Systems biology of osteoarthritis
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General introduction and scope

Osteoarthritis: the need for better diagnosis and treatment

Scientific discoveries from the past decades have increased our understanding of what constitutes health and disease, resulting in effective diagnosis and treatment for a number of illnesses. However, for most diseases that display complex interactions of multiple factors, diagnosis and treatment are still unsatisfactory or even non-existent.

One such a disease, and subject of investigation in this thesis, is osteoarthritis (OA). OA is one of the most frequently occurring rheumatic diseases, which mostly but not only affects the elderly (1). A recent Dutch demographic study found that 10% of the population above twenty years of age has diagnosed osteoarthritis, and as the population ages the prevalence is expected to increase further (2). The main characteristic of the disease is progressive loss of articular cartilage which is thought to originate from an imbalance between synthesis and degradation of the cartilage matrix (see Figure 1) (3;4). However, its precise aetiology is still far from understood and recent studies support the view that OA is a disease resulting from multiple pathophysiological mechanisms in which local and systemic factors, as well as biomechanical triggers are interplaying (1).

As a consequence of the limited understanding of the disease's complexity, no disease modifying treatments are currently available. Therefore the only existing therapies primarily comprise analgesics and joint replacement surgery. In addition, there is a lack of adequate biomarkers for early diagnosis, prediction of eventual joint damage, assessment of disease progression and for monitoring the efficacy of experimental treatments (5-7). The discovery of novel biomarkers for OA will help to improve OA diagnosis and will also help to find new leads for actual disease modifying treatments.
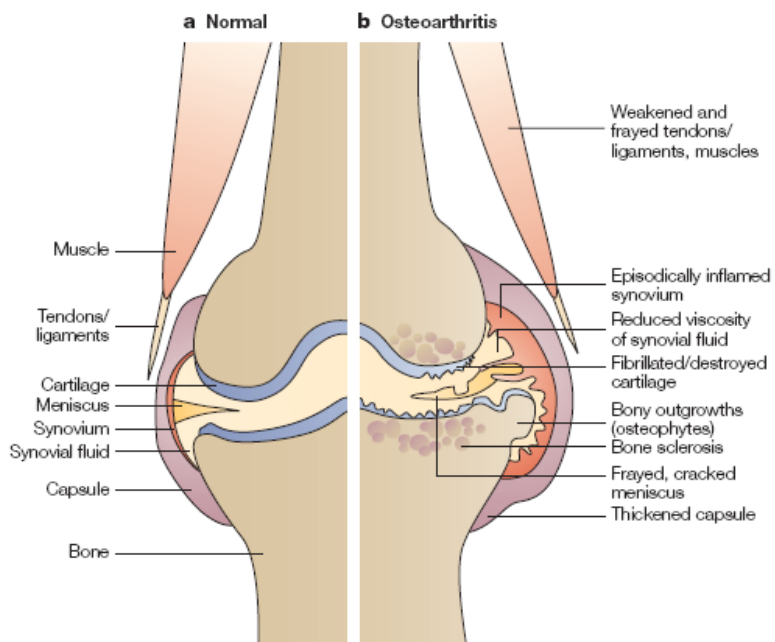
Figure 1. Joint damage resulting from osteoarthritis. (a) shows a healthy joint with intact cartilage. (b) illustrates OA with amongst others degradation of the cartilage, synovial inflammation, and the formation of bony outgrowths. Reprinted from reference (1).

The emergence of 'omics' and the search for biomarkers and systems-level understanding

Biological research has traditionally been focused on studying biochemical components/pathways in relative isolation (i.e. their interaction with other components/pathways was not considered). This has led to numerous new insights and has yielded most of the knowledge that we have today. However, in recent years analytical chemistry has evolved to a degree that it is feasible to measure dozens or even more compounds simultaneously (8;9). This development culminated in the emergence of the 'omics' disciplines, with the most notable variants (downstream of genomics) being transcriptomics, proteomics, and metabolomics, which comprehensively study the expression of many gene transcripts, proteins, and metabolites respectively (see Figure 2) (10). While genomics technologies like genome-wide linkage approaches

