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Progressive rise of c fos expression from premalignant to malignant lesions of oral cavity

Ritesh Sachdev, Ashish Kumar Mandal, Ishwar Singh, Arun Kumar Agarwal

Department of Pathology and ENT, Maulana Azad Medical College, Bahadur Shah Jafar Marg, New Delhi. India

Correspondence:

Dr. Ritesh Sachdev
Flat No A 803, Plot No 7 A,
Navrattan apartments,
Sector 23, Dwarka,
New Delhi 110075
India
E-mail: sachdev05@gmail.com

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Abstract

Objectives: Oral cancer is a worldwide phenomenon. It is thought to develop in a number of premalignant lesions. Protooncogene c fos is a known transformer of premalignant to malignant lesions. However, its role in oral carcinogenesis is not yet known.

Study design: A total of 130 cases were studied comprising of premalignant lesions (n=50), squamous cell carcinoma (n=50) and controls (n=30). c fos expression was studied by immunohistochemistry.

Results: Premalignant lesions of oral cavity occurred at a lower age group (mean 39.2 years) compared to squamous cell carcinoma (mean 51.8 years), $p < 0.001$. Mean c fos percentage positivity in squamous cell carcinomas (SCC), premalignant lesions and controls was $44.5 \pm 36.9\%$, $11.4 \pm 18.8\%$ and $1.23 \pm 2.6\%$ respectively, the differences were highly significant ($p < 0.001$). c fos positivity also increased from mild ($10.85 \pm 14.23\%$) to moderate dysplasia ($19.64 \pm 26.05\%$).

Conclusions: The serially increasing c fos expression from normal mucosa to premalignant lesions to SCC and in dysplasias suggests that it could be an early gene to get activated and form transcription factor activator protein -1 (AP-1).

Key words: c fos, squamous cell carcinoma, oral cavity, premalignant lesions.

Introduction

Oral cancer is one of the ten most common cancers in the world (1). Its high frequency in Central and South East Asian countries has been well documented. Epidemiological studies have shown that the worldwide incidence and mortality rates for oral cancer have increased considerably over the last decade. There were annual increases in oral cancer mortality from 1975-94, of 25% and 9% in males and females, respectively (2).

Oral cancer is preceded by a number of precursor lesions; these include leucoplakia, erythroplakia, lichen planus and oral submucous fibrosis. Various factors such as tobacco,

alcohol and diet (1) have been implicated in the causation of these premalignant and malignant lesions.

Oral cancer is amenable to primary prevention. In India and its subcontinent, tobacco is the single most important causative agent. It is used in various forms, most popular being chewing, either alone or in combination with areca nut and betel leaves.

The proto-oncogene c-fos is a major nuclear target for signal transduction pathways involved in the regulation of cell growth, differentiation and transformation (3). c fos protooncogene has a cellular homologue of v fos which is the transforming gene identified originally in the FBJ mu-

rine osteosarcoma virus(3). Under normal circumstances c fos plays an important role in cell differentiation in various organs. The flip side to differentiation is tumorigenesis, as happens when c fos gets over expressed, leading to development of osteosarcomas and chondrosarcomas(3). Thus, this paper aims to find whether similar carcinogenic process is involved in oral carcinogenesis.

Materials and Methods

A total of 130 cases were biopsied. These cases were further divided into premalignant (n=50) and malignant lesions (n=50) of oral cavity. 30 cases of normal oral mucosa with no evidence of dysplasia served as controls. The premalignant lesions were clinically diagnosed as leucoplakia and oral submucous fibrosis. These lesions were graded into mild and moderate dysplasia (4). None of the premalignant cases had histological evidence of severe dysplasia. A detailed history in a printed questionnaire form with special emphasis on tobacco, betel quid and betel nut chewing and cigarette and bidi smoking was recorded. All the biopsies were processed routinely for histopathological diagnosis and multiple sections were cut from paraffin embedded blocks for c-fos immunohistochemical staining.

- Immunohistochemistry of c fos

Immunohistochemistry for c fos was done on 4-micron thick paraffin sections using the standard peroxidase anti-peroxidase method. Positive and negative controls were put up with every batch. 4-micron paraffin sections were brought to water. Blocking was done by 0.03% hydrogen peroxide for 30 minutes. Non-specific blocking was done by 5 % skimmed milk for 30 minutes. After washing with PBS, primary antibody (Novocastra, lyophilized mouse anti-human, dilution 1in 20) was put and kept overnight at 4 degrees in a moist chamber. Secondary antibody (Novocastra) was added at room temperature for 30 minutes followed by tertiary antibody (Novocastra) and chromogen DAB. The sections were counterstained with hematoxylin. Only nuclear staining was considered positive. A total of 500 cells were counted at 450 magnifications and percentage of positive cells was calculated in each case. This was expressed as percentage positivity of c fos. Adding the c fos percentage positivity of individual cases and dividing by the total number of cases also calculated the mean percentage c fos positivity of the group (control, premalignant and malignant).

