R248W Mutations in p53 Gene are Rare among Indian Patients with Head-and-Neck Cancer

Abstract
Aim: Cancer is one of the curses to humankind, decades of research in eradicating the disease from the society is proven difficult. Close interaction between clinicians and scientists helps us to translate clinical observations into molecular mechanism of the disease. The Cancer Genome Atlas data suggest that genetic alterations in p53 gene play a crucial role in head-and-neck squamous cell carcinoma (HNSCC) tumorigenesis. Understanding p53 aberrations and their impact on other cellular activities can help with the design of new, more effective therapeutic strategy that target p53 mutation-bearing HNSCC, thereby producing a personalized medicine approach for the disease.

Materials and Methods: In an effort to identify the role of R248W mutation of p53 gene in HNSCC patients of Indian origin, tumor samples were collected from 55 patients (n = 55), and polymerase chain reaction–restriction fragment length polymorphism technique was used to screen for the mutation using genomic DNA isolated from the tumors. Results: The results reveal that except for one patient (heterozygous), all the patients were negative for the mutation. Conclusion: These results suggest that p53 R248W mutations are less prevalent in HNSCC Indian patients.

Keywords: Head-and-neck cancer, mutation, p53, polymerase chain reaction–restriction fragment length polymorphism

Introduction
Head-and-neck squamous cell carcinoma (HNSCC), a major form of head-and-neck cancer, is an important clinical challenge in oncology and is the sixth most common cancer in the world today.[1] More than 600,000 new head and neck cancer cases are diagnosed annually in the world, with about 350,000 deaths.[2] The Cancer Genome Atlas data suggest that genetic alterations in p53 gene play a crucial role in HNSCC tumorigenesis.[3] Identification of various gene mutations in cancer patients and relating their impact on cancer progression and recurrence will give us an insight into the role played by these mutations in cancer.

The p53 gene product is involved in regulating several key events in the cell that are essential for cell growth and suppression of malignancy. Thus, mutation in p53 induces malignancy.[4] When DNA gets damaged, wild-type p53 protein accumulates and stops replication to repair the DNA. This is followed by triggering specific cell cycle arrest in the G1/S phase. In case of tumor cells bearing mutated p53, this cell cycle arrest is not induced, which corresponds to an increase in various somatic mutations in cells that help the progression of cancer.[5] Inactivation of tumor suppressor gene p53 plays a key role in cancer development. The p53 gene is frequently lost or mutated in several kinds of tumors, including the colon, lung, breast, brain, ovary, and esophagus implicating p53 as an important tumor suppressor gene.[6] In head-and-neck cancer, alterations of p53 gene have been implicated at a high incidence of the disease.[7]

In several forms of cancer, p53 is mutated and codon 248 is one of the hot spot regions which represents 60% of known p53 mutations.[8] In HNSCC also, 75% to 85% of human papillomavirus-negative patients possess mutations in TP53 and most of the mutations are frequently observed within the DNA binding domain of the p53 protein.[9] All p53 mutations described in HNSCC occur in highly conserved regions between exon 5 and 8.[10] In head-and-neck cancer, p53 mutations are
significantly associated with short survival rate and often resistant to standard treatments including radiotherapy and chemotherapy. This indicates that for head-and-neck cancer patients, p53 mutations can be used as a prognostic biomarker. In p53 protein, since codon 248 is one of the hot spot mutations observed in several forms of cancer and there is no report available on the prevalence of this mutation in Indian HNSCC patients, this study was carried out.

**Materials and Methods**

**Patients and specimens**

Tumor samples used in this study were surgically excised from 55 head-and-neck cancer patients at Apollo hospital, Chennai, India. After resection, the tumors were snap-frozen and then transported to VIT University, Vellore. All patients were followed up and the data concerning cancer recurrence and patient survival were collected. We obtained informed consent from all the individuals who participated in the study as well as approval from the institute’s human ethical committee (IEC/IRB No: IECH/2013/Dec18-006). All patients were Indian residents from the states of Tamil Nadu, West Bengal, Andhra Pradesh, Manipur, and Pondicherry.

**DNA extraction**

DNA from 55 tumor samples was extracted by a high salt method. The tumor tissue was processed by grinding the fresh sample using a motor and pestle in liquid nitrogen. For protease digestion, the tissue was transferred into a microfuge tube containing 1 ml of Tris NaCl EDTA SDS (10 mM Tris, 6M NaCl, 100 mM ethylenediaminetetraacetic acid, and 0.6% sodium dodecyl sulfate) buffer with 60 µl of proteinase-k (20 mg/ml) and the tubes were incubated overnight at 45°C. At the end of the incubation period, 277 µl of 6M NaCl was added, mixed, and centrifuged for 12,000 RPM for 10 min. The supernatant was transferred to a fresh microfuge tube, and DNA was precipitated by adding equal volume of 100% ethanol. The DNA was pelleted by centrifugation at 12,000 RPM for 10 min. After centrifugation, the supernatant was discarded and the pellet was washed with 70% ethanol and air-dried. The recovered DNA was suspended in 20–100 µl of sterile distilled water. The DNA was quantitated by using NanoDrop instrument (Thermo Fisher scientific) and quality of the DNA was tested on agarose gel electrophoresis.

