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Original research article

The expression of survivin immune histochemically in oral epithelium dysplasia and various grades of oral squamous cell carcinoma

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Abstract

Background: Apoptosis inhibitory protein (API) survivin is not expressed in most normal adult tissues but is found in many types of cancer. Higher levels of survivin expression correlate with a more aggressive disease and a worse clinical result in cancer, and survivin expression in cancer has been linked to a poor prognosis, cancer progression, and medication resistance.

Methods: Present study was performed between April 2021 to March 2022, at Department of Pathology, Kakatiya Medical College, Warangal. Pre-diagnosed paraffin-embedded sections of NOE, OED, and OSCC across all three classes were analyzed for patterns of survivin immune depression and immune reactivity.

Results: The NOE tissues did not show any signs of immune reactivity for survivin. The immune expression pattern of survivin was predominately nuclear in the basal cells of OED tissues and cytoplasmic and nuclear in OSCC tissues. Moderately-differentiated OSCC had the greatest IRS, followed by poorly- and well-differentiated OSCC and OED, with a statistically significant difference between the normal and study groups.

Conclusion: It is possible that the expression of survivin proteins is an early stage in the progression of oral squamous cell carcinoma (OSCC) as well as a predictor of a bad prognosis for the disease.

Keywords: Survivin, oral epithelium dysplasia, oral squamous cell carcinoma

Introduction

Oral cancer is the third most common cancer in developing countries, and has the highest prevalence in India. An estimated 19% of all cancer cases in men and 7% of all cancer cases in women in India originate in the oral cavity, making it one of the five most common sites of cancer in the country. Squamous cell carcinomas, which begin in the mucosal lining, account for close to 90% of oral malignancies. Oral cancer is especially common in India since so many people there smoke and use tobacco products [1, 2].

To better predict the outcome of a patient's condition would be clinically useful, especially when the initial treatment plan may be adapted to the level of tumour aggression. The lack of distinct and substantial molecular tumour markers for risk assessment and prognosis in OSCC is a major contributor to the disease's dismal prognosis [3]. To aid clinicians in more precisely staging and grading lesions and predicting prognosis, it is crucial to identify improved prognostic tumour markers. Oral cancer forms when oncogenes are activated and tumour suppressor genes are silenced, leading to a loss of genomic stability. Most human malignancies share a common feature in their early stages: a breakdown in cellular mechanisms that control the rate at which cells divide, the ratio of cells that die to those that are created, and the occurrence of apoptosis [4, 5].

Survivin belongs to the family of proteins known as inhibitors of apoptosis (IAP), all of which have been proven to block the activity of activated caspases. When compared to other members of the IAP family, survivin mRNA is more commonly present in foetal development than in adult tissues. As an added bonus, survivin expression has been found in many different types of human cancer, such as those of the bladder, colon, liver, brain, lung, and prostate. Expression of survivin is correlated with a poor prognosis in the vast majority of malignancies investigated to date [6, 7].

Studies have shown that the role of survivin in cancer cells is not just limited to inhibition of apoptosis, but may also be associated with aggressive characteristics of cancer, such as angiogenesis and invasiveness, which has shifted the focus of relevant research towards survivin being exploited as a target in cancer therapy. However, research on the function of survivin in the development of OSCC and the relationship between survivin expression and OSCC differentiation is lacking [8, 9]. In the current investigation, we analyzed and compared the immune expression patterns and immune reactivity of surviving in tissue samples of NOE, OED, and OSCC of three different stages.

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Methods

Present study was performed between April 2021 to March 2022, at Department of Pathology, Kakatiya Medical College, Warangal. Pre-diagnosed paraffin-embedded sections of NOE, OED, and OSCC across all three classes were analyzed for patterns of survivin immune expression and immune reactivity. In this study, we categorized survivin expression as either cytoplasmic, nuclear, or both. The immune reactive score was used to qualitatively evaluate the level of survival protein survivin immune reactivity.

