THE EXON 5, 6, 7, 8 OF P53 MUTATIONS IN ORAL SQUAMOUS CELLS CARCINOMA

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ABSTRACT
Genetic instability may underlie the etiology of multistep carcinogenesis. The altered p53 gene observed in tumors may represent the expression of such instability and may allow the accumulation of other gene alterations caused by multiple mechanisms. p53 gene is the guardian of the genome, and why we pay more attention to this gene. In this study, we evaluated the significance of p53 mutation in 55 patients with oral squamous carcinoma, while the remaining 25 patients underwent poorly differentiated carcinoma. The mutations were detected by PCR-SSCP (Single Strand Conformational Polymorphism) analysis in the region between exon 5 and exon 8. The results indicated that the p53 mutation in exon 5 (40%), exon 6 (28%), exon 7 (24%) and exon 8 (8%) were associated with poorly differentiated carcinoma, whereas mutation in exon 5 (10%), exon 6 (30%), exon 7 (40%) and exon 8 (20%) were associated with well-differentiated carcinoma. These observations suggest that p53 mutations in exons 5, 6, and 7 have a strong correlation with poorly differentiated oral squamous carcinoma while well-differentiated level was related with mutation in exons 6, 7, and 8.

Keywords: p53, oral squamous carcinoma, mutation, PCR-SSCP

INTRODUCTION
Squamous cell carcinoma is the most common intraoral malignancy and is the most frequently occurring malignant tumor of the oral structures. In the malignant oral lesions, the squamous cell carcinoma is about 82–90%, and mainly affects male patients with ages varying between 40–80 years, although recent studies have reported the occurrence of this malignancy in younger patients.1 Tobacco and betel use, alcohol consumption, viruses, and other occupational and environmental factors have been implicated in the etiology of the disease. Epidemiological studies have shown that the incidence of oral squamous cell carcinoma varies significantly among the continents and within developed and developing countries.2
Altemations in tumour suppressor genes like p53 gene are the most commonly implicated genetic events in a number of human tumors and are frequently found in various types of cancer and have been considered as molecular markers of cancer.3

Mutation or increased expression of the p53 protein has also been reported in the head and neck squamous cell carcinoma. However, only few data are available on the frequencies of p53 mutation in oral squamous cell carcinoma correlated with poorly and well differentiated tumor grades. The different exon on p53 may be mutated in certain stages because it may serve as a regulatory marker in the process of tumorigenesis. Polymorphism in p53 codon 72 may contribute to oral cancer susceptibility.4 One possibility is that p53 alteration can enhance genomic instability and thus augment the accumulation of subsequent genetic event necessary for tumor development.3,5

Alteration of the specific gene, such as p53, p27, p16 and cyclin D-1, will induce oral cancer development through mutation, amplification, or deactivation mechanism.4 Therefore, Hsieh and his research team suggest that the pattern of p53 gene mutation in oral squamous cell carcinoma conduct further study.3

Single-Strand Conformation Polymorphism (SSCP) is a screening method for detection of unknown point mutations. SSCP analysis was orginally described by Orita et al. (1989). The general idea is to take a small PCR product, denature it, and electrophorese it through a non-denaturing polyacrylamide gel. Thus, as the PCR product moves into and through the gel (and away from the denaturant), it will regain secondary structure that is sequence dependent.6 The mobility of the single-stranded PCR products will be different at different sequences. Therefore, PCR-SSCP can be used to detect mutational products. The major advantage of SSCP is that many individual PCR products may be screened for variation simultaneously. Many of PCR products may be analyzed on each full-size sequencing gel (depending upon the comb size used).7 Most researchers use SSCP to reduce the amount of sequencing necessary to detect new alleles at loci of interest or to estimate allele frequencies of populations better.

The main purpose of the study is to evaluate the significance of p53 mutation in various stages of patients with oral squamous cell carcinoma. To determine whether mutation of p53 gene was associated with degree of tumorigenesis, we used PCR-SSCP method to detect the mutation in exons 5-8 of p53 gene.

