Systems biology of osteoarthritis
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Citation
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Note: To cite this publication please use the final published version (if applicable).
Conclusions and perspectives

Osteoarthritis (OA) is one of the most prevalent rheumatic diseases, especially affecting the elderly. As it reduces the quality of life of millions, there is a need for better diagnostics and more effective treatments. However, their development has been hampered by a lack of understanding of the disease aetiology. While the main characteristic of the disease is progressive loss of articular cartilage, the view that OA results from multiple pathophysiological mechanisms in which local and systemic factors, as well as biomechanical triggers are interplaying, is gaining support. To what extent these processes are triggers, modulators, or merely secondary effects and how these processes influence each other, remains elusive to date.

An approach promising to provide a deeper insight in this respect is systems biology, which is defined as studying biology as an integrated system of genetic, protein, metabolite, cellular, and pathway events that are in flux and interdependent. It focuses on the interaction of multiple biochemical components in networks, and as such, heavily relies on ‘omics’ technologies, which allow the simultaneous measurement of hundreds or even more transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics). The development of these ‘omics’ technologies has become attainable by amongst others innovations in separation science, mass spectrometry and computational approaches.

The aim of this project was to investigate the application of ‘omics’ technologies to improve understanding of OA from a systems biology perspective. As a first step, in Chapter 2, the most profound knowledge gaps of OA pathology were identified that prevent a comprehensive systems view, and subsequently the relevant ‘omics’ methods were developed and applied to contribute to the elucidation of these gaps. Two knowledge gaps involved the (endogenous) peptide and lipid compound classes, which are important in cell signalling and lipid homeostasis/metabolism, respectively.
Chapter 3 describes the development and performance of a nanoLC-MS-based platform for the study of endogenous peptides in human synovial (joint) fluid. As synovial fluid has a high viscosity due to the abundantly present hyaluronic acid, a rigorous sample preparation was necessary. Solid-phase extraction (SPE, reversed phase) and subsequently ultrafiltration was found to be effective in this respect. Also, as endogenous peptides are only present in low concentrations, especially the interesting ones, employing nanoLC was crucial to achieve the desired sensitivity.

This combination of selective sample preparation and sensitive analysis enabled for the first time a comprehensive view on a large number of endogenous peptides in SF. The performance of the nanoLC platform was satisfactory for our purposes and allowed the identification of disease-associated variations in the levels of multiple endogenous peptides in synovial fluid, as is described in Chapter 4. In total ~1000 peptide features could be compared among all samples. While fibrinogen fragments contributed considerably to this grouping, significant disease associated alterations were also found in the levels of peptide fragments derived from collagens (I, II, III), osteopontin, kininogen, complement components 3 & 4, serum amyloid A, and various histones. However, among the most interesting findings was the elevation of the inflammation mediator bradykinin in OA, also when compared to RA. Therefore it might be a selective biomarker for OA and is definitely worth further investigation.

While the nanoLC platform provided high quality separations and sensitive analyses, it was not always found to be robust during the analysis of large sample sets (more than hundred sample analyses). This is caused by variations introduced by the way the difficult-to-connect fittings are tightened and by the limited stability of the nanospray interface. This could be addressed by using chip-based emitters of a commercial system, where the emitters can be automatically exchanged if the spray is stopping due to for example clogging. However, recent developments in chip-based nanoLC including a nanospray emitter may provide a more promising alternative for future improvements of the peptidomics platform (Appendix of Chapter 4), as it provides improved robustness and a separation performance that approaches that of the nanoLC setup described in Chapter 3. In addition, the
choice of the mass spectrometer is important for the eventual sensitivity and specificity obtained. Currently, targeted MS/MS using a triple quadrupole MS system is still unrivalled in terms of dynamic range and sensitivity, and has great potential for targeted peptidomics. However, recent innovations in Orbitrap and ion cyclotron resonance Fourier transform (e.g. higher field strength) and high resolution time of flight technology might change that over time. In addition, new hybrid MS systems (dual pressure ion trap-Orbitrap, ion mobility-TOF) allow new profiling protocols to improve selectivity and/or sensitivity.

