Serpin B5 as a Diagnostic and Prognostic Molecular Biomarker for Oral Squamous Cell Carcinoma

Maryam Salik[1], Katy Flannery[2], Hilal Arnouk M.D., Ph.D.[1,2]

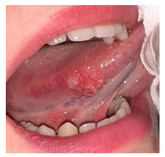
[1]Chicago College of Osteopathic Medicine, [2]Department of Pathology, Midwestern University

Abstract:

Oral squamous cell carcinoma is one of the most frequent oral neoplasms worldwide and remains to be associated with poor prognosis. The increased prevalence of late detection, tumor migration, and aggressiveness in conjunction with increasing incidences of oral cancer highlights the need for molecular biomarkers to be used as prognosticators and as a diagnostic tool for disease progression assessment. Serpin B5 was shown to have statistical significance in samples of normal oral mucosa in comparison to cancer, correlating with lymph node involvement, clinical staging, and differentiation status. This underscores its potential utility as a diagnostic and prognostic molecular biomarker for detecting oral squamous cell carcinoma.

Oral squamous cell carcinoma (OSCC) is a common malignancy of the head and neck region and remains to have relatively low five-year survival rates. Preliminary recognition of OSCC remains challenging as many patients remain asymptomatic until there is advanced progression emphasizing the importance of earlier detection and diagnosis. Existing diagnosis is based solely on histological morphology, which has high variability among examiners. Serpin B5 is a serine protease inhibitor that functions as a tumor suppressor to inhibit growth, invasion, and metastasis of malignant cells.

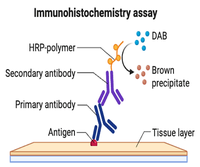
Previous studies have shown varying trends in the expression of Serpin B5, and we aim to better understand the role of Serpin B5 expression in the diagnosis and prognosis of OSCC.



Methodology

This study utilized tissue microarrays samples of normal oral mucosa and OSCC with varying histopathological grades and had undergone immunohistochemistry staining with a specific antibody to Serpin B5. The investigators were blinded to the TNM clinical staging of the tissue samples during IHC staining and analysis. Subsequently, quantitative analysis of Serpin B5 was completed using high-resolution Nikon A1R inverted microscope at 10x magnification with a pixel counting algorithm to eliminate subjectivity in sample analysis that is often seen in manual assessment of IHC samples staining intensity.

A “Histo-score” was calculated based on the staining intensity and the percentage of positively stained cells in each sample. These scores were used to determine significance using statistical analysis with a two-tailed T-test.



Results

SERPIN B5 AS A DIAGNOSTIC BIOMARKER FOR OSCC

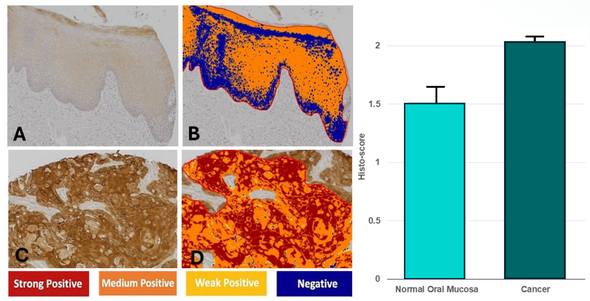


Figure 1: Computer-Assisted Analysis of Serpin B5 expression in Normal Oral Mucosa vs Cancer. The top row are IHC staining (A) and computer-assisted analysis (B) of normal oral mucosa. The bottom row shows IHC staining (C) and computer-assisted analysis of OSCC.

Figure 2: Quantitative Analysis of Serpin B5 expression in Normal Oral Mucosa vs Cancer. The average Histo-score of normal oral mucosa (N=22) is 1.516 and the average Histo-score of OSCC (N=172) is 2.028. Unpaired T-test generated a p value of 0.001, demonstrating statistical significance.

SERPIN B5 CORRELATES WITH LYMPH NODE INVOLVEMENT

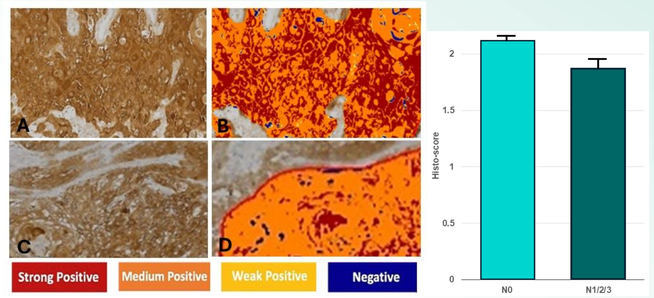


Figure 3: Computer-Assisted Analysis of Serpin B5 expression in N0 vs N1/2/3 OSCC samples. The top row are IHC staining (A) and computer-assisted analysis (B) of an N0 sample. The bottom shows IHC staining (C) and computer-assisted analysis (D) of an N2/3 sample. N represents the lymph node involvement of each sample.