MATERIALS AND METHODS

Tissues

Mucosal biopsies of 55 patients with oral squamous cell carcinomas (OSCC) from the floor of the mouth were obtained from the surgical pathology files of the Department of Oral Pathology, Faculty of Dentistry, Universitas Airlangga, Surabaya. The 55 cases of squamous cell carcinoma comprised of poorly and well differentiated tumor grades. The a verage of the patient ages was 58.7 (ranging from 21–74) years. Specimens from these patients, from 29 men and 26 women, were screened for adequacy of lesional tissue by staining the tissue sections with hematoxylin and eosin. The histological diagnosis of each specimen was independently reviewed based on the established histological criteria. Sixteen biopsies of normal control epithelium from the floor of the mouth were obtained from non-smoker 6 men and 10 women with the average age of 52.2 years (ranging from 35 to 73).

PCR-SSCP

The sequences of all primers were obtained from Laboratoires Eurobio, Paris for the amplification of oral squamous cell carcinoma DNA. The PCR conditions were denatured at 94°C for 1 min, annealed at 59°C for 1 min and extended at 72°C for 1.5 min, with the total number of cycles of 35. The amplified fragments were analyzed by PCR-SSCP. Single strand of the PCR product was made by snap-freeze technique and loading on 6% polyacrylamide containing 5% glycerol. The gel was running on TBE buffer at 20 W constant power at room temperature for 12–16 h, until the xylene cyanol dye reached 3 cm from the top of the gel. The gel was stained with silver nitrate staining.

RESULTS

In this study, we evaluated the significance of p53 mutation in 55 patients with oral squamous carcinoma. The male to female distribution in this study was ignored. Thirty among them underwent well-differentiated carcinoma, while the remaining 25 patients underwent poorly differentiated carcinoma. The mutations were detected by PCR-SSCP analysis in the region between exon 5 and exon 8. The results indicated that the p53 mutation in exon 5 (40%), exon 6 (28%), exon 7 (24%) and exon 8 (8%) were associated with poorly differentiated carcinoma whereas mutation in exon 5 (10%), exon 6 (30%), exon 7 (40%) and exon 8 (20%) were associated with well-differentiated carcinoma.
Oral squamous cell carcinoma (OSCC) is the most common cancer found approximately 80% of all cancer in the oral cavity. In the developed country like US, which has managed to eradicate infectious disease, cancer is the second cause of death after cardiovascular diseases. In Indonesia, cancer was recorded as the cause of death besides heart attack, diabetes and hepatitis.

Oral squamous cell carcinoma is one of the most common cancers worldwide, with incidences of more than 30 per 100000 population in India (oral cancer) and in France and Hong Kong (nasopharyngeal cancer). Epidemiological studies have shown that the sites of oral cancer differ widely. Tongue, lip and floor of the mouth are the most frequent sites of lesions of oral squamous cell carcinoma.2

Table 1. Prevalence of mutation in p53 gene associated with tumor grade in oral squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Position of mutation</th>
<th>Number of patients with mutation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Poorly differentiated (25 patients)</td>
</tr>
<tr>
<td>Exon 5</td>
<td>10 (40%)</td>
</tr>
<tr>
<td>Exon 6</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>Exon 7</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>Exon 8</td>
<td>2 (8%)</td>
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</tbody>
</table>

These observations suggest that p53 mutation in exon 5, 6, and 7 have strong correlation with poorly differentiated in oral squamous carcinoma while the well-differentiated level was related with mutation in exon 6, 7 and 8.

PCR-SSCP analysis showed that mutation on p53 gene could be detected by extra band eruption (Fig.1). The mobility of the single-stranded PCR products depended upon their secondary structure. Therefore, PCR products that contained change sequence differences as well as insertions, deletions or other mutation, had different mobilities in poly-acrylamide gel electrophoreses.

![Lane 1 2 3 4 Extra band](image)

Figure 1. PCR-SSCP analysis of p53 gene was drawn in lane 1, 2, 3, 4, respectively. Mutations were detected and signed by extra bands.

