

RESEARCH COMMUNICATION

OGG1 Gene Sequence Variation in Head and Neck Cancer Patients in Pakistan

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Abstract

In Pakistani culture tobacco use is very high and a well known risk factor for developing head and neck cancer (HNC), tobacco smoke containing high quantities of chemical carcinogens such as aromatic amines and reactive oxygen species. OGG1 is the primary enzyme in the base excision repair (BER) pathway, responsible for the excision of 7, 8-dihydro-8-oxoguanine, a mutagenic base byproduct that occurs as a result of exposure to reactive oxygen species. Groups of 300 already diagnosed HNC patients along with normal controls were included in this study. PCR-single-strand conformation polymorphism and DNA sequencing were used to analyze the whole coding region of OGG1 gene. Sequence analysis revealed eight novel mutations (six missense and two frame shift mutations). Frequencies of missense mutations, Asp267Asn, Ser279Gly and Ile253Phe were 0.12, 0.13 and 0.06 respectively. Frequencies of other missense mutations, 1578A>T, 1582C>T and Ala399Glu (1542C>A) were 0.13, 0.13 and 0.16, whereas values for the frame shift mutations 1582insG and 1543_1544delCT were 0.13 and 0.16. In our study, incidence of these mutations was found higher in oral cancers ($p<0.002$) and in smokers ($p<0.002$) when compared with other sites of HNC and nonsmokers, respectively. Our finding suggests that these germline mutations in OGG1 gene contribute to risk of developing HNC.

Key words: Head and neck cancer - OGG1 - polymorphism - mutation analysis - germline mutation

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Introduction

Head and neck cancers (HNC) are among the most common cancers worldwide (Blons and Laurent-Puig, 2003; Perez-Ordóñez et al., 2006). They also have one of the lowest 5-year survival rates of all cancers (Bosch et al., 2004). In Pakistan the situation is alarmingly high as head and neck cancer (HNC) has been ranked second in both genders, highest among all other types of cancer (Bhurgi et al., 2006). Etiology of head and neck cancers is relatively well defined in terms of risk factor association like tobacco and alcohol consumption (Crowe 2002; Vassallo et al., 2008) High incidence of HNC in Pakistan is also due to the widespread habits of chewing tobacco and smoking (Bhurgi et al., 2006). Tobacco smoke contains high quantities of chemical carcinogens such as polycyclic aromatic hydrocarbons, aromatic amines, N-nitroso compounds and reactive oxygen species (ROS) (Crowe 2002; Vassallo et al., 2008). These chemical carcinogens can form bulky adducts after activation by specific enzymes, and can induce a variety of oxidative damage (Valko et al., 2006)

The reaction of ROS results in numerous forms of both cellular and DNA damage (Poulsen et al., 1998).

Base excision repair (BER) is the primary mechanism for repairing small base lesions in DNA resulting from oxidation and alkylation damage (Mittra et al., 2002) OGG1 is a key BER enzyme which recognize and excise 8-hydroxy-deoxyguanine (8-oxo-dG) thus causes G>A transversion (Martinez et al., 2003). The OGG1 gene is located on human chromosome 3p26.2, a region that frequently shows loss of heterozygosity (LOH) in several human cancers (Kohno et al., 1998). The OGG1 gene belongs to a family of DNA glycosylases the signature of which is an active site HhH-GPD motif composed of a helix-hairpin-helix (HhH) element followed by a Gly/Pro-rich loop and terminating in an invariant aspartic acid residue (Scharer et al., 2001) OGG1 is a bifunctional glycosylase because it is able to cleave the glycosidic bond of the mutagenic lesion and can cause a strand break in the DNA backbone (Radicella et al., 1997).

OGG1 gene is highly polymorphic and mutations of OGG1 have been implicated in the development of certain human diseases including cancer (Parker et

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al., 2003). Gene knockout mice defective in OGG1 accumulates higher levels of 8-oxoG lesions compared with wild-type controls (Minowa et al., 2000) and exhibits elevated spontaneous mutations, especially when exposed to higher levels of oxidative stress (Arai et al., 2003). These observations suggest that OGG1 acts as a major pathway responsible for 8-oxoG removal (Friedberg et al., 2006). Mutations and polymorphisms of OGG1 may affect its enzymatic activity which can be associated with increased risk of several cancers. With respect to involvement of OGG1 gene in human cancer, significant efforts have been made to link the inactivation of OGG1 gene with cancer risk [Park et al., 2001; Sugimura et al., 1999; Kohno et al., 1998; Hardie et al., 2000; Xu et al., 2002]. However, the data, such as somatic mutation, loss of heterozygosity, and germ line polymorphism, are contradictory. The aim of this study was to evaluate the role of polymorphisms or mutations of OGG1 in the etiology of HNC in Pakistani population.

