Original Article

KAI-1 and p53 expression in oral squamous cell carcinomas: Markers of significance in future diagnostics and possibly therapeutics

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Abstract

Context: KAI-1/CD82 is a tumor suppressor gene with decreased gene expression being associated with increased invasive ability of oral squamous cell carcinomas (OSCCs). p53 protein functions in the G1-S phase of the cell cycle to allow repair of damaged DNA. In the present study, p53 and KAI-1 expression was investigated using monoclonal antibodies in OSCC.

Aims: The aim of this study was to detect KAI-1 and p53 expression in OSCCs and to assess the relation between both in OSCCs.

Materials and Methods: The present study included histopathologically diagnosed thirty cases of well- and moderately differentiated OSCCs to study the expression of KAI-1 and p53 antibodies.

Statistical Analysis: The results obtained were tabulated and statistically analyzed using descriptive statistical analysis; one-way ANOVA; least square difference method and independent *t*-test.

Results: OSCCs exhibited 41.62% positivity for KAI-1 while p53 positive cells were recorded to an extent of 60.82%. A significant positive correlation was observed between KAI-1 and p53 expression in OSCCs.

Conclusions: Although a significant amount of work is still required to uncover the mechanisms of action and regulation of KAI-1 and p53 expression, control of the complex metastatic processes would be of interest in controlling the tumor biology in OSCCs as well as other types of malignancies to enhance prognosis in the affected patients and to help protect against future metastasis in the going to be treated and treated patients.

Key Words: KAI-1, oral squamous cell carcinomas, p53

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INTRODUCTION

Head and neck cancers include cancers originating in the oral cavity, the oropharynx, the hypopharynx and the larynx. Oral cancer is the sixth most common cancer for both sexes in the general population and the third most common cancer in developing nations. Carcinomas account for 96%

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of all oral cancers, 91% of which are oral squamous cell carcinomas (OSCCs) (Silverman and Gorsky, 1990). The 5-year survival rate for oral cancers is one of the lowest, far below the rate for many other general body cancers, including skin melanomas and cancer of the testis, breast, colon, rectum and kidneys.[1] KAI-1 is a tumor suppressor gene which is inversely related to the progression and invasion of several tumors (metastasis) as was observed by Guo *et al*.,[2] who found upregulation of KAI-1 expression in early pancreatic carcinomas with decreased expression in the presence of metastasis. Wu *et al*.[3] identified the role of KAI-1 in digestive tract carcinomas and predicted it as a useful predictor of prognosis. Farhadieh *et al*.[4] and Imai *et al*.[5] identified the role of KAI-1 in OSCCs and suggested that a decreased gene expression is associated with increased invasive ability of OSCCs. The expression of KAI-1/CD82 gene is inversely related to tumor progression and can thus be taken as a favorable prognostic indicator.[6,7] Genetic and molecular events underlying the development of metastasis have been studied extensively in the past. At present, a number of oncogenes and tumor suppressor genes, including p53, RAS, β-catenin and PTEN, have been implicated in various cancers.[8,9] p53 protein is a product of the tumor suppressor gene p53 which functions in the G1-S phase of the cell cycle to allow repair of damaged DNA and to prevent the cell from entering S phase, or alternatively, in guiding the damaged cells to apoptosis.[10-14] p53 gene has a short life span in normal cells and cannot be detected immunohistochemically (IHC); however, when mutated, p53 protein is more stable and can be detected using immunohistochemistry. Therefore, p53 protein is expressed in actively proliferating cells. While positive staining for p53 may be correlated with a genetic mutation, wild-type p53 protein can also be retained in the tissues by, for example, binding to other proteins or by some defect in the normal degradation pathway and can therefore, be identified by immunohistochemistry. Wild-type p53 protein, acting as a tumor suppressor, downregulates cell growth, but mutations in p53 can inactivate its tumor suppression activity allowing the dominant oncogenic factor to lead to malignant transformation.[10-14] In the present study, p53 and KAI-1 expression was investigated using monoclonal antibodies in OSCCs.

Aims and objectives

- To detect KAI-1 and p53 expression in OSCCs; and
- To investigate if any correlation exists between the expressions of p53 and KAI-1 in OSCCs.

