FRACTURE MANAGEMENT STRATEGIES INFLUENCE THE FRACTURE HEMATOMA PROTEOME AFTER MULTIPLE TRAUMA.

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Introduction

Multiple trauma can lead to impaired fracture healing [1]. The subsequent surgical intervention is important in stabilizing the patient and is considered the so-called 'second hit'. However, invasive, prolonged surgery can cause excessive inflammation and may be detrimental to a patient's condition [2]. This dilemma lies at the base of two main trauma-treatment strategies: Early-Total-Care (ETC) and Damage-Control-Orthopaedics (DCO). ETC aims at early, permanent fixation of all long bone fractures during primary surgery, whereas DCO focusses on temporary fixation, using e.g. external fixators for later definitive fracture fixation [3]. Both treatment methods have pros and cons, but exact cellular mechanisms that underlie their differential effects on fracture healing are not yet fully known. The fracture hematoma (fxH) has proven to be a key element in adequate initiation and prolongation of the fracture healing cascade [4]. These cellular communication mechanisms depend in great part on proteins. Proteomics is increasingly applied in trauma research, but mainly on circulatory proteins. The aim of this study was to develop a protocol for the determination of proteins in fxH samples from a porcine multiple trauma model in which two surgical treatments were compared.

Methods

The porcine multiple trauma model consisted of bilateral femur fracture, blunt chest trauma, liver laceration, and controlled hemorrhagic shock. Animals were operatively and medically stabilized and monitored under ICU standards for 72 hours prior to sacrifice. Three experimental groups were defined; control (n=6), intramedullary nailing (ETC; n=7), and external fixation (DCO; n=7) (figure 1). FxH was sampled from the fracture site, snap-frozen and stored at -80°C to avoid molecular degradation. Samples were sectioned at 15µm at – 20°C using a cryostat to facilitate protein extraction. Per sample, 10 sections were collected in Eppendorf tubes and dissolved in 100µL of 50mM ammonium bicarbonate buffer containing 5M urea, followed by 3 freeze–thaw cycles for protein isolation. A Bradford protein quantification assay was performed and 20µg protein were loaded on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The SDS-PAGE ran for 10 min at 50V followed by 4 min at 180V. The gel was stained with Coomassie-blue for protein visualization. Protein bands were collected from the gel and digested using trypsin using a MassPREP robot. An HSA/immunoglobulin depletion was performed with 100 µg of the isolated protein. The digested samples were injected and separated on an Acclaim PepMap C18 analytical column (2µm, 75µm × 500mm, 100Å) coupled to a Thermo Fisher Scientific HPLC system. The HPLC system was coupled to an Orbitrap MS Q-Exactive instrument equipped with a nano electrospray Flex ion source. Raw data files were processed with proteome discoverer software for protein identification, and abundance and fold change calculations. The swiss-prot Sus scrofa database was used (TaxID 9823).

Results

Label-free proteomics analysis was performed to investigate the differences in protein expression between the ETC and DCO groups. For the first time in literature, the FxH proteome was described, finding a total of 2311 proteins. Protein interaction networks were generated using STRING software. The networks with the large enrichment effects were related to the cell cycle, electron transfer and hemoglobin complex go-term. Of these proteins, 30 proteins showed a statistical difference (adjusted p-value≤0.05; FC cutoff set at 1.5-fold) between the groups. Among those, 19 and 11 proteins showed higher abundance in the ETC and DCO groups respectively. These proteins are involved in cell cycle pathways and complement activation.

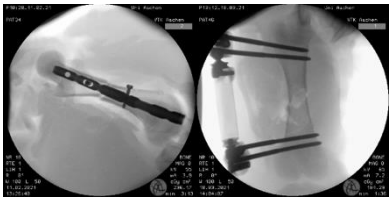

Figure 1: radiograph of fracture fixation strategies from the two treatment groups. Intramedullary nailing for ETC, and external fixation for DCO.

Discussion

This study shows that label free proteomics is a suitable analytical tool for protein analysis in fxH. The invasiveness of the surgical intervention had a clear effect on the fxH proteome at the injury site. Treatment-specific proteome changes were identified, linked to key processes in inflammation and fracture healing.

References

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