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EPIGENETIC AND PROTEOMIC SIGNATURES FOR CHRONIC PAIN PATIENTS AFTER TOTAL KNEE REPLACEMENT

BY
ROCCO GIORDANO

DISSERTATION SUBMITTED 2021

AALBORG UNIVERSITY
DENMARK

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Dissertation submitted

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CV

Rocco received a B.Sc. degree in health biotechnologies and a M.Sc. in medical biotechnology from the Catholic University of Sacred Heart, Rome, Italy. Subsequently, he worked as research assistant in several laboratories at the Catholic University of Sacred Heart, Rome, Italy, acquiring expertise in new high throughput molecular techniques to evaluate the expression of non-coding RNA and proteins. In 2016, he worked at IRCCS Fondazione Don Carlo Gnocchi, Rome, Italy increasing his interest in pain research and genomic biomarkers. In September 2016, he enrolled as Ph.d. fellow at Center for Neuroplasticity and Pain under the supervision of Prof. Lars Arendt-Nielsen at Aalborg University, Denmark. He is interested in epigenetics, transcriptomics, proteomics and molecular biology behind the pain process. He focused his research on evaluating potential circulating genomic biomarkers for chronic pain after surgery in patients affected by knee osteoarthritis. He has been involved in several dissemination activities through conferences, abstract and poster submission for international congresses.

ENGLISH SUMMARY

Osteoarthritis (OA) is the most frequent painful musculoskeletal diagnosis in the older population and the most prominent cause of disability. Total knee replacement (TKR) is expected to provide pain relief for patients with severe OA, however, around 20% of knee OA (KOA) patients will experience chronic post-operative pain after TKR surgery. Several studies have found high preoperative pain intensities and sensitization of central pain pathways as predictors for chronic post-operative pain following TKR. Recently a study found preoperative proinflammatory cytokines to be associated with chronic post-operative pain following TKR. Preclinical data shows that proinflammatory cytokines sensitize the peripheral nerve endings, which may eventually lead to central sensitization, indicating that preoperative increase levels of inflammatory markers could act as prognostic biomarkers for chronic post-operative pain following TKR. It is now apparent that epigenetic modifications, like the actions of noncoding RNAs (e.g. microRNAs, lncRNAs, siRNAs, circRNAs), may confer susceptibility to OA, which could open up new avenues for alternative therapeutic approaches. Several studies have illustrated the possible diagnostic potential of circulating non-coding RNAs in OA, indicating that this family of molecules may act as potential predictors for the development and progression of knee and hip osteoarthritis.

Real-time poly-chain reaction (RT-qPCR), quantitative and qualitative techniques allowed the evaluation of the circulating ncRNAs in serum samples, trough the isolation and retro-transcription of total RNAs. This methodology reduces non-specific results and reduces the difficult handling of the big amount of data obtained through other molecular biology techniques. Furthermore, a new proteomic approach, i.e. the proximity extension assay (PEA), has been used which gives a specific and standardized overview of inflammatory markers involved in the pathology.

This PhD-project includes three original studies that focus on the transcriptional, post-transcriptional and translation processes in the serum of patients with osteoarthritis, investigating factors that modulate the perception of pain.

The first study aimed to investigate the preoperative association of long non-coding RNAs (lncRNAs) and chronic post-operative pain, showing that patients with pain 1-year after surgery exhibited down-expression of three lncRNAs preoperatively when compared with patients with post-surgical full recovery (Study I).

The second study evaluated preoperative microRNA's (miRNA's) profile as potential predictive biomarkers for post-operative pain. Twenty-one miRNAs were analyzed,

and three miRNAs showed a pre-operative differential level of expression in patients with low pain relief after surgery compared to patients with high pain relief. The miRNAs dysregulated showed a predictive value for the pain relief after surgery pointing to possible use for these miRNAs as potential biomarkers for post-operative pain (Study II).

The third study aimed to evaluate the preoperative serum expression of a panel of 92 inflammatory cytokines and highlight correlation with pain intensity in patients with KOA. Patients with KOA showed ten cytokines with significantly lowered expression in the serum and five cytokines with higher expression levels when compared to healthy participants. Specifically, FGF-21 and 4E-BP1 were associated with pain in KOA, and TWEAK, FGF-21, CSF-1, IL6 were identified as independent predictors for pain intensity (Study III).

In conclusion, the current Ph.d. thesis provides the first evidence of the interaction between post-transcriptional modifications and post-operative pain, which is an important step forward and offers advantages for future exploration of the involvement of epigenetic modifications in the pain field.

DANSK RESUME

Slidgigt er den hyppigste muskuloskeletale lidelse hos ældre og er desuden den største årsag til smerter og funktionsnedsættelse i denne populationsgruppe. Hos patienter med svær slidgigt vil en operation med total udskiftning af knæet (TRK) forvente at lindre smerten, dog vil ca. 20% opleve at få kronisk post-operativ smerte efter TKR-operationen. Flere nye studier har vist, at der er en mulig sammenhæng mellem en høj præ-operativ smerteintensitet, tilstedeværelsen af inflammatoriske stoffer og udviklingen af kronisk post-operativ smerte efter TKR. For nyligt viste et studie, at præ-operative proinflammatoriske stoffer var forbundet med udviklingen af kronisk post-operativ smerte. Endvidere, prækliniske data viser, at proinflammatoriske stoffer sensibiliserer de perifere nerveender, hvilket til sidst kan føre til central sensibilisering af nervesystemet og deraf ændring af de nociceptive signalveje. Dette indikerer, at en øget tilstedeværelse af inflammatoriske markører præ-operativt kan fungere som mulige prognostiske biomarkører for udviklingen af kronisk post-operativ smerte efter TKR.

Det er påvist, at epigenetiske modifikationer, så som ikke-kodende RNA'er (fx mikroRNA'er, lncRNA'er, siRNA'er, circRNA'er) også kan have en mulig medvirken i udviklingen af slidgigt. Dette kan på sigt åbne op for nye alternative terapeutiske tilgange til at diagnosticere patienter, der kan være i risiko for at udvikle slidgigt. Bl.a. har flere studier demonstreret, at cirkulerende ikke-kodende RNA'er er tilstede i forskellige vævsvæsker (f.eks. serum) hos patienter med slidgigt, og deraf antydet, at denne familie af molekyler muligvis kunne fungere som potentielle biomarkører for udviklingen af knæ- og hofte-slidgigt.

Real-time poly chain reaction (RT-PCR) er en kvantitativ og kvalitativ metode, som gør det muligt at identificere og evaluere tilstedeværelsen af cirkulerende ikke-kodenden RNA'er i serumprøver via isolering og derefter retro-transkription af den totale mængde RNA. Denne metode reducerer ikke-specifikke resultater såsom falsk-positive fund og reducerer den vanskelige håndtering af store mængde data, som man opnår gennem andre molekylærbiologiske teknikker. Endvidere, en ny proteomisk metode, Proximity Extesnsion Array (PEA) anvendt til at identificere samt give et specifikt og standardiseret overblik over de inflammatoriske markører, der er involveret i patologien.

Dette Ph.d.-projekt inkluderer tre originale studier. Studie 1 og studie 2 undersøgte, om der var en sammenhæng mellem tilstedeværelsen af mulige epigenetiske modifikationer (ikke-kodende RNA'er) hos patienter med slidgigt præ-operativt og deres smerteintensitet post TKR. Studie 3 undersøgte desuden, om der er en

sammenhæng mellem niveauet af inflammatoriske stoffer præ-operativt og smerteintensiteten.

Studie 1 viste at patienter med kronisk smerte efter TKR (>1 år) havde en nedregulering i ekspressionen af 3 ikke-kodende RNA'er i serum præ-operativt sammenlignet med patienter, der ikke havde smerter efter TKR. I studie 2 var 21 miRNAs analyseret og ud af disse udviste 3 miRNAs en anderledes ekspression præ-operativt hos patienter med lav smertelindring efter TKR sammenlignet med patienter med høj smertelindring (ingen smerte) efter TKR. En sådan dysregulering i disse miRNA's kan om muligt have en potentialbetydning for at identificere patienter, der er i risiko for at udvikle smerter efter TKR. Studie 3 evaluerede ekspressionen af 92 inflammatoriske stoffer i serum fra patienter med slidgigt. Heraf var ekspressionen at 10 cytokiner signifikant nedreguleret, mens 5 andre var opreguleret i for hold til ekspressionen af samme cytokiner i serummet fra raske personer. Studiet 3 viste også at FGF-21 og 4E-BP1 især var associeret med smerteintensiteten hos patienter med slidgigt, hvorimod TWEAK, FGF-21, CSF-1 og IL6 var associeret med smerteintensiteten generelt.

Dette PhD-projekt fremviser signifikante detaljer omkring sammenhængen mellem tilstedeværelsen af forskellige post-transkriptionelle modifikationer og post-operativ smerte efter TKR og deres potentiale som mulige biomarkører hos patienter med slidgigt, hvilket er vigtigt i for hold til senere studier, der vil undersøge involvering af epigenetiske modifikationer indenfor smerteområdet.

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"The perfect being, huh? There is no such thing as perfect in this world. That may sound cliché, but it's the truth. The average person admires perfection and seeks to obtain it. But, what's the point of achieving perfection? There is none. Nothing. Not a single thing. I despite perfection! If something is truly perfect, then there is nothing left. There is no room for imagination. No place left for intelligence or for a person to gain additional knowledge or abilities. Do you know what that means? For scientists such as ourselves, perfection is a dead end a condition of hopelessness. It is our job to create things more wonderful than anything before them, but never to obtain perfection. A scientist must be a person who finds ecstasy while suffering from that antimony."

Tite Kubo

PREFACE

The work for this Ph.d. thesis was performed between September 2016 and August 2019 at the center for neuroplasticity and pain (CNAP), SMI®, department of Health Science and Technology, Aalborg University, Aalborg, Denmark. The Danish National Research Foundation (DNRF121) is gratefully acknowledged for stipend and project support.

The Ph.d. thesis aimed to evaluate the expression of potential circulating biomarkers and their association with the post-operative pain as well as the preoperative low systemic grade of inflammation in patients with knee osteoarthritis.

The first chapter gives an introduction to the burden of osteoarthritis, how the pathology is characterized at a clinical level and which treatments are available at present time. Moreover, an introduction about the characterization of painful knee osteoarthritis and how post-surgical chronic pain need biomarkers will be given. This is further sustained by the introduction of new targets represented by epigenetic modifications. The second chapter gives an overview of methodological procedures and presents the cohort of patients and healthy subjects recruited for the project. The third, fourth, and fifth chapters present, in details, the results obtained in the three studies; with main focus for the third chapter in the evaluation of lncRNA as signature for post-operative pain, for the fourth chapter in highlighting miRNA as potential circulating biomarkers for post-operative pain and the fifth chapter in the evaluation of a new inflammatory panel in serum of patient with osteoarthritis versus healthy subject.

The Ph.d. thesis is based on three original manuscripts two published and one submitted in international peer-reviewed journals.

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CHAPTER 1. INTRODUCTION

1.1. OSTEOARTHRITIS

Osteoarthritis (OA) is considered a degenerative and progressive disease affecting synovial joints of e.g. the knees, hips, hands, and spine [55]. Many factors can influence the outbreak and progression of OA such as joint alignment alteration, trauma, or obesity, biomechanical, biochemical, and genetic factors [25,49,68,82]. Specifically, obesity is associated with a higher risk of diabetes type-2, which is correlated with increased inflammation in the OA affected joint [65]. The result of these factors is manifested by alterations of the joints which includes cartilage disruption, sclerosis of subchondral bone, osteophyte formation, and high levels of synovitis [88]. Radiological evaluations are the most applied for the diagnosis of osteoarthritis, and the degeneration of the joint is evaluated by X-ray imaging using the Kellgren & Lawrence (KL) [138] system.

1.1.1. PREVALENCE OF OSTEOARTHRITIS

OA is defined as one of the most frequent musculoskeletal diseases in older populations and the most common cause of impairment [11]. It mostly affects women (around 40%) and in a lower incidence men (around 25%) in the age range of 60-70 years [3]. The future projection of a global increase in the elderly population and the higher rates of sedentary lifestyle means that the incidence of OA in the coming years is predicted to dramatically increase [18,23,27]. Within the highest contributor of global disability, OA holds the 11th place[47,256]. The burden of OA is also most likely underestimated due to the ongoing methodological issues regarding a conservative case definition and the restriction of diagnosis which only includes the hip and the knee [48]. Moreover, OA of hip and knee are included together with some major pathologies in a group of conditions with a high risk of mortality [195]. This underlines the great impact of OA on present and future society. The incidence of symptomatic OA has increased exponentially in men and women in the past two decades [192], and is becoming even more clear a substantial increase of this pathological condition in the future [111].

1.1.2. TREATMENT OF KNEE OSTEOARTHRITIS

In terms of treatment for knee OA (from here on KOA), no definitive cure has been found. For this reason, any kind of intervention aims to reduce the effects of characteristic symptoms and prevent an additional progression of the disease. Early

stages of KOA can be treated by several pharmacological and non-pharmacological remedies [110] and exercise in association with weight loss has been shown to yield pain relief [178]. Treatment for KOA patients can be subdivided into three major ways that are defined as first-line, second-line, and third-line of intervention [71,173]. International organizations active in the OA's research (EULAR and OARSI) recommends as first-line of treatment a combination of exercises aimed to achieve weight loss that can be associated with a second-line of treatments, which includes non-surgical treatments, while only after unsuccessful results the third-line of treatment can be considered [131,173]. At the moment, the current end-treatment for a late stage of KOA is the total knee replacement (TKR). TKR is considered as a surgical effective treatment for KOA [31], which consists of replacing the bone terminations part of the knee joint with metal and polyethylene prosthetic implants [31,168]. The rising number of incidences associated with more patients in need of treatment as well as the potential for the development of pain after surgery underscore the importance of new preoperative prediction methods for individual treatment.

1.1.3. PAIN AND SENSITIZATION IN KNEE OSTEOARTHRITIS

Specific pathways underlying pain mechanism in OA are unfortunately still poorly investigated [96], although many types of research have demonstrated that a large contributor is the sensitization of the pain system [8,73,91,135,236]. The pathogenesis of OA includes all joint tissues [53] and although pain cannot arise from this structure due to the absence of nociceptive fibers in the cartilage [60,70], KOA is diagnosed by using radiological evaluation of the cartilage alterations. The nociceptive input is possibly started by the occurrence of intense inflammation at the synovial level, or other features such as increased pressure in the subchondral bone, osteophyte growth, and sensitization of the periarticular tissues with central sensitization of the nervous system [54,70]. The inflammatory state leads to the sensitization of peripheral nociceptors [229], and this sensitization in the specific synovitis (inflammation of the synovial membrane) [264] could lead to sensitization of peripheral nociceptors in KOA [70], where factors such as sensitization in the periarticular tissues have been proved associated with maintenance of pain [3,7,8,91].

Previous studies suggest that musculoskeletal pain is influenced by the persistence of pain and its intensity, which finds a possible cause in the central sensitization [7,109]. This indicates that a certain pain condition or damaged tissue moves from a localized area to a more expanded area and ends in a condition of chronicity and widespread pain [7]. A generalized sensitization (e.g the knee area) has been demonstrated to be associated with high pain [8,67,73,122,265], poor quality of life [122], modest outcome after TKR surgery [166], and high concentration of proinflammatory mediators in the circulation of patients with KOA [147].

1.2. PAIN AFTER SURGERY

1.2.1. ACUTE AND CHRONIC POST-OPERATIVE PAIN

Acute post-operative pain and its severity are correlated with chronic post-operative pain [136]. For the treatment of acute pain, the last decade has produced several guidelines but with limited results[57,170,246]. Earlier studies have shown that the 24% of patients of major surgery, such as urological, cardiovascular, plastic, and orthopaedical, reports at six months an incidence of pain defined as mild (1,2 out 10 of the VAS), and 16% of them reported moderate/severe pain (more than 3 out 10 of the VAS), values that after 1 year are still present with the incidence of mild pain at 23% and the for the severe pain to a 12% [44]. In the particular case of TKR, patients have reported pain for 3 months in around 5% and for 6 months in the 9% of the cases [27]. More studies highlighted how 58% of OA patients developed chronic post-operative pain, where 11% showed a neuropathic component, and 22% of those presented moderate/severe chronic post-operative pain [44,208]. At present, a definitive reason for the development of chronic post-operative pain in some of the patients, while others recover fully in absence of pain, is lacking. In this direction are also missing clear explanation regarding the pathways that lead the acute pain to become chronic post-operatively.

