PvuII (for codon 194), RsaI(for codon 280), and MspI (for codon 399) (the restriction enzymes from B.M.). The products was resolved on 2% agarose gels (FMC Bioproducts Rockland. ME). The PCR amplification produced a PvuII restriction site for the *Trp* allele of codon 194. After digestion, the *Arg* allele of codon 194 gave a segment of 138-bp, while the *Trp* allele give the products of 63-bp and 75-bp . For the PCR product of codon 280, a restriction site of RsaI was created in the *Arg* allele and gave rise to products of 63, 201and 597-bp, while the *His* allele give the products of 201and 660-bp . For the PCR product of codon 399, a restriction site of MspI was created in the *Arg* allele and give the products of 115, 285 and 461-bp, while the *Gln* allele give the products of

285 and 576-bp .
The primers of 5'-TGAATTCGGAAGGTGCTTGGGGAAT-3' and 5'-ACTGTCACTAGTC-TCACCAG-3' was used to identify the polymorphisms of the Ser326Cys polymorphism in exon 7. Primers without an *Eco*RI restriction site (forward, 5'-GGAAGGTGCTTGGGGAAT-3', backward, 5'-ACTGTCACTAGTCTCACCAG-3') also used for the same DNA. The PCR amplification was carried under conditions of: denatured with formamide at 95°C for 15 min, quenched on ice, and loaded to polyacrylamide gels under several conditions. The product was visualized with a silver stain kit (Wako, Osaka, Japan). The two allelic bands was differentiated by the unambiguous migration in electrophoresis performed at room temperature and 4°C with a glycerol concentration of 12%. In addition, each allelic PCR product was cloned and sequenced to confirm the genotypes inferred from SSCP analysis. Sequencing involved a dye-primer cycle sequence kit (Amersham, Cleveland, OH) and an ABI 301 automated sequencer (Applied Biosystems, Foster City, CA).

Laser Capture Microdissection. (LCM) and alleotyping of hOGG1

Laser capture microdissection, i.e. LCM was performed to ensure the purity of tumor sample. Usually, more than 90% of purity of the tumor cells can be attained with this technique. LCM was performed on the immunostained slides using a PixCell laser capture microscope according to the technical quidance of the manufacturer (Arcturus Engineering, Mountain View, CA). Briefly, the stained, dehydrated tissue section was overlaid with a thermoplastic film mounted on an optically transparent cap. The visually selected areas (tumor cells) were bound to the membrane by short, low-energy laser pulses resulting in focal melting of the polymer, The cells was immersed in 50-100 μl Tris buffer, pH 8.0, containing 0.5 mmol/L EDTA and 400 μl /ml proteinase K , and digested for 24 hours at 55^{o}C. After digestion, the enzyme was heat-inactivated and the extract was used directly for alleotyping PCR (LOH detection). Multiple efforts was made to optimize LCM conditions, including to improve capture efficiency by overnight pretreatment of slide with xylene, and to ensure successful PCR amplification by avoiding fragmentation of DNA in captured tissue using a modified protocol of tissue fixation. DNA of microdissected tumor specimens and blood samples was used for alleotyping PCR with fluorescently labeled primers covering the polymorphic areas of hOGG1 from Applied Biosystems PRISM Linage Mapping Set-MD10 (PE Biosystems, Foster City, CA). Too reduced the effort in carrying out this arduous task of genome wide study, these markers was arranged in panels of compatible markers, which facilitates concurrent. The primers used for amplifying the hOGG1 gene were: 5'-GGCCACATGCTGCCCTTC-3', sense and 5'-CAGATGCAGTCAGCCACC-3', antisense for intron 4 (Ser 326 Cys), and 5'-CAGACTCCACCCTCCTAC-3', sense and 5'-CGCTTTGCTGGTGGCTCC-3', antisense for the exon 7. [Fan et al, 2001] PCRs were run in a GeneAmp PCR 9600 thermocycler (PE Biosystems, Foster City, CA). Reaction conditions for some markers yielding unsatisfactory amplification was modified, and, if necessary, these particular markers was run singly. PCR products was electrophoresed on a 377 ABI PRISM sequencer, and the fluorescent signal of different allelic size was recorded and analyzed by the GENOTYPER version 2.1 and GENESCAN version 3.1softwares.