Materials and Methods

Identification of patients and blood sample collection

The study group consisted of 300 patients with histologically confirmed head and neck cancer and 300 cancer-free controls. All subjects were age, gender and ethnicity matched. The HNC patients were recruited from October 2008 to February 2010 from National Oncology and Radiotherapy Institute (NORI) and Pakistan Institute of Medical Sciences (PIMS). The inclusion criterion for the controls was absence of any history of cancer or pre-cancerous lesions. Patients and controls suffering from any other familial disease (diabetes, blood pressure and cardiovascular impairment) were also excluded from this study. During recruitment, informed consent was obtained from each subject after whom participants were interviewed to seek detailed information on demographic characteristics and lifetime history of tobacco use. The present study was conducted with prior approval from ethical committees of both CIIT and hospitals.

Primer designing

Human OGG1 gene sequence was taken from Ensembl. Primers were designed by using primer 3 input software and obtained by BLAST. Exonic regions and their exon intron boundaries of approximately 60 bp sequence of OGG1 were also investigated to identify any splice site variation.

DNA isolation and amplification

Genomic DNA was isolated from the blood using standard phenol-chloroform extraction method (Baumgartner-Parzer et al., 2001, Vierhapper et al., 2004) and stored at -200C for further processing. PCR was performed in a 20 µl reaction mixture containing approximately 20 ng DNA, 10 mM each primer, 25 mM each dNTP, 0.2µl Taq DNA polymerase with 2 µl 10 × reaction buffer. PCR profile consisted of an initial

melting step of 94°C for 5 min, 35 cycles of 94 °C for 45sec, annealing temperature for 1min and 72 °C for 1min and a final extension step of 72°C for 10 min and hold at 4°C. PCR products were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

Mutation detection and data analysis

Single stranded conformational polymorphism (SSCP) was used for the mutational analysis of the PCR products. Samples displaying an altered electrophoretic

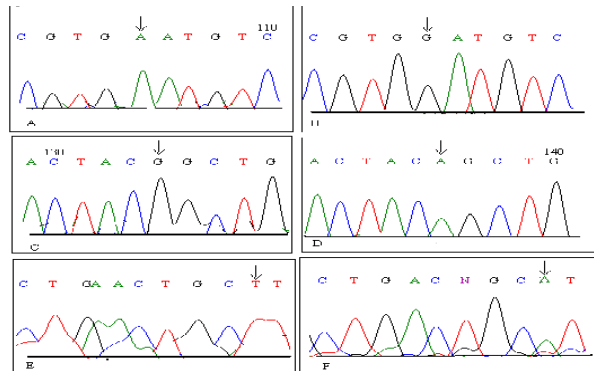


Figure 1. Nucleotide Sequence of Fragments of OGG1 Exon 5. (A) mutant codon (B) wild type codon of first missense mutation, Asp267Asn showing G to A substitution resulting in change of DNA sequences from GAT to AAT encoding the amino acid asparagine instead of aspartic acid. (C) mutant codon, (D) wild type codon of second missense mutation Ser279Gly, showing A to G substitution resulting in change of DNA sequence from AGC to GGC encoding the amino acid glycine instead of serine. (E) mutant codon, (F) wild type codon of third missense mutation, Ile253Phe showing A to T substitution resulting in change of DNA sequence from ATC to TTC encoding the amino acid isoleucine instead of phenylalanine.

