Global proteomics profiling of serum and synovial fluid identifies biomarkers of outcome after intraarticular gold for management of painful knee osteoarthritis

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Global proteomics profiling of serum and synovial fluid identifies biomarkers of outcome after intraarticular gold for management of painful knee osteoarthritis.

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Background and aims:

In a pilot study, we recently showed that intra-articular gold micro-particles can reduce knee osteoarthritis (KOA) pain for up to two years and found associated significant proteomic changes in serum and synovial fluid within eight weeks (1). This current study aimed to identify serum and synovial proteomic profile associations to patient-reported effect outcomes after intra-articular injection of gold microparticles.

Dissolucytotic metallic gold (DMG) ions have an immune-suppressive effect in laboratory testing (1). Gold may decrease inflammation because of various mechanisms such as regulation of the NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway (1). (Figure 1 - 4).

Methods:

Thirty patients with moderate KOA were included and intra-articular 20 mg gold microparticles (72.000 particles, 20-40 µm in diameter) were injected using the patient's synovial fluid (SF) as the carrier. Proteomic profiling on SF and serum samples before and after eight weeks of treatment. The patients evaluated the effect on an 11-point scale from very much worse (-5) to complete recovered (5) with a score of "0", indicating no changes.

Synovial fluid and serum samples were trypsin-digested and analyzed using a discovery proteomics LC-MS workflow on a timsTOF Pro mass spectrometer operated in diaPASEF mode. Raw mass spectrometry data were then matched against the human UniProt database using the directDIA algorithm in Spectronaut for the identification and quantification of proteins. A sparse Partial Least Squares (sPLS) model was adapted to integrate proteomics data (expression of up to 600 proteins) with subjective measures of treatment effect and explore relationships between the two datasets. The linear combination of proteins with the highest correlation to treatment effect was identified and used to predict treatment response. Finally, a functional enrichment analysis was made in Metascape to assess the biological relevance of the synovial fluid proteins that were covarying with the treatment effect. Biological processes/terms were regarded as significantly enriched at a $p < 0.01$.

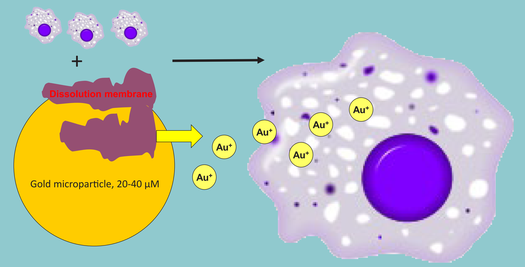

Figure 1. *Macrophages controls the dissolution membrane which liberate the gold ions by oxidation of the surface. Once the ions are liberated, most likely as Au(CN) , they are free to diffuse through the immediate microenvironment. The gold-loaded molecules are taken up into the cells, primarily macrophages, mastcells and histocytes.*

Figure 2. *Figure 2. Close to the gold implant gold-loaded molecular clusters are located outside cells. The two loaded cells are believed to be macrophages loaded with gold ions. The gold ions accumulate primarily in the lysosymes (3).*

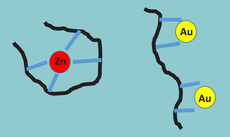

Figure 3. *Once in the intercellular fluid and the intracellular compartments, the gold ions act in the same ways that have been demonstrated for systemically administered gold ions. The effect is related to the ability of the gold ions to unfold the protein structures.*

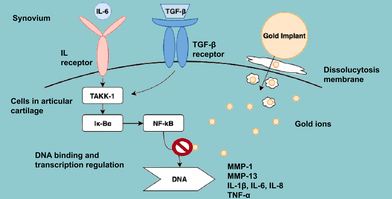

Figure 4. *Gold ions suppress inflammation locally by affecting certain signalling molecules and binding enzymes essential for the inflammatory process. The DNA binding activity and transcription regulation of NF-kB is abolished when AU- ions replace Zn2+ ions.*

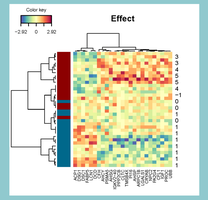

Figure 5. *Synovial fluid heat map*

Results:

The Pearson correlation coefficient between the treatment effect and the linear combination of 25 selected synovial fluid proteins was $r(34) = 0.93$, $p < 0.001$. The linear combination of synovial fluid proteins and the treatment effect explained the difference between baseline and follow-up samples. The functional enrichment analysis revealed that the 25 proteins were associated with 1) neutrophil degranulation, 2) regulation of IGF transport and uptake by IGF binding proteins and platelet degranulation, 3) plasma lipoprotein clearance and multiple intracellular signaling pathways related to cell cycle and apoptosis among others, 4) protein stabilization and cell growth, 5) EPH-Ephrin signaling and axon guidance, 6) negative regulation of peptidase activity and proteolysis, 7) signaling by receptor tyrosine kinases and ossification and 8) cell-cell adhesion (Figure 5).

The Pearson correlation coefficient between the treatment effect and the linear combination of seven selected serum proteins was $r(46) = 0.75$, $p < 0.001$. Both the linear combination of serum proteins and treatment effect explained the difference between baseline and follow-up samples. The functional enrichment analysis revealed that the seven proteins were associated with 1) complement cascade, 2) regulation of proteolysis and 3) NABA_MATRISOME_ASSOCIATED which is a collection of extracellular matrix-associated proteins.

Conclusions:

We identified linear combinations of serum or synovial biomarkers that changed significantly alongside the effect measures of gold micro-particle treatment for KOA. Both the serum and synovial fluid biomarkers were associated with inflammatory processes and regulation of proteolysis. The synovial fluid biomarkers were additionally associated with multiple biological processes that are collectively suggestive of tissue regeneration and nervous system remodeling following gold micro-particle treatment. The study further demonstrates the feasibility of utilizing protein biomarker signatures in future clinical decision-making.

COI statement: none

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