are powerful with regard to finding disease-associated patterns in the DNA, our focus is rather on the systems behaviour of DNA's expression products and metabolites. In combination with state-of-the-art bioinformatics applications it is possible to study for example changes in the levels of hundreds of biochemical components between diseased and healthy state. The 'omics' disciplines allow researchers to achieve a 'holistic', or wide-screen view of the processes involved in the disease, and provide a complementary approach to the classical reductionist approach. The 'omics' disciplines exert their impact on health care via two approaches: biomarker profiling and systems biology.
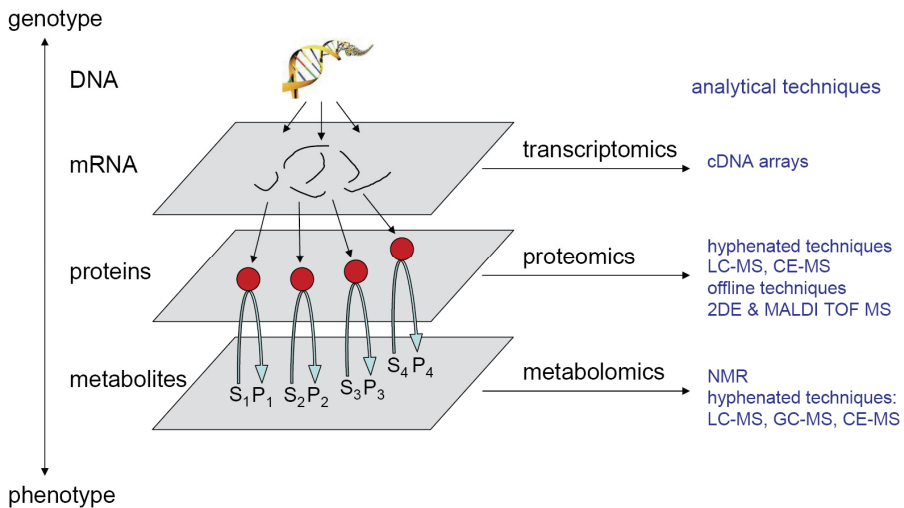

Figure 2. The most notable 'omics' disciplines related to the expression of genes, transcriptomics (closest to the genotype), proteomics, and metabolomics (closest to the phenotype), allow the simultaneous analysis of hundreds of transcripts, proteins, and metabolites respectively. For the analysis of transcripts cDNA array technology is used. For the detection of proteins either two-dimensional electrophoresis (2DE) is used followed by enzymatic digestion and analysis by MALDI-TOF MS, or a hyphenated approach of separation by liquid chromatography (LC) or capillary electrophoresis (CE) coupled to mass spectrometry (MS) detection after digestion. For the analysis of metabolites in addition to these hyphenated techniques, gas chromatography (GC) coupled to mass spectrometry is used, as well as nuclear magnetic resonance (NMR) (10).

Biomarker profiling

As the 'omics' disciplines enable the profiling of a multitude of compounds for the comparison between for example healthy and disease state, these approaches bear much promise for the discovery of new biomarkers. A biomarker is defined as a parameter that is objectively measured and evaluated as an indicator of normal biological or pathological processes, or pharmacological responses to a therapeutic intervention (11). It is hypothesized that biomarkers reflect the biological system's (in)ability to maintain equilibrium (see Figure 3). This equilibrium is termed homeostasis and this is the basis of human physiology, and thus essential for survival and health. During disease this homeostasis is impaired, leading to uncontrolled up-or down regulation of some of the biological system's components (the biomarkers), and finally leading to symptoms of the disease. Good examples of such biomarkers are glucose for diabetes and C-reactive protein for cardiovascular disease (12).

In this light, depending on its role in the biological system, a biomarker can reflect different stages of the biological system's derailment during disease development and/or progression. Multiple biomarker classification schemes exist that have subtle differences and overlaps (13;14). A popular method proposes three types of biomarkers: predisposition/risk, prognostic, and diagnostic biomarkers (14). Predisposition or risk biomarkers can inform about a subject's sensitivity for developing a disease in the future, even though the biological system is functioning perfectly normal at that moment. Good examples are the proto-oncogenes for certain types of cancer. Prognostic biomarkers represent actual changes in pathway dynamics to maintain homeostasis and predict whether the subject is likely to develop a certain condition. A well known example is LDL cholesterol which is used as a measure to determine susceptibility towards developing heart disease. Finally, diagnostic biomarkers report the incidence and progression of an already established disease (lost homeostasis), such us microalbuminuria levels reflect the degree of kidney injury/damage for diabetic patients (15;16). Diagnostic biomarkers can be further divided into early and late diagnostic biomarkers, which can indicate the early or late development of disease subsequently, and surrogate biomarkers that can substitute for a

clinical endpoint. A clinical endpoint is a measure for how a patient feels, functions, or survives, be it in the presence or absence of therapy.

