

RESEARCH ARTICLE

Analysis of ARID2 Gene Mutation in Oral Squamous Cell Carcinoma

Lakshmi Prabha Das^{1*}, Raghuram Hari Pitty¹, Kannan Asokan¹, Krithika C-L¹, Anandi M-S¹, Arvind Ramanathan²

Abstract

Background: The ARID2 gene, encoding a sub unit of the chromatin remodelling complex, has a possible tumour suppressor function and has been found to be frequently mutated in various tumours, including gingivo buccal oral squamous cell carcinomas. The present study was designed to analyse the presence of ARID2 gene mutations in the distinct genetic South Indian (Dravidian) population. **Materials and Methods:** Genomic DNA from thirty biopsy tissue samples of histopathologically confirmed cases of oral squamous cell carcinoma (OSCC) were subjected to PCR amplification with intronic primers encompassing exons 19 and 20 of ARID2. Subsequently, the PCR amplicons were purified and subjected to Sanger sequencing using forward primers for analysis of mutational status. **Results:** Our study yielded a 6% occurrence of mutations in the ARID2 gene among the thirty OSCC samples. Two samples showed a C(5174)A nonsense mutation whereby the “C” nucleotide was substituted with an “A” nucleotide at position 5174, resulting in the conversion of serine amino acid at codon 1725 to a premature STOP codon. **Conclusion:** Identification of ARID2 gene mutations in OSCCs in this distinct ethnic population reaffirms that aberrations in the chromatin remodelling complex could indeed also contribute to tumorigenesis, thus providing new insights for future research.

Keywords: Oral squamous cell carcinoma- ARID2- chromatin remodelling complex- PBAF- mutation

Asian Pac J Cancer Prev, **18** (10), 2679-2681

Introduction

Oral cancer is sixth most common cancer worldwide (Krishnamurthy and Ramshankar, 2013). In India, it is one of the leading cancers contributing significantly to the disease burden in the country. A prevalence rate of 22.9% and 9.8% has been estimated among men and women from rural and urban areas in the age group of 30-69 years respectively, making oral cancer one of the foremost fatal cancers among men (Dikshit et al., 2012). In India and in many other populations, more than 90% of oral cancers are oral squamous cell carcinomas (OSCC) (Wangpermtam et al., 2011; Gupta et al., 2013).

A multitude of factors combining an individual's genetic predisposition as well as chronic exposure to environmental factors such as tobacco in any form, alcohol consumption, poor oral and general health, aging and viral infections lead to the accumulation of multiple genetic alterations developing into oral squamous cell carcinoma (Choi and Myers, 2008; Wangpermtam et al., 2011). Genetic alterations predisposing to cancer are commonly detected in oncogenes, tumour suppressor genes and stability genes. If any genetic alteration is found recurrently in any specific tumour type, these can be used as biomarkers for detection and developing targeted

therapies and also predict responses to various treatment modalities (Bhatt et al., 2010).

Oral cavity sub-sites have distinct biological features and it is likely that genes driving cancers in these sub-sites may be different. Among the spectrum of genes associated with oral squamous cell carcinoma, ARID2 was a novel gene to be found mutated in site specific gingivo-buccal oral squamous cell carcinoma (GB-OSCC) (IPT-ICGC, 2013). However ARID2 has been reported to be frequently mutated in various other cancers like hepatocellular carcinoma (HCC) such as HCV associated HCC, HBV associated HCC, alcohol associated HCC and HCC with no known etiology, malignant mesothelioma, pancreatic cancer, primary urethral clear cell adenocarcinoma, microsatellite unstable colorectal cancer, non small cell lung carcinoma and malignant melanoma (Zhao et al., 2011; Biankin et al., 2012; Fujimoto et al., 2012; Hodis et al., 2012; Manceau et al., 2013; Cajuso et al., 2014; Duan et al., 2016).

The human ARID2 gene is located on chromosome 12q and consists of 21 exons. ARID2 is one among the many subunits of the SWI/SNF chromatin remodelling complexes (polybromo- and BGR1-associated factor [PBAF]), which facilitates ligand-dependent transcriptional activation by nuclear receptors. The SWI/SNF multiprotein chromatin

¹Department of Oral Medicine and Radiology, SRM Dental College, Ramapuram, ²Enable Biolabs, Madurai Meenakshipuram Extension, Urapakkam, Chennai, India. *For Correspondence: lakshmi.prabhadas@gmail.com

remodelling complex is an important determinant of genomic plasticity that modulates the accessibility of transcription factors to DNA and controls many biological processes including cell proliferation and growth arrest (Zhao et al., 2011; Manceau et al., 2013). ARID2 encodes a protein that is involved in transcriptional activation and repression of genes by chromatin remodelling (IPT-ICGC, 2013). ARID2 has been shown to have tumour suppressor action (Zhao et al., 2011; Cajuso et al., 2014).

Studies from the Indian Genome Variation Consortium (IGVC, 2008) have suggested that the genetic basis of diseases in most of the populations in the Indian subcontinent to be distinct. Based on this observation, the mutation profile of ARID2 gene from oral squamous cell carcinoma subjects from South India were explored to uncover any variations in the mutational profile of ARID2 gene.

