Quantitative SRM assay development for oral cancer prognosis in a clinical routine

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Quantitative SRM assay development for oral cancer prognosis in a clinical routine Daniela C. Granato1*, Carolina M. Carnielli1*, Alan R. Santos-Silva2, Márcio A. Lopes2, Estela K. Tango3, Thaís Brandão4, Ana Carolina Prado-Ribeiro4, Adriana Franco Paes Leme1#1Laboratório Nacional de Biociências, LNBio, CNPEM, Campinas, São Paulo, Brazil; 2Departamento de Diagnóstico Oral, Faculdade de Odontologia de Piracicaba Universidade Estadual de Campinas (UNICAMP), Piracicaba, SP, Brazil; 3Instituto do Câncer do Estado de São Paulo, Octavio Frias de Oliveira, São Paulo, Brazil; *authors contributed equally, # corresponding author.Introduction: Mass spectrometry-based targeted proteomics for absolute peptide and protein quantification in biological matrices is a powerful tool and has many advantages that make it promising for clinical oncological applications. Previous study from our group1 has revealed through discovery and targeted proteomics a four-peptide signature (COL6A1-pep, LTA4H-pep1, LTA4H-pep2, CSTB-pep) capable of distinguishing low risk from high-risk oral cancer (OSCC) patients. To validate this signature, we have developed a methodology that allows an accurate quantification of a prognostic signature in saliva for further application in oral cancer patients cohorts. Methods: Saliva samples from healthy individuals were used for development and optimization of an absolute quantification (AQ)-MS isotopic labeled workflow2,3 which will be further applied to OSCC patients (N0, without and N+, with lymph node metastasis). LC-MS SRM analysis was performed on triple-quadrupole Xevo TQ-XS coupled with Acquity UPLC-Class M LC (Waters). Results: First, we have evaluated the ability of different reagents such as urea, TFE, DDM and SDS to extract, solubilize and recover targeted peptides from saliva. The effectiveness of the protocols was evaluated regarding endogenous peptide recovery, providing a 2-fold increase under LysC and trypsin sequential digestion. Protocol without any desalt or acetone precipitation also improved peptide recovery. For transition selection, 3 and 10 transitions per peptide were evaluated, and the total area was improved in 50% for both LTA4H and CSTB peptides, while no differences were observed for COL6A1-pep when considering 10 transitions per peptide for quantification. Collison energies (CE) were optimized in steps of 3eV, under a range of 4 different steps, and resulted in higher peak area in CE different from the default4. We also compared 20-, 30- and 60-minute total gradients (11.5 min, 22.5 min and 45min at 40% MeCN, respectively), and total peak area did not change considerably while number of points per peak was impaired in shorter gradients, but still obtained 8 points per peak in 30 min. We compared the performance in signal to noise ratio of BEH, CSH and HSS iKey separation devices, and HSS showed an improvement of 50%. The response curves with the range 500 amol to 200 fmol SIS peptide concentration was performed with the optimized sample preparation and SRM-MS method using saliva, HeLa and BSA matrices, for LOD calculation of COL6A1, LTA4H, and CSTB peptides. For all the optimization steps, simpler, cheaper, and faster protocols were prioritized to ease the transference to the clinical routine. Conclusions: In summary, the present study has been able to develop a quantitative SRM-MS quantification workflow applied to prognostic signature for oral cancer patients in the clinical routine. References: 1-doi:10.1038/s41467-018-05696-2; 2- doi: 10.1186/s12014-024-09452-1; 3-doi: 10.3389/fimmu.2021.765898. 4- doi: 10.1021/ac102179j. Abbreviations: COL6A1: Collagen Type VI, alpha 1; LTA4H: Leukotriene A4 hydrolase; CSTB: Cystatin B; TFE: 2,2,2-Trifluoroetanol; DDM: n-Dodecyl-Beta-Maltoside.

Agradecimentos: