Decreased Expression of MYH in Oral Cancer

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Abstract

BACKGROUND: Oral cancer is one of the fastest increasing malignancies in Taiwan. An estimated 2 million Taiwanese are habitual chewers of betel quid, which contains many known carcinogens, capable of inducing DNA mutation and predisposes chewers to oral cancer. Defects in MYH, a DNA repair enzyme, are present and linked to colorectal cancer in patients with adenomatous polyposis coli. This study aimed to compare the expression of MYH in non-cancerous and cancerous oral tissues from betel quid chewers.

METHODS: MYH expression in different types of oral tissue from patients with oral cancer and habitual betel quid chewing was measured using immunohistochemistry and western blot.

RESULTS: MYH expression in cancerous tissue, compared to that in neighboring normal oral mucosa from the same patients, increased in 8 patients (8/62), similar in 25 (25/62), and decreased in 29 (29/62). MYH expression in papilloma, a benign neoplasm, increased (3/3) in all patients; expression in leukoplakia, a premalignant lesion, increased (2/4), or remained similar (2/4), compared to that in neighboring normal mucosa.

DISCUSSION/CONCLUSIONS: MYH expression level remains similar or increased in oral tissue undergoing histological changes leading to either papilloma or leukoplakia. On the other hand, the expression is impaired in half of the patients diagnosed of oral cancer. MYH expression may be important in the prevention as oral carcinogenesis in habitual betel quid chewers.

Key words: Betel quid, oral cancer, MYH.

Introduction

Oral cancer is one of the fastest increasing malignancies in Taiwan. In 1982, oral cancer incidence stood at 5.12 per 10^5 men per year and 1.54 per 10^5 women per year.1 The rates
increased to 27.04 and 3.17, respectively, in 2001, an alarming 5.3-fold increase for men and a 2-fold increase for women in 2 decades. Oral cancer has been ranked the fourth most common type of cancer among men in Taiwan. More importantly, oral cancer is the leading type of cancer to cause death in men aged 25 to 44, inflicting a great impact on the society.

From studies in western countries, it has long been known that cigarette smoking and alcohol abuse play roles in the etiology of oral cancer and these two agents may act in synergy. A 35-fold increase in the incidence of oral cancer was reported for habitual consumers of both alcohol and tobacco than for non-users. In Taiwan, another etiological factor may play a more important role. Betel quid chewing is practiced in Taiwan for centuries, and an estimated 2 million people on the island are habitual chewers of betel quid. A case control study in Taiwan reported a 123-fold increase in the incidence of oral cancer for those who consume tobacco, alcohol, and betel quid habitually than nonusers.

Betel quid contain many known carcinogens, some of which are shown to generate free radicals, and contribute to the induction of chromosomal aberrations and DNA damage. The extent of damage is higher at longer exposures. One study screening the coding region of the adenomatous polyposis coli (APC) gene in cancer patients showed that missense mutations and base pair deletion were detected in patients with both betel quid chewing and smoking.

MYH is a DNA repair enzyme, important in the repair of DNA strands after exposure to harmful free radicals. The most frequent mutagenic lesion is 7,8-dihydro-8-oxoguanine (8-oxo-G). 8-oxo-G can mispair with adenine during DNA replication, and cause transversions from G:C to T:A. MYH scans DNA strands during replication and removes adenines mispaired with 8-oxo-G. MYH deficiency leads to an increased frequency of G:C to T:A mutations in genes such as APC in MYH-associated polyposis tumors.

In this study, we measured MYH expression in different types of oral tissue in patients diagnosed as oral cancer. Most of them are both habitual betel quid chewers and smokers. Immunohistochemistry and Western blot were used to measure the amount of MYH protein in malignant and premalignant lesions and compared with the expression amount of MYH in surrounding normal oral tissue from the same patients.

**Materials and Methods**

**Tissue collection**

All patients included in this study had either oral cancer or papilloma. They have given informed consent. Specimens of normal oral tissue or leukoplakia were obtained from the excised surgical specimens adjacent to the tumor.