Definition of Allelic Loss (LOH).

For a given informative marker, it was considered to display LOH when a 2-fold or greater difference in the relative allele intensity ratio between tumor DNA and normal DNA was observed.

Immunohistochemistry

Immunohistochemical staining was performed on the paraffin-embedded section using the specific anti-hOGG1 (Novus Biologicals, Littleton, Colorado; Cat# NB 100–106). The binding antibodies was visualized with the system of streptavidin-biotin peroxidase and an aminoethylcarbazole chromagen. Negative control with tissue section incubated without the primary abtibodies was also run for each sample.

Statistical analysis

Logistic regression was used to evaluate the effect of environmental exposure on the frequency of genetic mutation. The data were analyzed using the SAS statistical package. Chi square was used to evaluate the statistical significance. The generalized additive model used to adjust for other confounding factors without imposing a rigid parametric assumption about the dependence of confounders on esophageal cancer risk. Unconditional logistic regression was used to assess the association between case/control status and substance use after adjusting for other significant predictors found in a univariate analysis. Odds ratios (OR) and 95% confidence intervals (CI) was used for the estimated risk of these potential factors for developing esophageal cancer. The data was analyzed using the SAS and S-plus statistical packages.

結果

In the last year, we had evaluated the genetic polymorphism, LOH and immunohistochemical staining of hOGG1 in our series. The results were described as followed.

hOGG1 Ser326Cys Polymorphisms with Esophageal Cancer Risk

We have recruited 204 cases of esophageal squamous cell carcinoma and 266 persons of healthy controls from National Taiwan University Hospital (NTUH) as well as from Kaohsiung Medical University Hospital (KMUH) and Kaohsiung Veterians General Hospital (KVGH) in this study. There was no significant difference in distribution of these genotypes between the patients and controls. However, among the males, *the* genotypes carrying the Cys allele was found to associate a higher risk of esophageal cancer (OR: 1.87; 95% CI: 1.06-3.30). The difference was mainly attributed to the effects on individuals who did not smoke cigarette (OR = 3.09; 95% CI= 1.22-7.81), drink alcohol (OR = 2.40; 95% CI= 1.08-5.31), or chew areca (OR = 1.86; 95% CI= 0.99-3.49)(Table 3). Our results revealed that the *hOGG1* genetic polymorphism might modulate the risk of esophageal cancer of male in Taiwan who have a lower exposure to environmental toxin.

Allelic loss and immunohischemical staining of hOGG1

Using the primers located on the intron4 and exon 7, we examined the tumor and normal buffy

coat DNA from 90 patients. There were 89 and 78 informative cases in the intron 4 and exon 7 repectively. Of the informative cases, LOH was found in 56 (62.2%) cases for the intron 4 and 51 (56.7%) cases for the exon 7. As for the immunostaining, we had 67 patients with adequate specimen for examination of the hOGG1 protein expression (Table 2). We found the expression of hOGG1 was found in the basal layer of the epitheium of the esophagus, while 55 (82%) of the patients did not express or had less than 10% expressing the hOGG1 protein in the tumor. Four patients (6%) expressed hOGG1in 10% to 50% of the tumor cells, and 8 patients (11.9%) expressed hOGG1 in more than 50% in the tumor tissue (Table 2). **The pattern of protein expression and allelic deletion of hOGG1 was not significantly affected by the individual exposure to tobacco, alcohol, or areca nut. However, the deletion status intron 4 and exon 7 of hOGG1 can influence the survival of esophageal cancer after treatment. Those patients who had LOH in the exon 7 of hogg1 have better prognosis than the patients without LOH ($p<0.05$) (fig 1). This effect looked to be more evident in the cigarette smokers ($p<0.05$) (fig 2), and alcohol drinkers ($p<0.05$) (fig 3). The status of lymphnode metastasis was also associated with the deletion of hOGG1 in exon 7 (OR: 2.76; 95% CI: 1.03-7.38). On the contrast, those patient without LOH in the intron 4 of hogg1 have better prognosis after surgery for esophageal cancer ($p<0.05$) (fig 4). This effect was more prominent in the patients did not smoke cigarette ($p<0.05$) (fig 5) or drink alcohol ($p<0.05$) (fig 5). This implies that repair of the oxidative damaged DNA, 8-oxoG, is not only an important mechanism for prevention of neoplasm in the esophagus but also a significant factor affecting the tumor behaviour of esophageal cancer which might act with the factor of environmental exposure.**