Inclusion criteria

1. Age 20 to 80 years
2. Patients having oral squamous cell carcinoma

Exclusion criteria

1. Patients having other disease
2. Complicated disease conditions

Immunohistochemical procedure

Following deparaffinization with xylene and rehydration with progressively more diluted alcohol, the sections were examined. After washing in Tris buffer saline, antigens were retrieved in a microwave using a 10 mM citrate buffer at high power for 15 minutes and low power for 10 minutes. Following a 30-minute incubation with 4% hydrogen peroxide to prevent endogenous peroxidase activity, the slices were analyzed. Using paraffin-embedded samples, we looked for differences in survivin immune expression and immune reactivity between NOE, OED, and OSCC that had already been identified. Survivin expression was classified in this investigation as being either cytoplasmic, nuclear, or both. Quality assessment of survivin immune reactivity was performed using the immune reactive score. roxidase activity, and the slides were treated with primary anti survivin monoclonal antibody for 60 min at 37 °C in a humid atmosphere. After 30 minutes in a humid environment at room temperature, the sections were treated with secondary linking antibody (biotinylated anti immune globulins/super enhancer) to improve the efficacy of the subsequent polymer process. The slices were left in a room temperature incubation with a diluted secondary antibody (the conjugate) for 30 minutes. After that, we used DAB and Mayer's hematoxylin as counterstains. Tissue slices were treated with all of the reagents besides the main antibody as a negative control. Positive control tissue slices, which comprised the NOE tissues, were employed to guarantee consistent, reliable staining.

Immunohistochemical analysis

The expression of survivin was assessed in every group's immune histochemically stained slides (I, II, III, IV, and V). Brownish immune staining of the nucleus and cytoplasm was indicative of immune positivity for survivin. Survivin expression was catalogued across all categories according to its localization: cytoplasmic, nuclear, and mixed.

Results

This work documented and contrasted NOE, OED, and the three OSCC grades with regards to survivin immune expression patterns and IRS. There was no evidence of survivin expression in NOE. The majority of OED samples displayed nuclear expression, while MDSCC and PDSCC demonstrated higher levels of both cytoplasmic and nuclear expression. The lowest overall percentage of samples showed cytoplasmic expression. The immune expression patterns of survivin were observed to differ significantly amongst the five groups.

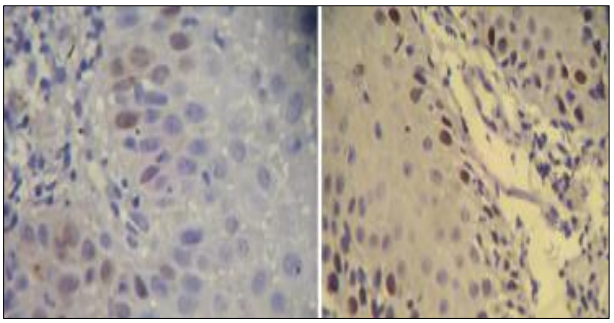

Fig 1: Oral epithelial dysplasia is associated with a positive immune reactivity for survivi

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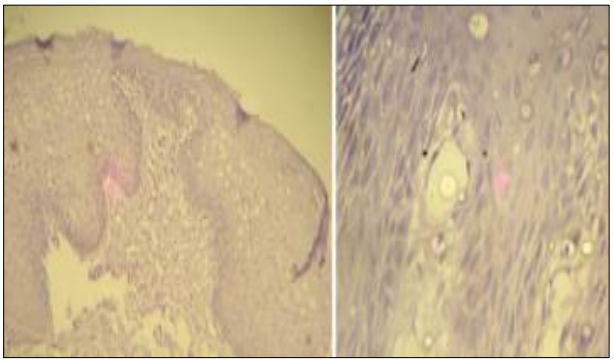

Fig 2: In the healthy oral epithelium, we do not see immune positivity for survivin

In contrast to 9 of WDSCC, 11 of MDSCC, and 6 of PDSCC, 28 of OED samples showed nuclear expression of survivin by immunohistochemical staining (IHC) and immunohistochemical staining by immunofluorescence microscopy (IFM).