1.2.2. RISK FACTORS FOR CHRONIC POST-OPERATIVE PAIN DEVELOPMENT

Previous studies showed how circulating markers such as pro-inflammatory cytokines and products of inflammatory cascades can be pointed out as potential risk factors of post-operative pain [6,121]. Further, evidence concords that epigenetic modifications, like the actions of noncoding RNAs (e.g. microRNAs, lncRNAs, siRNAs, circRNAs), might be highly related to the pathophysiology of OA, and this may pave the way for future treatments; in this regard several studies have illustrated the possible diagnostic potential of circulating non-coding RNAs in OA, indicating that this family of molecules may act as potential predictors for the onset and the clinical evolution of knee and hip osteoarthritis [15,24].

1.3. PROTEOMICS IN CHRONIC PAIN AND OA

The action of proteins has been long studied in relation to acute and chronic pain and molecular research has shown the complexity of chronic pain [90,193]. Mechanisms and molecular pathways under several pain syndromes are most probably different in the individuals and involves also a high number of molecular changes [217,240]. In the last twenty years, research has improved for the identification of receptors, signaling proteins, and ion channels involved in nociception and pain [217] but potential therapies that targeted single proteins showed low efficacy. For this reason, the upcoming clinical research studies focus on a molecular pathways based approach for pain therapy [16,108,225]. Interesting insights into modifications related to painful pathologies in preclinical and clinical studies have been described by research that aimed to evaluate the biological systems behind the complex molecular pathways that contribute to chronic pain, such as next-generation sequencing genomics [2,291] and epigenomic and transcriptomic studies [127,235]. Preclinical proteomic studies have focused on different aspects such as mice and rat models, which includes spinal nerve injury (SNI), spinal cord injury (SCI), Complete Freud's Adjuvant (CFA), and spinal nerve ligation (SNL) [142,146,224,244], and investigated changes in proteins levels in response to drug administration [189], highlighting several proteins dysregulation involved in response mechanism after painful and stress stimuli and inflammatory pathways [142,180]. In humans, only a few studies have focused on the proteomics approach to asses pain mechanisms and highlight potential biomarkers in tissue biopsies in order to study painful conditions like chronic widespread pain [202,203], Complex Regional Pain Syndrome [198], and myalgia [97,201]. In line with this, a study conducted on trapezius muscle micro biopsies from female patients with chronic widespread pain compared to healthy subjects showed 17 proteins differently regulated in the two groups, involved in multiple cellular functions which includes muscle damage, stress, and inflammation [202]. Moreover, proteome signatures in liquid biopsies such as saliva and CSF are becoming more relevant showing protein dysregulation in a pathological condition characterized by a painful state [14,288]. Other sources that are also evaluated are blood, plasma and serum due to their easy obtainment and for evaluation of changes in lifestyle, disease and treatment [20,84]. For instance, Ghafouri et al. evaluated plasma samples from subjects with work-related musculoskeletal disorders through mass spectrometry (MS) showing dysregulation of markers that can be used to prevent this kind of musculoskeletal pain [85]. Moreover, a study conducted in serum and synovial fluid (SF) from patients affected by KOA undergoing TKR, highlighted the association between inflammatory markers such as TNFα and IL6 and a predisposition to ongoing pain after TKR [79].

1.4. EPIGENETIC MODIFICATIONS

Epigenetics is the study of alterations in the phenotype, understood as gene functions, which are heritable and perpetuate alternative functional states in the presence of the same genomic DNA sequence [267]. In humans, some epigenetic changes are inherited by a new generation and can be detectable in it, but still, these changes could be phenotypically not expressed by the new generation [107]. Moreover, epigenetic changes, even though their potential persistent nature, can be a target of further modifications or be reversed at any time [193]. Generally, epigenetic changes and modifications processes include the action of transcription factors, chromatin modifying enzymes and chromatin-remodeling complexes and the action of non-coding RNAs [77,137,212,222,257]. These modifications that can activate or repress have been shown to be may also dependent on lifestyle and environmental factors [220]. Life experiences, behavior, nutrition, and exposure to toxins and pollutants are among the environmental factors associated with epigenetic modifications; for instance, identical twins sharing the same genome, although superficially phenotypically similar, are unique individuals with specific differences [250].

1.4.1. EPIGENETIC MECHANISMS, POST-TRANSCRIPTIONAL AND TRANSLATIONAL MODIFICATIONS

Epigenetics modifications include actions of several transcription factors and enzymes that change the way of reading the DNA double strand causing different expression of specific genes. These mechanisms mainly include histone modifications, DNA methylation and non-coding RNA.

Histone modifications

In eukaryotic cells, the DNA is folded around structural proteins called Histones, which combine two copies of each protein (H2A, H2B, H3 and H4) to form a stable structure, i.e. the nucleosome. Several histone modifications are important for gene expression regulation and DNA repair and replication [144,281]. These dynamic and reversible modifications are changes that involve histone acetylation catalyzed by the enzymes histone acetyltransferases and histone deacetylases. Histone methylation is regulated by histone methyltransferases (HMTs) and histone demethylases (HDMs) and plays a key role in gene expression depending on amino acid positions, the aminoacidic type residue, and levels of methylation [9,144].

DNA Methylation

Methylation of DNA is one of the principal epigenetic modifications of the DNA which consists of 5-methylation of cytosine at cytosine-guanine dinucleotides (CpG sites) by methyltransferases (DNMTs). Mostly, after methylation follows the inhibition and silencing of gene expression by physically blocking RNA polymerase II. Dysregulation of DNA methylation is a well know process associated with several pathophysiological conditions such as cancer, schizophrenia, addiction to opioids, and pain [56,92,194,232].

Non-coding RNA

In addition to histone modifications and DNA methylation, gene expression is regulated at the level of transcription and translation by noncoding RNAs, including microRNAs and long noncoding RNAs. miRNAs are a group of developmentally conserved small non-coding RNAs, with more than 7000 precursors and mature miRNAs recognized in humans [162]. Several studies have highlighted a part for miRNAs as a clinical signature and objectives for a wide array of diseases including lung malignancy, colorectal disease, and diabetes [34]. Despite many studies highlighting different functions of several miRNAs involved in various pain states [1], lncRNAs (ncRNAs with nucleotide lengths >200) are still to be investigated for the development of painful conditions. Recent studies indicate that more than 50% of the lncRNAs are present in the central nervous system (CNS), suggesting a prominent functional central role [102]. Consistent with this observation, several studies have documented the importance of the action of lncRNAs in CNS disorders, like Alzheimer's disease, brain tumors, and Multiple Sclerosis [227]. Only a single pre-clinical study has addressed an association between lncRNA and nociceptive modulation, identifying a novel antisense (asRNA) lncRNA for the potassium channel Kcna2 in rat dorsal root ganglion (DRG)[287]. Fu and colleagues in 2015 showed variable expression of lncRNAs by comparing the cartilage of healthy subjects and patients with OA, demonstrating upregulation of specific a lncRNA, uc.343, that by acting in *cis* on genes implicated in the development of cartilage caused degeneration and therefore faster progression in structural OA [78]. In a previous study, the possibility to detect lncRNAs in several body fluids such as whole blood, plasma, serum, and urine has been shown [218], and their levels have been evaluated in exosomes, structures that stabilize the half-life of these molecules in the circulation and make them potential molecular biomarkers [187]. For example, their stabilized structure and a good detectable amount in blood serum and plasma of patients affected by different kinds of cancer warrant the possibility of using them as a marker for cancer development [46,58,155]. Moreover, a recent study highlighted more than 1800 dysregulated lncRNA in patients with KOA with mild and severe pain; pointing at eight of the different

lncRNA identified as potential molecular biomarkers associated with the progression of KOA[36].

1.5. AIM OF PHD PROJECTS

Since these premises, which points clearly to a pivotal role for molecules such as non-coding RNA and inflammatory mediators in relation to inflammation and pain in knee osteoarthritis; the aim of this Ph.D. was to evaluate the expression of potential circulating biomarkers that could correlate with the post-operative painful state and the low systemic grade of inflammation in patients with knee osteoarthritis. The main aim was assessed through the achievement of three objectives:

1. Evaluation of circulating lncRNA, involved in several inflammation pathways and miRNA regulation, as a preoperative signature for the development of post-operative pain.

2. Evaluation of circulating miRNA, associated with pain, inflammation, and OA pathophysiology, as a preoperative signature and potential predictive biomarkers for post-operative pain.

3. Evaluation of serum inflammatory markers (e.g. interleukins, chemokines, etc.) as the signature for pain and evaluate their associations with pain intensity.

1.6. PAPERS AND DISSERTATION OVERVIEW

The current Ph.D. thesis includes two published manuscripts and one submitted manuscript to international peer-reviewed journals. The three papers had the aim to address the three objectives of the main project.

Study I: Giordano R, Petersen KK, Santoro M, Pazzaglia C, Simonsen O, Valeriani M, Arendt-Nielsen L. Circulating long non-coding RNA signature in knee osteoarthritis patients with post-operative pain 1-year after total knee replacement. *Scandinavian Journal of Pain 2020*. (Submitted)

Study II: Giordano R, Petersen KK, Andersen HH, Lichota J, Valeriani M, Simonsen O, Arendt-Nielsen L. Preoperative serum circulating microRNAs as predictive biomarkers of chronic post-operative pain after total knee replacement. *Molecular Pain 2020 (published).*

Study III: Giordano R, Petersen KK, Andersen HH, Simonsen O, Arendt-Nielsen L. Serum inflammatory markers in patients with knee osteoarthritis: a proteomic approach. *Clinical Journal of Pain* 2020 *(published)*.

Figure 1 conceptualizes the three studies and their relation to post-surgical pain and preoperative expression of circulation markers.

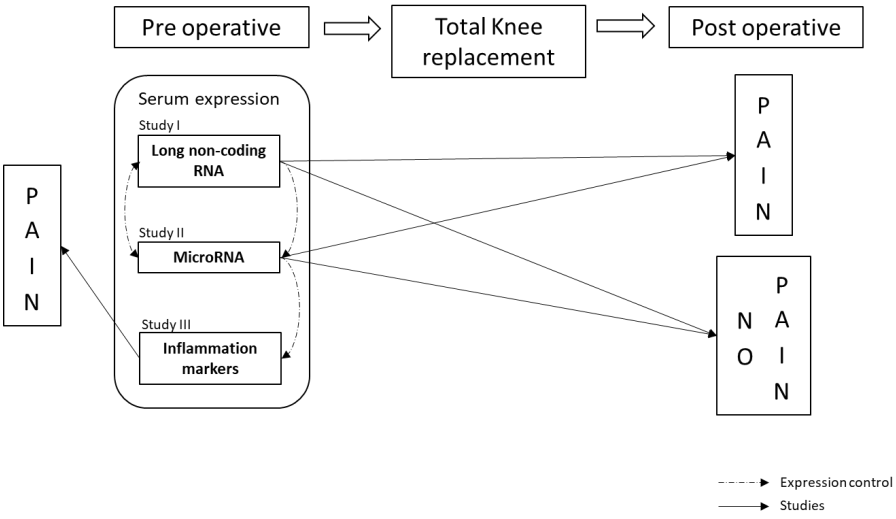

***Figure 1**. **A conceptual overview of the dissertation studies**. Study I, II aimed at the evaluation of non-coding RNA as potential biomarkers for post-operative pain. Study III evaluated inflammatory markers, potentially regulated by biomarkers from studies I and II, concerning knee OA and pain.*

CHAPTER 2. METHODOLOGICAL CONSIDERATIONS

The following considerations regarding cohort and methodologies applied are shared part of the original research articles on which this dissertation is based (Study I, II, III).

2.1. COHORT

Two hundred and two KOA patients, scheduled for TKR were recruited from the clinic at Hospital Vendsyssel, Frederikshavn, Denmark (Study I, II, III). Patients were excluded from participating in the study if mental impairment or diagnosis for other pain conditions, such as hip OA, RA, neuropathic pain and fibromyalgia were present (Study I, II, III). The degree of OA severity was based on radiological evaluation, quantified through the scale of Kellgren and Lawrence (KL) (Study I, II, III). The study was approved by The North Denmark Region Committee on Health Research Ethics (N-20120015) and conducted in accordance with the Helsinki Declaration (Study I, II, III).
In study III as a control group of 39 healthy controls were recruited from a database at CCBR/C4Pain Aalborg (Study III). To be included in the study the healthy participants had to not present any sign of painful OA (evaluated radiologically), be pregnant, have any history of drugs and alcohol abuse, or present clinical records of neurological and musculoskeletal disorders (Study III). All patients and healthy participants in the studies were asked to not use analgesic medications at least 24h before participation (Study I, II, III). All patients and healthy participants read and signed an informed consent form prior to enrollment (Study I, II, III).

2.2. PAIN INTENSITY ASSESSMENT

In order to facilitate the rating of pain sensation by the participants, visual analog scale (VAS) was used in all three studies. Patients and healthy participants had to evaluate their feeling of pain on a scale from zero to ten, where zero indicated the absence of pain, whereas ten was referring to the worst pain experienced. The usage of the VAS has been extensively adopted in clinical trials and pain experiments [126]. Associations between categorical pain ratings such as none, mild, moderate and severe, and VAS have been shown in previous studies, defining moderate pain to be above 3 and severe pain to be above 5 (Study I, II, III) [42,160,258]. For Study I and III, the highest pain felt in the past 24 hours and sensitivity profiles of patients with KOA were chosen. In

Study II percentage of pain relief after surgery was used for evaluation of postoperative pain. All these ways of evaluation were used due to their previously well-established correlations [8,91].

2.3. BLOOD AND SERUM COLLECTION

In order to isolate serum, whole blood was sampled by standard venipucture procedures from patients and controls between 07:30-09:00 in a standard untreated tube of 9ml (Study I, II, III). Subsequently, the collected blood was left to cloth at room temperature. Serum was isolated by centrifugation at 3000 RPM and stored in a -80˚C refrigerator. The serum samples isolated were used in Study I, II, and III.

2.4. ARRAY BASED SERUM EVALUATION

In the three studies, an array evaluation approach was used. To date, specific arrays approaches have been used for the evaluation of nucleic acid or protein in a specific (customized array) or in a more broad way using a list of markers validated to be involved in specific pathways [14,227,239]. All the arrays used for Study I, II, III were based on the evaluation of double strand cDNA or oligonucleotides through real-time polymerase chain reaction (real-time PCR). Main outcome of this technique is to monitor the amplification of a specific segments of DNA or RNA, retro-transcribed in cDNA, during a PCR but with a visual result obtained in real-time. This technique has two main ways of applications depending on the quantification of targeted sequence (quantitative real-time PCR), and semi-quantitatively (semi-quantitative real-time PCR). In Study, I, II, III, the semi-quantitative method was performed. In study I, RT^2 lncRNA PCR Array Human Inflammatory Response & Autoimmunity (QIAGEN, Germany) was used to evaluate the serum expression of 84 validated lncRNAs involved in the expression and regulation of inflammation marker genes and miRNA (Study I). In study II Custom miScript miRNA PCR Array (QIAGEN, Germany) was used to evaluate the levels of miRNA associated previously with OA pain and inflammatory response (Study II). In study III, collected serum samples were analyzed for the levels of 92 proteins through a pre-customized array of proteins related to inflammation (Olink Bioscience, Sweden) (Study III).

2.5. COHORT CONSIDERATIONS

Serum samples were obtained from patients and controls enrolled for previous studies [208,209] and because of signed informed consent were stored in a biobank at Aalborg University, Department of health science and technology, Aalborg for future studies. Potential loss in sample number was due to alterations in the serum quality or

insufficient amount of sample. In study I, the number of patients was chosen because it was reflecting the previously reported risk for chronic post-operative pain following knee surgery [18,208,209], and in order to express in the cohort the maximum effect of post-operative pain. In study III, a small number of samples was not included in the analysis due to internal standard settings at BioExpedia, Aarhus, Denmark facility who ran the laboratory tests.