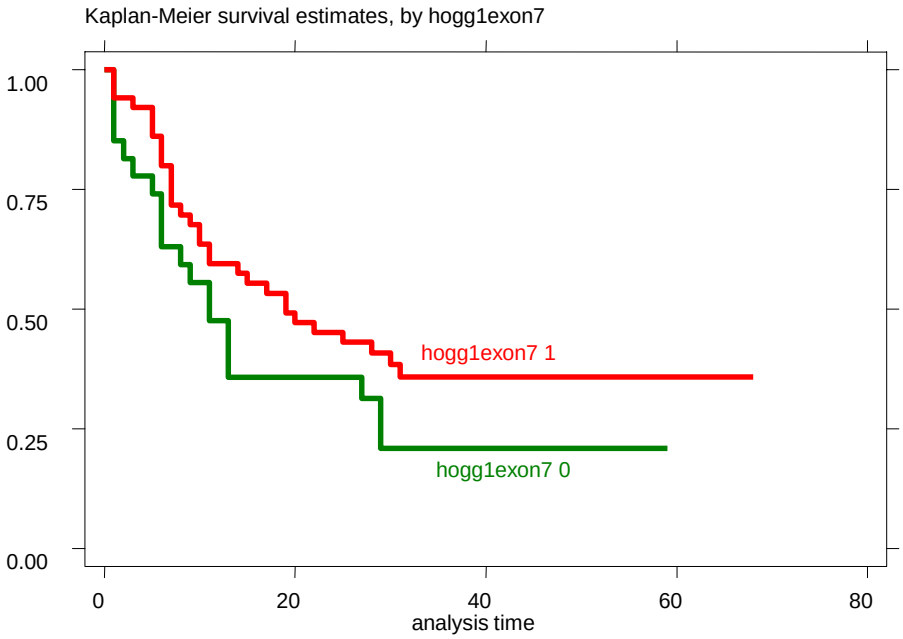
Table 1. Joint effects of *hOGG1* with cigarette smoking, alcohol drinking, and areca chewing in esophageal cancer in ***males.***

Variables*	hOGG1	Controls (%)	Patients (%)	ORs (95% CI)	AORs* (95% CI)
All	Ser/Ser	51 (19.1)	19 (11.2)	Referent	
	Ser/Cys+ Cys/Cys	215 (80.8)	150 (88.8)	1.87 (1.06-3.30)*	
Non-smoker	Ser/Ser	33 (22.3)	7 (9.1)	Referent	Referent
	Ser/Cys + Cys/Cys	115 (77.7)	70 (90.9)	2.87 (1.21-6.84)**	3.09 (1.22-7.81)**
Smoker	Ser/Ser	18 (15.3)	12 (13.0)	Referent	Referent
	Ser/Cys + Cys/Cys	100(84.7)	80 (87.0)	1. 2 (0.54-2.64)	0.97 (0.41-2.27)
Non-Drinker	Ser/Ser	32 (18.7)	9 (8.3)	Referent	Referent
	Ser/Cys + Cys/Cys	139(81.3)	100(91.7)	2.56 (1.17-5.59)**	2.40 (1.08-5.31)**
Drinker	Ser/Ser	19 (20)	10 (16.7)	Referent	Referent
	Ser/Cys + Cys/Cys	76 (70)	50 (83.3)	1.25 (0.54-2.91)	1.21 (0.47-3.13)
Non-areca chewer	Ser/Ser	49 (20.1)	15 (11.5)	Referent	Referent
	Ser/Cys + Cys/Cys	194(79.8)	115(88.5)	1.94 (1.04-3.61)	1.86 (0.99-3.49)#
Areca chewer	Ser/Ser	2 (8.7)	4 (9.3)	Referent	Referent
	Ser/Cys + Cys/Cys	21 (91.3)	39 (90.7)	0.83 (0.14-4.95)	1.34 (0.17-10.25)

