Systems biology of osteoarthritis
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Summary

Osteoarthritis (OA) is one of the most prevalent rheumatic conditions, affecting more than half of the population above sixty years of age. The main characteristic of the disease is the progressive loss of articular cartilage which is thought to originate from an imbalance between synthesis and degradation of the cartilage matrix. However, its precise aetiology is still far from understood, resulting in a lack of adequate diagnostics and therapies. Possibly, complex interactions of multiple pathophysiological mechanisms are involved. While these mechanisms are currently unknown to us, recent scientific developments, especially in the field of analytical biosciences may provide the means necessary to unravel them. In particular, the development of ‘omics’ techniques enables the analysis of hundreds of bio-molecules (transcripts, proteins, metabolites) simultaneously, providing an unprecedented coverage of the biochemical events taking place in healthy or diseased organisms.

It is expected that the use of ‘omics’ approaches will facilitate the discovery of novel biomarkers. A biomarker is an objectively measurable indicator of biochemical processes in relation to disease or therapeutical intervention. Additionally, with these methods it is possible to study diseases such as osteoarthritis from a systems biology perspective, i.e. studying biology as an integrated system of genetic, protein, metabolite, cellular, and pathway events that are in flux and interdependent.

The aim of the work described in this thesis was to better understand OA by approaching it from a systems biology perspective, and to study the applicability of ‘omics’ technology to this effort. As a first step in the project, through rigorous analysis of the literature, the most pronounced gaps in our knowledge of OA pathology were identified that prevent a comprehensive systems view. Subsequently, the relevant ‘omics’ methods were developed and applied.

In Chapter 2 the published articles comprising ‘omics’ data for osteoarthritis were reviewed from a systems biology (hence data integration) point of view, and gaps in the available data that prevent full-fledged
systems biology evaluation of OA were pinpointed. The three most promising mechanisms that have been reported in multiple studies and on multiple levels (transcript, protein, and/or metabolite), but have been relatively unstudied in their own right, were cell signaling, carbohydrate & lipid metabolism, and oxidative stress defense. Based on these findings our efforts focused on peptidomics and lipidomics approaches.

Peptidomics or peptide profiling methods enable the simultaneous analysis of hundreds of endogenously present peptides. These endogenous peptides are important signaling mediators as well as proteins degradation products. In **Chapter 3** analytical aspects were discussed of a method that allows for the analysis of endogenous peptides in synovial (joint) fluid (SF), a compartment that is derived directly from where the disease occurs. Major hurdles to be overcome in the development of this method were the highly viscous nature of SF due to the presence of high concentrations of hyaluronic acid and the inherently low concentrations of the endogenous peptides. It was found that a combination of ultrafiltration and solid-phase extraction provided sufficient sample treatment to eliminate hyaluronic acid and other contaminants, and together with the highly sensitive nanoLC-MS system, hundreds of peptides could be analyzed. The analytical performance was found to be satisfactory for biological studies, with within-day relative standard deviations of 5-15%, between-day relative standard deviations of 6-16%, a linear response in the analyzed 50-800 nM range (R² = 0.930-1.000), a limit of detection in the femtomole range, and reproducible recoveries of 14-67%.

In **Chapter 4** the developed method was used to analyze differences in peptide composition among synovial fluid samples from patients with OA, rheumatoid arthritis (RA), and from controls. In total ~1000 datapoints were compared among all samples and principal component analysis revealed clustering of the three groups. While fibrinogen fragments contributed considerably to this grouping, significant disease associated alterations were also found for peptide fragments derived from collagens (I, II, III), osteopontin, kininogen, complement components 3&4, serum amyloid A, and various histones. Amongst the most interesting findings was that levels
of the pain and inflammation mediator bradykinin and its hydroxylated form were elevated in OA, when compared to control and RA.

While the sample preparation by ultrafiltration and solid-phase extraction (as discussed in Chapter 3) proved efficient in extracting peptides from a difficult matrix, it is relatively laborious therefore less suited for large biomarker studies. Therefore, in Chapter 5, an alternative sample preparation method was explored that is based on electrodialysis. This method has the advantage easily being automated, miniaturized, and configured for high-throughput applications. A custom-made device was developed from the low-binding material Kel-F. It consisted of two compartments separated by a dialysis membrane, over which a voltage was applied. One compartment served as donor (containing the sample), while the other (smaller) compartment collected the peptides. Using model peptides, the procedure was optimized by investigating the effect of the applied voltage over the two compartments, the ammonium acetate concentration of the buffer in the compartments, and the duration of the electrodialysis process. Optimum conditions were found at 300 V (150 V/cm), 25 mM ammonium acetate buffer (pH 3.8) with 20% v/v DMSO, and a duration of 10 min, respectively. With these optimized parameters, recoveries for the model peptides were found to be 35%-85% (average 64%). Additionally, electrodialysis was successfully applied to a synovial fluid sample from a rheumatoid arthritis patient, in which 27 peptides originating from 12 proteins were identified, of which a considerable fraction was not identified before with other methods. This demonstrated the usefulness and complementary nature of combining electrodialysis with nanoLC-MS for biomarker discovery. These results indicate that electrodialysis is promising as a fast and selective sample preparation method for the profiling of endogenous peptides.

In Chapter 6 the performed lipidomics experiments were discussed that follow-up on the finding that lipid metabolism plays a role in OA. The lipidomics analysis of SF and plasma facilitated a first-time view on both local and systemic OA-related lipid changes. The plasma and SF samples underwent an approach similar to Bligh and Dyer extraction. Subsequently, the extracted samples were analyzed using liquid chromatography separation
(C₈), and mass spectrometry detection (QTOF). In total 115 lipids in plasma and 72 lipids in SF from 8 lipid classes could be integrated and compared among all samples. Principal component analysis revealed OA related changes in lipid composition, both locally (in SF) and systemically (in plasma). In plasma, a general lowering of lipid levels was observed. This lowering was most pronounced for lipids with shorter fatty acids and correlated with disease severity. For SF, the most pronounced variation between control and OA samples were changes in the relative abundance between lipid classes. Cross-compartment analysis revealed that plasma and SF of OA patients are more similar in their lipid class composition than those of controls. The results from this lipid profiling study indicate that OA associated changes in the lipid metabolism occur, both locally (SF) and systemically (plasma).

In conclusion, the work described in this thesis converged on the borders of analytical biosciences and the clinical sciences, by providing the first experimental systems biology view on the disease. The development and application of peptidomics and lipidomics approaches have led to interesting leads and it is very likely that these and other ‘omics’ approaches will point the way to new biomarkers and increase understanding of OA’s disease mechanisms. However, that will only be realized when analytical methods will further improve, and the ‘omics’ and systems biology approaches are fully integrated in the medical research paradigm.