A22 Forensic Proteomics: A New Perspective for Age of Death and Postmortem Interval (PMI) Estimation in Porcine Bone Within the Burial Context

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After attending this presentation, attendees will better understand the new potential applicability of proteomics to the world of forensic sciences. This presentation will introduce attendees to the basics of proteomic analyses and to the study of post-translational protein modifications related to the decay process. New biomolecular markers occurring both *in vivo* and postmortem may shine a light on the progress of tissue aging and on the estimation of the PMI from skeletonized remains.

This presentation will impact the forensic science community by illustrating the potential of a new proteomic method to address important forensic questions such as the age of death and the PMI estimation from a new, unexplored perspective. Some protein post-translational modifications, and in particular protein oxidation, deamidation, and racemization, have already been suggested as "molecular clocks" for protein turnover, development, and aging, both *in vivo* and postmortem; however, none of these modifications have been used until now on bones for forensic purposes.[1-3] This presentation will describe their new potential applications in this field to improve understanding of the biological and taphonomic decay of bone.

One of the most debated themes in forensic anthropology is the estimation of PMI as well as the approximation of the age of death from skeletal remains.[4-6] Many analytical and morphological methods have been published so far to address these goals, but the inherent limits regarding the accuracy of the current methods warrant the application of possible alternatives, including relatively new techniques, such as proteomics, to forensics. This study sought to apply proteomic methods to pig skeletal remains to look for new biomarkers that can help in estimating the animal's age of death as well as its PMI.

The first element of the study compares porcine skeletal remains from different aged animals to verify any proteomic difference between the various specimens, as well as to compare different skeletal elements within the same animal to evaluate any intra-skeletal difference. Bones from five different juvenile pigs were sampled, and their proteome was analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) techniques. The proteomes of these buried bones with one fresh bone purchased from a local butcher were also compared to evaluate the impact that the burial environment can have on proteome recovery. For the second portion of the study, an experimental burial environment in the field was created, in which four piglets of similar biological age were buried and the bones were recovered after different time intervals. The goal of this second stage was to focus on proteome decay postmortem, in order to look for biomarkers to correlate PMI with particular peptide modifications.

The results reveal intriguing differences within the proteome of bones from different aged piglets, paving the way for the estimation of the age using proteomics. Surprising differences within the same piece of bone tested in different areas across the length of the bone were also noted. While the midshaft gave the least variance, biological replicates collected at the ends of long bones (especially the proximal end) exhibited significant differences in terms

of their proteome composition. Differences were also present within different bones from the same individual, underlining the intra-skeletal variability of the proteome. Furthermore, important differences between the buried bone and the fresh bone were also observed, confirming that the burial environment can affect the proteome, as these lost a notable amount of proteins due to leaching. Hence, results are proving the applicability of these types of studies to forensic remains, and that they are promising approaches for future studies on age estimation. This presentation will conclude with the comparison of these results to those obtained from the burial experiments to evaluate applicability to PMI estimation.

Reference(s):

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Postmortem Interval, Proteomics, Aging