Proteomic Biomarkers in Proliferative Verrucous Leukoplakia (PVL)

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Introduction

Proliferative verrucous leukoplakia (PVL) is an oral potentially malignant disorder (OPMD) with high risk of malignant transformation. Oral leukoplakia (OL) share some clinical and microscopic features with PVL but have different clinical behavior with significantly lower rate of malignant transformation. Therefore, the aim of this study was to analyze the proteomic prolife of PVL in tissue and saliva samples to identify diagnostic biomarkers with therapeutic potential to differentiate between PVL and OL.

Materials and Methods

Altogether, 113 PVL, 49 OL, and 32 inflammatory fibrous hyperplasia (IFH) samples and 7, 8, and 5 saliva samples, respectively, were collected from Brazil, Spain, and Finland. PVL samples were compared with OL and IFH patients through label-free liquid chromatography with tandem mass spectrometry (LC-MS/MS) followed by qualitative and quantitative analysis to identify differentially expressed proteins. Potential biomarkers were further validated by immunohistochemistry (IHC), subsequently, marking intensity (MI) scan analyses. (Figure 1)

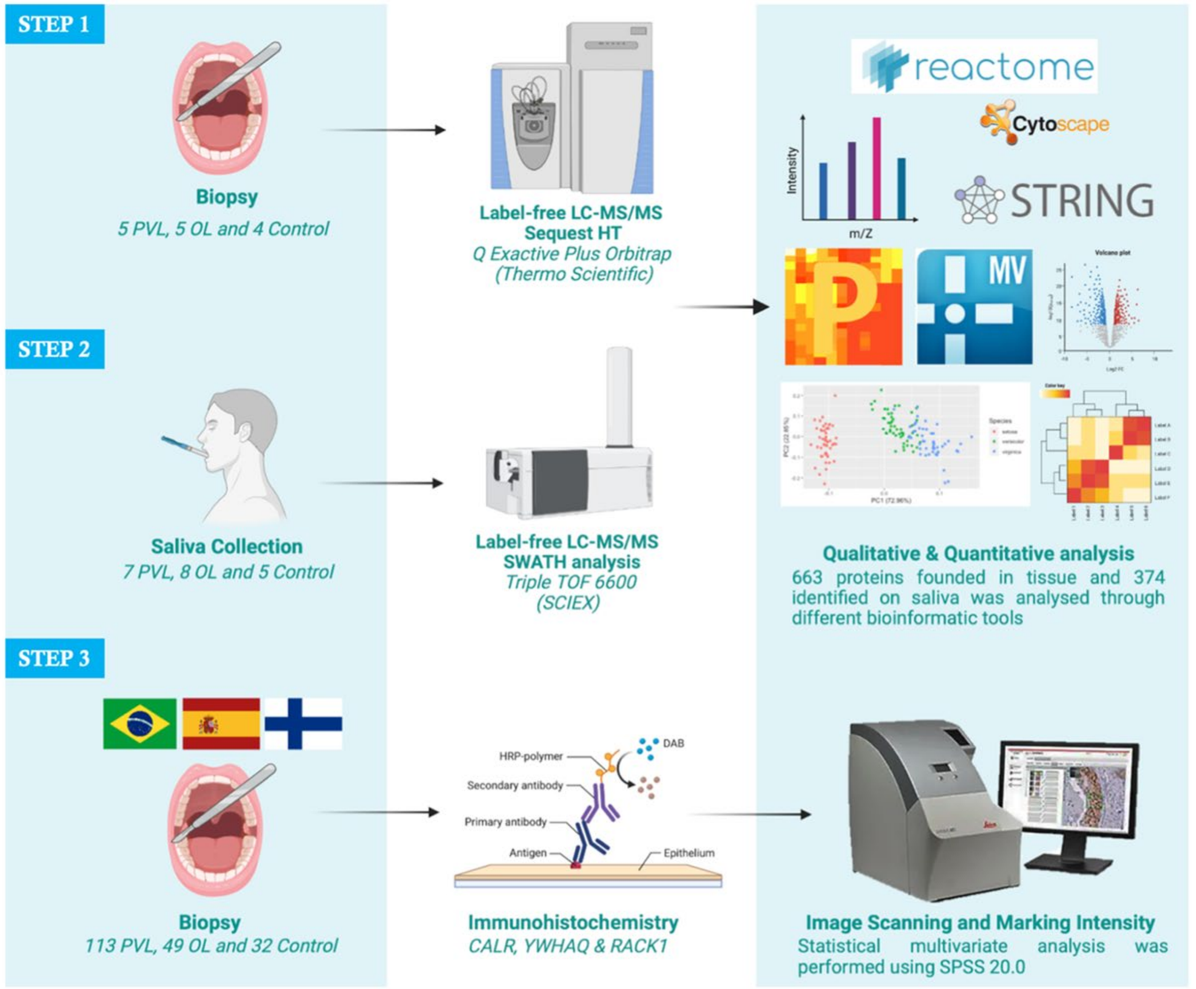

Figure 1. Study design. This study was conducted in 3 steps. Step 1 is the mass spectrometrybased label-free quantitative analysis conducted in the epithelial layer from laser capture microdissected frozen tissue samples gathered from patients with proliferative verrucous leukoplakia (PVL), oral leukoplakia (OL), and control group. Step 2 includes the label-free quantitative proteomic analysis using unstimulated saliva collected from the same groups. In step 3, the differentially expressed proteins identified in the quantitative proteomic analysis were validated using immunohistochemistry in paraffin-embedded specimens gathered from 3 countries. LC-MS/MS, liquid chromatography with tandem mass spectrometry; SWATH, Sequential Window Acquisition of all Theoretical Mass Spectra.

