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Immunoexpression of p53 and hMSH2 in oral squamous cell carcinoma and oral dysplastic lesions in Yemen: Relationship to oral risk habits and prognostic factors

Thanaa El.A. Helal [a,*], Mona T. Fadel [a], Abdalla K. El-Thobbani [b], Amira M. El-Sarhi [c]

[a] Department of Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[b] Department of Pathology, Faculty of Medicine, Sana'a University, Yemen
[c] Department of Pathology, Al-Thawra General Hospital, Sana'a, Yemen

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SUMMARY

Although several studies analyzed p53 and mismatch repair (MMR) gene expression separately in oral squamous cell carcinoma (SCC), no reports of combined assessment of both proteins in this cancer. The aim of this study was to investigate the roles of p53 and hMSH2 proteins in oral SCC as well as in oral dysplastic lesions (DL) in Yemen.

Immunohistochemistry was used to examine the pattern of expression of p53 and hmsh2 proteins in 70 oral SCC and 21 oral DL obtained from Yemeni patients.

p53 Immunoexpression was detected in 24 of the 70 oral SCC (34.3%) and 3 of 21 DL (14.3%) with no significant difference between the two groups. On the other hand, reduced expression of hMSH2 was detected in 26 of the 70 oral SCC (37.1%) and 2 of 21 oral DL (9.5%) with a statistically significant difference ($P = 0.03$). Both proteins were significantly related to the grade of tumor differentiation ($P = 0.007$ and 0.02, respectively). There was an inverse correlation between the levels of p53 and hMSH2 immunoexpression in the oral SCC ($r = 0.42$, $P = 0.01$).

This study suggested that p53 may play a role in the early stages of oral carcinogenesis, while hMSH2 may be altered in the late stages. More importantly, the roles of p53 and hMSH2 in oral carcinogenesis seem to be interrelated in the pathogenetic pathway of oral SCC. Such a relationship has not been published previously in this type of cancer and needs to be clarified in future studies.

Introduction

Oral squamous cell carcinoma (SCC) is the sixth most common malignancy worldwide.[1] Its incidence is relatively higher in Yemen due to high prevalence of tobacco smoking and qat chewing.[2,3] Oral carcinogenesis is a multistep process, but the specific molecular events involved and the sequence in which they occur is not well settled.[4,5] Several studies investigated the role of p53 mutation in oral cancer and its relation to oral risk habits especially smoking and qat chewing.[6–9]

Mismatch repair (MMR) genes are essential in reducing the accumulation of mutation and maintaining genomic stability.[10] hMSH2 is one of the mismatch repair genes that has been studied comprehensively. Inactivation of hMSH2 induces a decrease in function of mismatch repair and thus leads to microsatellite instability (MSI). MSI induces activation of oncogenes or inactivation of tumor suppressor genes, which can initiate cellular carcinogenesis.[11] The biological implication of MMR genes in oral carcinogenesis has been investigated in several studies.[12–16]

The relationship between MMR genes and the function of the tumor suppressor gene p53 is of interest. It has been extensively studied in various cancers as colorectal carcinoma,[17,18] cervical carcinoma,[19,20] hepatocellular carcinoma,[21,22] sporadic digestive tract tumors,[23] lung carcinoma[24] and gliomas.[25] The simultaneous investigation of p53 and MMR in oral cancer has been reported only by two studies,[26,27] which were based on culture and mutational analysis, respectively. The expression of hMSH2 by immunohistochemistry correlates with its gene expression alterations. Because antibody staining is more available than DNA analysis in a clinical setting, the use of immunohistochemistry may offer a relatively convenient and rapid technique for detecting the defects in the expression of hMSH2 gene.[28]

The aim of the current study was to analyze the immunoexpression pattern of p53 and hMSH2 in oral SCC and oral dysplastic lesions (DL) in Yemeni patients in an attempt to assess the relation of each protein to oral risk habits and other demographic and histologic factors. In addition, the relationship between the expression pattern of

* Corresponding author.
E-mail address: thanaahelal@hotmail.com (T.El.A. Helal).

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p53 and hMSH2 was investigated. Up to our knowledge, this immunohistochemical study has not been published previously.

Materials and methods

Specimen collection

This retrospective study was performed on formalin-fixed, paraffin-embedded resection specimens from 70 oral SCC and 21 biopsies from oral DL. These cases were retrieved from the Pathology Department, Al-Thawra General Hospital, Sana'a, Yemen. The patients' files were reviewed to know the patient's age, sex, smoking and qat chewing habits.