Table 2.LOH and immunohistochemical staining of hOGG1

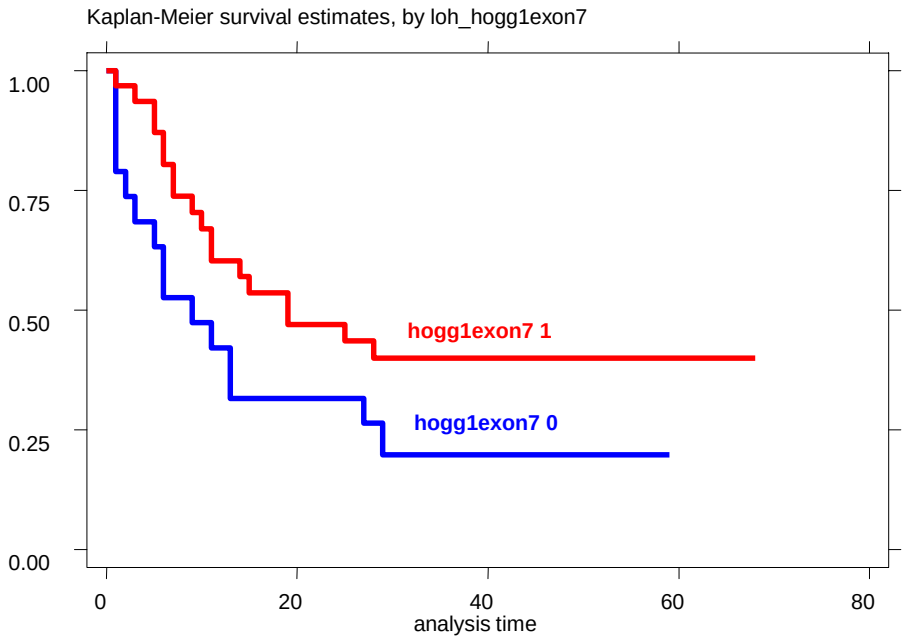
LOH variables	LOH on exon 7	LOH on intron 4	IHC No (%)	HOGG1 IHC expression
Normal	27	33	55 (82.1%)	<10%
LOH	51	56	4 (6.0%)	10%-50%
Non-informative	12	1	8 (11.9%)	> 50%
Total	90	90	67 (100%)	Total
LOH %	56.7%	62.2%		

Fig 1: Survival and hogg1 exon 7 deletion for the overall patients of esophageal cancer



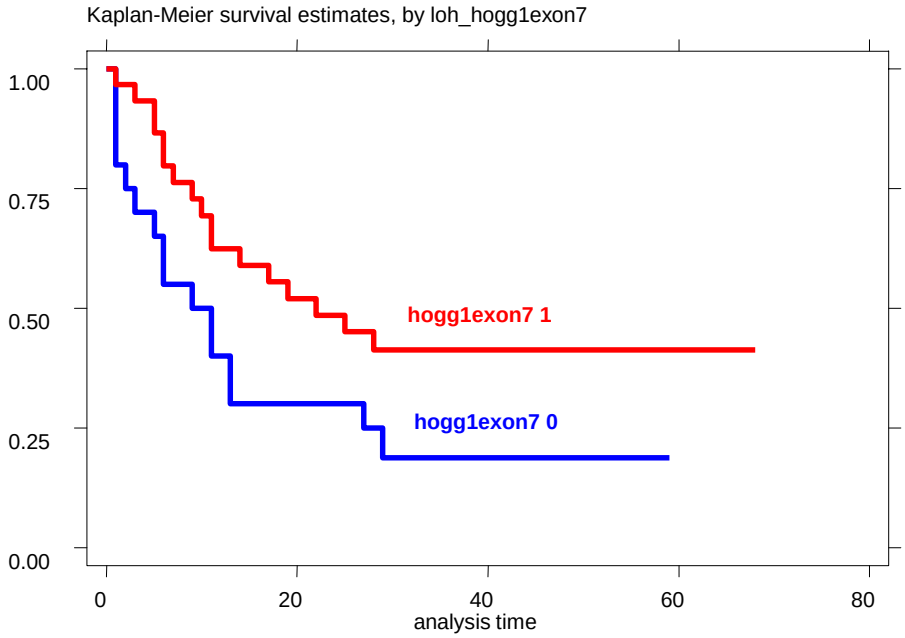
(p=0.06) (1= LOH; 0=normal)