CHAPTER 3. CIRCULATING LNCRNA AS SIGNATURE FOR POST-OPERATIVE PAIN IN KOA PATIENTS

The content of this chapter is the results of one of the original articles on which this dissertation is based (Study I).

Long non-coding RNA (lncRNA) are single strand macro molecules, that don't codify for protein, composed of more than 200 nucleotides (nt) involved in gene expression control regulation and other post- transcription modifications [45,219], with both nuclear and cytoplasmic localization, which can act on specific genes in *cis* and *trans* [215,219]. Active functions of these molecules are well known to happen at a developmental level, e.g. the action of the gene XIST that encodes for a lncRNA critical for the inactivation of the X chromosome in eutherian mammals [59] or at neuronal level where animal studies showed that knocking out genes that encode for lncRNAs causes alteration in the normal development of the neural system [22,251]. Levels of lncRNAs that are circulating intracellularly can also be evaluated, by the isolation of microvesicles in multiple biological fluids such as blood, cerebrospinal fluid, and others [155,218]; increasing the discoveries linking lncRNAs to different pathologies and identifying in them novel targets to diagnose and treat human diseases [19]. Many of these lncRNA targets are still to be validated, and the clinical applications of lncRNA as a biomarker remain in their early stages. LncRNA has barely been explored in the pain field and therefore opens potential new avenues to approach post-operative pain. In this respect, few preclinical studies showed the involvement of these ncRNA in an experimental condition of neuropathic pain or correlating their dysregulation to a painful behavior [175,278,287].

3.1. STUDY I: SERUM CIRCULATING LNCRNA IN KOA PATIENTS

In study I, we combined the evaluation of circulating lncRNA in the serum of KOA patients and the expression of 84 lncRNA as a potential signature for post-operative pain (Study I). Twenty serum samples were chosen from KOA patients based on their post-operative pain intensity and divided into two groups "pain" and "no pain" in order to explore their preoperative expression of lncRNA (Study I). The two groups (both groups n = 10) did not differ on gender (5m;5f versus 6m; 4f); years of age (64.3 versus 61.8); Kellgren Lawrence (3.7 ± 0.48 versus 3.3 ± 0.69); BMI (kg/m^2) (25.5 ±

3.23 versus 33.3 ± 6.52); preoperative VAS (8.0 ± 0.67 versus 6.9 ± 2.16); all *p*-values > 0.05 (StudyI).

In Study I, 3 out of 84 lncRNAs were significantly downregulated in serum of patients who presented with post-operative pain higher than 5 on VAS, one year after surgery (figure 2.) (Study I).

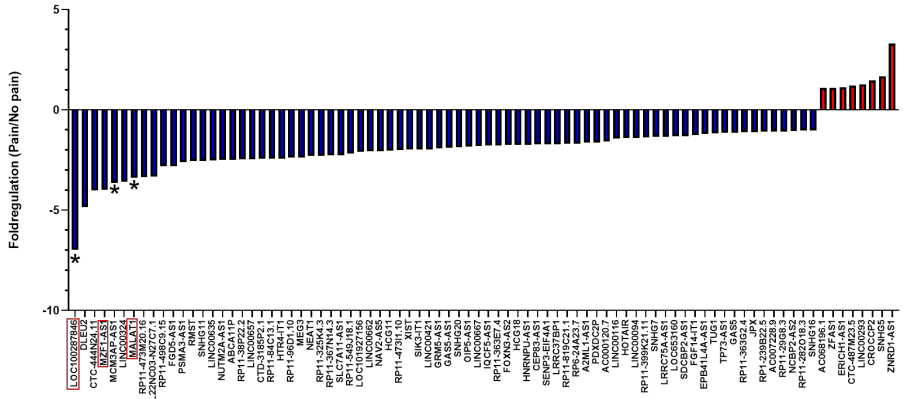

Figure 2. Bar chart lncRNA expression. *Bar chart of upregulations (red bars) and downregulations (blue bars) of 84 lncRNA evaluated (Study I). Data expressed as fold regulation for "pain group" compared with the "no pain" group. Fold regulation reports the biological meaning of fold-change (Study I). Asterisks (*) indicate significance p<0.05 from the "no pain" group (Study I).*

Dysregulated lncRNAs included Patched 1 pseudogene (LOC100287846, ENST00000528139) with a -6.99 fold regulation (p-value: 0.029); myeloid Zinc Finger 1 Antisense RNA 1 (MZF1-AS1, ENST00000593642) with a -3.99 fold regulation (p-value: 0.038) and Metastasis associated lung adenocarcinoma transcript 1(MALAT1, NR_002819) with a -3.39 fold regulation (p-value: 0.044) (Study I).
In study I, we demonstrated a preoperative downregulation of LOC100287846, an uncharacterized lncRNA, in patients with post-operative pain one year after TKR (Study I). No previous studies have demonstrated the involvement of this lncRNA in relation to OA pathophysiology (Study I). The lncRNA showing the highest downregulation was the myeloid Zinc Finger 1 Antisense RNA 1 (MZF1-AS1), which is a regulating factor of the transcription factor MZF1, a member of the SCAN-Zinc

Finger (SCAN-ZF) transcription factor family [63]. Recent researches have highlighted the association of MZF1 in different types of cancers such as hematopoietic, breast, and lung cancers and its dysregulation in several myeloid lineages [63,216]. Unfortunately, little is known about MZF1-AS1, but downregulation of this lncRNA could cause upregulation of MZF1, due to its role as antisense (Study I). Preclinical study have demonstrated action of MZF1 in the regulation of pathways involved in neuropathic pain genesis in rat with nerve injury [287]. In study I, down-regulation of MZF1-AS1 was found preoperatively in KOA patients with high pain intensity suggesting that MZF1-AS1 like its sense strand might be involved in processes that lead to altered regulation of various voltage ion channels causing the painful post-operative pain state (Study I). More research has been conducted on the lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1), which is a widely and stably expressed lncRNA, demonstrated to be associated with metastatic potential and cancer invasiveness in lung cancer [128]. In many types of cancer, MALAT1 is upregulated, which enhances proliferation and inhibits apoptosis, conversely, a down-regulation of this lncRNA induces a reduction of cellular proliferation by promoting apoptosis [157,268,289]. Few publications on the involvement of this lncRNA in relation to pain and pathways of osteoarthritis exist. A recent *in vitro* study showed the involvement of MALAT1 in OA progression [285]. These authors showed that the downregulation of MALAT1 increases the level of expression of metalloproteinase-13 (MMP-13) and ADAMTS-5 while decreasing collagen II and *aggrecan* in OA chondrocytes, indicates that down regulation of MALAT1 may contribute to extracellular matrix disruption and so to the pathogenesis of OA [285]. A preclinical study conducted in spinal cord neurons of a pain rat model showed that downregulation of MALAT1 could result in neuropathic pain by increasing the excitability of spinal cord dorsal horn neurons because of potential involvement in the regulation of the transmembrane flow of calcium ions [175]. The data presented in this study on lncRNA expression and pain intensity corroborates those of others who reported that these non-coding macromolecules are involved in several regulatory pathways that might lead to pain and progression of OA, pointing at the potential use for some of them as a therapeutic target (Study I).

3.2. LNCRNA IN SILICO TARGET PREDICTION

Accurate characterization of lncRNA targets is considered fundamental to elucidate their regulatory roles. In study I, a prediction analysis was run using an on-line database that allows the alignment of the lncRNA's sequences based on their ID name with all potential miRNA (Study I). Different applications are available online and companies offer specific software that allows to highlight specific pathways in which the lncRNA could be involved. In study I, we decided to evaluate the potential targets

of the significant lncRNA through starBase v. 3.0 software (http://starbase.sysu.edu.cn/), a web-based tool that predicts miRNA-lncRNA an lncRNA-RNA alignment by imposing lncRNA sequences that could merge with specific RNA targets [153,275] (Study I). Results of this in silico target prediction analyses were 357 miRNA targets for MALAT1 and 68 for MZF1-AS1. MiRNA included in the potential targets were e.g. hsa-miR-146-5p and hsa-miR-145-5p which are well known to be involved in the regulation of several pathways for pain and inflammation [1](Study I). LncRNA-RNA analysis showed 1637 genes regulated by MALAT1, 9 genes for MZF1-AS1. The functional enrichment analysis of GO biological processes, showed 26 significant pathways (FDR $\leq$ 0.05) regulated by MALAT1, which revealed involvement of this lncRNA in the inflammation cascade and his activity in response to stimuli (Study I). Unfortunately, due to the uncharacterized nature of LOC100287846, it was not possible to evaluate any eventual alignment with a sequence of specific miRNA targets (Study I).

CHAPTER 4. MIRNA AS POTENTIAL BIOMARKERS FOR POST-OPERATIVE PAIN

The presented findings in this chapter are the results of one of the original articles on which this dissertation is based (Study II) [86].

MicroRNAs (miRNAs) are a group of small noncoding RNA, with a peculiar length of 18 to 25 nucleotides [100]. Currently, the isoforms annotated in humans reaches numbers above 7000, and their main function involves the post-transcriptional regulation of several mRNAs [162]. MiRNAs carry out their function thanks to their ability to target, by complementary sequence with the RNA messenger, around 30% of genes that codify for proteins [162]. The action of targeting by miRNA results in translational suppression or degradation of the mRNA bind [183]. The majority of miRNAs are located intracellularly but many studies have investigated circulating or extracellular miRNA that can be detected in extracellular space, in cell media, and in biofluids like the serum, saliva, and cerebrospinal fluid[10,35,119,182,253]. Moreover, studies show how through the embedding in microvesicles, or their binding with protein such as Argonate2, nucleophosmin or high density lipoprotein, circulating miRNA can evade the degradation process driven by RNAse enzymes [10,253,255,260]. This structural protection, stabilize the miRNAs' half-life, allowing the evaluation of their levels that can be used as biomarkers in several conditions [259]. Dysregulation of this non-coding RNA has been shown at the developmental level, in cancer pathogenesis, viral infection, and cardiovascular alterations [66,124,134,245]. The past few decades have seen an increase in investigating the multiple pathways, physiological and pathological, in which the miRNAs regulates several genes pain associated [193]. Direct miRNAs involvement in relation to pain was reported for the first time in 2010, when by using a knockdown model for the protein Dicer (crucial protein for miRNAs production) it was demonstrated a dysregulation in pain related gene expression and an alteration in the sensation of pain [286]. Later more evidence showed altered expression of circulating miRNAs in patients affected by different pathologies characterized by the chronicization of pain, such as migraine, complex regional pain syndrome and fibromyalgia [148,204,247]. In recent years, more research has come up with evidence supporting the use of levels of circulating miRNAs as a diagnostic and predictive tool for different pain conditions [1], but unfortunately, there are still missing studies that evaluate the use of circulating biomarkers for prediction of chronic post-operative pain.

4.1. STUDY II: PREOPERATIVE CIRCULATING MICRORNA SIGNATURE FOR POST- SURGICAL PAIN

In Study II the dysregulation of 21 serum circulating miRNA was evaluated in KOA patients in order to highlight their pre-operative profile and their predictive value for pain one year after TKR. Patients included in the study were assigned to two groups in relation to their percentage of post-operative pain relief (the difference between pre- and post-operative VAS scores divided by preoperative VAS score) (Study II). Patients were part of the group defined as high pain relief if their value of post-operative pain relief was above 30%, instead of patients with values of pain relief inferior to a cut-off of 30% were defined as low pain relief (Study II). The two groups did not differ on preoperative VAS (6.5 ± 1.8 versus 6.3 ± 1.8); years of age (69 ± 8.7 versus 68.00 ± 10.1); gender % of female (58.7 versus 68.1); Kellgren Lawrence (range) (3.7 (2-4) versus 3.6 (2-4)); all *p*-values > 0.05, whereas the groups were significantly different on BMI (kg/m^2) (28.8 ± 4.6 versus 30.7 ± 4.6, p < 0.05) (Study II).

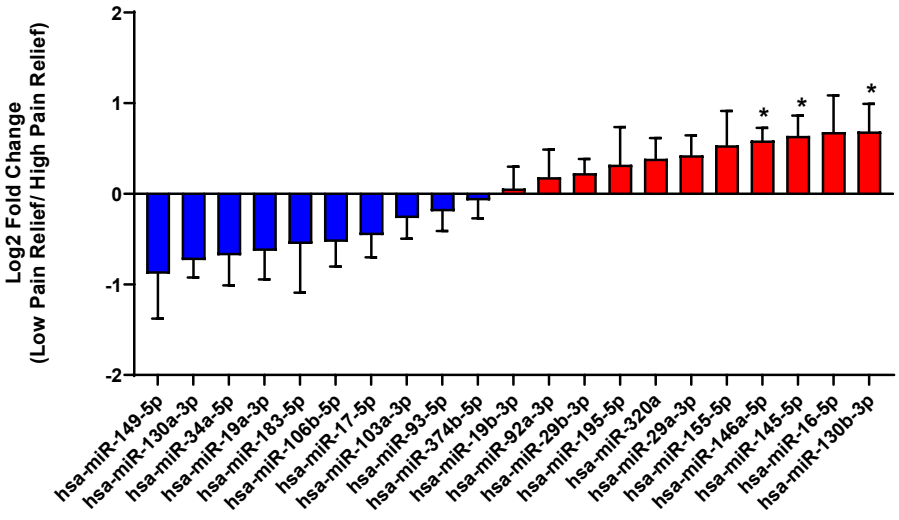

Figure 3. Bar chart miRNA expression. *The bar chart represents the fold change of miRNAs evaluated in the two groups. Asterisk indicates the significant upregulation of three miRNAs (p<0,05) (Study II).*

Study II demonstrated that patients with low pain relief exhibited higher preoperative levels of hsa-miR-146a-5p, hsa-miR-145-5p and hsa-miR-130b-3p. Hsa-miR-146a-5p (fold change = 1.50 ± 0.86 SD, P-value = 0.021), hsa-miR-145-5p (fold change = 1.55 ± 1.24 SD, P-value = 0.037) and hsa-miR-130b-3p (fold change = 1.61 ± 1.4 SD, P-value = 0.039)) (Figure 3.) (Study II). The current analysis corroborates previous studies which highlights the higher expression of hsa-miR-146a-5p, hsa-miR-145-5p and hsa-miR-130b-3p in relation to pain pathways, inflammation and OA [115,151,167] (Study II).

Study II showed dysregulation of three specific miRNA involved in regulating inflammatory processes, which is important since inflammation is participating in the peripheral sensitization which leads to pain onset (Study II) [30]. First miRNA (Hsa-miR-146a-5p) reported in Study II has been shown by earlier studies to be involved in relation to pain process, inflammatory response and pathophysiology of OA [156,273,276] (Study II). This miRNA, has is codifying gene situated on chromosome 5, and its mature sequence is shared by the other members of the miRNA-146 cluster [93]. Functions of this miRNA have been shown to be regulated by nuclear factor kappa B (NFκB), a factor involved in the inflammatory response which once activated by innate immune response induces the activity hsa-miR-146-5p [248]. This miRNA is also involved in the regulatory expression of specific cytokines that leads to the enhanced action of enzymes (e.g. metalloproteinase), involved in cartilage degradation, one of the most common features of OA [156]. In addition, a preclinical study conducted on rats model showed how the negative regulation of miR-146-5p on TNF receptor-associated factor 6 (TRAF6), reduced neuropathic pain in the study animals [165]. The same authors suggest this miRNA as a future target as pharmaceutical therapy for neuropathic pain [165]. Involvement of this miRNA has been shown also in relation to arthritis, where in synovial tissue of patients with RA, up-regulation of it was induced by pro-inflammatory mediators like Interleukin-1β and TNF-α [191]. In relation to OA, a study showed that miRNA 146-a-5p was over expressed in cartilage of OA patients when compared with normal cartilage [273]. Moreover, increased expression of miR-146-5p has been shown in OA patients at an early stage of the pathology, suggesting the underlying role of this non-coding RNA in OA pathophysiology [200]. The data presented in Study II highlights how circulating over-expression of hsa-miR-146a-5p in patients with pain after surgery is in line with previous findings regarding the involvement of this miRNA for pain process in OA, and give insight for the possibility

to detect its dysregulation at serum level that could lead to an easier pre-operative evaluation for pain after surgery (Study II).