Histopathologic examination

Hematoxylin and eosin (H&E)-stained sections were prepared from the paraffin blocks and examined to:

1. Determine the histologic tumor type, grade of tumor differentiation[29] and depth of tumor invasion.
2. Assess the degree of dysplasia in the oral dysplastic lesions.[30]

Immunohistochemistry

The primary antibodies used were the monoclonal anti-p53 antibody (Do7) and the monoclonal anti-hMSH2 antibody (FE11). Both antibodies were supplied in a concentrated form (Zymed, San Francisco, USA) and diluted 1:100 in PBS. All retrieved paraffin blocks were used to cut 5 μm-thick sections and mounted onto positively charged slides. These tissue sections were dried overnight at 37 °C. Prior to immunostaining, the tissue sections were deparaffinized and rehydrated. Immunohistochemical technique was performed according to Hsu et al.[31] Briefly, the tissue sections were microwave pre-treated using citrate buffer solution. After cooling down at room temperature, endogenous peroxidase activity was blocked. Then, the sections were incubated with the monoclonal anti-p53 antibody for 2 h at room temperature and with the monoclonal antibody hMSH2 antibody for 18 h at 4 °C. The antibodies were detected with the avidin–biotin detection kit (Zymed, San Francisco, USA) using diaminobenzidine (DAB) as the chromogen. For each case, another tissue section was processed along all steps, except for omission of the primary antibody, served as the negative control. A tissue section from breast carcinoma known to be positive for p53 was used as a positive control for p53 immunostaining and the normal squamous epithelium included in the specimens was used as an internal positive control for hMSH2.

Interpretation of immunostaining

Only cases with clear nuclear staining for p53 and hMSH2 were considered positive. For p53, positive immunostaining results were evaluated according to Ralhan et al.[32] into: 0: negative, 1+: <10% positive cells, 2+: 10–50% positive cells and 3+: >50% positive cells. Zero and 1+ were considered negative. For hMSH2, the immunohistochemical results were scored according to Fernandes et al.[16] into: 0: negative, 1: ⩽75% positive cells and 2: >75% positive cells. Negative and 1+ cases were grouped as having reduced expression, and 2+ cases as having preserved expression.

Statistical analysis

The data were statistically analyzed using SPSS statistical software. Chi-square test and Fisher's exact test were applied to know the association of each protein expression with other factors, and also to evaluate the difference between the cancer and dysplastic groups regarding the frequency of expression of both proteins. Correlation between the levels of p53 and hmsh2 expression was calculated using Spearman's correlation coefficient (r). $P < 0.05$ was considered significant.

Results

The 70 patients with oral SCC included 40 males and 30 females. Their ages ranged from 38 to 90 years (mean age 59.4 ± 14.2). Sixty-six patients (94.3%) were smokers and 58 (82.9%) were qat chewers. Verrucous carcinoma was diagnosed in 16 of the 70 oral SCC (22.9%). Forty-six tumors (65.7%) were well differentiated, 10 (14.3%) moderately differentiated and 14 (20.0%) poorly differentiated SCC. Muscle invasion by the tumor was observed in 48 of the 52 (92.3%) specimens which included muscle tissue. The patients with oral DL were 10 males and 11 females and their ages ranged from 37 to 88 years (mean age 60.2 ± 15.2). All patients except one were smokers (95.2%) and 16 were qat chewers (76.2%). Seven lesions were mild dysplasia, 13 moderate dysplasia and 1 severe dysplasia.

P53 immunohistochemistry

Of the 70 oral SCC, 24 (34.3%) showed positive nuclear staining for p53 (Fig. 1). The pattern of 53 immunoexpression was significantly related to the grade tumor differentiation, less differentiated tumors (grades 2 and 3) showed a significantly higher frequency of p53 expression than better differentiated tumors (grade 1) ($P = 0.007$). Other factors were not significantly related to the pattern of p53 expression (Table 1).

In the 21 oral DL p53 protein was immunoexpressed in three specimens (14.3%) (Fig. 2). It was not significantly related to the patients' age or sex, smoking, qat chewing or grade of dysplasia (Table 2). No significant difference was noted between oral SCC and DL regarding the pattern of p53 expression.

hMSH2 immunohistochemistry

Twenty-six of the 70 oral SCC carcinoma (37.1%) showed reduced expression of hMSH2 protein (0 in 14 cases and 1+ in 12 cases). The remaining 44 tumors (62.9%) had preserved hMSH2 expression (2+ in 24 cases, 3+ in 20 cases) (Fig. 3). The grade of tumor differentiation was the only parameter associated with the immunoexpression pattern of hMSH2. Less differentiated tumors (grades 2 and 3) showed a higher rate of reduced hMSH2 expression than better differentiated tumors (grade 1) ($P = 0.02$). Reduced expression of hMSH2 was noted in 2 of the 21 oral DL (9.5%). The rate of reduced hMSH2 expression was significantly higher in the oral SCC than oral DL (Odds ratio = 1.6, 95% CI: 1.1–2.3, $P = 0.03$).

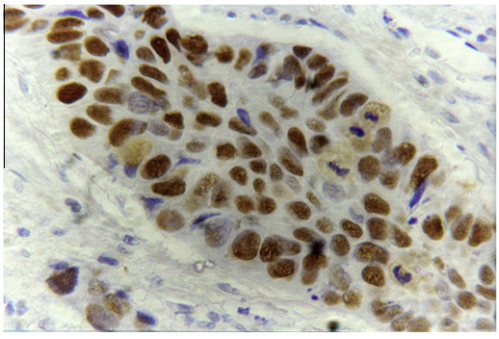

Fig. 1 A case of oral squamous cell carcinoma showing positive nuclear positivity for p53 (immunoperoxidase × 400).