Fig 2: Survival and hogg1 exon 7 deletion for the patients of esophageal cancer smoking cigarette



(p=0.04); (1=LOH, 0=normal)

Fig 3: Survival and hogg1 exon 7 deletion for the patients of esophageal cancer drinking alcohol

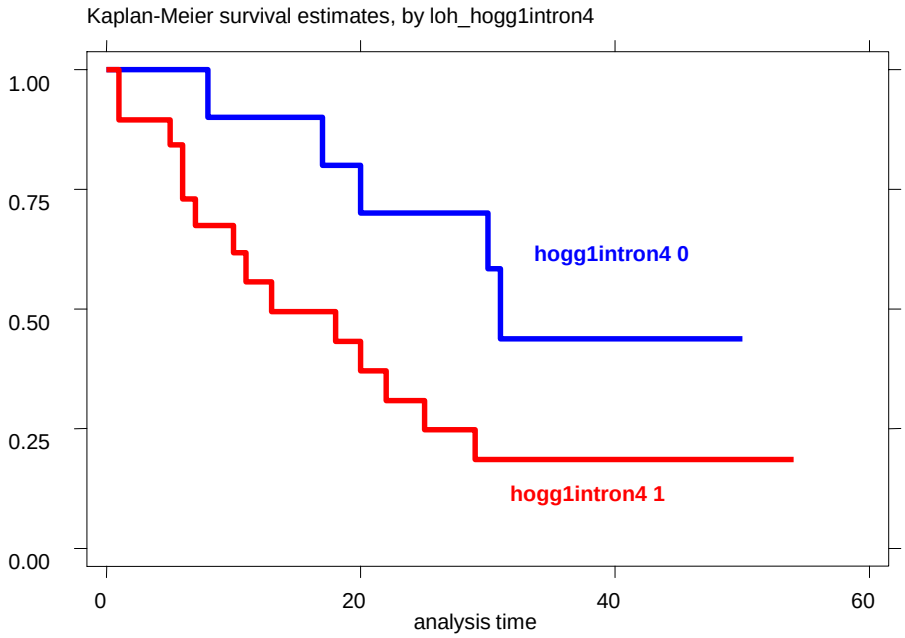


(p=0.03); (1: LOH; 0: normal)

Fig 4: Survival and hogg1 intron 4 deletion for the overall patients of esophageal cancer

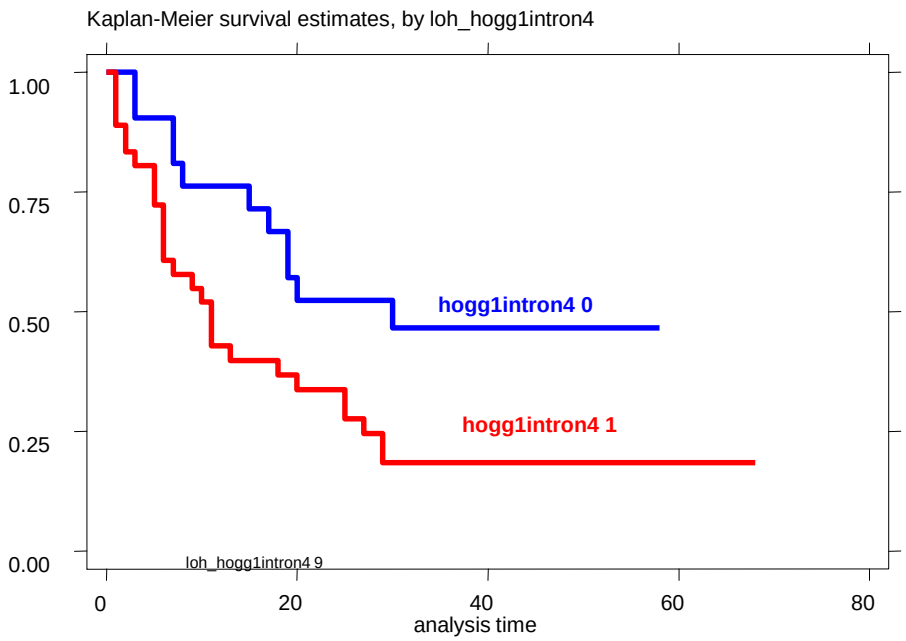
(p=0.08) (1: LOH; 0: normal)

