Abstract
Tumor Budding—A Histomorphological Representation of Epithelial–Mesenchymal Transition Mechanism in Metastasis of Oral Squamous Cell Carcinoma †

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Background: Over 90% of oral cancer deaths are due to metastases, facilitated by a mechanism, epithelial–mesenchymal transition (EMT), that demonstrates a mesenchyme-like phenotype of epithelial cells. Prompt metastasis prediction would navigate surgeons in avoiding unwanted or aggressive treatment for low- and high-risk patients. Recently, the use of image processing is gaining popularity.

Aim: To assess the metastatic risk of Oral Squamous Cell Carcinoma (OSCC) by the quantification of tumor buds and epithelial–mesenchymal transition biomarker expression using MATLAB software 2016 Ra 9.0 software (The MathWorks, Inc., Natick, MA, USA) with an integrated Image Processing Toolbox.

Objectives:
• To evaluate pan CK-stained tumor buds and the expression of epithelial–mesenchymal transition biomarkers, E-Cadherin, β-Catenin, MMP-2 and MMP-9, using immunohistochemistry;
• To quantify and correlate tumor buds and the protein expression of EMT biomarkers using MATLAB software.

Methodology: Pan CK-stained tumor buds and E-Cadherin, β-Catenin, MMP-2, and MMP-9 expression were evaluated immunohistochemically on 40 archival tissue samples of OSCC (20 metastatic and non-metastatic groups). Photomicrographs of immunostained OSCC cases were captured and subjected to texture and color segmentation using image processing in MATLAB software.

Statistical analysis: Statistical analysis using Statistical Package for the Social Sciences (SPSS) software 20.0 was carried out for the mean scores.

Results: Metastatic OSCC showed a statistically significantly high number of tumor buds (90%). There was a significant decrease in the proportion and intensity of positive cells in E-Cadherin and β-Catenin (p = 0.00) and an increase in MMP2 and MMP9 protein expression in metastatic OSCC (p = 0.00).

Conclusions: This study is the first of its kind wherein image processing using texture and color segmentation in MATLAB has been used effectively to quantify the phenotypic protein expression of immunostained OSCC tumor buds and EMT markers. The correlation of tumor buds with EMT expression supports the mechanism of EMT responsible for OSCC metastasis, thereby facilitating surgeons in arriving at a definitive treatment plan.

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