MATERIALS AND METHODS

The present study included histopathologically diagnosed 30 well [Figure 1] and moderately differentiated [Figure 2] cases of OSCCs along with ten cases of normal buccal mucosa used as controls which were subjected to IHC staining for the expression of KAI-1 [Figure 3] and p53 [Figure 4] antibodies.

Principle of immunohistochemical staining

Sections were hydrated with increasing grades of alcohol and brought to distilled water and treated with hydrogen peroxide (H_2O_2) to eliminate endogenous peroxidase activity. The tissues were then incubated sequentially with:

- Primary antibody (KAI-1, C-16, sc-1087, primary antibody, rabbit polyclonal antihuman antibody, Santa Cruz Biotechnology, Inc., p-53, clone DO-7, primary antibody, mouse monoclonal anti-human antibody, DAKO), which binded to specific tissue antigens
- Secondary antibody (Biotinylated secondary antibody, DAB Chromogen, DAB Substrate Buffer, Hematoxylin, DAKO), which binded to the primary antibody; it is a polyvalent antibody that binds to primary antibodies derived from rabbit, mouse, rat and guinea pig; and (Both the antibodies were diluted to the concentration of 1:500)
- Addition of peroxidase substrate (hydrogen peroxidase) and chromogen resulted in the formation of a colored precipitate at the tissue antigen sites. Counterstaining with hematoxylin aided in visualization.

Positive and negative controls

Normal oral mucosa samples showing KAI-1 labeling for p-53 expression acted as positive controls. One positive control was included for each IHC cohort. One section from each positive control was used as a negative control by omitting the primary antibody and by incubating with tris-buffered saline.

Assessment of immunohistochemically stained sections

Sections stained with KAI-1 and p53 antibodies were examined under light microscopy. The positive controls were examined for the presence of a colored end-product at the site of the target antigen (DAB chromogen brown end-product). The presence of these colors was interpreted as positive staining, indicating proper performance of the kit reagents. The absence of nonspecific staining in the negative controls confirmed the specificity of the primary antibody. Cells were considered positive for KAI-1, when they revealed cytoplasmic membrane staining, and p53, when they showed nuclear staining (brown color), respectively. The stained cytoplasmic membranes and nuclei were scored positive regardless of the intensity of staining. Cells that lacked a clear staining were excluded. A minimum of 1000 cells were counted in each section. Tissue sections positive for KAI-1and p53 were evaluated by locating the epithelial linings which were most heavily labeled on scanning the sections at ×100. Cell counts were made at ×400 with a conventional light microscope in five randomly selected fields. KAI-1 and p53 labeled cell counting was done among all groups. The constituent cells of the lining epithelium were divided into basal, suprabasal/intermediate and surface layers. Cuboidal/columnar cells located in one row at the basement membrane were considered the basal layer. This method of counting the KAI-1 positive cells was according to Iezzi *et al*.[15]

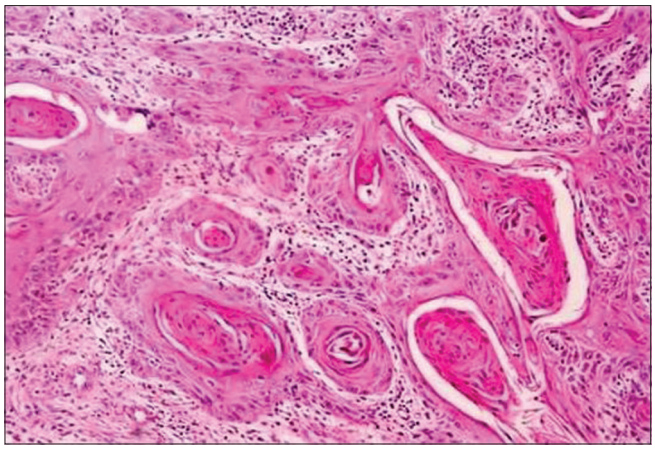

Figure 1: Photomicrograph showing a case of a well-differentiated oral squamous cell carcinoma in a patient (H&E stain, ×40)