Fig 5: Survival and hogg1 intron 4 deletion for the patients of esophageal cancer without smoking cigarette



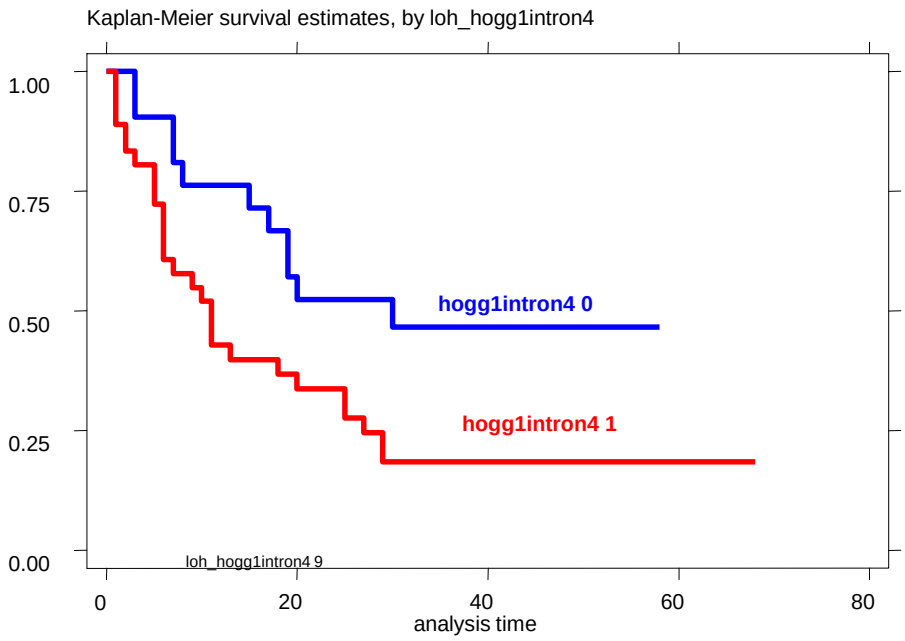
(p=0.03) (1: LOH; 0: normal)

Fig 6: Survival and hogg1 intron 4 deletion for the patients of esophageal cancer without drinking alcohol.



(p=0.02) (1: LOH; 0: normal)

Fig 7: Survival and hogg1 intron 4 deletion for the patients of esophageal cancer without chewing areca



(p=0.02) (1: LOH; 0: normal)

Discussion

Our data demonstrated that the presence of hOGG1 Cys allele will increased the risk of esophageal cancer in males, especially in those who did not regularly consumed tobacco, alcohol, or areca nut. The tendency for the impact of the hOGG1 326 Cys allele is compatible with that found by Xing et al in the high-risk areas of esophageal cancer in China (Xing DY et al., 2001). However, they did not find interaction of cigarette smoke with the hOGG1 genotype on the esophageal cancer risk. Noteworthy is that a more obvious effect of the hOGG1 326 Cys allele was found in the males with lower environmental toxin exposure, indicating that the genetic effect was evident only in the individual under low burden of damaged DNA. High dose of environmental toxin exposure seemed to overwhelm the difference in DNA repair arising from the polymorphic gene. It is also obvious that the loss of function of hOGG1 is a common event in esophageal cancer, as evidenced by the allelic loss or downregulation of the protein expression. The survival of patients of esophageal cancer was also influenced by allelic deletion of hOGG1. This means that repair of the oxidative damaged DNA, 8-oxoG, is an important mechanism for prevention of neoplasm in the esophagus. Through the genetic variation produced by the polymorphism, the function of the hOGG1 might have a further detriment once allele was lost under the continuous challenge of the DNA damage. This is compatible with the ‘two hit” hypothesis proposed by Knudson (Knudson et al,1985). The function of hOGG1 was also important for the biological behavior of esophageal cancer. This observation remained to be elucidated by further studies in mechanism.

Reference:

Brooks, P.J. DNA damage, DNA repair, and alcohol toxicity--a review. Alcohol Clin Exp Res 1997; 21:1073-82.

