

## **Poor clinical response to Autologous Chondrocyte Implantation is associated with a unique synovial fluid proteome profile**

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### **Purpose (the aim of the study)**

Autologous chondrocyte implantation (ACI) in the treatment of cartilage injuries has reported success rates ranging between (71-95%). However, we are yet to understand fully why some individuals do not respond well to this intervention. This study has aimed to profile the synovial fluid (SF) proteome of clinical responders or non-responders to ACI, both prior to cartilage harvest (Stage I) and at the time of cell implantation (Stage II). The proteome profiles were then studied to: (1) assess potential protein biomarkers able to predict which patient suitability for cell therapy, (2) assess the protein response to a defined cartilage injury (cartilage harvest; Stage I) and (3) provide greater understanding of whether the underlying biology differs between the two clinical outcome groups.

### **Methods**

SFs were derived from 14 ACI responders (mean Lysholm improvement of 33 (range of 17-54) and 13 non-responders (mean Lysholm decrease of 14 (4-46)) at the two stages of surgery (cartilage harvest and chondrocyte implantation). Two independent Liquid chromatography- tandem mass-spectrometry (LC-MS/MS) approaches were used to profile the SF proteome in an attempt to identify predictive markers of ACI success. The first approach used protein dynamic range compression coupled with label-free quantitation (LF) LC-MS/MS with the aim of identifying lower abundance SF proteins. The second approach used isobaric tag for relative and absolute quantitation (iTRAQ)-LC-MS/MS on pooled SFs without dynamic range compression, aiming to assess higher abundance SF proteins. Three of the biologically relevant proteins were validated using enzyme linked immunoassay (ELISA) (R&D biosystems). The proteomic profiles were assessed using Ingenuity™ Pathway Analysis software (Qiagen) to investigate the pathways involved in this ACI response.

### **Results**

LF with dynamic range compression and iTRAQ approaches identified few significant differences in the baseline SF proteomic profiles of ACI responders versus non-responders (1 and 17 proteins with  $p \leq 0.05$  and  $\geq 2.0$  fold differential abundance identified by LF and iTRAQ, respectively). There was, however, a marked proteome shift in response to cartilage harvest (Stage I) (116 and 41 proteins with  $\geq 2.0$  fold differential abundance identified by LF and iTRAQ, respectively), though only four of these protein changes were detected with both techniques.

Protein datasets were combined for a comprehensive analysis of the proteome shift between stages I and II of ACI. Non-responders and responders demonstrated 96 and 45 uniquely differentially abundant proteins ( $\geq 2.0$  fold change), respectively and 12 proteins were differentially abundant irrespective of clinical response between stages I and II.

Focussing on our subsequent analyses on the non-responder shift we have described that the pathways associated with their unique response to cartilage harvest included Liver X Receptor/Retinoic X Receptor activation ( $p = 3.4 \times 10^{-7}$ ) and acute phase response signalling ( $p = 8.96 \times 10^{-6}$ ). We have also validated three biologically relevant protein changes in response to cartilage harvest in non-responders using ELISAs, confirming that both matrix metalloproteinase (MMP) 1 (9.7 fold;  $p = 0.006$ ) and MMP3 (6.9 fold;  $p = 0.001$ ) were prominently elevated, whereas S100 Calcium Binding Protein A13 (S100A13) was reduced (2.6 fold;  $p = 0.02$ ).

## **Conclusions**

The use of two independent proteomic techniques has highlighted a differential proteome response to cartilage harvest in clinical responders versus non-responders that has not previously been described. The use of dynamic compression of proteins for LF but not for iTRAQ proteomic analysis has allowed for increased coverage of the SF proteome, particularly for study of lower abundance proteins. Our analyses have suggested several pathways that appear to be altered in non-responders which are worthy of further investigation and may enable the elucidation of the mechanisms of ACI failure. Moreover, three candidate biomarkers, MMP1, MMP3 and S100A13, have been identified which may be useful for selecting patients suitable to progress to Stage II of the ACI procedure.