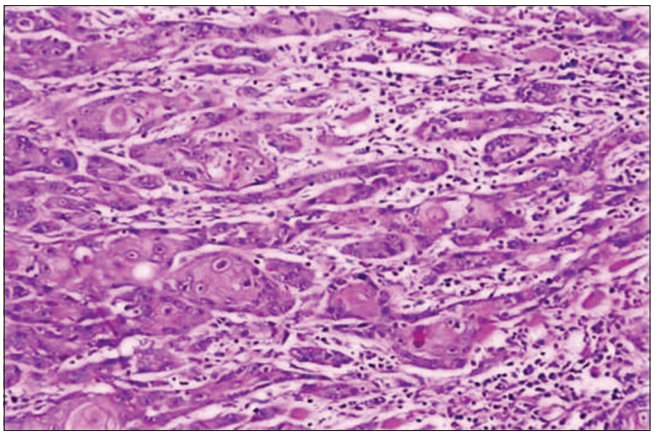

Figure 2: Photomicrograph showing a case of a moderately differentiated oral squamous cell carcinoma in a patient (H&E stain, ×40)

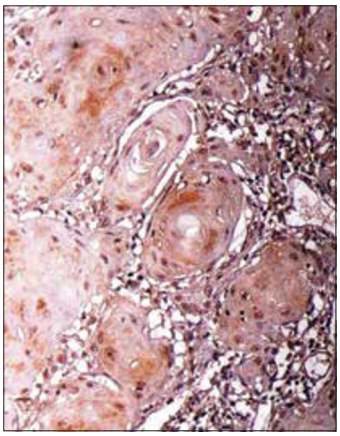

Figure 3: KAI-1 expressivity in oral squamous cell carcinoma (IHC stain, ×100)

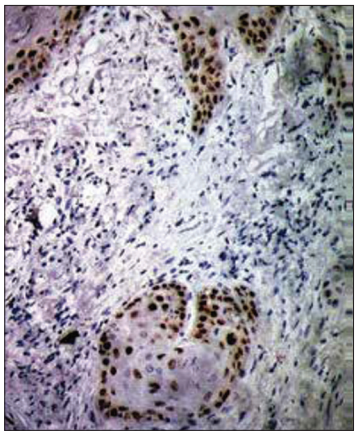

Figure 4: p53 expressivity in oral squamous cell carcinoma (IHC stain, ×100)

The surface layer constituted flattened or polygonal cells consisting of one to five layers, localized just beneath the surface of the lining epithelium. The suprabasal/intermediate layer was composed of relatively large round cells between the basal and the surface layers. KAI-1 expression in the epithelium was converted into score defined by Farhadieh *et al*.,[4] with score 1 assigned for <10% of cells, 2 for 11–30%, 3 for 31–50% and 4 for >51% of the total number of cells with positive staining. The cells which were positive for KAI-1 expression were divided according to their scores. These scores were given after the inspection of neoplastic fields at higher magnification. The numbers of positively stained nuclei were expressed as a percentage of the total number counted for the individual layer and in the complete epithelium.

$$\text{KAI-1 / p53 labeling index} = \frac{\text{Number of IHC Positive Cells (KAI-1 / p53)}}{\text{Total number of cells observed}} \times 100$$

Statistical analysis

The results obtained were tabulated and statistically analyzed using descriptive statistical analysis; one-way ANOVA; least square difference (LSD method) and independent *t*-test.

RESULTS

An IHC analysis was carried out for the evaluation of KAI-1 and p53 expression in histopathologically diagnosed 30 well- [Figure 1] and moderately differentiated [Figure 2] cases of OSCCs along with 10 cases of normal buccal mucosa that were used as controls. KAI-1 expression in OSCC cases showed no cases recorded with score 1. The number of cases recorded with score 2 was 10 with their mean of 21 (±5.23) and confidence interval (CI) of 17.77–24.22. The number of cases with score 3 was 9. The mean, standard deviation (SD) and CI were 41.38, ±7.29 and 36.65–46.12, respectively. The number of cases with score 4 was 11 and was highest among the other scores with their mean of 60.56 (±6.25) and CI of 56.89–64.24 [Table 1]. p53 expression in OSCC cases showed no cases recorded with score 1 and 2. The number of cases recorded with score 3 was 5 with the mean of 36.63 (±6.59)

