Prognostic Implication of Survivin Expression in Oral Squamous Cell Carcinoma—An Immunohistochemical Study

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ABSTRACT

Aim: Immunohistochemical expression of survivin was analyzed among the three histological grades (well differentiated, moderately differentiated, and poorly differentiated) of oral squamous cell carcinoma (OSCC).

Materials and methods: The study material consisted of 60 formalin-fixed paraffin-embedded tissue samples: 15 cases each of well, moderate, and poorly differentiated OSCC and normal oral mucosal (NOM) tissues as the control. Survivin expression was evaluated immunohistochemically and statistical analysis of data was performed using Fisher's Chi-square and analysis of variance (ANOVA) tests.

Results: Survivin was expressed in all grades of OSCC, but absent in normal oral tissue samples. Poorly differentiated OSCC exhibited 51 to 75% immunopositivity (53.3%) and severe staining intensity (46.7%) for survivin, predominantly in nuclear areas. While moderately differentiated OSCC had 26 to 50% immunopositivity (40%) and moderate staining intensity (80%), 5 to 25% immunopositivity (40%) with moderate staining intensity (86.7%) was observed in well-differentiated OSCC. Overall, there was a statistically significant difference among the three grades of OSCC in relation to survivin immunopositivity and immunoreactivity ($p < 0.01$).

Conclusion: This study supports the use of survivin as a potent diagnostic and prognostic marker for OSCC.

Clinical significance: Increased survivin expression and its nuclear localization appeared to correlate with a higher grade of malignancy suggesting unfavorable prognosis.

Keywords: Immunohistochemistry, Oral cancer, Prognostic marker, Survivin.

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INTRODUCTION

Oral and pharyngeal cancer, grouped together, is the sixth most common cancer in the world. According to the International Agency for Research on Cancer of the World Health Organization (IARC-WHO), cancer rates are expected to increase at an alarming rate, from 10 million new cases globally in 2000 to 15 million in 2020.¹ In the oral cavity, squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm. The major risk factors for oral SCC (OSCC) are the use of tobacco or betel quid chewing, regular alcohol consumption, high-risk human papilloma virus (HR-HPV) infection, and diet containing lesser amounts of fresh fruits and vegetables. According to WHO, SCC of head and neck can be graded based on epithelial differentiation into well, moderate, and poorly differentiated types.²

Survivin is one of the seven members of inhibitors of the apoptosis protein (IAP) family. The gene encoding human survivin was cloned by Ambrosini in 1997. All survivin isoforms contain one of the characteristic N-terminal BIR (Baculovirus IAP Repeats) domains and an alpha-helical region. The BIR domain is supposed to be important for antiapoptotic function, whereas the alpha helical domain interacts with tubulin structures. Survivin is expressed highly at the G2/M phase and declines rapidly in the G1 phase of the cell cycle. IAP family members inhibit apoptosis by inhibiting one or more caspases. Survivin is expressed diffusely during fetal development but is not generally found in normal adult tissues. It has been detected in various human cancers including bladder, colon, liver, brain, lung, and prostate and its expression levels correlate with their aggressiveness, implying poor prognosis and increased resistance to radiotherapy and chemotherapy.³ Nuclear survivin is assumed to control cell division, whereas cytoplasmic survivin is considered cytoprotective. Its selective

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expression makes it a lead target for tumor diagnosis, prognosis, and anticancer therapies. Therefore, the present study aims to analyze the immunohistochemical expression of survivin in all the three histological grades of OSCC (well, moderate, and poorly differentiated OSCC) to evaluate its prognostic implication.

MATERIALS AND METHODS

Prior to the commencement of the study, ethical approval was obtained from the institutional review board. This retrospective study was done by retrieving formalin-fixed paraffin-embedded tissue specimens (FFPE) of OSCC from the archives of Oral Pathology Department, Best Dental Science College & Hospital, Madurai, India. The study material was composed of 60 FFPE tissue specimens that are divided into 4 groups: 15 cases each of well-differentiated (WDSCC), moderately differentiated (MDSCC), and poorly differentiated OSCC (PDSCC) and normal oral mucosal (NOM) tissue specimens.