DISCUSSION

Oral cancer constitutes about 4% of all cancers in the United States and 5% in the United Kingdom. A total of 2940 new cases of lip, mouth, and pharyngeal cancer in men were reported in the United Kingdom in 1996: an incidence of 10.2 per 100000 population. Early detection should be a priority, given the excellent prognosis of early stage disease compared with the poor results in advanced stages. In Indian screening programmes, community health workers have been trained in primary prevention and early detection of oral cancer and premalignant lesions, but no evidence indicates that this reduces mortality. Screening is the most cost effective procedure when targeted at high risk groups, for example, heavy drinkers and smokers. Oral squamous carcinogenesis is a multistep process in which multiple genetic events occurring, alter the normal functions of oncopgenes and tumour suppressor genes. This can result in increased production of growth factors or numbers of cell surface receptors, enhanced intracellular messenger signalling, and/or increased production of transcription factors. Genetic alterations are known to occur during carcinogenesis including point mutations, amplifications, rearrangements, and deletions. Point mutations (single base changes) can lead to overactivity or inactivity of gene products. These are common in genes such as K-ras and p53. Alterations of these genes have frequently been reported in malignant neoplasms.

Oncogenes themselves are not sufficient to cause oral cancer and appear to be initiators of the process. The crucial event in the transformation of a premalignant cell to a malignant cell is the inactivation of cellular negative regulators tumour suppressor genes, and is regarded to be a major event leading to the development of malignancy. Tumour suppressor genes are mostly inactivated by point mutations, deletions, and rearrangements in both gene copies. The p53 gen mutation will not cause cells apoptotic therefore the cancer cells will proliferate. If p53 gene mutated, there was no repair into the apoptosis cell which underwent DNA damage, that mutant cell could enter the cell circulation and it will happen with uncontrolled cell development. One of the important tumor suppressor genes is p53, mainly roles as the guardian of the genome in apoptotic gene. This protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis. These functions are achieved by the ability of p53 to modulate the expression of several genes. The p53 protein transcriptionally activates the production of the p21 protein, encoded by the WAF1/CIP gene, and p21 become an inhibitor of cyclin and cyclin dependant kinase complexes. p21 transcription is activated by wild-type p53, not mutant p53. However, WAF1/CIP expression is also induced by p53 independent pathways such as growth factors, including platelet derived growth factor, fibroblast growth factor, and transforming growth factor β. Wild-type p53 has a very short half life (4–5 minutes) whereas mutant forms of protein are more stable, with a six hour half life.
Rahayu: The Exon 5, 6, 7, 8 of p53 Mutations

the wild-type protein, thereby inactivating its suppressor activity, or by deletion, which leads to a reduction or loss of p53 expression and protein function. The tumour suppressor gene p53 is known to be mutated in approximately 70% of adult solid tumors.15 Mutations of exons 4 to 9 of the p53 gene were found in 72 of 106 patients with Head and Neck Squamous Cell Carcinoma (HNSSC).15 p53 mutations were associated with loss of heterozygosity at chromosome 17p. The prevalence of p53-mutated tumors was higher in the group of patients with nonresponse to neoadjuvant chemotherapy than that in the group of responders (81% v 61%, respectively).4

According to these research the p53 mutation on Poorly differentiated level mostly occurs in exon 5 (40%), whereas the least mutation occurs in exon 8 (8%), followed by exon 6 (28%) and exon 7 (24%). However the least number of p53 mutation in Well differentiated mutation occurs in exon 5 (10%), and most of the mutation occur in exon 7 (40%), followed by exon 6 (30%) and exon 8 (20%). A high prevalence of p53 mutations on Poorly differentiated level was found in exons 5 to 7. But high prevalence of p53 mutations on well differentiated level was found in exons 6 to 8. It is relevant to some studies which state that mutation in Oral Squamous Cell Carcinoma occurs between exon 5 and 8. These observation suggest that p53 mutation in exon 5, 6, and 7 have strong correlation with poorly differentiated level in oral squamous carcinoma while well-differentiated level was related with mutation in exon 6,7 and 8.

REFERENCES