**Polymerase chain reaction–restriction fragment length polymorphism**

Polymerase chain reaction (PCR) amplification of p53 gene at codon 248 was performed in 50 µl of reaction mixture containing 5 µl of 10x buffer, 2 µl of each primer, 4 µl of dNTP mixture (2.5 mM), 0.4 µl of Taq polymerase (5units/ml) (Takara, Japan), 1 µl of template DNA (50 ng/µL), and 35.6 µl of H₂O. The profile used in the PCR (Eppendorf Master cycler Nexus cycler) was 30 s at 94°C, 45 s at 68°C, 72°C for 50 s for 35 cycles, and 5 min at 72°C. The PCR product was digested with Msp1 restriction enzyme (New England Biolabs) and the digested products were visualized under ultraviolet light on the gel documentation system (Syngene) after electrophoresis on a 2% agarose gel containing ethidium bromide. The primers used was Forward: 5’TGG TGC TGG GCA CCT GTA GTC CCA GCT ACT CG3’ and Reverse: 5’ ACT ACT CAG GAT AGG AAA AGA GAA GCA AGA GGC3’.[11]

**Results**

p53 mutation using polymerase chain reaction–restriction fragment length polymorphism

p53 mutation detection was assessed for 55 samples using PCR-restriction fragment length polymorphism (PCR-RFLP) method. For determining the mutations in the codon 248, PCR-amplified fragments of p53 gene (680 base pair) were digested with restriction enzyme Msp1 for 1 h at 37°C and electrophoresed in 2% agarose gel containing ethidium bromide. Upon restriction, the wild type resulted in 170, 220, and 290 base pair product for the CGG (R/R) in codon 248. Samples with 170, 220, 290, and 460 base pairs represent the heterozygous mutation (HTM) since the CGG (Arg) codon gets mutated to TGG (Trp) or other sequences. The p53 mutational analysis in all 55 samples is shown in Figure 1. Of 55 samples, only one HTM was observed. The p53 mutation rate in a total of 55 samples was 98% (54/55)
wild type and 2% (1/55) was heterozygous. Differences in the categorical variables including age, gender, anatomical location of the tumor, stage, histopathology, grade, tobacco usage, and treatment type between patients with and without p53 mutations were evaluated. The p53 mutations and the clinicopathological factors of all the patients are shown in Table 1.

### Discussion

Our laboratory has been interested in identification of mutations in various genes of HNSCC patients using DNA isolated from the tumor samples. Most of the properties of cancer can be related to mutations in the genome. p53 protein is one of the essential proteins required for maintaining the integrity of genome by preventing proliferation of cells when the genomic DNA is mutated. Mutation in p53 will lead to production of cancer cells with high rate of somatic mutations in the tumor. For these reasons, monitoring mutations in the p53 gene in cancer patients is of interest. Several mutations are reported in p53 gene in various cancers; among this, R248W is one of the common mutations. Considering these factors, using PCR-RFLP technique, R248W mutations in p53 gene were screened in 55 HNSCC patients.

From the results, it was interesting to note that, of 55 samples analyzed in this study, there was only one heterozygous R248W mutation, suggesting that R248W mutation is not prevalent in HNSCC patients of Indian origin. In Chinese population also, of 35 patients with HNSCC, only two mutations were detected in p53 gene at codon 248. Similar studies carried out with American population of HNSCC patients also reveal a lower percentage (3.5%) of R248W mutation. Depending on the type of cancer, the site of mutations also differs; hence, studies directed toward identification of mutations in the whole p53 coding sequence will give the complete status of p53 mutations in these patients. Furthermore, similar studies carried out with more patients representing other parts of India should give us more details about the status of R248 mutations in Indian HNSCC patients.

### Conclusion

Identification of mutations in cancer patients will give an insight into molecular mechanism behind the cause of the disease, and also the correlation of the mutation to patient’s disease progression, response to treatment, and recurrence of the disease will help the clinicians to design novel and effective treatment protocols to the cancer patients as well as these data can be used for preclinical counseling for individuals having deleterious germ line mutations. In this study, we screened for one of the widely reported mutations in p53 gene, R248W, and report that this mutation is less prevalent in HNSCC patients of Indian origin.

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### Conflicts of interest

There are no conflicts of interest.

### References