Blot WJ, McLaughlin JK.1999. The changing epidemiology of esophageal cancer. Semin Oncol 26(Suppl 15):2-8

Brown LM, Hoover RN, Greemberg, RS, Schoenberg JB, Schwartz AG, Swanson GM, Liff JM, Silverman DT, Hayes RB, Pottern LM. 1994. Are racial differences in squamous cell esophageal cancer explained by alcohol and tobacco use? J Natl Cancer Inst 86:1340-1345.

Caldecott, K.W., Aoufouchi, S., Johnson, P. and Shall, S., XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick- sensor' in vitro. *Nucleic Acids Res.*, 24, 4387-4394 (1996).

Caldecott, K.W., Tucker, J.D. and Thompson, L.H., Construction of human XRCC1 minigenes that fully correct the CHO DNA repair mutant EM9. *Nucleic Acids Res.*, 20, 4575-4579 (1992).

Castelli, E., Hrelia, P., Maffei, F., Fimoganari, C., Foschi, F.G., Caputo, F., Cantelli-forti, G., Stefanini, G.F., and Gasbarrini, G.. Indicators of genetic damage in alcoholics: reversibility after alcohol abstinence. Hepatogastroenterol 1999; 46:1664-8.

Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K. and Frosina, G., Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J. *Biol. Chem.*, 272, 23970-23975 (1997)

Cheng KK, Duffy SW, Day NE, Lam TH. 1995. Oesophageal cancer in never-smokers and never-drinkers. Int J Cancer 60:820-822.

C, Li Z, Wang J, Takezaki T, Tajima K, Varga T, Sawaguchi T, Lum JK, Martinson JJ, Tsugane S, Iwamasa T, Shinmura K, Yokota J. hOGG1 Ser326Cys polymorphism and lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1999 Aug;8(8):669-74

Daiker, D.H., Shipp, B.K., Schoenfeld, H.A., Klimpel, G.R., Witz, G., Moslen, M.T., and Ward, J.B., Jr. Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure. J Toxicol Environ Health 2000; 59:181-96.

Dhenaut A, Hollenbach S, Eckert I, Epe B, Boiteux S, Radicella JP. Characterization of hOGG1 promoter structure, expression during cell cycle and overexpression in mammalian cells. Adv Exp Med Biol 2001;500:613-6

Fan CY, Liu KL, Huang HY, Barnes EL, Swalsky PA, Bakker A, Woods J, Finkelstein SD. Frequent allelic imbalance and loss of protein expression of the DNA repair gene hOGG1 in head and neck squamous cell carcinoma. Lab Invest 2001 Oct;81(10):1429-38

Franceschi S, Talamini R, Barra S, Baron AE, Negri E, Bidoli E, Serraino DLA, Vecchia C. 1990. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. Cancer Res 50:6502-6507.

Gao YT, Mclaughlin JK, Blot WJ, Ji BT, Benichou J, Dai Q, Fraumeni JF, JR. 1994. Risk factors for esophageal cancer in Shanghai, China. I. Role of cigarette smoking and alcohol drinking. Int J Cancer 58:192-196.

Garidou A, Tzonou A, Lipworth L, Signorello LB, Kalapothaki V, Trichopoulos D. 1996. Life-style factors and medical conditions in relation to esophageal cancer by histologic type in a low-risk population. Int J Cancer 68:295-299.

Hollenbach S, Dhenaut A, Eckert I, Radicella JP, Epe B., Overexpression of Ogg1 in mammalian cells: effects on induced and spontaneous oxidative DNA damage and mutagenesis, Carcinogenesis 1999 20:1863-8.

Knudson, A.G., Jr. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res 1985; 45:1437-43.

Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H, Yokota J. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 1998 Jun 25;16(25):3219-25

Kubota, Y., Nash, R.A., Klungland, A., Schar, P., Barnes, D.E. and Lindahl, T., Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. *EMBO J.*, 15, 6662-6670 (1996).

Ko, Y.C., Huang, Y.L., Lee, C.H., Chen, M.J., Lin, L.M., and Tsai, C.C. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J Oral Pathol Med 1995; 24:450-53.

Lasko, D., and Cavenee, W.K., Nordenskjöld, M. Loss of constitutional heterozygosity in human cancer. Ann. Rev. Genet. 1991; 25:281-314.