Immunohistochemical Staining (IHC)

Four micron thickness tissue sections were prepared and mounted on poly L-lysine-coated glass slides. Antigen retrieval was performed by heating the slides immersed in citrate buffer at pH 6 in a microwave oven. Primary rabbit monoclonal antibodies against survivin (path *in situ*) were used. Biotinylated anti-rabbit IgG (Biogenex Supersensitive Detection system) was used as a secondary antibody and the bound antibody was detected using streptavidin-conjugated horseradish peroxidase (Biogenex Supersensitive Detection system) with 3,3'-diaminobenzidine as a substrate and Harris' hematoxylin as the counterstain. The high-grade human breast carcinoma specimen showing a strong expression for survivin was used as a positive control. Negative control was prepared by staining the positive control breast cancer specimen with the secondary antibody alone.

Criteria for Evaluation

Immunohistochemically stained slides were viewed by two observers using an Olympus BX 53 light microscope and compared with their respective H and E sections. Photomicrography was done with Prog Res Speed XT Core 3 software. Survivin immunopositivity was assessed by the presence of a brown color immunostaining of the nucleus and cytoplasm.

The intensity of staining was estimated based on the criteria followed by Nakagawa et al.⁴: no staining (0); mild (1); moderate (2); and severe (3). The percentage of survivin immunopositivity was categorized based on the criteria adopted by Muzio et al.⁵ It was estimated and graded, in five random fields, on a scale of 0 to 4: <5% immunopositive cells (0); 5 to 25% immunopositive cells (1); 26 to 50% immunopositive cells (2); 51% to 75% immunopositive cells (3); and >75% immunopositive cells (4). The immunoreactivity of survivin was assessed semi-quantitatively by calculating the immunoreactive score (IRS) which is a product of the percentage of immunopositive cells and staining intensity. It was scored as negative (0–1); mild (2–3); moderate (4–8); and strongly positive (9–12).

Statistical Methods

Statistical analysis was done using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 22.0 for Windows). Fisher's Chi-square test was opted to compare the variance in distribution, intensity, percentage of immunopositivity, and also to assess pairwise comparison between the study groups. ANOVA was used to compare the immunoreactivity score of survivin within the four groups. "*p*" value less than 0.05 denotes a significant relationship ($p > 0.05$ —not significant, $p \leq 0.05$ —significant, $p \leq 0.01$ —highly significant, and $p \leq 0.001$ —very highly significant).

RESULTS

Survivin expression was found predominantly in the nuclear region of malignant epithelial cells in most cases of WDSCC (66.7%) (Figs 1A and B), MDSCC (60%) (Figs 2A and B) and PDSCC (66.7%) (Figs 3A and B). Both cytoplasmic and nuclear expressions of survivin were found in 26.7% of each of WDSCC and MDSCC and 33.3% of PDSCC cases. However, cytoplasmic expression of this marker was observed only in few cases of WDSCC (6.7%) and MDSCC (13.3%). The intensity of the survivin reaction was found to be severe in 46.7% of PDSCC cases. Nevertheless, the majority of cases of WDSCC (86.7%), MDSCC. (80%), and PDSCC (53.3%) showed moderate staining

Figs 1A and B: Mild immunopositivity for survivin in malignant epithelial cells in the nuclear region of WDSCC [IHC, $\times 100$ (A); $\times 400$ (B)]

Figs 2A and B: Moderate immunopositivity for survivin in malignant epithelial cells in the nuclear region of MDSCC [IHC, $\times 100$ (A); $\times 400$ (B)]

Figs 3A and B: Strong immunopositivity for survivin in malignant epithelial cells in the nuclear and few cytoplasmic regions of PDSCC [IHC, $\times 100$ (A); $\times 400$ (B)]

Figs 4A and B: Absence of survivin expression in NOM epithelium [IHC, $\times 100$ (A); $\times 400$ (B)]

Graph 1: The comparison of the immunoreactivity score for survivin among the study groups

intensity which was presented in Table 1. Survivin expression was totally absent in NOM epithelium (Figs 4A and B).