While new biomarkers are destined to provide significant benefits to health care in the future, the current lack of understanding of the underlying biochemistry's complexity often makes it difficult to assess the value of a potential biomarker. hile extensive validation studies can circumvent this issue, the effectiveness of biomarker studies would be much improved if we could understand biology at a systems level.
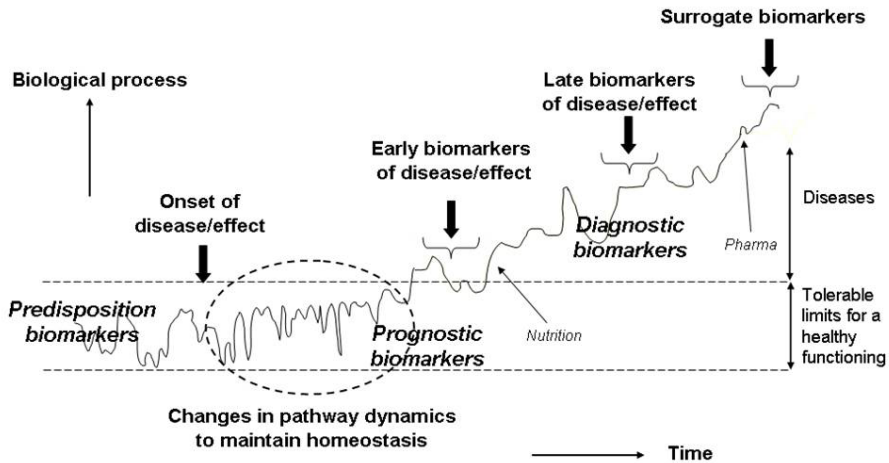

Figure 3. The collection of biomolecules in an organism functions as a system that seeks to maintain equilibrium (homeostasis). Biomarkers reflect disruptions in this equilibrium by, for example, a disease and can be used for prognosis, diagnosis, of progression of the disruption. Reprinted with permission from Jan van der Greef.

Systems biology

A biological entity, such as a human being, consists of myriads of cells, which in turn contain many genes, transcripts, proteins, and metabolites. However, it is not their mere presence that constitutes a living person, but rather the intricate interaction of these biological components. These molecules participate in specific networks and systems of interactions, and it

is the aberrations in network behaviour that causes disease. The discipline that seeks to reconstruct how molecules interact with each other in networks is systems biology. It is defined as 'studying biology as an integrated system of genetic, protein, metabolite, cellular, and pathway events that are in flux and interdependent' (9;17). As such, it enables integration of interactions between multiple levels (transcripts, proteins, metabolites) and compartments (plasma, urine, etc.), with or without pre-existing knowledge.

While the systems-based approach emerged across various scientific domains in the last century, it only recently gained considerable momentum in the biological sciences and pharmaceutical research, particularly as awareness grew for the need to look at biology from a different perspective, together with advances in multiple technologies. The realization grew that the classical reductionist approaches to biology have only limited usability for the multifactorial, complex diseases, as is evidenced by the lack of new diagnostics and therapies, and that these approaches need to be amended with tools that look at biology from a network/system perspective. This new, holistic way of looking at biology started to become attainable by technical advances in analytical chemistry and computational approaches (9;18;19). At present, modern systems biology approaches are still in the developmental stage, but bear much promise.

Aim of this thesis

This thesis investigates the potential of 'omics' technologies to improve understanding of OA from a systems biology perspective. There is still little knowledge about the cause (or trigger) of OA. As a first step the most pronounced gaps in our knowledge of OA pathology were identified that prevent a comprehensive systems view, and subsequently relevant 'omics' methods for metabolites and peptides were developed and explored.

Outline of this thesis

Each chapter represents an individual step towards realizing the aim formulated in this thesis. In **chapter 2**, the envisioned systems biology approach to OA is discussed and the most pronounced OA knowledge gaps that prevent a full-fledged systems biology view are identified. These

knowledge gaps may be caused by the lack of proper analytical platforms, a lack of priority of scientists to venture into the specific topic, or a combination thereof. As will be shown, two important knowledge gaps involve endogenous peptides and lipids, respectively. Therefore, subsequent work was focused on the development and application of both peptidomics (peptide profiling) and lipidomics (lipid profiling) approaches.

Chapter 3 discusses the analytical aspects of the newly developed method for the profiling of endogenous peptides in synovial (joint) fluid. Synovial fluid is of interest as it is situated directly at the site of the disease. However, due to its high viscosity and high concentration of hyaluronic acid, selectively extracting the low-concentration endogenous peptides is a challenge. This issue was solved with a combination of selective sample preparation and highly sensitive analysis, and the validation of the platform is discussed.

In **chapter 4** this platform is applied to synovial fluid samples of OA and rheumatoid arthritis (RA) patients, and of a control group, to find potential candidate biomarkers and to improve understanding of the disease. Therefore, in this chapter the emphasis is on how the results were statistically evaluated and biological interpretation.

While the sample preparation procedure from chapter 3 is effective, it tends to be laborious for large-scale biomarker studies. As this is the case for alternative sample preparation procedures as well, the challenge was to find a viable alternative for these methods. In **chapter 5** a feasibility study of a novel sample preparation technique is presented for the fast and selective extraction of low concentration endogenous peptides from biological samples.

Chapter 6 reports the findings from a lipidomics study of OA human plasma and SF samples. As the plasma samples' donors had various degrees of diagnosed OA, potential correlations were examined between the plasma lipid profiles and progression of disease. Also a comparison was made between the lipid profiles of the plasma and SF compartments.

In **chapter 7**, conclusions and perspectives are presented.

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