Materials and Methods

Study setting

The study was approved by the Institutional Review Board of SRM Dental College, Ramapuram, Chennai, India. OSCC patients visiting tertiary centres of the geographical zone of South India were explained about the details of the study and due informed consent were obtained. Tissue biopsy samples of OSCC were obtained and stored in RNA Save (cat#01-891-1B, Biological Industries, Israel) under optimum temperature until being processed for DNA extraction.

DNA extraction and Sanger sequencing

DNA was extracted from histopathologically confirmed biopsy tissues using column based DNA extraction kit (Cat# P4850, Sigma-Aldrich, St.Louis, Missouri, USA) in accordance to manufacturer's protocol. A 529 base pair region encompassing both exons 19 and 20 of ARID2 gene were amplified in a single reaction

with the following intronic primers: Forward primer: GGCAGGGTGGTCATAGTTGTC, Reverse primer: ATGGCCTGACCTAGGCATAAAC. PCR was performed on 50ng of DNA samples under the following conditions: Initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 62°C for 30 seconds, primer extension at 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. The PCR amplicons were purified from agarose gel by column based gel extraction kit (Cat# NA1111, Sigma-Aldrich, St.Louis, Missouri, USA) and subjected to sanger sequencing with forward primer only. Sequenced data were analyzed by BLAST nucleotide analysis program, which is available at www.ncbi.nlm.nih.gov/BLAST.

Results

Exons 19 and 20 of ARID2 gene were screened to understand the mutational status of this region in patients with well-differentiated OSCC. A 50ng aliquot of the total genomic DNA was used as template to amplify both exons 19 and 20 with intronic primers in a single reaction. The primer combination showed specific amplification of a 529 base pair band, which was purified from agarose gel and subjected to direct sequencing.

Sequence analysis identified a mutation C(5174)A within exon 19 of ARID2 in two OSCC samples from among the thirty study samples in this study yielding a 6% occurrence. The substitution of "C" nucleotide with "A" nucleotide at position 5174 causes substitution of serine amino acid at codon 1725 from TCA (coding for serine) to TAA (coding for a STOP codon) (Figure 1).The schematic representation of the mutation is shown in Figure 2.

Discussion

To the best of our knowledge, the role of ARID2 gene

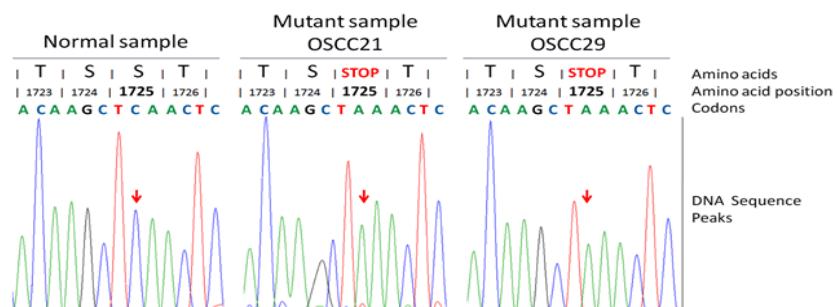


Figure 1. Electropherogram Data of Normal and Mutant Exon19 of ARID2 Gene. The mutant nucleotide is indicated by a bold red arrow in sequence image. The mutation changes serine amino acid into a premature STOP codon.

WILD TYPE ARID2: CAG CCA ACT GTA GGG GGC ACA AGC TCA ACT CCT AGACTG CAG TGA
Amino acid sequence: Q P T V G G T S S T P R L Q STOP

MUTANT ARID2: CAG CCA ACT GTA GGG GGC ACA AGC TAA
Amino acid sequence: Q P T V G G T S STOP

Figure 2. Schematic Representation of the Normal and Mutant ARID2 and Its Effect on Protein is Shown. Normal ARID2 produces a full length ARID2 protein, whereas mutant ARID2 produces a truncated protein due to premature occurrence of STOP codon.

mutation in Oral squamous cell Carcinoma (OSCC) has not been elucidated in any other ethnic population other than the Indian population. The India Project Team of the International Cancer Genome Consortium (IPT-ICGC) investigated sub-site specific gingivo buccal OSCC (GB-OSCC), in which only patients from Northern part of India were included (IPT-ICGC, 2013). It has been observed that high levels of genetic divergence exists between groups of populations in India that cluster largely on the basis of ethnicity, as quoted from the study conducted by the Indian Genome Variation Consortium (2008). Therefore the reference of the people of India as 'Indian' in many population studies as a genetically homogenous population cannot be justified (IGVC, 2008). Hence, we designed the present study to understand the genetic status of the mutational hotspot region of ARID2 gene in South Indian Dravidian race. Our study yielded a 6% occurrence of mutation in exon 19 of ARID2 gene from 30 study samples of OSCC which is comparably significant to the 10% occurrence from 50 GB OSCC samples from the previous study (IPT-ICGC, 2013).