Most cases (40%) of WDSCC had 5 to 25% immunopositive cells, while 33.3% of cases showed less than 5% positivity. However, the number of immunopositive cells was higher in MDSCC with around 40% of cases showing positivity in the range of 26 to 50% and 33.3% of samples with 51 to 75% immunopositivity. Comparatively, PDSCC exhibited greater percentage of survivin positivity with 53.3% of cases showing about 51 to 75% and 26.7% of samples with greater than 75% of positive cells (Table 2).

It was observed that the IRS for survivin increases with the grade OSCC. WDSCC showed mild (40%) to moderate (26.7%) immunoreactivity, whereas negative immunoreactivity was observed in 33.3% of cases. Most cases of MDSCC had moderate immunoreactivity (60%) followed by mild (26.7%) and strong (13.3%) expressions. In the case of PDSCC, moderate (53.3%) to strong immunoreactivity (46.7%) was observed (Table 2 and Graph 1).

There exists a very high statistically significant difference in staining intensity and immunoreactivity of survivin among the study groups ($p < 0.001$). Pairwise comparison of survivin expression between the study groups is presented in Table 3.

DISCUSSION

Several mechanisms have been proposed for the pathogenesis of OSCC.⁶⁻⁹ One of the mechanisms through which the tumor cells are believed to acquire resistance to apoptosis is by overexpression of the inhibitor of IAP. Survivin is one of the seven members of IAPs and its expression signals more aggressive and disseminated disease. Increased survivin expression is commonly associated with reduced levels of apoptosis and survival rate, increased proliferative index, recurrence rate, and resistance to chemotherapy and radiotherapy, thereby identifying it as a significant independent prognostic indicator of the poor outcome in patients with most tumor types.

Table 1: Comparison of immunohistochemical distribution and staining intensity of survivin among the study groups

Category	Immunohistochemical distribution*				Staining intensity*			
	Absent	Cytoplasmic	Nuclear	Cytoplasmic and nuclear	Negative	Mild	Moderate	Severe
WDSCC	0 (0%)	1 (6.7%)	10 (66.7%)	4 (26.6%)	0 (0%)	0 (0%)	13 (86.7%)	2 (13.3%)
MDSCC	0 (0%)	2 (13.3%)	9 (60%)	4 (26.7%)	0 (0%)	0 (0%)	12 (80%)	3 (20%)
PDSCC	0 (0%)	0 (0%)	10 (66.7%)	5 (33.3%)	0 (0%)	0 (0%)	8 (53.3%)	7 (46.7%)
NOM	15 (100%)	0 (0%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)	0 (0%)
<i>p</i> value	<0.001 [†]				<0.001 [†]			

*ANOVA test. [†]*p* value < 0.001 (very highly significant)

Table 2: Comparison of immunopositivity and immunoreactivity scores of survivin among the study groups (WDSCC, MDSCC, PDSCC, and NOM)

Category	Immunopositivity*					Immunoreactivity score*			
	<5%	5–25%	26–50%	51–75%	>75%	Negative	Mild	Moderate	Strong
WDSCC	5 (33.3%)	6 (40%)	4 (26.7%)	0 (0%)	0 (0%)	5 (33.3%)	6 (40%)	4 (26.7%)	0 (0%)
MDSCC	0 (0%)	4 (26.7%)	6 (40%)	5 (33.3%)	0 (0%)	0 (0%)	4 (26.7%)	9 (60%)	2 (13.3%)
PDSCC	0 (0%)	0 (0%)	3 (20%)	8 (53.3%)	4 (26.7%)	0 (0%)	0 (0%)	8 (53.3%)	7 (46.7%)
NOM	15 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)	0 (0%)
<i>p</i> value	<0.001 [†]					<0.001 [†]			

*ANOVA test. [†]*p* value < 0.001 (very highly significant)

Table 3: Pairwise comparison of survivin expression among the study groups

Compared groups	Distribution <i>p</i> value*	Intensity <i>p</i> value*	Immunopositivity <i>p</i> value*	Immunoreactivity score <i>p</i> value*
WDSCC vs MDSCC	0.824	0.624	0.011 [†]	0.004 [†]
MDSCC vs PDSCC	0.69	0.121	<0.001 [†]	0.002 [†]
WDSCC vs PDSCC	0.69	0.046 [†]	<0.001 [†]	<0.001 [†]

