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A PRELIMINARY STUDY TOWARDS THE DEVELOPMENT OF AN INNOVATIVE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY-BASED PROTEOMICS STRATEGY FOR SKIN WOUND AGE ESTIMATION IN FORENSIC MEDICINE

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In forensic pathology wound age estimation is a classic but still modern issue that meets the need of establishing a causal relationship between any wound and death. In fact, if in autoptical observation it is quite straightforward to localize and identify wounds, on the other hand the assessment of the time intervening between the injury and death is anything but trivial. In the last decades many efforts have been paid for the selection of wound healing-related molecular biomarkers as the most effective for wound age estimation [1, 2] and adequately supported by scientific evidence to be a proof value in court. Immunohistochemical analysis is the commonly exploited method applied to formalin-fixed paraffin-embedded tissue, offering the possibility to localize the biomarker within the tissue or cell substructures, giving only qualitative or semi-quantitative results. In addition, it suffers from operator-related manual variability, subjective data interpretation, artefact generation risk, low sensitivity, and difficulty to visualize target that are co-localized [3, 4].
For these reasons, forensic pathologists urgently require more reliable and sensitive analytical approaches for wound age estimation. In this context, to the best of our knowledge, the present investigation represents the first attempt to develop a high-throughput bottom-up proteomics strategy based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) applied to autoptic skin for the development of a target method for the simultaneous determination of biomarkers for wound age estimation related to cutaneous ecchymoses. Thanks to the collaboration with the Institute of Legal Medicine, University of Milan, the development of the present strategy is carried out on human autopsy skin samples that from an analytical point of view represent the most adequate and realistic model, compared to tissues from sacrificed animals or synthetic skin. Both ecchymotic wound tissues and uninjured skin tissues (reference/control samples) were collected.

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Preliminary results on the development of a procedure for protein extraction from dermal-epidermal tissues with subcutaneous layer and on protein analysis using LC-high resolution mass spectrometry with data-dependent acquisition are here reported. Tissue disruption and homogenization prior protein extraction resulted the first challenging issue due to hard skin texture and very reduced dimensions of specimens. Moreover, the high heterogeneity of the investigated dermal-epidermal tissues with subcutaneous layer is another aspect to be addressed. For this purpose, different strategies, applied also in combination, were compared. After a defatting step with hexane, they involved the use of keratolytic agents, a stainless steel home-made mortar immersed in liquid nitrogen, beads-based tissue homogenization, and a sonicator immersion probe. Different protein extraction buffers, i.e. UTC and RIPA, were tested, extracting about 10 mg of proteins/g of skin tissue. Taking into account the presence of matrix interferents, detergent residues and highly abundant proteins, such as keratin, different protocols for proteolysis were applied involving a final purification step on C18 cartridges before analysis. The different approaches were evaluated in terms of the total protein extraction by Bradford assay, gel electrophoretic protein separation, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and LC-/high resolution MS analysis of the digested extracts. These results represent the starting point towards a broader and ambitious interdisciplinary research project aimed at developing a reliable and robust strategy for would age estimation based on the application of target LC-MS/MS method and on multivariate data processing.

References

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