Second miRNA found upregulated in Study II was hsa-miR-145-5p (Study II). Previous studies indicate this miRNA being induced by the high levels of inflammatory mediators such as TNF-α and IL-1β, and to regulate the feedback levels of TNF-α and metalloproteinase, both involved in the alteration of the cartilage in OA[114,274]. Previous findings, obtained from human chondrocytes of patients with KOA, found a higher regulation of miR-145-5p in the late stage of OA and after induction by IL- 1β [69,104,161]. Furthermore, the up-regulation of the same miRNA in the cerebrospinal fluid (CSF) correlated with high scores for pain and fatigue [21]. The data concerning the preoperative up-regulation of hsa-miR-145-5p, presented in Study II, corroborates previous findings, pointing out a possibility for high levels of this miRNA to promote and prolong the action of inflammatory response in KOA, which may lead to a bad recovery after surgery.

In regards to hsa-miR-130b-3p, there is still a lack of evidence concerning the action of it in relation to pain conditions, but established is its involvement at different levels in the pathophysiology of several types of tumors [94,282]. Moreover, the dysregulation of this miRNA has been associated to several pathological conditions such as obesity, or resistance to insulin, but has been also found involved in physiological process which includes osteo and chondrogenesis [101,169,171]. In Study II the results find hsa-miR-130b-3p positively dysregulated in the preoperative circulation of KOA patients with bad postoperative recovery, but more evidence is necessary to validate the involvement of this miRNA for post-operative pain conditions in patients with KOA and show whether it can serve as a potential biomarker (Study II).

4.2. MIRNA IN SILICO TARGET PREDICTION

In Study II the characterization of miRNA targets was run in order to elucidate their regulatory roles and highlight the specific pathways where the potential RNA messenger (mRNA), targeted by the significant miRNA, was involved. In Study II the between-group analysis was performed only for the dysregulated miRNA, with an opensource on-line software (DIANA-Tool TarBase v.8) [133] (Study II). The predicted targeted mRNA genes were chosen by their prediction score with a value between 0.5-1.0 and if they have been validated with a high/low throughput experiment (Study II). Moreover, a gene ontology analysis through two online software PANTHER and Reactome was run to discover the process in which the identified mRNA, potentially bind by the specific miRNAs (146, 145, 130b), are involved [179] (Study II). Detailed

analysis of genes involved with biological regulation, immune system, and cellular functionality and response to stimuli was performed (Study II). Study II showed that targets were 103 for hsa-miR-146a-5p, 35 for hsa-miR-145-5p and 337 for hsa-miR-130b-3p (Study II).

Most of the identified mRNA related genes were associated with to cells pathways, but Study II was focused on those associated with pathways in the inflammatory response (Study II).

One of the genes predicted to be targeted by hsa-miR-146a-5p, was interleukin-1 receptor-associated kinase 1 (IRAK1) gene which has been validated for being associated mostly with cytokine signaling and toll-like receptor signaling pathways [112,276] (Study II). In addition, hsa-miR-145-5p was found to be involved in inflammatory pathways through the regulation of transcription factor JunB (JUNB) (Study II). However, as reported in Study II, evidence demonstrastred its role in the regulation of IL-2, IL-4, IL-6, and TNF-α [80,89,152] and the promotion of its action due to external or endogenous stimuli leads to a cellular control, regulates the pathway of differentiation or cell death [32,213,263] (Study II).

4.3. PREDICTIVE VALUE OF MIRNA FOR POST-OPERATIVE PAIN

In Study II several linear regressions were run to evaluate the predictive value of serum miRNA expression for post-operative pain relief. In Study II two models were established; model 1 which consisted of all significant miRNA and the preoperative VAS score as variables, showed a predictive value (R2) of 30% and identified preoperative pain intensity (p-value <0.001) as a significant factor (Study II). Model 2 was constructed with a backward identification of the parameters included in model 1 and showed preoperative pain intensity (p-value <0.001) as a significant independent parameter for post-operative pain relief prediction with a value of R2 of 30%, and showed a trend of hsa-miR-146a-5p (p-value 0.06) (Study II).

Moreover, were highlighted significant correlations between pain relief and preoperative pain intensity (R=0.500, p-value <0.001) and hsa-miR-146a-5p (R=0.300, p-value=0.006) (Study II).

CHAPTER 5. INFLAMMATORY MARKERS IN SERUM OF KOA PATIENTS

Evidence reported in this chapter are results of one of the publications on which this dissertation is based (Study III)[87].

The inflammatory response in osteoarthritis is debilitating and intensively represented but is in most cases defined as sub-clinical [69], even though its actions in conjunction with process of synovial degradation, are well known factors at the base of progression of OA from the early stage of the disease [69,230].
Inflammation can occur locally at the synovium level due to disturbed metabolism and enhanced catabolism of joint tissue involved leading to the secretion of inflammatory factors such as proinflammatory cytokines. Several studies are showing the involvement of proinflammatory and anti-inflammatory markers in the onset and progression of OA in clinical and preclinical models, as well as contributor to the pathogenesis of OA pointing out the action of markers such as interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, IL-10, IL-13 and IL-4 [33,76,132,233].
The local production of inflammatory mediators is the main actor for the cartilage disruption and alteration of the synovial membrane with the release of cells activated (e.g. neutrophils, lymphocytes, macrophages) in the synovial fluid. Additionally, the inflammatory response that occurs in the joint tissue can result in a systemic response in patients with OA [221,238]. A systemic inflammation, defined as cold or low grade, has been found recently in patients affected by OA [221,234]. Evidence showed serum levels of several inflammatory mediators are higher in OA than healthy subjects [12,72,237].
In this case, has been demonstrated an upregulation of IL-6 in patients which reported intensive synovitis [65], that is commonly related to increased pain intensity in these patients [117] and is one of the few interleukins that correlates with OA pain [4]. Preclinical studies have found that both the central and peripheral sensory nervous systems can be sensitized by pro-inflammatory cytokines and several recent human studies have found that highly pain sensitive preoperative OA patients are at a greater risk of developing chronic post-operative pain [208,209,211,252]. The origins of the increased inflammation are poorly understood but the associations between dysregulated serum inflammatory markers in OA and pain might be pivotal to increase our understanding of the pathways of this low-grade inflammation.

5.1. INFLAMMATION SYSTEMIC MARKERS IN KOA PATIENTS COMPARED WITH HEALTHY CONTROLS

In Study III in order to evaluate this systemic inflammation, 92 markers (List 3. Appendices) were assessed through multiplex OLIK inflammation panel in the serum of 127 KOA patients comparing them with 40 serum of healthy subjects (Study III). The two groups were no different on gender (% females) (57,8% versus 61,5%); years of age (68.82 ± 9.15 versus 68.46 ± 3.13); BMI (kg/m2) (29.37 ± 4.79 versus 26.71 ± 4.21), whereas significant difference was present for the Osteoarthritis score Kellgren and Lawrence scale (1-4) (3.74 ± 0.49 versus 1.43 ± 0.59) (Study III).

In Study III 72 markers out of 92 were evaluated, and levels of 15 cytokines were found dysregulated in serum of KOA patients (Figure. 4) (Study III). The proteins are identified and listed in the table. 1 (Study III).

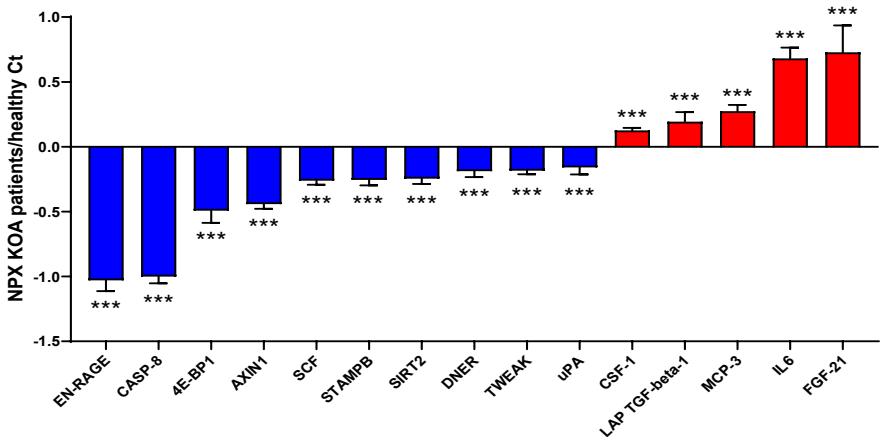

Figure 4. Bar chart of inflammatory markers expression. *Bar chart of upregulations (red bars) and downregulations (blue bars) of significant markers evaluated in the PEA array* (Study III)*. Data are expressed as Normalized Protein eXpression (NPX). Asterisks indicate significance p<0.001 from controls* (Study III)*. Error bars report SEM. All data were corrected for FDR* (Study III)*.*

Downregulated markers	*p-value*	*Upregulated markers*	*p-value*
(CASP-8)	< 0.001	(IL-6)	< 0.001
(EN-RAGE)	< 0.001	(CSF-1)	≤ 0.001
(DNER)	< 0.001	(FGF21)	≤ 0.001
(AXIN1)	< 0.001	(MCP-3)	< 0.01
(STAMBP)	< 0.001	(LAP TGF-beta-1)	< 0.01
(SIRT2)	< 0.001		
(SCF)	< 0.001		
(4E-BP1)	< 0.001		
(TWEAK)	< 0.01		
(uPA)	< 0.01		

Table 1. *Inflammatory markers significant in patients with knee OA. The P-values showed are corrected using the False Discovery Rate (FDR) methods* [272].

5.1.1. CHEMOKINES

MPC-3

Chemokines are small cytokines that act as signaling proteins secreted by cells. Because of their ability to generate chemotaxis on the responsive closer cells. In Study III, monocyte chemotactic protein 3 (MCP-3, known as CCL7), members of chemokines CC subfamily, was upregulated in patients with painful KOA (Study III). An earlier animal study showed the involvement of this chemokine in neuropathic pain pathways and pain plasticity in a rat model of nerve ligation [129]. Its upregulation has been demonstrated to promote central sensitization and facilitate pain transmission in the dorsal root ganglion, in which MPC-3 codifying gene is upregulated by proinflammatory cytokines [120]. In Study III, the upregulation of MPC-3 agrees with previous literature indicating a cooperative action of proinflammatory cytokine and MPC-3 in pain sensation (Study III).

5.1.2. INTERLEUKINS

IL-6

Interleukin 6 (IL-6) is an interleukin, codified by the IL6 gene, which acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In arthritic conditions, Interleukin-6 (IL-6) has been shown to be involved in innate immunity pathways and the general inflammation of the join [28]. This interleukin has been shown to activate and be involved in multiple signaling pathways and to have as targets seral cell types [13,28]. Preclinical studies show how IL-6 undertakes its function as pain regulator on nociceptive neurons [50,197]. In relation to arthritis, preclinical and clinical studies find high expressed levels of this protein in painful KOA [4,65,117]. Study III supported the evidence regarding higher levels of IL-6 in KOA patients, finding this dysregulation at systemic level which confirms the inflammation typical of KOA (Study III).

5.1.3. GROWTH FACTORS

FGF-21

Fibroblast growth factor-21 (FGF-21) part of FGF superfamily is a hormone with an endocrine function which regulates metabolic process [163]. Previous research showed this growth factor to be present at systemic level and in synovial fluid (SF), and highlighted that its levels are lower in osteoarthritic cartilage in OA patients [116]. In this regard, a recent study demonstrated that high levels of FGF-21 in the circulation of patients with OA are correlated with severity of KOA [158]. In Study III higher levels of FGF-21 were shown in serum of patients with KOA, adding evidence to previous studies and showing the association of this hormone in the pathophysiology of KOA and its correlation to pain (Study III).

LAP TGF-beta-1

The growth factor TGF-β1 is produced and released as a homodimer binding its latency-associated pro-peptide LAP. The connection with the factor LAP avoids the release of TGF-β1 defining the moment of secretion to the active function of the hormone [231]. Recent evidence shows the involvement of LAP TGF-beta-1 in plasma of female patients with widespread pain and fibromyalgia with inflammatory levels of these factors and several pain outcomes [14,83]. Study III shows high levels of LAP TGF-beta-1 at systemic level of KOA patients, suggesting that release of the active factor could happen in the knee area (Study III).

DNER

Delta/Notch-like EGF-related receptor (DNER) is a transmembrane protein that acts as a growth factor and is expressed in the central nervous system (CNS) in dendrites and cell bodies of CNS neurons [64] It is heavily involved in several cancer types, in which regulation of certain pathways of the factor Notch leads to increased malignancy and metastasis formation [261,262]. Unfortunately, no evidence exists connecting the action of DNER to KOA pathways or pain perception but Study III, demonstrated a down-regulation of this receptor in KOA patients and warrants further consideration (Study III).

5.1.4. CYTOKINES

SCF

The stem cell factor (SCF) is produced by cells like fibroblasts and it is involved in cell differentiation and proliferation [266]. Preclinical studies have shown SCF acting as a ligand for specific receptors, such as c-Kit, expressed on sensory neurons suggesting an involvement of this factor on pain and its transmission at central and peripheral level [181,249]. In Study III, SCF was found negatively expressed in KOA patients, supporting the evidence that sees this factor associated with pain sensitivity highly present in these patients (Study III).

TWEAK

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is part of the TNF superfamily, expressed in epithelial cells with a pro-inflammatory function [37]. This factor has been shown also to induce proliferation of multiple types of cells, such as astrocytes and endothelia cells [52,271]. An *in vitro* study highlighted how high levels of TWEAK increase the release of pro-inflammatory markers (cytokines and chemokines) in culture media of fibroblasts and synoviocytes isolated from RA and OA patients[38]. On the contrary with previous evidence, Study III found decreased levels of this factor in the circulation of KOA patients (Study III).

5.1.5. OTHER MARKERS

4E-BP1

The eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) is a protein involved in the inhibition of the interaction between translation factor eIF4E and different elongation factors, which regulate protein production and regulation of cell homeostasis [125]. This factor action is induced by several stimuli, including glutamate, growth factors, and cytokines, which leads to the proliferation and cellular differentiation but also synaptic plasticity [105,150]. In this regard, authors have shown levels of 4E-BP1 in neurons, and downregulation of it which cause hypersensitivity due to external stimuli of mechanical, inflammatory, or chemical nature [130,139,174]. Study III demonstrated that serum of KOA patients down-expressed 4E-BP1 suggesting that these patients with lower expression of this factor report more pain in phase of mechanical evaluation of pain intensity (Study III).

STAMPB

STAMBP is a deubiquitinase enzyme which through the binding with SRC Homology 3 domain of the signal-transducing adaptor molecule (STAM), activates the singling cascade of JAK-STAT and consequentially inflammatory response cytokine-mediated [17,41]. Evidence shows the association of STAMBP for the modulation of differentiation and maturation of cytokines, and silencing of these factors leads to a decrease in the expression of cytokines such as IL-1b [17,164]. Study III, highlighted that STAMBP was downregulated in serum of KOA patients.

AXIN1

Axis inhibition protein (AXIN1) is a protein express at cytoplasmic level of cells involved in the cartilage formation at the embryonic state and regulates negatively cell transduction pathways of Wnt factor [140,280]. A previous animal study in an Axin1 KO mice model showed that depletion of this protein in the chondrocytes led to OA-like degeneration [290]. Study III showed that this protein was negatively expressed in the patients with KOA adding knowledge to the involvement of this protein in OA's pathogenesis (Study III).

CASP-8

Caspases are proteins usually involved in the process of cellular death, but previous evidence has proven the association of this protein in pathways leading to inflammation [74], cell motility, T-cell activation, and osteoblast regulation [190]. Study III showed down regulation of CASP-8 pointing to a possible negative regulation of osteoblast activity (Study III).

SIRT2

Sirtuin2 is a member of the sirtuin family, group of nicotinamide adenine dinucleotide-dependent deacetylases [113], regulating cellular process which includes metabolism, proliferation, inflammation or cellular death [103,123]. Studies have reported the action of SIRT2 in the regulation of neuroinflammation and being involved in neurological diseases [277,279]. The SIRT2 pathway leads to inhibition of NF-κB-dependent pro-inflammatory cytokine, through de-acetylation of the NF-κBp65 protein, consequentially reducing inflammation response [205,223]. Previous evidence has proven the function of SIRT2 as an arthritis blocker, demonstrating in mice model how severe collagen-induced arthritis is established when this protein is downregulated [159]. In addition, involvement of SIRT2 in neuropathic pain modulation has been proven in animal models, where up-regulation of this protein reduced pain sensation through the negative modulation of factors of NF-κB-mediated neuroinflammation cascade [284]. In Study III, significant downregulation of SIRT2 was showed in serum from patients compared with healthy controls, supporting the evidence that downregulation of SIRT2 might be associated with pain sensation and high levels of inflammatory markers in these patients (Study III).

EN-RAGE

Recent identified extracellular receptors involved in advanced glycation end-products (EN-RAGE or Protein S100-A12) is a protein highly represented in cells involved in the immune response, which recruits or stimulates inflammatory mediators, and cooperates in cell networks that influence immunological response [214,269]. Moreover, high levels of this protein are expressed in synovial tissue [207]. EN-RAGE higher expression has been found in samples of rheumatoid arthritis patients, this receptor is secreted by granulocytes which promote localized inflammation resulting in chronic arthritis [75]. High levels of these proteins have been evaluated in synovial fluid OA patients, and have been correlated with the high disruption of the joint cartilage [149]. Conversely, Study III demonstrated downregulation of EN-RAGE in patients with KOA, taking into account that expression levels in two different body fluids could be sometimes inverse [210]. Nonetheless, this indicates that EN-RAGE may be involved in the low-grade systemic inflammation in KOA patients (Study III).

uPA

Urokinase plasminogen activator (uPA) is one of the two enzymes, that regulates the changes that lead the plasminogen to turn in plasmin, and is also involved in cell transduction signals that stimulate cellular proliferation and survival [254]. Animal studies highlighted an association of the action of uPA in the regeneration of axons in CNS and its release after ischemia[176,177]. In relation to arthritis, authors suggested that levels of uPA are not evaluable in the synovium of OA patients when compared with RA patients and healthy controls [26]. Study III reported significant downregulation of uPA in KOA patients, which may confirm previous evidence on this enzyme and its correlation with OA (Study III).

CSF-1

CSF-1 is a factor member of the hematopoietic CSF family involved in several biological functions of macrophages and osteoclasts such as differentiation, proliferation and survival [40,141]. Several studies highlight the expression of CSF-1 receptor (CSF-1R) on microglia and neurons [39,43,99] and signaling of CSF-1/CSF-1R is involved in several pathological conditions [98,99] and active in an arthritis animal model [81]. Previous studies have demonstrated the critical role played by CSF-1 for arthritic diseases and pain intensity [99,188] and have been shown the effect on microglia by the control of neuropathic pain due to nerve injury [95,199]. Evidence demonstrated that acting peripherally CSF-1 regulates pain, through activation of macrophages and possibly in nerves [172]. Study III showed significantly higher CSF-1 presence in patients affected by KOA, supporting what has been shown in previous research [226] (Study III).

5.2. INFLAMMATORY MARKERS ASSOCIATED WITH PAIN INTENSITY IN KOA PATIENTS

In study III associations between the significant markers highlighted in patients with KOA and pain intensity were evaluated. Markers such as FGF-21 and 4E-PB1 were positively associated with pain scores in patients in line with previous studies that look at these new markers as involved in the pathways of pain in preclinical and clinical models [116,130,139,158,174]. Additionally, inflammatory markers such as IL-1b, IL-6, IL-8, TNFα and IL-10 have been shown to be associated with the severity of KOA and OA pain [61,65,79,242]. The linear regression demonstrated that TWEAK (standard coefficient (SC) -0.355, p-value 0.001), FGF-21 (SC 0.264, p-value 0.002), CSF-1(SC 0.264, p-value 0.015), and IL6 (SC -0.406, p-value <0.001) were independent factors predicting pain intensity in KOA patients with a predictive value (R^2) of 16% (Study

III). Although previous studies in synovial fluid and serum of KOA patients showed higher levels of IL-6 positively correlated with pain outcomes [79,121], Study III showed a negative association of IL-6 serum levels with pain intensity, suggesting that IL-6 might act differentially at local site (the synovial fluid) and systemically (e.g. in serum) but confirming the low-grade inflammation present at systemic levels in patients with OA [221] (Study III).

CHAPTER 6. GENERAL DISCUSSION

The need for finding biomarkers for OA is growing, due to the necessity to identify OA at an early stage, better definition of the clinical stages of the pathology and identify risk factors of progression that could be targeted for intervention [145,185]. Previous studies focused on the clinical features of the pathology looking at imaging outcomes in order to achieve an understanding of the natural history of the progression through the visualization of cartilage morphology and composition [106]. To date, research in OA is referring to the so-called "wet biomarkers", molecules that can be easily accessible through blood, serum, plasma, synovial fluid (SF) and cerebral spinal fluid (CSF) [186]. A recent study showed how tumor necrosis factor-alpha (TNFα) in the serum of pre-OA patients is significantly different when compared with healthy participants [62]. Moreover, Kosek et. al showed dysregulation of pro-inflammatory cytokines in CSF, serum and SF of patients with KOA indicating that IL-6 and IL-8 are associated with reduced symptom severity centrally and augmented peripherally [143]. Also, research on the action of miRNA and lncRNA is increasing which indicates a key role for these regulatory nucleic acids in the alteration and regulation of pathways related to OA progression and pathogenesis [78,241,270]. For example, studies have demonstrated that miRNAs, in both preclinical and clinical studies, are involved in processes of cartilage homeostasis [184] or cartilage apoptosis-like the action of miR-145[114,151,274]. Furthermore, studies showed their involvement in the regulation of the inflammatory response in OA that leads to a release of pro-inflammatory cytokines such as the action of has-miR-146 on the factor NF-kβ [154,283]. Regarding the lncRNA, few studies have explored these in relation to OA but emerging evidence has demonstrated that, in an OA animal model, MALAT1 is involved in the regulation of a specific miRNA (miR-19) which leads to inactivating Wnt/β-catenin and NF-κB pathways involved in inflammatory injuries [206]. Moreover, the action of maternally expressed gene 3 (MEG3) has been proven to be downregulated in chondrocytes of KOA patients and its dysregulation is associated with higher levels of vascular endothelial growth factor (VEGF) suggesting a role that enhanced angiogenesis may be a mechanism by which inactivation of MEG3 contributes to OA development [243]. Within this expanding body of research, the results presented in this thesis give more evidence regarding the relationship between the action of ncRNA and proteins with OA and increase our knowledge on the use of these molecules as future potential biomarkers for KOA. In study I, a signature of three lncRNA was found in the serum of patients who develop chronic pain after 1-year from the surgery. The results highlighted the downregulation of MALAT1, MZF1-AS1 and LOC100287846 involved at different levels in the pathogenesis of KOA (see *section 3.1*). Moreover, the significant lncRNA targets miRNA that we found dysregulated in serum of KOA

patients in study II such as hsa-miR-146a-5p, hsa-miR-145-5p and hsa-miR-130b-5p (see *section 3.2*); our data shows that these miRNAs are preoperatively upregulated in KOA patients with low pain relief after surgery and their involvement in many inflammatory pathways and chondrocyte homeostasis regulation suggests a possible use of these as potential intervention target (see *section 4.1*). Moreover, in study III, using a proteomic approach, we showed the link of several inflammatory markers with KOA, showing the higher expression levels of IL-6 and introducing new serum inflammatory mediators that could partly explain the low-grade inflammation present in osteoarthritic patients (see *section 5.1*).

Pain in OA has been the main focus of past research since it is the primary complaint from OA patients [118]. Previous studies have shown preoperative pain intensities [208,209] and sensitization of central pain pathways [5] as predictors for chronic postoperative pain following TKR. Besides a growing number of studies have explored epigenetics [51,56,193] and other genes regulatory and transcriptomic changes in painful pathologies [79,196]. In this thesis, the association between pain and the expression profile of ncRNA and inflammatory markers was shown. In study I lncRNA were dysregulated in patients who developed pain after 1 year from the surgery showing a signature for these molecules to be involved in pain process and corroborating data already present in the literature that indicates that these molecules take part in the regulation of neuropathic pain pathways (see *section 3.1, Study I*). More research is available on the relationship between miRNA and pain and our data show the expression of this short single stranded RNA in a pain condition, showing not only an upregulation in serum of patients that showed lower post-operative pain relief but also a trend in predicting the 30% of the post-operative pain relief in KOA patients (see *section 4.3, Study II*). Moreover, Study III indicated that markers found at serum level can be associated with high pain intensity in KOA patients and predicting 16% of the pain highlighting new inflammatory markers, like TWEAK, FGF-21, CSF-1, and IL6 as independent factors (see *section 5.2, Study III*).

CHAPTER 7. LIMITATIONS AND FUTURE PERSPECTIVES

7.1. LIMITATIONS

Due to the exploratory nature of the three studies is necessary to point out some limitations related to our approach. The type of sample, in which all three studies were conducted, has been chosen in order to evaluate systemic alterations in terms of ncRNA and proteins looking at the so-called "cold-inflammation" [29], knowing that the majority of the previous research has been conducted in cells and specific tissues probably closer related with the pathogenesis of KOA or the sensitization of peripheral nerves leading to pain process [228]. Moreover, it remains unknown which specific cell type is involved in the production and secretion of circulating free and exosomal lncRNA and miRNA, although several are involved in this process [10,253,259], making it difficult to define the origin tissue and also the cells that receive them. In this respect, more studies on the transporting mechanisms of circulating ncRNA are needed. In addition, all the data obtained and presented within the dissertation can be identified at the moment only as a signature feature of OA pathology and pain intensity; for this reason, validation of specific pathways, in which all the molecules presented are involved, through luciferase assays on suspected ncRNA-mRNA interactions of interest has to be focus of future studies.

7.2. FUTURE PERSPECTIVES

Confirmation of the action of all these post-transcription and translational modifications is needed before validating the pathways. Moreover, focus in the future should be on the evaluation of timing within all the modifications and action of ncRNA and mediators happening in order to highlight a potential intervention that could block the results of this modification or enhance the protective effect of them.

CHAPTER 8. CONCLUSION

The present Ph.D. project addressed three objectives: 1) Evaluation of circulating lncRNA, involved in several inflammation pathways and miRNA regulation, as a preoperative signature for development of postoperative pain; 2) Evaluation of circulating miRNA, associated with pain, inflammation and OA pathophysiology, as a preoperative signature and potential biomarkers for the development of postoperative pain; and 3) Evaluation of serum inflammatory markers (e.g. interleukins, chemokines, etc.) as a signature for pain and evaluate their associations with pain intensity.

Study I was the first study, to demonstrate an association between down-regulation of preoperative lncRNA in the specific case of MALAT1, MZF1-AS1 and LOC100287846 and chronic post-operative pain after total knee replacement, giving the first insight into the dysregulation of preoperative circulating lncRNAs, and how they can serve as a potential signature for post-operative pain condition due to their regulation of multiple pathways in relation to pain and inflammation (Study I).

Study II showed that postoperative pain is related with specific preoperative serum alteration in the expression of circulating miRNA (Study II). Patients with poor recovery after surgery showed positive increased levels of expression for hsa-miR-146a-5p, hsa-miR-145-5p and hsa-miR-130b-3p (Study II). Moreover, linear regression analysis highlighted preoperative pain intensity as an independent predictive factor for postoperative pain and demonstrated a trend for hsa-miR-146a-5p (Study II). Although explorative, Study II gives the first evidence into the preoperative alteration of miRNAs, and how those can be used as potential biomarkers for post-operative pain condition (Study II).

Study III found, a thorough evaluation of a new high throughput panel, serum of patients with painful KOA to have ten inflammatory markers downregulated and five markers with higher expression levels, when compared to healthy participants (Study III). In patients specifically, correlations were found between expression levels of FGF-21 and 4E-BP1 and pain intensity (Study III). A linear regression model showed that TWEAK, FGF-21, CSF-1 and IL6 were independent predictors for pain intensity showing a contribution of some of the involved inflammatory markers in OA and to the systemic low-grade inflammation in these patients that could be detected at serum level (Study III).

In summary, the studies presented in this dissertation have contributed to increasing the knowledge regarding the action of non-coding RNA and pain, giving proves that thorough evaluation of these type of epigenetic changes there could be a possibility to use such biomarkers for understanding pain in OA and for taking possible precautions to prevent the development of chronic postoperative pain after knee replacement.

LITERATURE LIST

[1] Andersen HH, Duroux M, Gazerani P. MicroRNAs as modulators and biomarkers of inflammatory and neuropathic pain conditions. Neurobiol Dis 2014;**71**:159–68.

[2] Antunes-Martins A, Perkins JR, Lees J, Hildebrandt T, Orengo C, Bennett DLH. Systems biology approaches to finding novel pain mediators. Wiley Interdiscip Rev Syst Biol Med 2013;**5**:11–35.

[3] Arendt-Nielsen; PBBG-N, Bajaj P, Graven-Nielsen T, Arendt-Nielsen L. Osteoarthritis and its association with muscle hyperalgesia: an experimental controlled study. Pain 2001;**93**:107–114.

[4] Arendt-Nielsen L. Joint pain: More to it than just structural damage? Pain 2017;**158**:S66–S73.

[5] Arendt-Nielsen L. Pain sensitisation in osteoarthritis. Clin Exp Rheumatol 2017;**35**:S68–S74.

[6] Arendt-Nielsen L, Eskehave TN, Egsgaard LL, Petersen KK, Graven-Nielsen T, Hoeck HC, Simonsen O, Siebuhr AS, Karsdal M, Bay-Jensen AC. Association between experimental pain biomarkers and serologic markers in patients with different degrees of painful knee osteoarthritis. Arthritis Rheumatol 2014;**66**:3317–26.

[7] Arendt-Nielsen L, Graven-Nielsen T. Translational musculoskeletal pain research. Best Pract Res Clin Rheumatol 2011;**25**:209–226.

[8] Arendt-Nielsen L, Nie H, Laursen MB, Laursen BS, Madeleine P, Simonsen OH, Graven-Nielsen T. Sensitization in patients with painful knee osteoarthritis. n.d.

[9] Arrowsmith CH, Bountra C, Fish P V., Lee K, Schapira M. Epigenetic protein families: A new frontier for drug discovery. Nat Rev Drug Discov 2012;**11**:384–400.

[10] Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci 2011;**108**:5003–8.

[11] Attal N, Rouaud J, Brasseur L, Chauvin M, Bouhassira D. Systemic lidocaine in pain due to peripheral nerve injury and predictors of response. Neurology

2004;**62**:218–225.

[12] Attur M, Statnikov A, Aliferis CF, Li Z, Krasnokutsky S, Samuels J, Greenberg JD, Patel J, Oh C, Lu QA, Ramirez R, Todd J, Abramson SB. Inflammatory genomic and plasma biomarkers predict progression of symptomatic knee OA (SKOA). Osteoarthr Cartil 2012;**20**:S34–S35.

[13] Axmann R, Böhm C, Krönke G, Zwerina J, Smolen J, Schett G. Inhibition of interleukin-6 receptor directly blocks osteoclast formation in vitro and in vivo. Arthritis Rheum 2009;**60**:2747–2756.

[14] Bäckryd E, Tanum L, Lind AL, Larsson A, Gordh T. Evidence of both systemic inflammation and neuroinflammation in fibromyalgia patients, as assessed by a multiplex protein panel applied to the cerebrospinal fluid and to plasma. J Pain Res 2017;**10**:515–525.