*Fischer's Chi-square test, [†]*p* value ≤ 0.05 (statistically significant)

The immunopositivity for survivin was completely negative in normal oral epithelium, as observed in other studies by Gayathri et al.,¹⁰ Lin et al.,¹¹ Khan et al.,¹² Li et al.,¹³ and Jinbu et al.¹⁴ Survivin is generally expressed at high levels in embryonic tissues, but present at lower or non-detectable levels in normal adult tissues due to the presence of differentiated cells.¹¹ In contrast to our findings, few studies have reported mild nuclear and cytoplasmic expression among sporadic cells of basal and parabasal layers.^{15,16} However, Negi et al.¹⁷ had observed survivin positivity in almost 20% of normal tissue specimens due to high proliferative capacity of the tissues. It was established that survivin immunoexpression in normal cells was due to the presence of active mitotic cells.¹⁸ Survivin was also shown to be present in normal hematopoietic cells, vascular endothelial cells, polymorphonuclear neutrophils, T lymphocytes, immune cells, melanocytes, keratinocytes, and neurons.¹⁹ Significant expression of survivin in normal oral epithelium can also occur due to processing errors during fixation, antigen retrieval techniques, types of primary antibodies, and detecting systems used.²⁰

The present study reported a predominant nuclear positivity for survivin in all grades of OSCC though cytoplasmic and nuclear distribution was observed in few cases. Conversely, studies by Jinbu et al.,¹⁴ Lippert et al.,²¹ Li et al.,²² and Pannone et al.²³ have demonstrated greater expression of survivin in both cytoplasmic and nuclear regions of keratinocytes in OSCC. It was suggested that the nuclear pool of survivin is involved in promoting cell proliferation,²³ whereas the cytoplasmic pool participates in controlling cell survival.¹⁰ The intensity of survivin expression was found to be moderate in most cases of WDSCC and MDSCC; nevertheless, PDSCC showed moderate to strong immunoreactivity, in concurrence with other studies by Gayathri et al.¹⁰ and Jane et al.²⁴

A markedly increased expression of survivin was found in OSCC in comparison to normal oral epithelium, analogous to earlier studies by Preuss et al.,²⁵ Ciesielski et al.,²⁶ and Liping et al.²⁷ This suggests that survivin can be a lead target for tumor diagnosis, prognosis, and anticancer therapies. Survivin was highly expressed in PDSCC compared to WDSCC and MDSCC which is statistically significant (*p* < 0.01). Though Muzio et al.⁵ observed increased survivin expression in poorly differentiated OSCC, the differences were not statistically significant.

Further studies with larger sample size are required to assess the expression of survivin in OSCC cases to validate the significance of the study. Moreover, studies with clinical follow-up of patients are needed to evaluate the prognostic significance to confirm the role of survivin as a prognostic marker for OSCC. Survivin may act as a specific therapeutic target due to its unique expression in tumor cells and its absence in normal tissues. Various therapies involving survivin as a lead target are transcriptional inhibitors, small-molecule antagonists, Hsp90 inhibitors, cyclin-dependent kinase (CDK) inhibitors, promoter inhibitors, gene therapy, and immunotherapy.²⁶ Transfection with dominant-negative mutants of survivin has led to increased apoptosis in gastric cancer cell lines

and in breast cancer in animal models.^{28,29} Various survivin targeting immunotherapeutic strategies using survivin vaccines (SurVaxM) have been developed.³⁰

CONCLUSION

Survivin expression and its nuclear localization appeared to correlate with a higher grade of malignancy. The greater immunoreactivity of survivin in poorly differentiated OSCC compared to well and moderately differentiated tumor suggests unfavorable prognosis. Based on the present study, it was concluded that survivin can be used as an important diagnostic and prognostic marker for an aggravated form of OSCC. To further validate survivin as a prognostic marker, a large-scale study with greater sample size along with clinical follow-up data is needed.

Due to its selective expression in tumor cells and absence in normal tissues, it has the potential to be used as a therapeutic target in various malignancies and is currently under phase I and II clinical trials. This might increase the effectiveness of radiation and chemotherapeutic agents in oral cancer patients and help in developing modified treatment strategies.

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