- Statistical analysis

Kruskal – Wallis one-way analysis of variance was done for comparison of percentage positivity between various groups. The p value of <0.05 was taken as significant while p < 0.01 was highly significant.

Results

- Age and sex distribution:

All the cases were Indians. In the squamous cell carcinoma (SCC) group there were 45 males and 5 females. The ages

ranged from 30-80 years with a mean age of 51.8 years. Largest number of cases was in the age group more than sixty years (Table 1). The premalignant group comprised of 40 males and 10 females. The ages ranged from 18-65 years, the mean age being 39.2 years. The maximum number of cases was in the age group 20-29 years (Table 1). This difference in age distribution between SCC and premalignant lesions was statistically significant (p<0.001). In the control group there were 25 males and 5 females. The mean age was 36.6 years (range 22-62 years). Largest number of cases was in the age group 30-39 years (Table 1). There was no relation of c fos with age and gender.

- c-fos Expression

** Squamous cell carcinoma group*

Thirty five out of fifty (70 %) cases of squamous cell carcinoma showed c fos positivity; the mean percentage positivity of the group being 44.5±36.9 % (Table 2, Figure1).

Table 1. Age and sex distribution in squamous cell carcinoma, premalignant lesions and controls.

Age group (Years)	SCC		Premalignant		Controls	
	M	F	M	F	M	F
10-19	0	0	1	1	0	0
20-29	0	0	13	3	7	0
30-39	4	2	5	1	9	1
40-49	13	2	8	2	6	2
50-59	11	1	8	2	2	1
≥ 60	17	0	5	1	1	1
Total	45	5	40	10	25	5

SCC: Squamous cell carcinoma, M: Males, F: Females

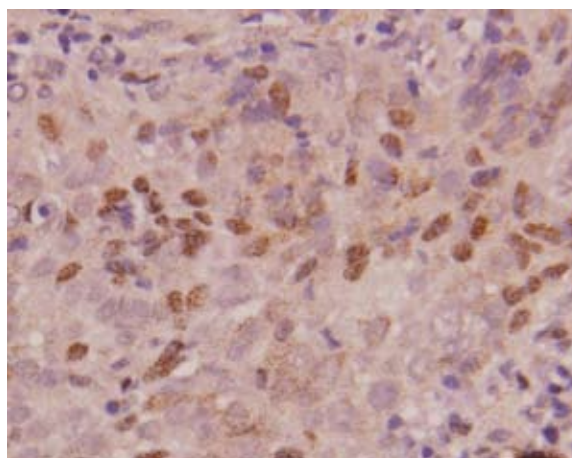


Fig. 1. c fos positivity in squamous cell carcinoma (High power 400X)

Table 2. c-fos expression in squamous cell carcinoma, premalignant lesions and controls.

Group	Number of cases (Number of positive c)	c fos Percentage positivity Mean±S.D
Squamous cell carcinoma	50(35)	44.5±36.9%
Premalignant	50(19)	11.4±18.8%
Mild dysplasia	36(12)	10.85±14.23%
Moderate dysplasia	14(7)	19.64 ± 26.05%.
Controls	30(5)	1.23±2.6%

p<0.001, Kruskal Wallis test

* Premalignant group

Nineteen out of fifty (38%) premalignant cases showed c-fos positivity; mean positivity was 11.4±18.8 % (Table 2). The premalignant cases showed thirty-six cases of mild dysplasia, twelve of which (33%) showed c fos positivity; mean positivity being 10.85% ±14.23%. Out of remaining fourteen cases with moderate dysplasia, seven (50%) were c fos positive, mean positivity being 19.64 ±26.05%. There was no case of severe dysplasia.

* Controls

Five out of thirty (16%) cases of controls (normal mucosa) were positive for c fos. However the intensity and percentage positivity was low as compared with dysplastic and malignant cases. The mean c fos percentage positivity was 1.23 ± 2.6%.

- Statistical comparison between different lesions

The mean percentage c fos positivity in squamous cell carcinoma group (44.5 ± 36.9%) was significantly higher than the controls (1.23 ± 2.6%) p<0.001 (Table 2). c fos expression of squamous cell carcinoma group was also significantly higher than that of premalignant group (p<0.001).