and CI of 30.88–42.38. The number of cases recorded with score 4 was 25 with the mean of 65.66 (±9.05) and CI of 62.13–69.19 [Table 1]. Descriptive statistical analysis was performed for KAI-1 and p53 expression and further for comparing their expression in OSCCs and normal buccal mucosa. KAI-1 counts were observed in OSCCs with the mean of 41.62 (±17.87) as against normal buccal mucosa with the mean of 24.28 (±4.15). [Table 2]. In case of p53 counts in OSCCs, a mean of 60.82 (±13.96) as against a mean of 3.44 (±2.32) for normal buccal mucosa was obtained [Table 2]. The LSD *post hoc* tests for KAI-1 and p53 expression in OSCCs and normal buccal mucosa were also found to be highly significant with a mean difference of -3.36 (±4.14) in case of KAI-1 expression [Table 2] while 0.61 (±2.50) for p53 expression [Table 2]. Comparison between the expressivity of KAI-1 and p53 in OSCCs and normal buccal mucosa, carried out, was again found to be statistically significant and with a positive correlation [Table 3].

Table 1: KAI-1 and p53 expression in oral squamous cell carcinomas samples

Score	Number of cases positive	Mean	SD	CI
KAI-1				
1	Nil	Nil	Nil	Nil
2	10	21.00	5.23	17.77-24.22
3	9	41.38	7.29	36.65-46.12
4	11	60.56	6.25	56.89-64.24
p53				
1	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil
3	5	36.63	6.59	30.88-42.38
4	25	65.66	9.05	62.13-69.19

CI: Confidence interval, SD: Standard deviation

Table 2: Least significant difference *post hoc* test analysis between KAI-1 expression in OSCC and normal buccal mucosa samples

Variable 1	Variable 2	Mean	SE	*P*	95% CI	
					Upper bound	Lower bound
OSCC	Normal buccal mucosa	17.34 57.39	4.14 2.56	0.00** 0.00**	9.15 52.33	25.54 62.45

**Significance at $P<0.01$. CI: Confidence interval, SE: Standard error, OSCC: Oral squamous cell carcinomas

Table 3: Comparison between KAI-1 and p53 expression between the groups by independent *t*-test

Sample	Variable	Mean	SD	SEM	*t*	*P*	95% CI	
							Lower bound	Upper bound
OSCC	KAI-1	41.62	17.87	3.26	−4.64	0.00**	−27.50	−10.90
	p53	60.82	13.96	2.55				
Normal buccal mucosa	KAI-1	24.28	4.15	1.31	13.86	0.00**	17.62	24.06
	p53	3.44	2.32	0.74				

**Significance at $P<0.01$, CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

DISCUSSION

OSCCs are the most common malignant neoplasms of the oral cavity. OSCCs are the sixth most common malignancy in the world today. Despite advances in treatment, the overall 5-year survival rate of these patients remains relatively low. Metastasis, the main cause of death in most cancer patients, remains the most important but the least understood aspect of cancers. The main reason for treatment failure and death of patients with OSCCs is the locoregional recurrence and metastasis. The high incidence of oral cancer and precancerous lesions has been linked to the chronic use of tobacco and smoking.[1] Identification of groups at high risk for tumor metastasis, thus, is an important part of the research for cancer management. There are several methods for predicting the metastatic potential of cancer cells but none is completely reliable. Advances in molecular biology have made it possible to investigate tumor growth and metastasis at the molecular level with a certain degree of accuracy. KAI-1 has been detected in normal human tissues as a regulator of cell behavior. The expression of KAI-1 is supposed to decrease in cancer cell lines derived from metastatic prostatic tumors, pancreatic carcinoma, bladder carcinoma, breast carcinoma and esophageal carcinoma along with OSCCs.[3-7] Cancer cells expressing KAI-1 attach to vascular endothelial cells through direct interaction between KAI-1 and DAR (an endothelial cell surface protein) leading to the inhibition of tumor cell proliferation and induction of senescence.[16,17] The tumor metastasis is suppressed mainly by an inhibition of cancer cell motility and invasiveness.[6,18-20] p53 protein, on the other hand, functions in the G1-S phase of the cell cycle to allow repair of damaged DNA and to prevent the cell from entering S phase, or alternatively, in guiding the damaged cells to apoptosis.[10-14] In the present study, immunohistochemistry for KAI-1 and p53 was employed to evaluate cell proliferation and aggressive behavior in OSCCs. Totally, 30 cases of OSCCs along with 10 cases of normal buccal mucosa were subjected to immunohistochemistry for KAI-1 and p53 expressivity. IHC expression of KAI-1 and p53 protein in the epithelia of the included samples was done to correlate the expression of either of these biomarkers with their biological aggressiveness. There is a documented proof that downregulation of KAI-1 is associated with increased metastasis. In the present study, KAI-1 expression was significantly high in normal mucosa (24.28%) while very few densely stained cells were located in the basal cell layers in normal oral mucosa on p53 immunolabeling. Although a large body of work exists regarding the significance of p53 expression, the significance of increased or decreased KAI-1 expression in the aggressiveness in nonneoplastic lesions remains as yet unclear. Oral cancer is considered to be a multi-hit process which involves a number of aberrant genetic