Results

The proteomic profiles were different between PVL, OL, and IFH. Altogether, 48 proteins showed statistically significant differences in expression in the three groups through proteomic analysis ($q \leq 0.05$) (Figure 2, 3). IHC confirmed that biomarker CALR is significantly more expressed in lesional tissues of PVL than OL ($p = 0.001$) (Figure 4). In addition, biomarkers RACK1 and YWHAQ were differentially expressed in PVL and OL in relation to IFH.

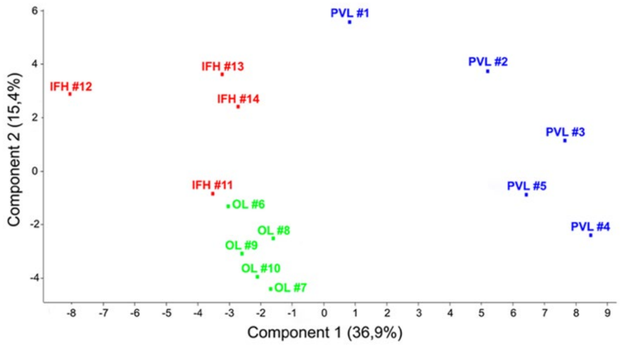

Figure 2. Principal component analysis separated PVL samples (right side of the graph) from the rest: OL and IFH samples (closer to each other on the left side of the graph).

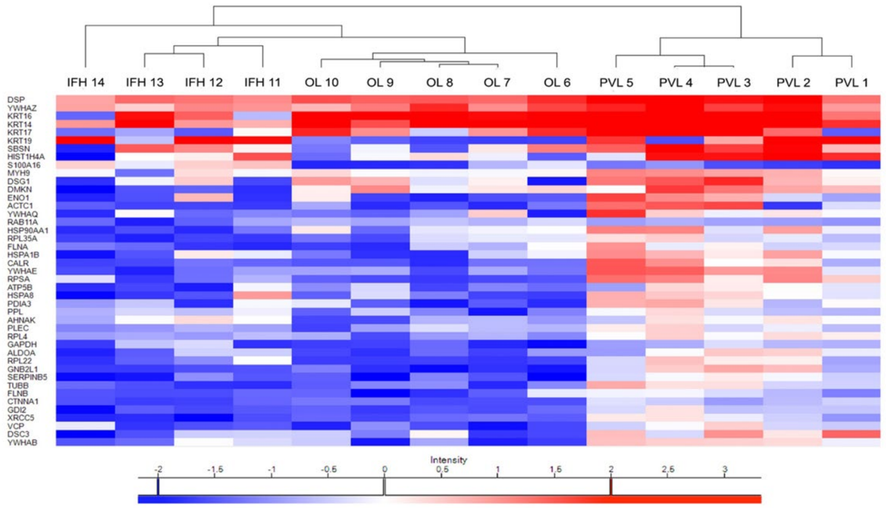

Figure 3. Heat map analysis showed differentially expressed proteins in PVL, OL, and IFH (upregulation in red and downregulation in blue).

Conclusions

PVL and OL have a different protein background. The identified biomarkers can help in early diagnosis of PVL distinguishing from OL. Furthermore, they provide insight into the underlying pathophysiological mechanisms of PVL and may offer therapeutic possibilities.

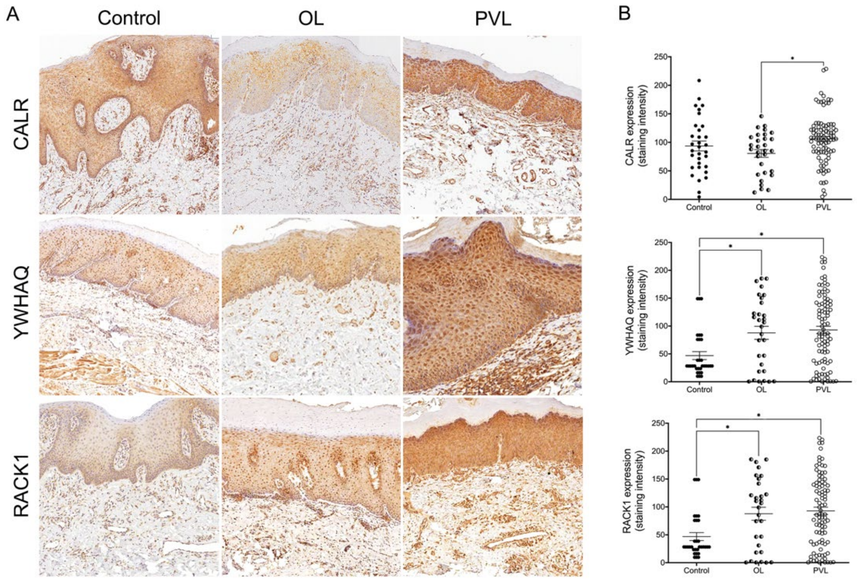

Figure 4. Immunohistochemical staining. CALR, YWHAQ, and RACK1 expression in proliferative verrucous leukoplakia (PVL), oral leukoplakia (OL), and control samples (inflammatory fibrous hyperplasia (IFH)).
(A) Representative photomicrographs demonstrate the immunostaining pattern of each biomarker in the respective groups (single stain, 200).
(B) Quantification and statistical analysis of staining intensity for CALR, YWHAQ, and RACK1 evaluated in the intraepithelial area in each group. *P < .05.

Arroyo E, …, Rintala M, Salo T, Rautava J, …, Bufalino A. Identification of Proteomic Biomarkers in Proliferative Verrucous Leukoplakia through Liquid Chromatography With Tandem Mass Spectrometry. Lab Invest. 2023 Jul 26;103(10):100222.

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