[15] Bali KK, Kuner R. Noncoding RNAs: Key molecules in understanding and treating pain. Trends Mol Med 2014;**20**:437–48.

[16] Baron R, Maier C, Attal N, Binder A, Bouhassira Di, Cruccu G, Finnerup NB, Haanpaä M, Hansson P, Hüllemann P, Jensen TS, Freynhagen R, Kennedy JD, Magerl W, Mainka T, Reimer M, Rice ASC, Segerdahl M, Serra J, Sindrup S, Sommer C, Tölle T, Vollert J, Treede RD. Peripheral neuropathic pain: A mechanism-related organizing principle based on sensory profiles. Pain 2017;**158**:261–272.

[17] Bednash JS, Weathington N, Londino J, Rojas M, Gulick DL, Fort R, Han SH, McKelvey AC, Chen BB, Mallampalli RK. Targeting the deubiquitinase STAMBP inhibits NALP7 inflammasome activity. Nat Commun 2017;**8**.

[18] Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. BMJ Open 2012;**2**:e000435.

[19] Bhartiya D, Kapoor S, Jalali S, Sati S, Kaushik K, Sachidanandan C, Sivasubbu S, Scaria V. Conceptual approaches for lncRNA drug discovery and future strategies. Expert Opin Drug Discov 2012;**7**:503–513.

[20] Bigoni M, Turati M, Sacerdote P, Gaddi D, Piatti M, Castelnuovo A, Franchi S, Gandolla M, Pedrocchi A, Omeljaniuk RJ, Bresciani E, Locatelli V, Torsello A. Characterization of synovial fluid cytokine profiles in chronic meniscal tear of the knee. J Orthop Res 2017;**35**:340–346.

[21] Bjersing JL, Lundborg C, Bokarewa MI, Mannerkorpi K. Profile of Cerebrospinal microRNAs in Fibromyalgia. PLoS One 2013;**8**:e78762.

[22] Bond AM, VanGompel MJW, Sametsky EA, Clark MF, Savage JC, Disterhoft JF, Kohtz JD. Balanced gene regulation by an embryonic brain non-coding RNA is critical for GABA circuitry in adult hippocampus. Nat Neurosci 2009;**12**:1020.

[23] Brown III EC, Scuderi GR, Clarke HD. The Painful Total Knee Arthroplasty: Diagnosis and Management. Orthopedics 2006;**29**:129–136.

[24] Buchheit T, Van de Ven T, Shaw A. Epigenetics and the Transition from Acute to Chronic Pain. Pain Med (United States) 2012;**13**:1474–1490.

[25] Buckwalter JA. Osteoarthritis and articular cartilage use, disuse, and abuse: experimental studies. J Rheumatol Suppl 1995;**43**:5–13.

[26] Busso N, Péclat V, So A, Sappino AP. Plasminogen activation in synovial tissues: Differences between normal, osteoarthritis, and rheumatoid arthritis joints. Ann Rheum Dis 1997;**56**:550–557.

[27] Buvanendran A, Kroin JS, Della Valle CJ, Kari M, Moric M, Tuman KJ. Perioperative oral pregabalin reduces chronic pain after total knee arthroplasty: a prospective, randomized, controlled trial. Anesth Analg 2010;**110**:199–207.

[28] Calabrese LH, Rose-John S. IL-6 biology: Implications for clinical targeting in rheumatic disease. Nat Rev Rheumatol 2014;**10**:720–727.

[29] Calay ES, Hotamisligil GS. Turning off the inflammatory, but not the metabolic, flames. Nat Med 2013;**19**:265–267.

[30] Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. Lancet Neurol 2012;**11**:629–642.

[31] Carr AJ, Robertsson O, Graves S, Price AJ, Arden NK, Judge A, Beard DJ. Knee replacement. The Lancet. Lancet Publishing Group, 2012, Vol. 379. pp. 1331–1340.

[32] Chalaux E, Lopez-Rovira T, Rosa JL, Bartrons R, Ventura F. JunB Is Involved in the Inhibition of Myogenic Differentiation by Bone Morphogenetic Protein-2. J Biol Chem 1998;**273**:537–43.

[33] Chauffier K, Laiguillon M, Bougault C, Gosset M, Priam S, Salvat C, Mladenovic Z, Nourissat G, Jacques C, Houard X, Berenbaum F, Sellam J. Induction of the chemokine IL-8/Kc by the articular cartilage: possible influence on osteoarthritis. Joint Bone Spine 2012;**79**:604–609.

[34] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo

X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;**18**:997–1006.

[35] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang YY, Chen J, Guo X, Li Q, Li X, Wang W, Zhang YY, Wang JJ, Jiang X, Xiang Y, Xu C, Zheng P, Zhang JJ, Li R, Zhang H, Shang X, Gong T, Ning G, Wang JJ, Zen K, Zhang JJ, Zhang C-YY. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;**18**:997–1006.

[36] Chen Y, Lin Y, Bai Y, Cheng D, Bi Z. A long noncoding RNA (LncRNA)-associated competing endogenous RNA (ceRNA) network identifies eight lncrna biomarkers in patients with osteoarthritis of the knee. Med Sci Monit 2019;**25**:2058–2065.

[37] Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, Garcia I, Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 1997;**272**:32401–10.

[38] Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, Dayer JM. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: Blocking and enhancing effects of anti-TWEAK monoclonal antibodies. Arthritis Res 2002;**4**:126–133.

[39] Chitu V, Gokhan Ş, Nandi S, Mehler MF, Stanley ER. Emerging Roles for CSF-1 Receptor and its Ligands in the Nervous System. Trends Neurosci 2016;**39**:378–393.

[40] Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. Curr Opin Immunol 2006;**18**:39–48.

[41] Clague MJ, Urbé S. Endocytosis: the DUB version. Trends Cell Biol 2006;**16**:551–559.

[42] Collins SL, Moore RA, McQuay HJ. The visual analogue pain intensity scale: What is moderate pain in millimetres? Pain 1997;**72**:95–97.

[43] Cook AD, Christensen AD, Tewari D, McMahon SB, Hamilton JA. Immune Cytokines and Their Receptors in Inflammatory Pain. Trends Immunol 2018;**39**:240–255.

[44] Correll D. Chronic postoperative pain: Recent findings in understanding and management. F1000Research 2017;**6**.

[45] Costa FF. Non-coding RNAs, epigenetics and complexity. Gene 2008;**410**:9–17.

[46] Crea F, Watahiki A, Quagliata L, Xue H, Pikor L, Parolia A, Wang YY, Lin D, Lam WL, Farrar WL, Isogai T, Morant R, Castori-Eppenberger S, Chi KN, Wang YY, Helgason CD. Identification of a long non-coding RNA as a novel biomarker and potential therapeutic target for metastatic prostate cancer. Oncotarget 2014;**5**:764–74.

[47] Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, Bridgett L, Williams S, Guillemin F, Hill CL, Laslett LL, Jones G, Cicuttini F, Osborne R, Vos T, Buchbinder R, Woolf A, March L. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;**73**:1323–30.

[48] Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, Bridgett L, Williams S, Guillemin F, Hill CL, Laslett LL, Jones G, Cicuttini F, Osborne R, Vos T, Buchbinder R, Woolf A, March L. The global burden of hip and knee osteoarthritis: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis 2014;**73**:1323–1330.

[49] Dai J, Ikegawa S. Recent advances in association studies of osteoarthritis susceptibility genes. J Hum Genet 2010;**55**:77–80.

[50] DeLeo JA, Colburn RW, Nichols M, Malhotra A. Interleukin-6-Mediated Hyperalgesia/Allodynia and Increased Spinal IL-6 Expression in a Rat Mononeuropathy Model. J Interf Cytokine Res 2009;**16**:695–700.

[51] Denk F, McMahon SB. Chronic pain: Emerging evidence for the involvement of epigenetics. Neuron 2012;**73**:435–444.

[52] Desplat-Jégo S, Varriale S, Creidy R, Terra R, Bernard D, Khrestchatisky M, Izui S, Chicheportiche Y, Boucraut J. TWEAK is expressed by glial cells, induces astrocyte proliferation and increases EAE severity. J Neuroimmunol 2002;**133**:116–123.

[53] Dieppe P. Developments in osteoarthritis. Rheumatology 2011;**50**:245–247.

[54] Dieppe PA. Relationship between symptoms and structural change in osteoarthritis: what are the important targets for therapy? J Rheumatol 2005;**32**:1147–9.

[55] Dieppe PA, Lohmander LS. Pathogenesis and management of pain in osteoarthritis. Lancet. Elsevier, 2005, Vol. 365. pp. 965–973.

[56] Doehring A, Geisslinger G, Lötsch J. Epigenetics in pain and analgesia: An

imminent research field. Eur J Pain 2011;**15**:11–16.

[57] Dolin SJ, Cashman JN, Bland JM. Effectiveness of acute postoperative pain management: I. Evidence from published data. Br J Anaesth 2002;**89**:409–423.

[58] Duan W, Du L, Jiang X, Wang R, Yan S, Xie Y, Yan K, Wang Q, Wang L, Zhang X, Pan H, Yang Y, Wang C. Identification of a serum circulating lncRNA panel for the diagnosis and recurrence prediction of bladder cancer. Oncotarget 2016;**7**.

[59] Duret L, Chureau C, Samain S, Weissenbach J, Avner P. The Xist RNA gene evolved in eutherians by pseudogenization of a protein-coding gene. Science 2006;**312**:1653–5.

[60] Dye SF, Vaupel GL, Dye CC. Conscious Neurosensory Mapping of the Internal Structures of the Human Knee Without Intraarticular Anesthesia. Am J Sports Med 1998;**26**:773–777.

[61] Ebbinghaus M, Segond von Banchet G, Massier J, Gajda M, Bräuer R, Kress M, Schaible HG. Interleukin-6-dependent influence of nociceptive sensory neurons on antigen-induced arthritis. Arthritis Res Ther 2015;**17**:334.

[62] Edd SN, Favre J, Blazek K, Omoumi P, Asay JL, Andriacchi TP. Altered gait mechanics and elevated serum pro-inflammatory cytokines in asymptomatic patients with MRI evidence of knee cartilage loss. Osteoarthr Cartil 2017;**25**:899–906.

[63] Eguchi T, Prince T, Wegiel B, Calderwood SK. Role and Regulation of Myeloid Zinc Finger Protein 1 in Cancer. J Cell Biochem 2015;**116**:2146–2154.

[64] Eiraku M, Hirata Y, Takeshima H, Hirano T, Kengaku M. Delta/notch-like epidermal growth factor (EGF)-related receptor, a novel EGF-like repeat-containing protein targeted to dendrites of developing and adult central nervous system neurons. J Biol Chem 2002;**277**:25400–7.

[65] Eitner A, Pester J, Vogel F, Marintschev I, Lehmann T, Hofmann GO, Schaible H-G. Pain sensation in human osteoarthritic knee joints is strongly enhanced by diabetes mellitus. Pain 2017;**158**:1.

[66] Esquela-Kerscher A, Slack FJ. Oncomirs — microRNAs with a role in cancer. Nat Rev Cancer 2006;**6**:259–269.

[67] Farrell MJ, Gibson SJ, McMeeken JM, Helme RD. Increased movement pain in osteoarthritis of the hands is associated with Aβ-mediated cutaneous

mechanical sensitivity. J Pain 2000;**1**:229–242.

[68] Felson DT. Osteoarthritis as a disease of mechanics. Osteoarthr Cartil 2013;**21**:10–15.

[69] Felson DT. Osteoarthritis of the Knee. N Engl J Med 2006;**354**:841–848.

[70] Felson DT. The sources of pain in knee osteoarthritis. Curr Opin Rheumatol 2005;**17**:624–628.

[71] Fernandes L, Hagen KB, Bijlsma JWJ, Andreassen O, Christensen P, Conaghan PG, Doherty M, Geenen R, Hammond A, Kjeken I, Lohmander LS, Lund H, Mallen CD, Nava T, Oliver S, Pavelka K, Pitsillidou I, da Silva JA, de la Torre J, Zanoli G, Vliet Vlieland TPM, European League Against Rheumatism (EULAR). EULAR recommendations for the non-pharmacological core management of hip and knee osteoarthritis. Ann Rheum Dis 2013;**72**:1125–35.

[72] Fernández-Puente P, Mateos J, Fernández-Costa C, Oreiro N, Fernández-López C, Ruiz-Romero C, Blanco FJ. Identification of a panel of novel serum osteoarthritis biomarkers. J Proteome Res 2011;**10**:5095–5101.

[73] Finan PH, Buenaver LF, Bounds SC, Hussain S, Park RJ, Haque UJ, Campbell CM, Haythornthwaite JA, Edwards RR, Smith MT. Discordance between pain and radiographic severity in knee osteoarthritis: Findings from quantitative sensory testing of central sensitization. Arthritis Rheum 2013;**65**:363–372.

[74] Fischer U, Jänicke RU, Schulze-Osthoff K. Many cuts to ruin: A comprehensive update of caspase substrates. Cell Death Differ 2003;**10**:76–100.

[75] Foell D, Kane D, Bresnihan B, Vogl T, Nacken W, Sorg C, FitzGerald O, Roth J. Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis. Rheumatology 2003;**42**:1383–1389.

[76] Fransès RE, McWilliams DF, Mapp PI, Walsh DA. Osteochondral angiogenesis and increased protease inhibitor expression in OA. Osteoarthr Cartil 2010;**18**:563.

[77] Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;**19**:92–105.

[78] Fu M, Huang G, Zhang Z, Liu J, Zhang Z, Huang Z, Yu B, Meng F. Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. Osteoarthr Cartil 2015;**23**:423–432.

[79] Gandhi R, Santone D, Takahashi M, Dessouki O, Mahomed NN. Inflammatory predictors of ongoing pain 2 years following knee replacement surgery. Knee 2013;**20**:316–18.

[80] Garaude J, Farrás R, Bossis G, Charni S, Piechaczyk M, Hipskind RA, Villalba M. SUMOylation Regulates the Transcriptional Activity of JunB in T Lymphocytes. J Immunol 2008;**180**:5983–90.

[81] Garcia S, Hartkamp LM, Malvar-Fernandez B, van Es IE, Lin H, Wong J, Long L, Zanghi JA, Rankin AL, Masteller EL, Wong BR, Radstake TRDJ, Tak PP, Reedquist KA. Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis. Arthritis Res Ther 2016;**18**:75.

[82] Garnero P, Aronstein WS, Cohen SB, Conaghan PG, Cline GA, Christiansen C, Beary JF, Meyer JM, Bingham CO. Relationships between biochemical markers of bone and cartilage degradation with radiological progression in patients with knee osteoarthritis receiving risedronate: the Knee Osteoarthritis Structural Arthritis randomized clinical trial. Osteoarthr Cartil 2008;**16**:660–666.

[83] Gerdle B, Ghafouri B, Ghafouri N, Bäckryd E, Gordh T. Signs of ongoing inflammation in female patients with chronic widespread pain: A multivariate, explorative, cross-sectional study of blood samples. Med (United States) 2017;**96**:e6130.

[84] Geyer PE, Kulak NA, Pichler G, Holdt LM, Teupser D, Mann M. Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst 2016;**2**:185–195.

[85] Ghafouri B, Carlsson A, Holmberg S, Thelin A, Tagesson C. Biomarkers of systemic inflammation in farmers with musculoskeletal disorders; a plasma proteomic study. BMC Musculoskelet Disord 2016;**17**:206.

[86] Giordano R, Petersen KK, Andersen HH, Lichota J, Valeriani M, Simonsen O, Arendt-Nielsen L. Preoperative serum circulating microRNAs as potential biomarkers for chronic postoperative pain after total knee replacement. Mol Pain 2020;**16**:174480692096292.

[87] Giordano R, Petersen KK, Andersen HH, Simonsen O, Arendt-Nielsen L. Serum Inflammatory Markers in Patients with Knee Osteoarthritis: A Proteomic Approach. Clin J Pain 2020.

[88] Goldring MB. Articular cartilage degradation in osteoarthritis. HSS J 2012;**8**:7–9.

