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Immunohistochemical expression of Ki-67, CD34 and Podoplanin in hyperkeratosis and squamous cell carcinoma of the lower lip

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Background: Squamous cell carcinoma is the most common malignancy in the lower lip. Although lower lip squamous cell carcinoma is slow growing, up to 29% of tumors develop metastases to the cervical lymph nodes. Thus, identification of biological markers that could provide prognostic information about the invasive or metastatic potential of these lesions is important. It is known that angiogenesis, lymphangiogenesis and proliferation play an important role in tumor progression. Therefore, the goal of this study was to analyze the immunohistochemical expression of Ki-67, CD34 and Podoplanin in hyperkeratosis and in squamous cell carcinoma of the lower lip. It was intended to assess the possibility of using such markers as indicators of morphological aggressiveness of squamous cell carcinoma of the lower lip (LLSCC).

Materials and methods: Seventy-one cases of the lower lip lesions, obtained from the files of Novosibirsk Regional Oncology Center were selected for this study. The specimens were divided into three groups: a lower lip hyperkeratosis group consisting of 22 cases; LLSCC with keratinization consisting of 34 cases and LLSCC without keratinization consisting of 15 cases. To analyze angiogenesis, lymphangiogenesis and proliferation, we performed immunostains of the lower lip biopsy material for the CD34, vascular marker, Podoplanin, lymphatic-specific markers and Ki-67, marker of proliferation. Tissue samples were fixed in 10% neutral formalin, processed by standard histological techniques and embedded in paraffin. All steps of the immunohistochemical reaction were performed by using BENCHMARK/XT slide stainer (Ventana). The lymphatic and blood vessels volume density and Ki-67 cells numerical density were morphometrically analyzed in all groups and compared using the non-parametric Mann–Whitney test and the Wilcoxon signed rank test. A level of significance of 5% ($p < 0.05$) was adopted for all tests.

Results: All cases of a lower lip hyperkeratosis and LLSCC were positive for Ki-67, CD34 and Podoplanin. With respect to the pattern of staining, specimens exhibited a predominantly peripheral staining for CD34 and Podoplanin in inflammatory infiltrates and tumor sites. In contrast, staining for Ki-67 was predominantly central in inflammatory infiltrates and tumor sites in hyperkeratosis and LLSCC. When compared to lower lip hyperkeratosis, LLSCC (both with keratinization and without keratinization) showed a higher number of immunopositive Ki-67 cells (by 64% and 77%, respectively, $p < 0.05$). It was found that proliferative activity of tumor cells in LLSCC with keratinization was 2 times higher than that in LLSCC without keratinization. Comparison of the volume density of blood vessels showed that the density of CD34+ – blood vessels in hyperkeratosis was lower by 77% than in LLSCC without keratinization and lower by 64% than in LLSCC with keratinization. At the same time, volume density of blood vessels in LLSCC without keratinization was higher by 56% than that in LLSCC with keratinization. Investigation of lymphatic vessels showed that Podoplanin+ – lymphatic vessels volume density in hyperkeratosis was lower by 50% than that in LLSCC without keratinization and lower by 24% than that in LLSCC with keratinization. Whereas in LLSCC without keratinization the lymphatic vessels volume density was higher by 51% than that in LLSCC with keratinization.

Conclusion: This study has shown the greater development of blood and lymphatic vessels in LLSCC without keratinization in comparison with hyperkeratosis and LLSCC with keratinization, thus contributing to the development of metastasis.

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Advantages of acoustic focusing in flow cytometry and state of the art microscopy on your bench

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The work describes advantages of our latest instrument developments in flow cytometry and microscopy. Characteristics of newly launched flow cytometer, which uses acoustic focusing to align the cells in the focus of the laser for analysis, allows to analyze samples at a much higher throughput rate without losing precision and sensitivity. This opens up new possibilities regarding sample preparation and analysis. These advantages we want to demonstrate on 2 examples, whole blood analysis and rare event analysis. Analysis of biological samples in the most physiologic state with minimal sample preparation and manipulation is a key objective to any workflow. However as whole blood samples generally require significant manipulation, such as wash/centrifugation steps and/or red blood cell lysis, we have developed multiple no-lyse, no-wash assays to minimize these manipulation steps to obtain more physiological results. Here we show how we utilize the rapid sample collection capabilities of the our instrument to characterize phagocyte function in human whole blood with a phagocytosis/phagosome acidification assay and also a dihydrorhodamine 123 superoxide production assay, in a no-lyse, no wash format.

Another area where our technology can shift boundaries is the field of rare event analysis. Due to the low frequency of the target cell population a large number of total events (in the range of several millions) need to be acquired. Simply due to the enormous amount of data that needs to be acquired, time becomes a very limiting factor for rare event analysis. As Acoustic Focusing allows analyzing samples at an approximately 10× higher throughput rate without scarifying data quality and maintaining a low coincidence rate, it pushes the limits what can be done in this field. As an example we will show the characterization of human iNKT cells.