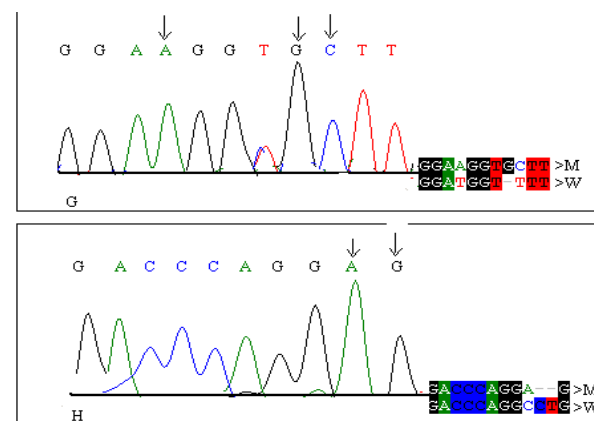


Figure 2. (G) Nucleotide sequence of a fragment of OGG1 3'UTR. First missense mutation, 1578A>T in 3'UTR region showing T to A substitution, second frame shift mutation, 1582insG in 3'UTR region showing insertion of G and third missense mutation, 1582C>T in 3'UTR region showing T to C substitution. (H) Nucleotide sequence of a fragment of OGG1 exon 8. First missense mutation, Ala399Glu showing C to A substitution resulting in change of DNA sequence from GCC to GAG encoding the amino acid glutamic acid instead of alanine. Second frame shift mutation, 1543_1544delCT showing deletion of two nucleotides C and T. (M) mutant sequence and (W) wild type sequence.

Table 1. Frequency of Missense and Frame Shift Mutations in 300 HNC Patients of Pakistani Origin

| Mutations | Type of mutation | No of patients | Allele Frequency | OR(95%CI) |
|-------------------------------|--------------------------|----------------|--|-----------------|
| Asp267Asn | Missense | 49 | A0.12, G0.88 | 8.79(4.91-15.8) |
| Ser279Gly | Missense | 53 | G0.13, A0.87 | 8.94(5.09-15.7) |
| Ile253Phe | Missense | 24 | T0.06, A0.94 | 8.00(3.54-18.1) |
| 1578A>T, 1582C>T, 1582insG | Missense, Frame shift | 50 | A0.13, T0.87 C0.13, T0.87 insG0.13 | 8.83(4.95-15.8) |
| Ala399Glu, 1543_1544delCT | Missense, Frame shift | 62 | A0.16, C0.84 delCT0.16 | 9.27(5.48-15.7) |
| Total | | 238 | | 27.4(19.7-38.0) |

Table 2. Frequency of Missense and Frame Shift Mutations in Smokers, Non-smokers and Different Sites of HNC

| Mutations | Smokers | | | Site of cancer | | |
|----------------------------|------------|------------|--------|----------------|--------|---------|
| | Betel quid | Cigarettes | Naswar | Oral Cavity | Larynx | Pharynx |
| Asp267Asn | 3 | 18 | 18 | 39 | 7 | 3 |
| Ser279Gly | 5 | 11 | 19 | 28 | 13 | 12 |
| Ile253Phe | 2 | 8 | 9 | 20 | 3 | 1 |
| 1578A>T, 1582C>T, 1582insG | 5 | 9 | 24 | 33 | 11 | 6 |
| Ala399Glu, 1543_1544delCT | 7 | 22 | 22 | 48 | 11 | 3 |
| Total | 22 | 68 | 92 | 168 | 45 | 25 |
| p value for t -Test | <0.007 | <0.008 | <0.002 | <0.002 | <0.007 | <0.06 |

mobility were reamplified in another reaction and were analyzed by direct sequencing to confirm and characterize the nature of mutations. Sequencing was carried out by MCLAB (U.S.A). Control (normal) samples were also sequenced along with cases to check the quality of sequencing. For data analysis, student t-test was used to compare the frequency of missense and frame shift mutations in patients with reference to smoking history and area of cancer.

Results

Using a combination of SSCP and direct genomic sequencing, we examined all eight exons of the OGG1 gene in 300 HNC patients. SSCP showing an electrophoretic mobility shift were identified as positive for OGG1 gene mutations. They were subsequently selected for DNA sequencing to confirm the SSCP results and to characterize the types and locations of mutations. Mutations of the OGG1 gene were identified in 238 of 300 (79%) patients, OR=27.37, 95% CI= 19.74-37.95 (Table 1). Out of the eight mutations, six were missense and two were frame shift mutations.

Germline mutations in HhH-GPD domain

Three missense mutations [Asp267Asn, Ser279Gly and Ile253Phe] were observed on exon 5 in HhH-GPD domain which were absent in control. First missense mutation; Asp267Asn (G to A substitution) was observed in 49 patients with frequency of 0.12, whereas second missense mutation; Ser279Gly (A to G transition) was observed in 53 patients with frequency of 0.13 and third missense mutation; Ile253Phe (A to T transition) was observed in 24 patients with frequency of 0.06 (Table 1, Figure 1).