[89] Gomard T, Michaud H-A, Tempé D, Thiolon K, Pelegrin M, Piechaczyk M. An NF-κB–Dependent Role for JunB in the Induction of Proinflammatory Cytokines in LPS-Activated Bone Marrow–Derived Dendritic Cells. PLoS One 2010;**5**:e9585.

[90] Gomez-Varela D, Barry AM, Schmidt M. Proteome-based systems biology in chronic pain. J Proteomics 2019;**190**:1–11.

[91] Graven-Nielsen T, Wodehouse T, Langford RM, Arendt-Nielsen L, Kidd BL. Normalization of widespread hyperesthesia and facilitated spatial summation of deep-tissue pain in knee osteoarthritis patients after knee replacement. Arthritis Rheum 2012;**64**:2907–2916.

[92] Grayson DR, Jia X, Chen Y, Sharma RP, Mitchell CP, Guidotti A, Costa E. Reelin promoter hypermethylation in schizophrenia. Proc Natl Acad Sci U S A 2005;**102**:9341–6.

[93] Griffiths-Jones S. The microRNA Registry. Nucleic Acids Res 2004;**32**:109D–11.

[94] Gu J-J, Fan K-C, Zhang J-H, Chen H-J, Wang S-S. Suppression of microRNA-130b inhibits glioma cell proliferation and invasion, and induces apoptosis by PTEN/AKT signaling. Int J Mol Med 2018;**41**:284–292.

[95] Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, Guan AK, Evans-Reinsch Z, Braz J, Devor M, Abboud-Werner SL, Lanier LL, Lomvardas S, Basbaum AI. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci 2016;**19**:94–101.

[96] Gwilym SE, Pollard TCB, Carr AJ. Understanding pain in osteoarthritis. J Bone Joint Surg Br 2008;**90-B**:280–287.

[97] Hadrevi J, Ghafouri B, Larsson B, Gerdle B, Hellström F. Multivariate Modeling of Proteins Related to Trapezius Myalgia, a Comparative Study of Female Cleaners with or without Pain. PLoS One 2013;**8**:e73285.

[98] Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 2008;**8**:533–544.

[99] Hamilton JA, Cook AD, Tak PP. Anti-colony-stimulating factor therapies for inflammatory and autoimmune diseases. Nat Rev Drug Discov 2017;**16**:53–70.

[100] Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev 2015;**87**:3–14.

[101] Han J, Yang T, Gao J, Wu J, Qiu X, Fan Q, Ma B. Specific microRNA expression during chondrogenesis of human mesenchymal stem cells. Int J Mol Med 2010;**25**:377–84.

[102] Hart RP, Goff LA. Long noncoding RNAs: Central to nervous system development. Int J Dev Neurosci 2016:1–8.

[103] Harting K, Knöll B. SIRT2-mediated protein deacetylation: An emerging key regulator in brain physiology and pathology. Eur J Cell Biol 2010;**89**:262–269.

[104] Hashimoto M, Nakasa T, Hikata T, Asahara H. Molecular network of cartilage homeostasis and osteoarthritis. Med Res Rev 2008;**28**:464–81.

[105] Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev 2004;**18**:1926–45.

[106] Hayashi D, Roemer FW, Guermazi A. Imaging for osteoarthritis. Ann Phys Rehabil Med 2016;**59**:161–169.

[107] Heard E, Martienssen RA. Transgenerational epigenetic inheritance: Myths and mechanisms. Cell 2014;**157**:95–109.

[108] Van Hecke O, Kamerman PR, Attal N, Baron R, Bjornsdottir G, Bennett DLH, Bennett MI, Bouhassira D, Diatchenko L, Freeman R, Freynhagen R, Haanpää M, Jensen TS, Raja SN, Rice ASC, Seltzer Z, Thorgeirsson TE, Yarnitsky D, Smith BH. Neuropathic pain phenotyping by international consensus (NeuroPPIC) for genetic studies: A NeuPSIG systematic review, Delphi survey, and expert panel recommendations. Pain 2015;**156**:2337–2353.

[109] Herren-Gerber R, Weiss S, Arendt-Nielsen L, Petersen-Felix S, Stefano G Di, Radanov BP, Curatolo M. Modulation of Central Hypersensitivity by Nociceptive Input in Chronic Pain After Whiplash Injury. Pain Med 2004;**5**:366–376.

[110] Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, Towheed T, Welch V, Wells G, Tugwell P. American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis Care Res 2012;**64**:465–474.

[111] Holt HL, Katz JN, Reichmann WM, Gerlovin H, Wright EA, Hunter DJ, Jordan JM, Kessler CL, Losina E. Forecasting the burden of advanced knee osteoarthritis over a 10-year period in a cohort of 60–64 year-old US adults. Osteoarthr Cartil 2011;**19**:44–50.

[112] Hou J, Wang P, Lin L, Liu X, Ma F, An H, Wang Z, Cao X. MicroRNA-146a feedback inhibits RIG-I-dependent Type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. J Immunol 2009;**183**:2150–8.

[113] Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 2012;**13**:225–238.

[114] Hu G, Zhao X, Wang CC, Geng Y, Zhao J, Xu J, Zuo B, Zhao C, Wang CC, Zhang X. MicroRNA-145 attenuates TNF-α-driven cartilage matrix degradation in osteoarthritis via direct suppression of MKK4. Cell Death Dis 2017;**8**:e3140.

[115] Hu X-Y, Li L, Wu H-T, Liu Y, Wang B-D, Tang Y. Serum miR-130b level, an ideal marker for monitoring the recurrence and prognosis of primary hepatocellular carcinoma after radiofrequency ablation treatment. Pathol - Res Pract 2018;**214**:1655–1660.

[116] Hulejová H, Andrés Cerezo L, Kuklová M, Pecha O, Vondráček T, Pavelka K, Vencovský J, Haluzík M, Šenolt L. Novel adipokine fibroblast growth factor 21 is increased in rheumatoid arthritis. Physiol Res 2012;**61**:489–494.

[117] Hunter DJ, Guermazi A, Roemer F, Zhang Y, Neogi T. Structural correlates of pain in joints with osteoarthritis. Osteoarthr Cartil 2013;**21**:1170–8.

[118] Hunter DJ, McDougall JJ, Keefe FJ. The Symptoms of Osteoarthritis and the Genesis of Pain. Med Clin North Am 2009;**93**:83–100.

[119] Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee MLT, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008;**3**:e3694.

[120] Imai S, Narita M, Ikegami D, Yamashita A, Shimizu T, Narita M, Niikura K, Furuya M, Kobayashi Y, Miyashita K, Okutsu D, Kato A, Nakamura A, Araki A, Omi K, Nakamura M, James Okano H, Okano H, Ando T, Takeshima H, Ushijima T, Kuzumaki N, Suzuki T. Epigenetic transcriptional activation of monocyte chemotactic protein 3 contributes to long-lasting neuropathic pain. Brain 2013;**136**:828–843.

[121] Imamura M, Ezquerro F, Marcon Alfieri F, Vilas Boas L, Tozetto-Mendoza TR, Chen J, Özçakar L, Arendt-Nielsen L, Rizzo Battistella L. Serum levels of proinflammatory cytokines in painful knee osteoarthritis and sensitization. Int J Inflam 2015;**2015**:1–8.

[122] Imamura M, Imamura ST, Kaziyama HHS, Targino RA, Hsing WT, De Souza LPM, Cutait MaM, Fregni F, Camanho GL. Impact of nervous system

hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: A controlled analysis. Arthritis Rheum 2008;**59**:1424–1431.

[123] Inoue T, Hiratsuka M, Osaki M, Oshimura M. The molecular biology of mammalian SIRT proteins: SIRT2 in cell cycle regulation. Cell Cycle 2007;**6**:1011–1018.

[124] Ivey KN, Srivastava D. microRNAs as Developmental Regulators. Cold Spring Harb Perspect Biol 2015;**7**:a008144.

[125] Jaworski J, Sheng M. The Growing Role of mTOR in Neuronal Development and Plasticity. Mol Neurobiol 2006;**34**:205–220.

[126] Jensen MP, Karoly P, Braver S. The measurement of clinical pain intensity: a comparison of six methods. Pain 1986;**27**:117–126.

[127] Jeong H, Na YJ, Lee K, Kim YH, Lee Y, Kang M, Jiang BC, Yeom Y Il, Wu LJ, Gao YJ, Kim J, Oh SB. High-resolution transcriptome analysis reveals neuropathic pain gene-expression signatures in spinal microglia after nerve injury. Pain 2016;**157**:964–976.

[128] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H, Müller-Tidow C. MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003;**22**:8031–8041.

[129] Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. NIH Public Access, 2014 533–548 pp.

[130] Jiménez-Díaz L, Géranton SM, Passmore GM, Leith JL, Fisher AS, Berliocchi L, Sivasubramaniam AK, Sheasby A, Lumb BM, Hunt SP. Local translation in primary afferent fibers regulates nociception. PLoS One 2008;**3**:e1961.

[131] Jordan KM. EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). Ann Rheum Dis 2003;**62**:1145–1155.

[132] Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011;**7**:33–42.

[133] Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos

G, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-TarBase v8: A decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res 2018;**46**:D239-45.

[134] Kartha R V., Subramanian S. MicroRNAs in Cardiovascular Diseases: Biology and Potential Clinical Applications. J Cardiovasc Transl Res 2010;**3**:256–270.

[135] Kavchak AJE, Fernández-De-Las-Peñas C, Rubin LH, Arendt-Nielsen L, Chmell SJ, Durr RK, Courtney CA. Association between altered somatosensation, pain, and knee stability in patients with severe knee osteoarthrosis. Clin J Pain 2012;**28**:589–594.

[136] Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet 2006;**367**:1618–1625.

[137] Keller C, Bühler M. Chromatin-associated ncRNA activities. Chromosom Res 2013;**21**:627–641.

[138] Kellgren JH, Lawrence JS, Lawrance JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;**16**:494–502.

[139] Khoutorsky A, Bonin RP, Sorge RE, Gkogkas CG, Pawlowski SA, Jafarnejad SM, Pitcher MH, Alain T, Perez-Sanchez J, Salter EW, Martin L, Ribeiro-Da-Silva A, De Koninck Y, Cervero F, Mogil JS, Sonenberg N. Translational control of nociception via 4E-binding protein 1. Elife 2015;**4**.

[140] Kishida S, Yamamoto H, Ikeda S, Kishida M, Sakamoto I, Koyama S, Kikuchi A. Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. J Biol Chem 1998;**273**:10823–6.

[141] Kitaura H, Zhou P, Kim H-J, Novack D V, Ross FP, Teitelbaum SL. M-CSF mediates TNF-induced inflammatory osteolysis. J Clin Invest 2005;**115**:3418–27.

[142] Komori N, Takemori N, Kim HK, Singh A, Hwang S-H, Foreman RD, Chung K, Chung JM, Matsumoto H. Proteomics study of neuropathic and nonneuropathic dorsal root ganglia: altered protein regulation following segmental spinal nerve ligation injury. Physiol Genomics 2007;**29**:215–230.

[143] Kosek E, Finn A, Ultenius C, Hugo A, Svensson C, Ahmed AS. Differences in neuroimmune signalling between male and female patients suffering from knee osteoarthritis. J Neuroimmunol 2018;**321**:49–60.

[144] Kouzarides T. Chromatin Modifications and Their Function. Cell

2007;**128**:693–705.

[145] Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, Gendreau M, Gardiner J, Garnero P, Hardin J, Henrotin Y, Heinegård D, Ko A, Lohmander LS, Matthews G, Menetski J, Moskowitz R, Persiani S, Poole AR, Rousseau JC, Todman M. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthr Cartil 2011;**19**:515–542.

[146] Lee SC, Yoon TG, Yoo Y Il, Bang YJ, Kim HY, Jeoung D Il, Kim HJ. Analysis of spinal cord proteome in the rats with mechanical allodynia after the spinal nerve injury. Biotechnol Lett 2003;**25**:2071–2078.

[147] Lee YC, Lu B, Bathon JM, Haythornthwaite JA, Smith MT, Page GG, Edwards RR. Pain Sensitivity and Pain Reactivity in Osteoarthritis. n.d.

[148] Leinders M, Doppler K, Klein T, Deckart M, Rittner H, Sommer C, Üçeyler N. Increased cutaneous miR-let-7d expression correlates with small nerve fiber pathology in patients with fibromyalgia syndrome. Pain 2016;**157**:2493–503.

[149] van Lent PLEM, Blom AB, Schelbergen RFP, Slöetjes A, Lafeber FPJG, Lems WF, Cats H, Vogl T, Roth J, van den Berg WB. Active involvement of alarmins S100A8 and S100A9 in the regulation of synovial activation and joint destruction during mouse and human osteoarthritis. Arthritis Rheum 2012;**64**:1466–1476.

[150] Lenz G, Avruch J. Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. J Biol Chem 2005;**280**:38121–4.

[151] Li B, Ding C, Li Y, Peng J, Geng N, Qin W. MicroRNA-145 inhibits migration and induces apoptosis in human non-small cell lung cancer cells through regulation of the EGFR/PI3K/AKT signaling pathway. Oncol Rep 2018;**40**:2944–2954.

[152] Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. EMBO J 1999;**18**:420–32.

[153] Li J-H, Liu S, Zhou H, Qu L-H, Yang J-H. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014;**42**:D92-7.

[154] Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X, Dai K. MiR-146a, an IL-1β responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. Arthritis Res Ther 2012;**14**:R75.

[155] Li Q, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B, Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. Tumor Biol 2015;**36**:2007–2012.

[156] Li X, Gibson G, Kim J-S, Kroin J, Xu S, van Wijnen AJ, Im H-J. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. Gene 2011;**480**:34–41.

[157] Li Z, Xu L, Liu Y, Fu S, Tu J, Hu Y, Xiong Q. LncRNA MALAT1 promotes relapse of breast cancer patients with postoperative fever. Am J Transl Res 2018;**10**:3186–3197.

[158] Li ZC, Xiao J, Wang G, Li MQ, Hu KZ, Ma T, Wang WL, Liu Z De, Zhang JD. Fibroblast growth factor-21 concentration in serum and synovial fluid is associated with radiographic bone loss of knee osteoarthritis. Scand J Clin Lab Invest 2015;**75**:121–125.

[159] Lin J, Sun B, Jiang C, Hong H, Zheng Y. Sirt2 suppresses inflammatory responses in collagen-induced arthritis. Biochem Biophys Res Commun 2013;**441**:897–903.

[160] Littman GS, Walker BR, Schneider BE. Reassessment of verbal and visual analog ratings in analgesic studies. Clin Pharmacol Ther 1985;**38**:16–23.

[161] Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012;**64**:1697–707.

[162] Londin E, Loher P, Telonis AG, Quann K, Clark P, Jing Y, Hatzimichael E, Kirino Y, Honda S, Lally M, Ramratnam B, Comstock CES, Knudsen KE, Gomella L, Spaeth GL, Hark L, Katz LJ, Witkiewicz A, Rostami A, Jimenez SA, Hollingsworth MA, Yeh JJ, Shaw CA, McKenzie SE, Bray P, Nelson PT, Zupo S, Van Roosbroeck K, Keating MJ, Calin GA, Yeo C, Jimbo M, Cozzitorto J, Brody JR, Delgrosso K, Mattick JS, Fortina P, Rigoutsos I. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci 2015;**112**:E1106-15.

[163] Long YC, Kharitonenkov A. Hormone-like fibroblast growth factors and metabolic regulation. Biochim Biophys Acta - Mol Basis Dis 2011;**1812**:791–795.

[164] Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, Schröder GF, Fitzgerald KA, Wu H, Egelman EH. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell 2014;**156**:1193–1206.

[165] Lu Y, Cao D-LL, Jiang B-CC, Yang T, Gao Y-JJ. MicroRNA-146a-5p

attenuates neuropathic pain via suppressing TRAF6 signaling in the spinal cord. Brain Behav Immun 2015;**49**:119–29.

[166] Lundblad H, Kreicbergs A, Jansson KÅ. Prediction of persistent pain after total knee replacement for osteoarthritis. J Bone Jt Surg - Ser B 2008;**90**:166–171.