Literature evidences quote mutations in ARID2 in similar strengths in some primary human cancers such as 5-8% of hepatocellular carcinomas (HCC), particularly HCCs that are related to hepatitis C virus (HCV) (14% of HCV-related cases compared with 2% of hepatitis B virus-related cases) (Zhao et al., 2011; Planchon et al., 2015), non-small cell lung cancer (7.3%) (Manceau et al., 2013) and melanoma (7%) (Hodis et al., 2012) to quote a few. Complete loss of function of ARID2 by homozygous deletions or somatic mutations associated with a loss of heterozygosity, had been identified in these cancer types (Planchon et al., 2015).

For detecting the presence of mutation in our study, specific primers flanking exon 19 and 20 were designed and the genome region amplified by polymerase chain reaction (PCR). The quality of DNA for being amplified by polymerase chain reaction (PCR) was also determined to avoid procedural errors in which all samples showed a clear amplification of the ARID2 gene regions which indicated that the DNA extracted were indeed of high quality (data not shown). Subsequent to amplification, exons 19 and 20 were directly sequenced to detect mutations.

Comparatively lower occurrence of mutation of 6% observed in this study could be attributed to the fact that the entire ARID2 gene was sequenced in the study by India Project Team of ICGC (IPT-ICGC, 2013) when compared to this study where only two exons (19 and 20), which are proven to be mutational hotspots had been analysed. ARID2 gene mutation was one among the novel mutations uncovered in the Gingivo Buccal oral squamous cell carcinoma with a prevalence of 10% (IPT-ICGC, 2013), which hasn't been validated or replicated in any other varying ethnic population. Our study reaffirms that the chromatin remodelling complex (PBAF) could also be one among the aberrant key pathways leading to the genesis of oral squamous cell carcinoma.

The mortality and morbidity rates of oral squamous cell carcinoma still remains hardly changed despite the vast ongoing research in the field. In this era of precision

medicine where attempts are to tailor make therapy for cancer patients, ARID2 gene mutation can definitely not be overlooked. Research with a larger sample size across diverse ethnicity could only validate its role in the carcinogenesis of OSCC.

References

- Bhatt AN, Mathur R, Farooque A, Verma A, Dwarkanath BS (2010). Cancer biomarkers-Current perspectives. *Indian J Med Res*, **132**, 129-49.
- Biankin AV, Waddell N, Kassahn KS, et al (2012). Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*, **491**, 399-405.
- Cajuso T, Hänninen UA, Kondelin J, et al (2014). Exome sequencing reveals frequent inactivating mutations in ARID1A, ARID1B, ARID2 and ARID4A in microsatellite unstable colorectal cancer. *Int J Cancer*, **135**, 611-23.
- Choi S, Myers JN (2008). Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. *J Dent Res*, **87**, 14-32.
- Dikshit R, Gupta PC, Ramasundarahettige C, et al (2012). Cancer mortality in India: a nationally representative survey. *Lancet*, **379**, 1807-16.
- Duan Y, Tian L, Gao Q, et al (2016). Chromatin remodeling gene ARID2 targets cyclin D1 and cyclin E1 to suppress hepatoma cell progression. *Oncotarget*, **7**, 45863-75.
- Fujimoto A, Totoki Y, Abe T, et al (2012). Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nature Genet*, **44**, 760-4.
- Guichard C, Amaddeo G, Imbeaud S, et al (2012). Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nature Genet*, **44**, 694-8.
- Gupta B, Ariyawardana A, Johnson NW (2013). Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. *Int Dent J*, **63**, 12-25.
- Hodis E, Watson IR, Kryukov GV, et al (2012). A landscape of driver mutations in melanoma. *Cell*, **150**, 251-63.
- India Project Team of ICGC (2013). Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. *Nat Commun*, **4**, 2873.
- Indian Genome Variation Consortium (IGVC) (2008). Genetic landscape of the people of India: a canvas for disease gene exploration. *J Genet*, **87**, 3-20.
- Krishnamurthy A, Ramshankar V (2013). Early stage oral tongue cancer among non-tobacco users-An increasing trend observed in a South Indian patient population presenting at a single centre. *Asian Pac J Cancer Prev*, **14**, 5061-5.
- Manceau G, Letouzé E, Guichard C, et al (2013). Recurrent inactivating mutations of ARID2 in non-small cell lung carcinoma. *Int J Cancer*, **132**, 2217-21.
- Planchon JM, Bièche I, Guinebretière JM, Bourdeaut F, Delattre O (2015). SWI/SNF chromatin remodeling and human malignancies. *Annu Rev Pathol Mech Dis*, **10**, 145-71.
- Wangperm tam P, Sanguansin S, Petmitr S, Punyarat P, Weerapradist W (2011). Genetic alteration in oral squamous cell carcinoma detected by arbitrarily primed polymerase chain reaction. *Asian Pac J Cancer Prev*, **12**, 2081-5.
- Zhao H, Wang J, Han Y, et al (2011). ARID2: a new tumor suppressor gene in hepatocellular carcinoma. *Oncotarget*, **2**, 886-91.