Germline mutations in 3'UTR and exon 8

Two missense [1578A>T (T to A substitution), 1582C>T (T to C substitution)] and one frame shift [1582insG] mutation was observed in 3'UTR in 50 HNC patients with frequency of 0.13 whereas no controls showed this mutation. In addition to this, one missense [Ala399Glu (C to A substitution)] and one frame shift mutation [1543_1544delCT] was observed on exon 8 in 62 patients with frequency of 0.16 which was absent in controls (Table 1, Figure 2).

Association of germline mutations with area of cancer and smoking status

Significantly higher incidence of these mutations was observed in the patients of oral cavity cancer ($p<0.002$) when compared to patients of laryngeal cancer ($p<0.007$) and pharyngeal cancer ($p<0.06$) (Table 2).

In case of smoking status, all missense and frame shift mutations was observed significantly higher in the smokers ($p<0.002$) when compared to non smokers ($p<0.006$). In smoking habit, significantly higher incidence of these mutation was observed in patients using naswar ($p<0.002$) when compared to patients using cigarettes ($p<0.008$) and betel quid ($p<0.007$) (Table 2).

Discussion

DNA repair proteins are crucial in maintaining the stability of the genome and their proper functioning may prevent cancer transformation. Therefore changes in the sequence of DNA repair genes may influence the function of the corresponding protein and sponsor or inhibit carcinogenesis. Smoking is considered a risk factor in HNC. Tobacco smoke contains many chemical substances (Hecht, 2008). It also contains unstable

free radicals and reactive oxygen species, oxidative DNA damages may be induced in the cells of smokers (Valavanidis et al., 2008). The OGG1 protein is in front line of the cellular defense against oxidative DNA damage. Because HNC is often smoke related, it seemed reasonable to investigate the association of variability in OGG1 gene and the risk of HNC.

Six missense and two frame shift mutations were found in this study. A total of 238 mutations in a total of 300 samples were observed that result in a mutation frequency of 79%, OR=27.37, 95% CI= 19.74-37.95. While comparing mutation rates according to area of cancer, the rate of occurrence of mutation was found higher in area of oral cavity compared to area of larynx and area of pharynx. In case of smoking status a significantly higher difference was observed in smokers when compared to nonsmokers. All these mutations were found significantly higher in patients using naswar when compared to patients using the betel quid and cigarettes. In Pakistani culture chewing betel, paan, naswar and smoking are known to be strong risk factors for developing oral cancer. This is in a general agreement with results obtained in other studies (De Stefani et al., 2007; Werbrouck et al., 2008)

In present study three missense mutations, Asp267Asn, Ser279Gly and Ile253Phe on exon five were found in the important HhH-GPD domain (Bruner et al., 2000) a highly conserved domain (Nash et al., 1997) in OGG1. It has been also observed that regions conserved between species are likely to contain functionally important domains involved in the maintenance of normal protein function (Lamerdin et al., 1995). Mutation in this region can be pathogenic as this domain is important in both the catalytic and DNA-binding functions of the DNA glycosylase (Norman et al., 2003).

Two missense mutations leading to amino acids change at 253 and 267 in present study are part of C2H2 zinc finger domain (Arai et al., 1997). Amino acid 253 is also a part of extrahelical pocket composed of amino acid residues (319, 315, 42 and 253) of OGG1. In this active site pocket, 319 and 253 sandwich the 8-oxoG lesion via interactions with the lesion (Bruner et al., 2000). This active site pocket have significant role in repairing pathway as 8-oxoG lesion is extruded from the DNA helix and inserted into this pocket (Bruner et al., 2000).

In present study one frame shift mutations and one missense mutation was found on exon 8. This frame shift mutation observed in coding region is changing the whole downstream sequence of OGG1. Translation of wrong reading frame continues and the resultant premature RNA stability is compromised so either mRNA will not stable or protein degradation happens (Indu, 2009). Genetic changes in the 3' UTR of several genes have earlier been reported to be associated with higher susceptibility to particular tumor types (Vogelstein and Kinzler 2002). While screening UTR region of OGG1 gene, two missense and one frame shift mutation was observed in 3'UTR. Mutations in this region resulting in

either exon skipping or cryptic splice site utilization and can possibly deregulate protein levels by affecting protein half life (Campbell and Farrell 2009, Lheureux 2011).

In conclusion our results suggest that OGG1 is a highly polymorphic gene and genetic variations in this gene are associated with HNC carcinogenesis atleast in Pakistani population. Additional studies of these and other DNA repair-polymorphisms will provide essential information about the relationships between the DNA-repair mechanisms and carcinogenesis. These will be helpful in further illumination of the complex landscape of DNA repair and cancer risk.

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