The premalignant group also had a significantly higher c-fos percentage positivity (11.4 ±18.8%) than that of controls (1.23 ± 2.6%), p<0.001 (Table 2). c fos percentage positivity in mild dysplasia was 10.85% ± 14.23% which was also significantly higher than that of control group p<0.001, (Table 2). c fos expression increased from mild to moderate dysplasia (19.64 ± 26.05%). However the differences were not statistically significant.

Thus c fos expression increased serially from normal mucosa (1.23 ± 2.6%) to premalignant lesions (11.4 ± 18.8%) to squamous cell carcinoma (44.5 ± 36.9%). This increasing expression of c fos was statistically significant (p<0.001).

Discussion

Various risk factors have been implicated in the causation of oral cancer. These include use of tobacco in a variety of forms like smoking, chewing, etc (5, 6). Alcohol use has also been implicated in the etiology of oral cancer and has a synergistic effect on tobacco users (7). Other factors include diet, oral hygiene, etc. A continuous exposure to these carcinogenic substances leads to development of

various premalignant lesions. However, there is a time lag between the appearance of premalignant lesions and its progression to carcinoma as was seen in our study. The majority of premalignant lesions were in the age group of 20-29 years compared to squamous cell carcinoma where majority occurred in more than sixty years age group. The mean age of premalignant lesions was 39.2 years while SCC showed a mean age of 51.8 years. Thus squamous cell carcinoma developed almost after a decade of premalignant lesions. This is possibly the time taken by these premalignant lesions to progress to malignant transformation.

Unlike other countries oral and oropharyngeal carcinoma is the commonest carcinoma in Indian males, the male: female ratio being 2.3:1. We also found a male preponderance. This predilection for males is due to high use of tobacco in its various forms, chewing being the most popular. Tobacco is chewed alone or in combination with areca nut and betel leaves. This exposes the oropharyngeal mucosa to multiple carcinogenic substances not only from tobacco but also from areca nut (8, 9).

Carcinogens in tobacco smoke are known to cause squamous cell carcinoma of various organs and it probably requires repeated exposure (5). Tobacco derived carcinogens probably act in a multistep carcinogenesis to induce c fos proto oncogene (10). This is a major nuclear target for signal transduction pathways involved in the regulation of cell growth, differentiation and transformation (3). The proto-oncogene c fos encodes a nuclear DNA binding phosphoprotein that together with the product of the proto-oncogene c Jun or other members of Jun family forms the heterodimeric transcription factor AP-1(11) that plays a pivotal role during tumorigenic transformation. Over expression of c fos has been found in a number of malignancies such as cervical cancer (12), lung cancers (13), pancreatic carcinomas (14), osteosarcomas (15), hepatocellular carcinoma (16) and colorectal adenoma (17). There are scarce reports on the role of c fos in oral carcinogenesis. Turatti et al (18) reported intense c fos expression in normal mucosa, reduced in mild dysplasia and increased in moderate dysplasia and squamous cell cancer. Our study differs from that of Turatti et al. Ours is a larger study of 130 cases reflecting a more relevant statistical correlation. Furthermore, we report a serially in-

creasing c fos expression with normal oral mucosa having the lowest, premalignant lesions having intermediate and SCC having the highest expression. We found that c fos is over expressed in 70% of squamous cell carcinoma of oral cavity, 38% of premalignant lesions and 16% in normal oral mucosa. Similar study was done by Cheung et al (12) to evaluate the role of c fos in cervical malignant and premalignant lesions. They found that c fos is over expressed in cervical carcinoma (59%) compared to premalignant lesions (10%) and had no expression in normal cervical tissue. Similarly Lee et al (14) also found c fos expression in 75% of adenocarcinoma, 22% of chronic pancreatitis and 40% of normal pancreas. Thus, these studies along with ours prove that c fos expression increases significantly as we move from normal to premalignant to squamous cell carcinoma, the premalignant cases being intermediate. This variation in positivity is statistically significant. The result suggests a role of c fos in malignant transformation in oral cavity. It was further seen that moderate dysplasia had more c fos expression compared to mild dysplasia. Thus c fos plays an important role in malignant transformation and its progression and thus is an early event starting at the level of leucoplakia or oral submucous fibrosis and progresses to develop dysplasia and ultimately transforming to malignancy. This is different than that found by Saez et al (3) who found it to be a late event in mouse skin carcinogenesis. This being an animal model may not truly reflect the human carcinogenesis. Thus to conclude, various extra cellular and environmental stimuli such as tobacco and other tumour promoters (19), UV light (19), X-ray (20) and alkylating agents (21) induce expression of the immediate early genes such as c fos and c jun. Together they form a transcription factor AP-1(11) which plays a role in mediating tumorigenic transformation and growth, which in oral cancers takes almost a decade.

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