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# The Role of K-Ras and PI3Kcb Expression in Oral Squamous Cell Carcinoma

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## Abstract

**Background:** Oral squamous cell carcinoma is an aggressive malignancy. It arises from premalignant lesions followed by outgrowth of clonal cell populations with cumulative genetic alterations. Phosphoinositol 3-kinases are a family of related intracellular signal transducer enzymes capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). Phosphatidylinositol-3-kinases involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer. RAS is a signal transduction protein for many important cellular processes such as cellular proliferation, differentiation, and survival by mediating the cell's response to extracellular stimulations. Kras, is a protein within the RAS family, function in the same pathway of a Ras –Raf -Mek-Erk-map kinase pathway which plays a role in mediating cellular response to cell growth. Kras appears to be involved in signal transduction and cell cycle regulation. To dates there has been limited previous investigation of protein expression of both PI3Kcb and Kras in oral squamous cell carcinoma. This study was performed to examin the expression of PI3Kcb and kras in oral squamous cell carcinoma in comparison to normal oral mucosa and to correlate the expression of both markers with clinicopathological findings including tumor grade and stage and with each other.

**Materials and Methods:** This study included 31 selected cases of oral squamous cell carcinoma pro and retrospectively and 10 cases normal oral mucosa. PIK3cb and Kras expression has been detected by immunohistochemistry.

**Results:** Results of the present study revealed positive PI3Kcb and kras expression in all examined oral squamous cell carcinoma cases. Immunostaining results for kras & PI3kcb were detected as brown stain for cell membrane and cytoplasm in tumor cells while there was no expression of both markers in normal oral mucosa. No significant correlation was observed between both markers and the clinicopathological finding of oral squamous cell carcinoma while highly significant positive correlation was observed between the two markers.

**Conclusion:** The Present study provides additional evidence that Kras, and PIK3cb over expression are common in contribution to oral squamous cell carcinoma tumorigenesis, and that PI3Kcb signaling network plays an important role in oral squamous cell carcinoma and thus, pathway-specific therapies targeting these two pathways should be considered in the future treatment of oral squamous cell carcinoma patients.

**Key words: oral squamous cell carcinoma, signal transduction, Kras, and PIK3cb.**

## Introduction

Cancer of the oral cavity is a subset of head and neck squamous cell carcinoma that ranks 11th in frequency and 13th in cancer-specific mortality worldwide [(1)].

Although significant progress has been made in early detection, diagnosis, and treatment, the 5-year survival rates for patients with oral cancer have not improved in the past 25 years and remain ~50%. Consequently, there has been an increasing focus on identifying key genetic players that may contribute to OSCC pathogenesis, with the overall goal of preventing onset and progression of disease. To date, numerous oncogenes and tumor suppressor genes have been implicated in the development of OSCC. Of interest in this regard are mutations in the oncogenes KRAS and PIK3CA [(2)].

Since amplification of Kras in oral cancer results in an overactive mitogenic signal, in turn resulting in an increased proliferative response that contributes to the etiology of OSCC, this study conducted to characterize the biological role of Kras & PIK3cb expression in OSCC and provide evidence that Kras plays an important unrecognized growth-promoting role, to gain a comprehensive view about PIK3cb regulation, and attempt to compare the results with the clinicopathological data.

## Materials and Methods

Thirty one tissue samples (31) of parraffine-embeded blocks of OSCC were involved in this study. Twenty cases of them were prospective and eleven were retrospective obtained from all over Iraqi cities, Department of oral pathology, College of Dentistry, University of Baghdad and from Ghazi hospital lab (Baghdad) from the years 2000-2011.

Clinicopathological data in regard to age, gender, lesion site and clinical presentation were obtained from the case sheets. All Hematoxylin and Eosin stained tissue sections were reviewed, the best sections and those representing the original tumor site from each specimen were selected. Ten samples of histologically normal oral mucosa, from patients (matched for age and gender) who needed surgical removal of impacted wisdom teeth and frenectomy, were included in the present study as control group. The samples were fixed and processed as the test samples. Positive control is used for indication the properness of the staining techniques. One positive control was used for each set of test runs as well as all reagents were applied on the positive control. Normal skin was used as positive control for Kras and PI3Kcb. The region of staining viewed at 400 magnifications was scored as follows. Kras and PI3Kcb expression of three punches per each case were evaluated and regarded as a whole. The immunoreactivity was evaluated on a semi quantitative scale considering the percentage of positive cells (score: 0–4 for respectively, <5, 5–20, 20–40, 40–80, >80%) [(3)]. In tumors displaying heterogeneous staining, the predominant pattern was considered as a score.

All cases were divided into four expression groups according to their scores which were as follows: score 1=1-20 score 2=20-40 score 3=40-70 score 4=>70[(3)]. Statistical analyses

were done by using SPSS (statistical package for social sciences) V17 (2008).

## Results

Immunohistochemical staining with Kras & PI3Kcb monoclonal antibody showed that Kras & PI3Kcb expression was positive in all examined OSCC specimens. Kras and PI3Kcb expression scores were evaluated and calculated according to the scoring system and revealed a percentage results for each number of cases as illustrated in table (1) & (2).

Immunostaining results for Kras &PI3Kcb were detected as brown stain for cell membrane and cytoplasm in tumor cells as shown in (figure 1) while there was no expression for both markers in normal oral mucosa. Concerning correlation between Kras and PI3Kcb expression score, a highly significant correlation was found: (p=0.0005).

## Discussion

In the present study, immunohistochemical examination of kras in 31 cases of OSCC revealed positive expression in all studied cases with no expression in normal oral mucosa.