Figure 4: Quantitative Analysis of Serpin B5 expression in N0 vs N1/2/3. The average Histo-score of an N0 sample (N=113) is 2.118. The average Histo-score of an N1/2/3 (N=56) sample is 1.875. Unpaired T-test generated p value is 0.006, demonstrating statistical significance.

SERPIN B5 CORRELATES WITH CLINICAL STAGING

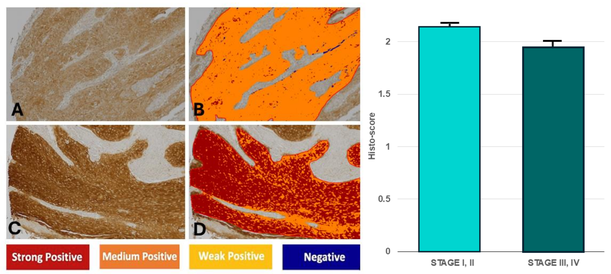


Figure 5: Computer-Assisted Analysis of Serpin B5 expression in early vs late clinical staging. The top shows IHC staining (A) and computer-assisted analysis (B) of Stage I/II. The bottom shows IHC staining (C) and computer-assisted analysis (D) of Stage III/IV.

Figure 6: Quantitative Analysis of Serpin B5 expression in early vs late clinical stages. The average Histo-score of Stage I/II (N=85) is 2.122. The average Histo-score of Stage III/IV (N=118) is 1.939. Unpaired T-test generates a p value of 0.0326, demonstrating statistical significance.

SERPIN B5 CORRELATES WITH DIFFERENTIATION STATUS

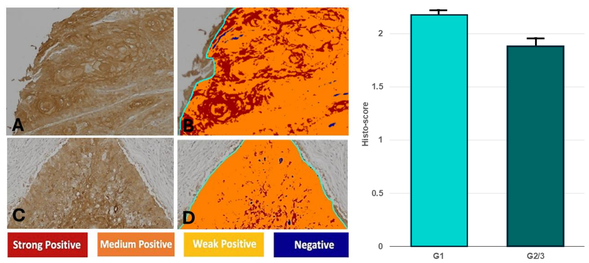
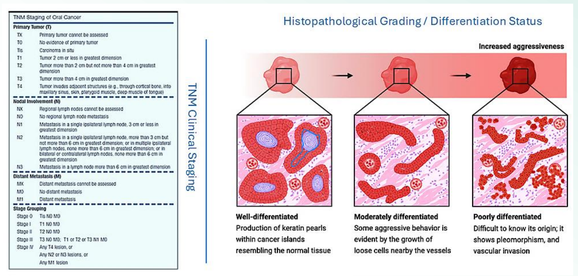


Figure 7: Computer-Assisted Analysis of Serpin B5 expression in low vs high histopathological grade samples. Top row shows IHC staining (A) and computer-assisted analysis (B) of Grade 1 OSCC sample. Bottom row shows IHC staining (C) and computer assisted analysis (D) of a Grade 2/3 OSCC sample.

Figure 8: Quantitative Analysis of Serpin B5 expression in low vs high histopathological grade. The average Histo-score of low grade/G1 (N=88) is 2.128, and the average for high grade/G2/3 (N=67) is 1.886. The unpaired T-test generates a p value of 0.0008, demonstrating statistical significance.

Conclusion

This study evaluated the utility of Serpin B5 as a diagnostic and prognostic marker for OSCC and discovered that Serpin B5 expression was significantly increased in oral cancer samples compared to normal mucosa tissues. Based on the AJCC TNM clinical staging criteria, there was significant correlation with lymph node metastases, where Serpin B5 was expression was downregulated. Moreover, there was significant downregulation of Serpin B5 expression in OSCC in the higher histopathological grades vs. low grades biopsies. Further, there was significant correlation with the clinical staging, where Serpin B5 was significantly downregulated in the advanced stages, which emphasizes to its role as a potential prognostic indicator since advanced clinical stages are associated with poor five-year survival rate. Ongoing studies in our lab aim to further explore Serpin B5 clinical applications.



References

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