Relative Protein Abundances and Biological Ageing in Whole Skeletal Elements

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Introduction

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Skeletal age estimation is vital part of identifying the dead and can be achieved by a variety of techniques, though their accuracy and applicability is affected by a range of factors, such as taphonomic state, sex and ancestry. Ageing juveniles is also considerably easier than adults as skeletal and dental development is predictable but completes around 25 years of age; moreover, adult age estimation is limited and methods are few, and elements do not always survive for analysis [1]. Proteins are noted for their survivability over DNA, surviving 1000s of years, and have shown to have potential as biological age markers in pigs [2]. The primary aims of this study were to analyze the proteomes of whole elements using a rat model. Our secondary aims were to consider the cross-species implications of the signals identified.

Method

-Whole tibiae were demineralised in EDTA and then extracted with GuHCl,
-Proteins isolated via ultrafiltration from GuHCl fraction,
-Digested with trypsin,
-Purified with C18 zip-tips,
-Analysed by OrbiTrap LC-MS/MS,
-Abundances calculated with Proteome Discoverer (Thermo Fisher),
-A query was used to order proteins by high coverage, followed by high abundance in the youngest age, and low abundance for the oldest, 9 proteins of interest were identified.

Results

Males (solid line) & Females (dotted line)

Discussion

Age related Proteins

-Though fetuin-A is generally seen to decrease throughout life[1], this study shows the importance of considering the animal analogue chosen in forensic research.
-There was a remarkable difference between males and females for chromogranin-A, especially after sexual maturity, it also increased throughout life.
-Serum albumin is known to decrease through life, age dependent differences in bone vascularity may have contributed to lower serum albumin in older samples.
-This study observed a steady increase in biglycan abundance up until adulthood in rats, followed by a plateau in abundance during adulthood; this may be due to only studying rats up to middle age.
-A generally steady decrease in ApoA-I was seen from the youngest age to the oldest for both sexes, which may be attributed to how aging slows the liver's rate of regeneration by affecting the regeneration pathways.
-Prothrombin displayed an increase in abundance with age in this study, similarly to biglycan and chromogranin-A, with both sexes showing a small decrease at 8–10 weeks, shortly after they reach sexual maturity.
-The study of vimentin from bone is very limited and has yet to be applied to age estimation but this study shows that age-related changes to vimentin may also be reflected within bone.
-There is a potential use of osteopontin to provide more precise proteomic age estimation in juvenile individuals when used in conjunction with other proteins, as a decrease in abundance is seen for both sexes from the youngest age up to reaching adulthood.

Sex Differences

Most abundance changes happen around sexual maturity (f=6-10 weeks, m=8-12 weeks). Female rats' skeletal mass is increased in preparation for the first estrus cycle and litter, accounting for the increase in bone development proteins and chromogranin-A, as its abundance is inversely linked to extracellular calcium abundance, this mass is also not recovered after their first litter.

Sample Preparation

Warm water was chosen as the maceration medium to prevent protein loss as much as possible, compared to chemical maceration methods that could easily break down the collagen matrix. Protein leaching may be more likely in younger, more porous bone, this study has shown that scaled maceration times may be beneficial for juvenile studies. The bones were also degreased in 83% chloroform/17% methanol solution, a common degreasing method in proteomics, to ensure the samples reflect a more representative bone proteome with the removal of residual soft tissue proteins upon degreasing.

Conclusion

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This study has shown the potential of proteomic age estimation and offering a wider age range than previous studies, as well as the potential to provide a more robust age estimation method for adults.
Though this method highlights differences between males and females, it cannot provide an accurate estimation of sex.
Furthermore, all the proteins studied should be analysed together to provide an age profile, similar to current methods.
Further research is needed to determine applicability to forensic contexts and in humans.

References

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