events culminating into malignant transformation. Recent advances in molecular biology provide unique possibilities for studying aberrations at genetic levels. These techniques have also provided the basis for possible treatment strategies including gene therapy. Cells normally go through different stages of the cell cycle in a well-regulated manner and a number of different proteins involved in this regulation have been identified, p53 protein being one among them. Studies of genetic progression have suggested that p53 alteration occurs at a greater frequency in invasive carcinomas than in noninvasive ones.[11,21] The mutant p53 gene protein has enabled to utilize IHC methods to demonstrate the mutant p53 protein product in the tumor tissue. In the present study, thirty cases of well and moderately differentiated OSCC were studied and all cases showed positivity for p53 expression. p53 immunolabeling was dense and scattered in the epithelium [Figure 4]; however, some weakly stained cells were also seen to be distributed along with the darkly stained cells. Crosthwaite *et al*.[21] studied the expression of p53 in OSCCs and found aberrant expression of p53 early in the pathogenesis of lip cancer. Solomon *et al*.[8] suggested that the accumulation of p53 in tumor cells indicated an alteration in the cell cycle and found 66.6% of cases positive for p53. KAI-1 immunoreactivity was seen within the invading epithelial islands as well as around keratin pearls with a diffusely positive cytoplasmic membrane staining. Among the thirty OSCC samples, all showed KAI-1 positivity [Figure 3] and these also stained positive for p53 [Figure 4] suggesting that the expression of KAI-1 has a significant correlation with expression of p53 in OSCC samples. These results were in accordance with the findings of Mashimo *et al*.,[22] who observed a significant correlation between KAI-1 and p53 expression in prostate tumors and proposed KAI-1 expression to be positively controlled by p53 at transcriptional level due to the existence of p53 consensus sequence in KAI-1 promoter. Thus it was proposed that p53 could directly activate KAI-1 expression in highly metastatic cell lines. This, however, was in contradiction to the conventional role of an antimetastatic molecule, attributed to KAI-1. This was, also, in contradiction with the findings of the study conducted by Jackson *et al*.,[20] who could not detect elevation of KAI-1 mRNA levels in urothelial cell lines after transfection of either wild-type or mutant p53 gene. Thus, keeping the results in mind, further studies become mandatory to speculate whether p53 activates cellular adhesion molecules such as KAI-1 during the process of cell cycle arrest so that the cells can be sustained from immediate death and allowed time to repair DNA damage. In OSCCs, it is hypothesized that p53 activity governs KAI-1 expression and, thus, the possibility that KAI-1 may be recruited by p53 in response to specific signals and/or, particular cell types, cannot particularly be discarded.[8] However, this hypothesis requires further investigation to understand the exact role KAI-1, as very limited studies, till date, have been conducted on the KAI-1 gene expression in OSCCs.

CONCLUSION

Although a significant amount of work is still required to uncover the mechanisms of action and regulation of KAI-1 and p53 expression, control of the complex metastatic processes would be of interest in understanding the tumor biology in OSCCs as well as other types of malignancies to enhance prognosis in the affected patients and to help protect against future metastasis in going to be treated and treated patients. Despite the novel nature of this area of research; pursuits, thus are worthwhile.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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