The sample preparation approach introduced in Chapter 3, based on a combination of ultrafiltration and solid phase extraction (SPE), provided sufficiently robust and repeatable peptide extractions to be applied to SF samples obtained in a clinical study. However, this sample preparation strategy was quite time consuming and cannot readily be automated. In addition, peptide recoveries from biological samples were reproducible but their extraction was not complete. Therefore, the possibilities of electrodialysis (ED) as a sample preparation technique for peptides was investigated, as is discussed in Chapter 5. The obtained results were encouraging and a proof-of-concept was achieved. However, this technique still requires some further development before it can be used for biomarker studies. The main advantages of ED are its speed, and its potential for miniaturization, automation, and high-throughput applications. Applications are foreseeable where chip-based ED is performed online with for example LC-MS, or where ED is performed in a 96-wells plate in a high-throughput application.

Chapter 6 of this thesis describes for the first time the application of lipidomics to SF. In addition it demonstrates that there is evidence for changes in lipid metabolism in OA, both locally and systemically, as was also suggested by our findings in Chapter 2. Specifically, in OA plasma samples a general lowering of lipid levels was observed with respect to control samples. This lowering was most pronounced for lipids with smaller fatty acid constituents and correlated with disease severity. For SF, the most pronounced difference between control and OA was the abundance of lipid classes (sum of all lipids in a class) relative to each other (triglycerides lower
in OA, sphingomyelins and glycerophosphocholines higher in OA). The results from this study indicate that changes in the lipid profiles are associated with OA, both locally and systemically. These results aptly demonstrate the potential of systems biology, but further research is necessary to better understand if and how lipid regulation contributes to the development of OA.

To study more in-depth the involvement of specific lipid species and pathways, more understanding of lipid biochemistry is needed. This understanding is currently limited as lipid molecules are structurally divers and the biochemistry complex. This is likely to change as novel developments (multidimensional separations, ion mobility-mass spectrometry hybrids, etc) in analytical chemistry are increasingly suited to address this complexity. One of the difficulties most of the lipidomics platforms have still to deal with is the inability to unequivocally identify the structure and position of the fatty acid constituents of the lipids, including the position of the double bond(s). When this is resolved, it will be easier to place the individual lipids within its biochemical framework and to better understand the changes that are associated with OA.

Overall, the collection of all transcriptomics, proteomics, and metabolomics methods should be regarded as a ‘toolbox’ for the study of biology and depending on the particular biological question and compartment of interest, the most appropriate method should be used. For example, for a not well studied disease it would be a sound strategy to start with untargeted transcriptomics, proteomics, and metabolomics profiling, followed by targeted (and more in-depth) analyses of affected pathways/networks, and subsequently flux analysis to study network behaviour in relation to intervention in detail. Intuitive bioinformatics programs that efficiently manage the heterogenic data will be important for this approach. However, of absolute essence for these interdisciplinary research projects is a good collaboration between the analytical chemist, who develops and applies the appropriate ‘omics’ methods, the bio-informatician who efficiently manages the data and extracts the relevant information, and the biologist and clinician who bring in the biomedical knowledge and questions and perform the biological interpretation.
Special consideration should be given to sampling and sample types. Proper optimization and validation of sampling procedures are often neglected but are of utmost importance, as some groups of compounds are liable to *ex-vivo* degradation, leading to artefacts. In addition, while in most cases we are interested in human pathology, it is not ethically feasible to obtain particular types of human samples (for example SF). In those cases, one often diverts to *in-vitro* and/or *in-vivo* disease models. As it is often not known which events are relevant to human biology or merely specific to that model, one should be prudent in extrapolating those results to the human case. However, when these issues are taken into account and experiments are carefully set up, systems biology studies will reveal a new level of biological information. This information will help us to design new and better treatments. It is becoming increasingly clear that multiple mechanisms are involved in OA, and that it is the combination of these mechanisms that produces the disease outcome. Therefore, it is unlikely that effective treatments will be based on single bioactive compounds, as is practised now, but rather on a combination of drugs that simultaneously modulate multiple targets. As such, progress in understanding disease biology will have to coincide with fundamental changes in the way drugs are developed and evaluated, and systems biology will certainly contribute to realizing these changes.