**Immunohistochemistry**

Fresh tissue samples were embedded in OCT and stored in -80°C refrigerator. Tissue sections were fixed with acetone, and stained with LAB-SA Detection System (ZYMED Laboratories, Invitrogen, San Francisco, US), according to the protocol suggested by the manufacturer. In brief, sections were first quenched with 3% hydrogen peroxide/methanol, and blocked with serum blocking solution before adding MYH antibody (polyclonal antibodies were from Dr. Wu-Tsai at the National Taiwan University). After overnight incubation, biotinylated second antibody was applied. Histochemical substrate for
horseradish peroxidase (3,3’-Diaminobenzidine tetrahydrochloride substrate kit, ZYMED Laboratories, Invitrogen, San Francisco, US) was used for color expression. Finally, sections were counter-stained with hematoxylin before reading.

**Western blot**

Fresh frozen tissue samples were homogenized with protein lysis buffer (1% v/v NP-40, 1 mM sodium orthovanadate, 2 mM EDTA, 10 mM NaF, 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM PMSF, 1 μM aprotinin, 1 μM pepstatin and 25 μM leupoptin), and centrifuged. The supernatant was then aspirated for analysis. The protein was separated by one dimensional electrophoresis of 10% SDS-PAGE, and transferred to a PVDF membrane. The PVDF membrane was blocked with 5% non-fat milk of TBST buffer. Overnight incubation with MYH antibody was performed at 4℃. Incubation with a secondary antibody (HRP conjugated antibody, Chemicon international, Inc, USA) was performed for 1 hour at 4℃. The antigen–antibody complexes were visualized by the enhanced chemiluminescence (ECL) system.

**Statistical analysis**

The McNemar test was applied to detect changes in the amount of MYH expression.

**Results**

Oral cancer patients are largely betel quid chewers and cigarette smokers. Tissue specimen from 62 patients with oral cancer and three with papilloma were collected at Changhua Christian Hospital in 2005. The clinical characteristics of cancer patients are summarized in Table 1 and 2. None of the female patients are habitual consumers of betel quid, cigarette, or alcohol. In contrast, 46 out of 54 male patients were betel quid chewers; 48 out of 54 were smokers; 20 out of 54 were drinkers. Almost all chewers were smokers. Of these 62 patients, 54 had squamous cell carcinoma, while the other eight had verrucous carcinoma.

As for the three patients with papilloma,

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<tr>
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<td>0</td>
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<tr>
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<tr>
<td>Smoking</td>
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<td>Total number</td>
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Table 1. Distribution of betel quid chewing, cigarette smoking, and alcohol drinking within the 62 cancer patients studied.

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<thead>
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Table 2. Distribution of cigarette smoking and alcohol drinking habits in regard to the habit of betel quid chewing within the 62 cancer patients studied.
there were all betel quid chewers and cigarette smokers.

MYH expression in oral cancer, papilloma, leukoplakia, and normal oral mucosa: comparison of MYH expression in different types of oral tissue from the same patients was studied and measured using immunohistochemistry and western blot. All tissue specimens from the same patients were mounted side by side on the same slide, and exposed to the same solution for the same period of time, so the differences in color intensity in immunohistochemistry can be a reliable indicator of the differences in the amount of MYH expression. To confirm the differences in MYH expression, western blot was performed.

As shown in Table 3 and Figure 1, MYH expression in cancerous tissue, compared to that in normal oral mucosa, increased in 8 patients (8/62), remained unchanged in 25 (25/62), and decreased in 29 (29/62). MYH expression in papilloma, a benign neoplasm, increased (3/3) in all patients. Expression in leukoplakia, a premalignant lesion, increased (2/4), or remained unchanged (2/4).

**Discussion**

In 1987, the International Agency Research against Cancer (IARC) classified betel quid without tobacco as a group 3 human carcinogen. In 2003, a re-evaluation had re-classified betel quid without tobacco as a group 1 carcinogen.

In Taiwan, the number of betel quid chewers was an estimated 2.0 million. A 123-fold increase in the risk of oral cancer was found in those who smoked, drank alcohol, and chewed betel quid, compared to abstainers. Without a surprise, oral cancer is increasing rapidly in Taiwan.