Table 1: Analyzing differences in immune expression between groups

Groups	n	Expression Pattern			
		No Expression	Cytoplasmic	Nucleus	C+N
NOE	12	10	0	2	0
OED	28	11	13	2	2
WDSCC	9	0	7	1	1
MDSCC	11	6	2	0	3
PDSCC	6	2	0	1	3
Total	60	29	22	06	09

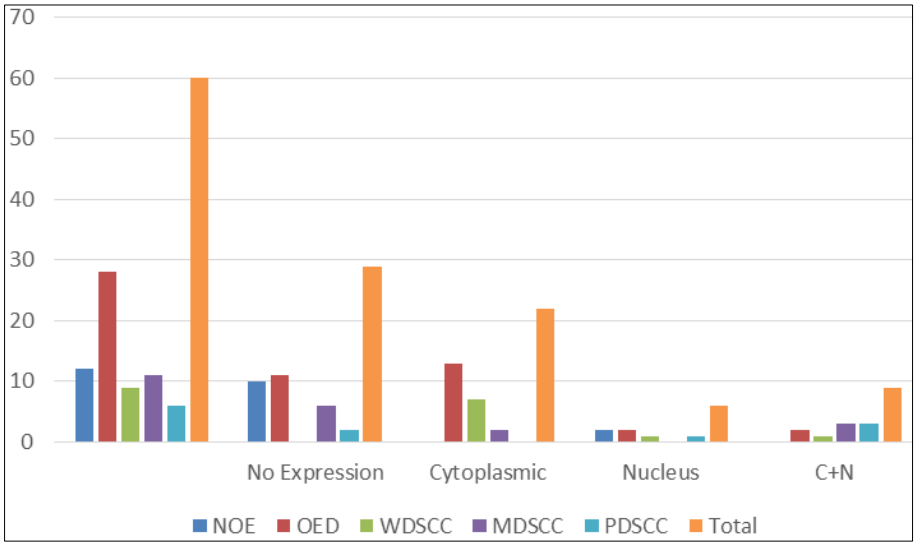

Fig 1: Analyzing differences in immune expression between groups

Table 2: IRS Comparison among the study groups

Groups	n	Expression Pattern			
		Strong	Moderate	Mild	Negative
NOE	12	1	10	1	0
OED	28	2	12	2	12
WDSCC	9	3	5	0	1
MDSCC	11	1	3	1	3
PDSCC	6	1	3	1	1
Total	60	08	30	05	17

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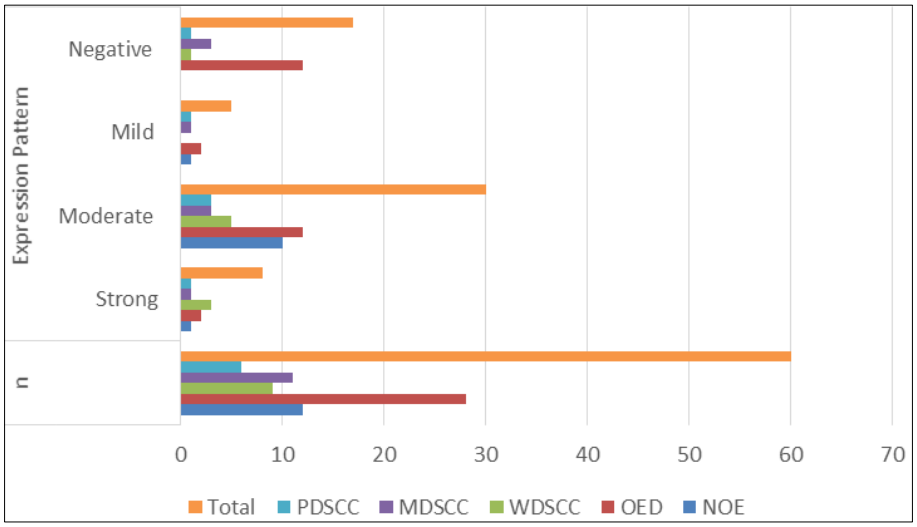

Fig 2: IRS Comparison among the study groups

All five groups differed significantly from one another in terms of survivin's individual relative survival (IRS) value. Although nuclear expression was more prevalent in WDSCC samples compared to MDSCC and PDSCC, no statistically significant differences in immune expression patterns or IRS of survivin were detected when comparing WDSCC to the other grades of OSCC. Finally, no statistically significant difference was identified between MDSCC and PDSCC when comparing the immune expression patterns and IRS of survivin, but cytoplasmic and nuclear expression was slightly higher in PDSCC than in MDSCC. Similarly, there was no statistically significant difference between MDSCC and PDSCC IRS, despite the fact that the majority of MDSCC samples displayed moderate immune reactivity compared to only five PDSCC samples, and only two MDSCC and five PDSCC samples displayed strong immune reactivity.

