ASSOCIATION OF CYCLIN D1 EXPRESSION AND MALIGNANCY GRADE OF TUMOURS OF ORAL SQUAMOUS CELL CARCINOMAS

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Introduction

Oral cancers are the most common cancers in Sri Lanka. Oral Squamous cell Carcinomas (OSCCs) represents 90% of the malignancies in the oral cavity (Bryne et al., 1989). In spite of modern diagnostic and treatment methods, the prognosis of OSCCs and the survival rates are poor. In clinical practice, accurate prognosis is essential since the treatment planning of OSCC is mainly based on the prognosis. At present clinical staging and histopathological classifications are used to predict the prognosis of OSCC. However, such predictions have not been consistent and contradictory results have also been observed. Therefore, it is important to look for new biological prognostic methods that predict the tumor aggressiveness more precisely. In the last decade, attention of researchers has been focused on understanding the molecular pathogenesis behind the development of OSSCs. It has been shown that cell cycle regulatory genes like, Cyclin D1 expressions are altered in oral cancers as a result of gene aberrations (Ronaldo et al., 1999). Possibly, studying these molecules may lead to new biomarkers that could be combined with histopathological grades for better prognosis. Previous studies have

indicated a correlation between the grade of malignancy and the expression of Cyclin D1 protein in head and neck SCCs, using different malignancy grading systems (Costa et al., 2004; Ronaldo et al., 1999; Sang et al., 2004). However, association between Bryne's histopathological malignancy grading and Cyclin D1 protein expressions has not been studied. The aim of this study is to find the association between the levels of expression of Cyclin D1 protein and grade of malignancy and assessment of the prognostic significance of CyclinD1 expression in OSCCs using patient survivals.

Materials and methods

One hundred primary oral squamous cell carcinomas which are formalin fixed and paraffin embedded (OSCCs) from the archives of the Department of Oral Pathology,

Two consecutive sections (4μ) were taken from each biopsy and mounted on sialin coated slides. One section was stained with H& E for histopathological grading. The second section was stained immunohistochemically using Cyclin D1 antibody (clone DCS-6) according to the manufacturer's instructions. Cyclin D1 positive breast cancer tissues were used as positive controls and omission of primary antibody served as negative controls. Bryne's grading system was used to asses the grade of malignancy (Bryne et al., 1989). Five histopathological parameters were assessed and each parameter was scored on a point scale running from 1 to 4. Total grade of each tumor was calculated by summing the 5 score in to a total. The total malignancy grades were grouped into three Byrne's prognosis groups; (grade I=5-8), (grade II=9-12), (grade III=13-20). Grade I tumors have a better prognosis than the tumors of Grade II. Grade III tumors show poor prognosis, compared to grade I and II (Bryne et al., 1989).

The immuno stained sections were examined under the light microscope. Cyclin D1 positive cells showed the brown stained signals in both cytoplasm and nuclei. One thousand cells were counted from the invasive front of each tumor and the numbers of positive cells were counted. The relationship between histopathological tumor grade and

number of cells positive for Cyclin D1 protein expression was statistically evaluated using Spearman's test (Ronaldo et al., 1999). Survival information was gathered up to a period of 3 years using data obtained from 42 patients individually. Association between Cyclin D1 expressions and survival time was studied using Cox Regression analysis.

Results

According to the statistical analysis there was a positive correlation between cyclinD1 expression and Bryne's grades and there was a statistically significant difference between Cyclin D1 expression in grade I and II ($p= 0.019$) and grade I and III ($p=0.002$). The Mean ranks of Cyclin D1 expression in each of the grade is given in the Table 1.

The survival data indicated that out of 42 patients 10 had died within 3 years from OSCC. Out of 32 surviving patients, 11 had recurrence within 3-year period, whereas 21 patients were disease-free. However, no statistically significant association were observed between Cyclin D1 expression and survival time of primary OSCC patients ($p>0.05$).

Discussion

Many studies have revealed a positive correlation of Cyclin D1 expression with different histopathological grades in head and neck tumours. However, these findings vary in their expression of Cyclin D1 depending on the location, grading and different sites within the tumour itself. We found a statistically significant positive correlation of Cyclin D1 protein expression with Bryne's grade of malignancy in OSCCs. Genetically damaged cells which have a high mutation rates at 11q p13 region, show high expression of Cyclin D1 and this is believed to cause damage to the cell signaling pathway resulting deregulation of the cell cycle and increasing cell proliferation rate. Recent studies have found that Cyclin D1genotype and the degree of aberration may determine the amount of Cyclin D1 expression in OSCCs. However, further studies are needed to understand the exact role played by Cyclin D1 genotypes in expression of the protein.

Some studies have found a positive correlation between CyclinD1 expressions and loco-regional recurrence/ decreased survival rates. Even though, there was a trend of decreased Cyclin D1 expression with increased survival time in the present study, the findings were not significant statistically.

Conclusion

In OSCC's Cyclin D1 protein expression increases with Bryne's grade of malignancy. A bigger sample size is needed to study the association between CyclinD1 expression and patient survival rates to reach further conclusions.

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Table 1. Cyclin D1 expression vs histopathological grading according to Bryne's classification

Bryne's Grades	Number of Tumors	CyclinD1	
		Mean rank	P-value
Grade I	21	33.1	0.019 (I-II)
Grade II	34	49.9	0.196 (II-III)
Grade III	45	58.1	0.002 (I-III)
Total	100		