Carcinogens in the daily diet are one of the important causes of human cancer. Some of these carcinogens may induce mutations in the genome with a fingerprint-like pattern. One well-known example is the p53 mutation found in hepatocellular carcinoma. A G:C to T:A transversion at the third base position of codon 249 of the p53 gene is strongly associated with chronic dietary intake of aflatoxin and hepatitis B virus infection. Similar findings are also reported in oral cancer. The G:C to T:A transversions in the p53 gene were found mainly in patients with both betel quid chewing and cigarette smoking habits. Persistence of these G:C to T:A transversions in the p53 gene may indicate a major defect in the system responsible for correcting this specific pattern of mutation.

MYH is an enzyme responsible for the correction of G:C to T:A transversions. MYH scans DNA strands during replication and removes adenes mispaired with 8-oxo-G. MYH deficiency leads to an increased frequency of G:C to T:A mutations in genes such as APC in MYH-associated polyposis tumors.

In view of these literature findings, our findings that MYH expression in cancer,
compared with that in normal oral mucosa, was increased in 5 patients (8/62), remained unchanged in 25 (25/62), and decreased in 29 (29/62), were informative. These patients have smoked on average for 22.98 years, and chewed betel quid for 19.74 years (data not shown here). Repeated stimulation on the oral mucosa from betel quid chewing and cigarette smoking is likely to have a great effect on various mechanisms in the cell. The ultimate development of oral cancer may imply that many defense mechanisms against cancer formation are impaired. MYH expression in nearly half of the cancerous tissue specimen tested is either decreased or absent. This may explain why the G:C to T:A transversions in genes like p53 and APC occurred mainly in cigarette smokers and betel quid chewers. On the other hand, MYH expression in papilloma, a benign neoplasm, or in leukoplakia, a premalignant lesion, either
increased or remained unchanged, compared to that in the normal counterpart. The changes from normal oral mucosa to papilloma, leukoplakia, or cancer are a result of repeated insults from betel quid chewing and cigarette smoking. However, cells can make different responses to the same challenge. There was no decrease or absence of MYH expression in papilloma or leukoplakia in this study, which may indicate that a functioning and intact MYH protein is an important defense against cancer development. This without surprise, the important role of MYH in the cell is already clearly demonstrated in a disease called adenomatous polyposis coli.

Although most of the patients in this study were both betel quid chewers and smokers, only a few male patient and all the female patients were not. We also analyzed the effect of betel quid chewing and smoking on MYH expression, and found no significant association. However, betel quid chewers are diagnosed as oral cancer, on average, 10 years earlier than non-chewers. Repeated chewing and smoking may hasten cancerous changes, but once cancer develops, major defenses, like MYH, are breached.

In conclusion, MYH expression in cancerous tissue, compared to that in normal oral mucosa, was decreased in half of the patients studied. On the other hand, MYH expression in papilloma or leukoplakia increased or remained unchanged compared to that in the normal counterpart. Maintenance of adequate MYH expression, and thus its function, is important in the prevention of oral carcinogenesis in betel quid chewers and smokers, non-chewers, and non-smokers alike. The mechanism of impairing MYH expression to promote earlier development of oral cancer for betel quid chewers and smokers requires further research.

References

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口腔癌組織中MYH基因表現有減弱的情形

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摘 要

口腔癌是台灣成長最快的惡性腫瘤之一。預計有兩百萬的台灣人有嚼食檳榔的習慣，而檳榔經研究發現含有致癌物，造成去氧核醣核酸(DNA)變異引發口腔癌。另一方面，MYH，一種去氧核醣核酸修復酵素，已知與大腸直腸癌病患的大腸息肉有關。本文研究檳榔食用者的非癌化與癌化口腔組織中MYH的表現。使用免疫化學法以及西方點墨法量測病患口腔組織的MYH表現。結果發現MYH表現在同一位病患癌化口腔組織中，與周圍正常口腔組織相比，呈現升高的有八位病患(8/62)；呈現相似的有二十五位病患(25/62)；而呈現減少的有二十九位病患(29/62)。此外，MYH表現在良性乳突瘤與周圍正常組織的比較，均呈現增加的情形(3/3)；在癌前病變白斑與正常組織的比較也呈現增加的情形(2/4)。總結以上，我們認為MYH的表現在檳榔嚼食者罹患口腔惡性腫瘤有相當的關聯性。

關鍵詞：檳榔，口腔癌，MYH。