This finding come in accordance with previous study performed by Hoa and his team as they noted over expression of the Kras protein by reverse transcriptase-polymerase chain reaction (RT-PCR) in the HNSCC cell lines [(4)].

Regarding immunohistochemical expression of Kras in OSCC there was only one previous study deal with analysis of the expression of H-ras, K-ras and N-ras oncogenes on frozen sections of squamous cell carcinoma of the head and neck by immunohistochemistry using anti-RAS monoclonal antibodies. They found that, Of 32 primary HNSCC, 15 (68%) stained positive for H-ras, 10 (45%) for K-ras and 7 (32%) for N-ras [(5)].

Thus, results of present study provide further evidence of the important role of RAS family in oral carcinogenesis.

As far as correlation of Kras expression with clinicopathological parameters of OSCC is concerned, results of the present study showed statistically nonsignificant difference neither among different ages nor between males and females. These findings agree with previous available one study on Kras expression in OSCC that revealed no correlation with age and gender [(5)].

Similarly non-significant correlation was found in the present study between Kras over expression and different tumor site, histological grade and stage of OSCC; again these findings come in accordance with previous study that showed no correlation with site of occurrence of tumor [(5)].

These findings may be attributed to the limited number of tumor cases included and method of sample collection.

In the present study, we employed immunohistochemistry method in an attempt to specifically detect abnormalities that affect PI3Kcb subunit in 31 OSCC cases; results revealed positive expression of PI3Kcb in all studied cases with no expression in normal oral mucosa. High expression scores formed 45.16% of positive cases.

Regarding correlation between Kras and PI3Kcb expression in OSCC, results of the present study revealed a highly significant positive correlation.

Although no previous study highlights this correlation, the present findings support the suggestion given by previous study about the importance

of evaluating the RAS - RAF-MEK - ERK - MAP kinase and PI3KCA - PTEN - AKT pathways together to improve their correlation in OSCC tumorigenesis [(2)].

The present study provides evidence that oncogenic activations of these pathways include additional mechanisms other than small mutations such as over expression.

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Table 1 Kras expression scores in 31 cases of OSCC:

| Kras expression | No. | % |
|---|---|---|
| Score 1 | 1 | 3.23 |
| Score 2 | 6 | 19.35 |
| Score 3 | 8 | 25.81 |
| Score 4 | 16 | 51.61 |

Table 2 PI3Kcb expression scores in 31 cases of OSCC:

| PI3Kcb expression | No. | % |
|---|---|---|
| Score 1 | 1 | 3.23 |
| Score 2 | 5 | 16.13 |
| Score 3 | 11 | 35.48 |
| Score 4 | 14 | 45.16 |

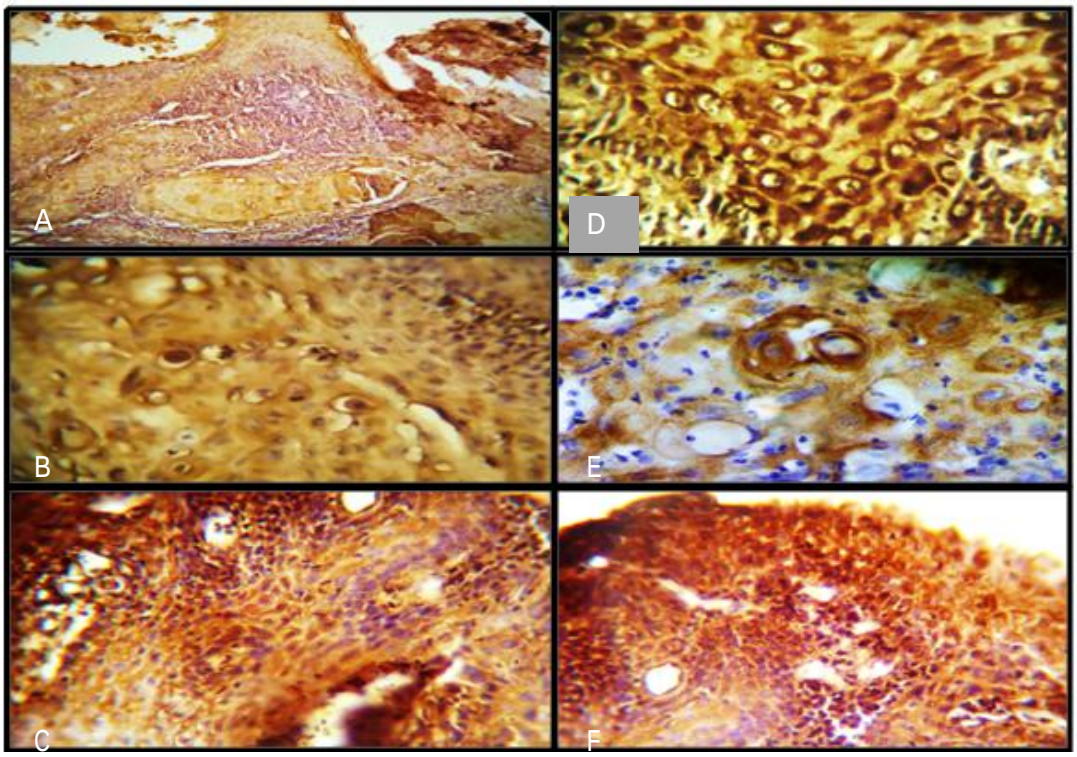


Fig 1: Immunohistochemical expression of Kras (A-C), and PI3Kcb (D-F) in well, moderate and poor differentiated OSCC respectively.