Discussion

In carcinogenesis, oncogenes are turned on and tumour suppressor genes are turned off in a complex multistep process. As a result, the hallmark of the vast majority of human malignancies is a dysfunction in the regulatory mechanisms that govern the cell cycle, the balance of cell death and viability, and apoptosis. The study of apoptosis and the implementation of new cancer therapy have become fundamental. As a physiological event that controls cell proliferation and eliminates damaged cells, apoptosis regulation is essential for embryonic development, tissue morphogenesis, and maintaining a stable internal environment, or homeostasis. Conversely, resistance to apoptotic stimuli is often involved in the initiation, progression, and maintenance of cancer and autoimmune diseases. Overexpression of IAPs, with survivin as one example, is thought to be a means by which tumour cells acquire resistance to apoptosis. Overexpression of survivin has recently been reported to be common in OSCC. Survivin protein has been found to suppress apoptosis *in vitro* by binding selectively to caspases 3 and 7 [9-11].

Survivin is expressed during the G2/M phase of the cell cycle, and Li *et al*. discovered that disruption of survivin microtubule connections leads to elevated caspase 3 activation and faster apoptotic cell death. Cells in the G0/G1 phase were significantly reduced while those in the S and G2M phases were significantly increased in hepatocellular carcinoma cell lines transfected with survivin, as reported by Ito *et al*. According to these results, the expression of the survivin protein may be linked not only to decreased apoptotic cell death but also to increased proliferative activity in cancer cells. Increased survivin expression is characteristic of poorly differentiated tumours. In addition, greater survivin expression has been linked to worse survival rates [12-14].

Here, we compared survivin immune expression patterns and IRS between NOE, OED, and the three OSCC grades. Consistent with previous reports by Jinbu *et al*., Khan *et al*., Lin *et al*., and Li *et al*., we observed no survivin immune expression in NOE samples. While Jinbu *et al*., Khan *et al*., Jane *et al*., and Lin *et al*. all report a cytoplasmic localization of survivin, we detected a nuclear localization of survivin in 93.3% of OED samples. The intracellular localization of survivin, predominately in the cytoplasm with focal nuclear expression, was also seen by Pannone *et al*. in all cases of oral pre malignant lesions they studied. We found that 73.3% of OED samples were survivin positive, while Negi *et al*. reported this in only 53.3% of instances with dysplasia [15-19].

Weak survivin expression was reported by Kim *et al*. Strong survivin expression was seen in 21% of OSCC biopsy specimens and 79% of his samples. According to the findings of Jane *et al*., survivin expression was low in WDSCCs and was found mostly in keratinocytes, low to moderate in MDSCCs, and moderate to strong in all PDSCCs, with two samples displaying unique nuclear expression of survivin. The overall level of immune reactivity in our analysis was higher than that seen in the

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aforementioned investigations; we found moderate immune reactivity in WDSCC and MDSCC samples, and about equal numbers of mild, moderate, and severe immune reactivity in PDSCC samples. Head and neck squamous cell carcinomas (HNSCCs) have been shown to express high levels of nuclear and/or cytoplasmic survivin, and Qi *et al*. have noted that survivin-positive cells are located primarily at the tumor's periphery in well-differentiated HNSCCs, but are found throughout poorly differentiated HNSCC tumour nests [17-20].

The authors Lo Muzio *et al*. found that while survivin expression was reportedly higher in poorly differentiated tumours, the observed differences were not statistically significant. None of the NOE samples tested positive for survivin expression, and the immune expression pattern of survivin in OED was markedly different from that in WDSCC and highly significant from that in MDSCC and PDSCC, according to our findings [20-22]. However, there was no discernible difference between OED and OSCC of any grade in terms of immune reactivity. Additionally, we found no statistically significant variations in the immune expression patterns or immune reactivity of survivin amongst the three OSCC grades in our investigation.

Conclusion

Although there was no discernible difference between OED and OSCC samples with regards to immune reactivity, the survivin protein expression patterns were very different between the two types of samples. There was moderate to strong survivin immune reactivity in all of the OSCC samples, supporting the possible function of survivin in dysplasia and malignancies. As survivin was not expressed in any of the NOE samples, its overexpression in OED and OSCC provides a potential diagnostic, prognostic, and therapeutic target.

Conflict of Interest

None

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Nil

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