Liu, T.Y., Chen, C.L., and Chi, C.W. Oxidative damage to DNA induced by areca nut extract. Mut Res 1996; 367:25-31.

Launoy G, Milan CH, Faivre J, Pienkowski P, Milan CI, Gignoux M. 1997. Alcohol, tobacco and esophageal cancer: effects of the duration of consumption, mean intake and current and former consumption. Br J Cancer 75:1389-1396.

Lunn, R.M., Langlois, R.G., Hsieh, L.L., Thompson, C.L. and Bell, D.A., XRCC1 poymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. *Cancer Res*., **59**, 2557-2561 (1999).

Miller, J.A., and Miller, E.C. The metabolic activation and nucleic acid adducts of naturally-occurring carcinogens: recent results with ethyl carbamate and the spice flavors safrole and estragole. Br J Cancer 1983; 48:1-15.

Nash, R.A., Caldecott, K.W., Barnes, D.E. and Lindahl, T., XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry*, 36, 5207-5211 (1997).

Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S. Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 1997; 94(15):8010-5

Ratnasinghe, D., Yao, S.X., Tangrea, J..A., Qiao, Y.L., Andersen, M.R., Barrett, M.J., Giffen, C.A., Erozan, Y., Tockman, M.S., Taylor, P.R. Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. *Cancer Epi. Biomark. Prev.* 10:119-23 (2001)

Sugimura H, Kohno T, Wakai K, Nagura K, Genka K, Igarashi H, Morris BJ, Baba S, Ohno Y, Gao Sankaranarayanan R, Duffy SW, Padmakumary G, Nair SM, Day NE, Padmanabhan TK. 1991. Risk factors for cancer of the oesophagus in Kerala, India. Int J Cancer 49:485-489.

Shen, M.R., Jones, I.M. and Mohrenweiser, H., Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res*., **58**, 604-608 (1998).

Sturgis, E.M., Castillo, E.J., LI, L., Zhen, R., Eicher, S.A., Clayman, G.L. Strom, S.S., Spitz, M.R. and WeI, Q., Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. *Carcinogenesis*, **20**, 2125-2129 (1999).

Tavani A, Negri E, Franceschi S, La Vecchia C. 1993. Risk factors for esophageal cancer in women in northern Italy. Cancer 72:2531-2536

Thompson, L.H., Bachinski, L.L., Stallings, R.L., Dolf, G., Weber, C.A., Westerveld, A. and SICILIANO, M.J., Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: a third repair gene on human chromosome 19. *Genomics*, 5, 670-679 (1989).

Thompson, L.H., Brookman, K.W., Jones, N.J., Allen, S.A. and Carrano, A.V., Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid

exchange. *Mol. Cell. Biol*., 10, 6160-6171 (1990).

Van Schooten, F.J., Godschalk, R.W., Breedijk, A., Maas, L.M., Kriek, E., Sakai, H, Wigbout, G., Baas, P., Van't Veer, L. and VAN Zandwijk, N.. 32P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures. Mut Res 1997; 378:65-75.

Wang, C.K., and Hwang, L.S. Phenol compounds of betel quid chewing juice. Food Sci 1993; 20:458-71.

Wikman H, Risch A, Klimek F, Schmezer P, Spiegelhalder B, Dienemann H, Kayser K, Schulz V, Drings P, Bartsch H. hOGG1 polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a caucasian population. hOGG1 polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a caucasian population. Int J Cancer 2000 Dec 15;88(6):932-7

Xing DY, Tan W, Song N, Lin DX. Ser326Cys polymorphism in hOGG1 gene and risk of esophageal cancer in a Chinese population. Int J Cancer 2001 May 20;95(3):140-3

Yang CS. 1980. Research on esophageal cancer in China: a review. Cancer Res 40: 2633-2644.
Yu MC, Garabrant DH, Peters JM, Mack TM. 1988. Tobacco, alcohol, diet, occupation, and carcinoma of the esophagus. Cancer Research 48:3843-3848.

Zdzienicka, M.Z., Van Der Schans, G.P., Natarajan, A.T., Thompson, L.H., Neuteboom, I. And Simons, J.W., A Chinese hamster ovary cell mutant (EM-C11) with sensitivity to simple alkylating agents and a very high level of sister chromatid exchanges. *Mutagenesis*, 7, 265-269 (1992).