[167] Luo J, Si Z-Z, Li T, Li J-Q, Zhang Z-Q, Chen G-S, Qi H-Z, Yao H-L. MicroRNA-146a-5p Enhances Radiosensitivity in Hepatocellular Carcinoma through Replication Protein A3 Induced Activation of the DNA Repair Pathway. Am J Physiol Physiol 2018:ajpcell.00189.2018.

[168] Lützner J, Kasten P, Günther KP, Kirschner S. Surgical options for patients with osteoarthritis of the knee. Nat Rev Rheumatol 2009;**5**:309–316.

[169] Lv C, Zhou Y hong, Wu C, Shao Y, Lu C lu, Wang QY. The changes in miR-130b levels in human serum and the correlation with the severity of diabetic nephropathy. Diabetes Metab Res Rev 2015;**31**:717–24.

[170] Lynch EP, Lazor MA, Gellis JE, Orav J, Goldman L, Marcantonio ER. Patient experience of pain after elective noncardiac surgery. Anesth Analg 1997;**85**:117–23.

[171] Ma Y, Shi J, Wang F, Li S, Wang J, Zhu C, Li L, Lu H, Li C, Yan J, Zhang X, Jiang H. MiR-130b increases fibrosis of HMC cells by regulating the TGF-β1 pathway in diabetic nephropathy. J Cell Biochem 2018:1–13.

[172] Martínez-Muriana A, Mancuso R, Francos-Quijorna I, Olmos-Alonso A, Osta R, Perry VH, Navarro X, Gomez-Nicola D, López-Vales R. CSF1R blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasion of macrophages into peripheral nerves. Sci Rep 2016;**6**:25663.

[173] McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, Hawker GA, Henrotin Y, Hunter DJ, Kawaguchi H, Kwoh K, Lohmander S, Rannou F, Roos EM, Underwood M. OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthr Cartil 2014;**22**:363–388.

[174] Melemedjian OK, Khoutorsky A, Sorge RE, Yan J, Asiedu MN, Valdez A, Ghosh S, Dussor G, Mogil JS, Sonenberg N, Price TJ. mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK. Pain 2013;**154**:1080–1091.

[175] Meng C, Yang X, Liu Y, Zhou Y, Rui J, Li S, Xu C, Zhuang Y, Lao J, Zhao X. Decreased expression of lncRNA Malat1 in rat spinal cord contributes to

neuropathic pain by increasing neuron excitability after brachial plexus avulsion. J Pain Res 2019;**Volume 12**:1297–1310.

[176] Merino P, Diaz A, Jeanneret V, Wu F, Torre E, Cheng L, Yepes M. Urokinase-type plasminogen activator (uPA) binding to the uPA receptor (uPAR) promotes axonal regeneration in the central nervous system. J Biol Chem 2017;**292**:2741–2753.

[177] Merino P, Yepes M. Urokinase-type Plasminogen Activator Induces Neurorepair in the Ischemic Brain. J Neurol Exp Neurosci 2018;**04**.

[178] Messier SP, Loeser RF, Miller GD, Morgan TM, Rejeski WJ, Sevick MA, Ettinger WH, Pahor M, Williamson JD. Exercise and Dietary Weight Loss in Overweight and Obese Older Adults with Knee Osteoarthritis: The Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum 2004;**50**:1501–1510.

[179] Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the panther classification system. Nat Protoc 2013;**8**:1551–66.

[180] Michaelevski I, Medzihradszky KF, Lynn A, Burlingame AL, Fainzilber M. Axonal transport proteomics reveals mobilization of translation machinery to the lesion site in injured sciatic nerve. Mol Cell Proteomics 2010;**9**:976–987.

[181] Milenkovic N, Frahm C, Gassmann M, Griffel C, Erdmann B, Birchmeier C, Lewin GR, Garratt AN. Nociceptive Tuning by Stem Cell Factor/c-Kit Signaling. Neuron 2007;**56**:893–906.

[182] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;**105**:10513–8.

[183] Miyaki S, Asahara H. Macro view of microRNA function in osteoarthritis. Nat Rev Rheumatol 2012;**8**:543–552.

[184] Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno-Kudo H, Asahara H. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev 2010;**24**:1173–1185.

[185] Mobasheri A. The future of osteoarthritis therapeutics: Emerging biological therapy. Curr Rheumatol Rep 2013;**15**:385.

[186] Mobasheri A, Henrotin Y. Biomarkers of (osteo)arthritis. Biomarkers 2015;**20**:513–518.

[187] Mohankumar S, Patel T. Extracellular vesicle long noncoding RNA as potential biomarkers of liver cancer. Brief Funct Genomics 2016;**15**:249–56.

[188] Monaco C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. Int Immunol 2015;**27**:55–62.

[189] Moore D, McCrory C. The Proteomics of Intrathecal Analgesic agents for Chronic Pain. Curr Neuropharmacol 2017;**15**:198–205.

[190] Mori G, Brunetti G, Collucci S, Oranger A, Ciccolella F, Sardone F, Pignataro P, Mori C, Karapanou V, Ballini A, Mastrangelo F, Tetè S, Grassi FR, Grano M. Osteoblast apoptosis in periodontal disease: Role of TNF-related apoptosis-inducing ligand. Int J Immunopathol Pharmacol 2009;**22**:95–103.

[191] Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M, Asahara H. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. Arthritis Rheum 2008;**58**:1284–92.

[192] Nguyen U-SDT, Zhang Y, Zhu Y, Niu J, Zhang B, Felson DT, Felson DT. Increasing prevalence of knee pain and symptomatic knee osteoarthritis: survey and cohort data. Ann Intern Med 2011;**155**:725–32.

[193] Niederberger E, Resch E, Parnham MJ, Geisslinger G. Drugging the pain epigenome. Nat Rev Neurol 2017;**13**:434–447.

[194] Nielsen DA, Yuferov V, Hamon S, Jackson C, Ho A, Ott J, Kreek MJ. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. Neuropsychopharmacology 2009;**34**:867–873.

[195] Nüesch E, Dieppe P, Reichenbach S, Williams S, Iff S, Jüni P. All cause and disease specific mortality in patients with knee or hip osteoarthritis: population based cohort study. BMJ 2011;**342**:d1165.

[196] Nugent M. MicroRNAs: Exploring new horizons in osteoarthritis. Osteoarthr Cartil 2016;**24**:573–80.

[197] Obreja O, Schmelz M, Poole S, Kress M. Interleukin-6 in combination with its soluble IL-6 receptor sensitises rat skin nociceptors to heat, in vivo. Pain 2002;**96**:57–62.

[198] Oki G, Wada T, Iba K, Aiki H, Sasaki K, Imai SI, Sohma H, Matsumoto K, Yamaguchi M, Fujimiya M, Yamashita T, Kokai Y. Metallothionein

deficiency in the injured peripheral nerves of complex regional pain syndrome as revealed by proteomics. Pain 2012;**153**:532–539.

[199] Okubo M, Yamanaka H, Kobayashi K, Dai Y, Kanda H, Yagi H, Noguchi K. Macrophage-Colony Stimulating Factor Derived from Injured Primary Afferent Induces Proliferation of Spinal Microglia and Neuropathic Pain in Rats. PLoS One 2016;**11**:e0153375.

[200] Okuhara A, Nakasa T, Shibuya H, Niimoto T, Adachi N, Deie M, Ochi M. Changes in microRNA expression in peripheral mononuclear cells according to the progression of osteoarthritis. Mod Rheumatol 2012;**22**:446–457.

[201] Olausson P, Gerdle B, Ghafouri N, Larsson B, Ghafouri B. Identification of Proteins from Interstitium of Trapezius Muscle in Women with Chronic Myalgia Using Microdialysis in Combination with Proteomics. PLoS One 2012;**7**:e52560.

[202] Olausson P, Gerdle B, Ghafouri N, Sjöström D, Blixt E, Ghafouri B. Protein alterations in women with chronic widespread pain - An explorative proteomic study of the trapezius muscle. Sci Rep 2015;**5**.

[203] Olausson P, Ghafouri B, Ghafouri N, Gerdle B. Specific proteins of the trapezius muscle correlate with pain intensity and sensitivity – An explorative multivariate proteomic study of the trapezius muscle in women with chronic widespread pain. J Pain Res 2016;**9**:345–356.

[204] Orlova IA, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ, Ajit SK. MicroRNA modulation in complex regional pain syndrome. J Transl Med 2011;**9**:195.

[205] Pais TF, Szegő ÉM, Marques O, Miller-Fleming L, Antas P, Guerreiro P, Oliveira RM de, Kasapoglu B, Outeiro TF. The NAD-dependent deacetylase sirtuin 2 is a suppressor of microglial activation and brain inflammation. EMBO J 2013;**32**:2603–2616.

[206] Pan L, Liu D, Zhao L, Wang L, Xin M, Li X. Long noncoding RNA MALAT1 alleviates lipopolysaccharide-induced inflammatory injury by upregulating microRNA-19b in murine chondrogenic ATDC5 cells. J Cell Biochem 2018;**119**:10165–10175.

[207] Panezai J, Ghaffar A, Altamash M, Sundqvist KG, Engström PE, Larsson A. Correlation of serum cytokines, chemokines, growth factors and enzymes with periodontal disease parameters. PLoS One 2017;**12**:e0188945.

[208] Petersen KK, Arendt-Nielsen L, Simonsen O, Wilder-Smith O, Laursen MB. Presurgical assessment of temporal summation of pain predicts the

development of chronic postoperative pain 12 months after total knee replacement. Pain 2015;**156**:55–61.

[209] Petersen KK, Graven-Nielsen T, Simonsen O, Laursen MB, Arendt-Nielsen L. Preoperative pain mechanisms assessed by cuff algometry are associated with chronic postoperative pain relief after total knee replacement. Pain 2016;**157**:1400–1406.

[210] Petersen KK, Siebuhr AS, Graven-Nielsen T, Simonsen O, Boesen M, Gudbergsen H, Karsdal M, Bay-Jensen AC, Arendt-Nielsen L. Sensitization and serological biomarkers in knee osteoarthritis patients with different degrees of synovitis. Clin J Pain 2016;**32**:841–848.

[211] Petersen KK, Simonsen O, Laursen MB, Arendt-nielsen L. The Role of Preoperative Radiologic Severity, Sensory Testing, and Temporal Summation on Chronic Postoperative Pain Following Total Knee Arthroplasty. Clin J Pain 2018;**34**:193–7.

[212] Petty E, Pillus L. Balancing chromatin remodeling and histone modifications in transcription. Trends Genet 2013;**29**:621–629.

[213] Piechaczyk M, Farràs R. Regulation and function of JunB in cell proliferation. Biochem Soc Trans 2008;**36**:864–7.

[214] Pietzsch J, Hoppmann S. Human S100A12: A novel key player in inflammation? Amino Acids 2009;**36**:381–389.

[215] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009;**136**:629–41.

[216] Powell ND, Sloan EK, Bailey MT, Arevalo JMG, Miller GE, Chen E, Kobor MS, Reader BF, Sheridan JF, Cole SW. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via -adrenergic induction of myelopoiesis. Proc Natl Acad Sci 2013;**110**:16574–16579.

[217] Price TJ, Gold MS. From mechanism to cure: Renewing the goal to eliminate the disease of pain. Pain Med (United States) 2018;**19**:1525–1549.

[218] Qi P, Zhou X-Y, Du X. Circulating long non-coding RNAs in cancer: current status and future perspectives. Mol Cancer 2016;**15**:39.

[219] Qureshi IA, Mehler MF. Long Non-coding RNAs: Novel Targets for Nervous System Disease Diagnosis and Therapy. Neurotherapeutics 2013;**10**:632–646.

[220] Reamon-Buettner SM, Mutschler V, Borlak J. The next innovation cycle in

toxicogenomics: Environmental epigenetics. Mutat Res - Rev Mutat Res 2008;**659**:158–165.

[221] Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, Sokolove J. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016;**12**:580–592.

[222] Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. Biochim Biophys Acta - Gene Regul Mech 2014;**1839**:627–643.

[223] Rothgiesser KM, Erener S, Waibel S, Lüscher B, Hottiger MO. SIRT2 regulates NF-κB dependent gene expression through deacetylation of p65 Lys310. J Cell Sci 2010;**123**:4251–8.

[224] Rouwette T, Sondermann J, Avenali L, Gomez-Varela D, Schmidt M. Standardized profiling of the membrane-enriched proteome of mouse dorsal root ganglia (DRG) provides novel insights into chronic pain. Mol Cell Proteomics 2016;**15**:2152–2168.

[225] Sacerdote P, Franchi S, Moretti S, Castelli M, Procacci P, Magnaghi V, Panerai AE. Cytokine modulation is necessary for efficacious treatment of experimental neuropathic pain. J Neuroimmune Pharmacol 2013;**8**:202–211.

[226] Saleh R, Lee M-C, Khiew SH, Louis C, Fleetwood AJ, Achuthan A, Förster I, Cook AD, Hamilton JA. CSF-1 in Inflammatory and Arthritic Pain Development. J Immunol 2018;**201**:ji1800665.

[227] Santoro M, Nociti V, Lucchini M, De Fino C, Losavio FA, Mirabella M. Expression Profile of Long Non-Coding RNAs in Serum of Patients with Multiple Sclerosis. J Mol Neurosci 2016;**59**:18–23.

[228] Schaible HG. Nociceptive neurons detect cytokines in arthritis. Arthritis Res Ther 2014;**16**:470.

[229] Schaible HG, Grubb BD. Afferent and spinal mechanisms of joint pain. Pain 1993;**55**:5–54.

[230] Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol 2010;**6**:625–635.

[231] Sengle G, Ono RN, Sasaki T, Sakai LY. Prodomains of transforming growth factor beta (TGFbeta) superfamily members specify different functions: extracellular matrix interactions and growth factor bioavailability. J Biol Chem 2011;**286**:5087–99.

[232] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2009;**31**:27–36.

[233] SHINGU M, MIYAUCHI S, NAGAI Y, YASUTAKE C, HORIE K. THE ROLE OF IL-4 AND IL-6 IN IL-1-DEPENDENT CARTILAGE MATRIX DEGRADATION. Rheumatology 1995;**34**:101–106.

[234] Siebuhr AS, Petersen KK, Arendt-Nielsen L, Egsgaard LL, Eskehave T, Christiansen C, Simonsen O, Hoeck HC, Karsdal MA, Bay-Jensen AC. Identification and characterisation of osteoarthritis patients with inflammation derived tissue turnover. Osteoarthr Cartil 2014;**22**:44–50.

[235] Simonetti M, Hagenston AM, Vardeh D, Freitag HE, Mauceri D, Lu J, Satagopam VP, Schneider R, Costigan M, Bading H, Kuner R. Nuclear Calcium Signaling in Spinal Neurons Drives a Genomic Program Required for Persistent Inflammatory Pain. Neuron 2013;**77**:43–57.

[236] Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. Pain 2013;**154**:1588–1594.

[237] Sohn DH, Sokolove J, Sharpe O, Erhart JC, Chandra PE, Lahey LJ, Lindstrom TM, Hwang I, Boyer KA, Andriacchi TP, Robinson WH. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. Arthritis Res Ther 2012;**14**:R7.

[238] Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: Latest findings and interpretations. Ther Adv Musculoskelet Dis 2013;**5**:77–94.

[239] Solheim N, Östlund S, Gordh T, Rosseland LA. Women report higher pain intensity at a lower level of inflammation after knee surgery compared with men. PAIN Reports 2017;**2**:e595.

[240] Sommer C. Exploring pain pathophysiology in patients. Science (80-) 2016;**354**:588–592.

[241] Sondag GR, Haqqi TM. The Role of MicroRNAs and Their Targets in Osteoarthritis. Curr Rheumatol Rep 2016;**18**:56.

[242] Stannus OP, Jones G, Blizzard L, Cicuttini FM, Ding C. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. Ann Rheum Dis 2013;**72**:535–540.

[243] Su W, Xie W, Shang Q, Su B. The long noncoding RNA MEG3 is downregulated and inversely associated with vegf levels in osteoarthritis.

Biomed Res Int 2015;**2015**.

[244] Sui P, Watanabe H, Ossipov MH, Bakalkin G, Artemenko K, Bergquist J. Proteomics of Neuropathic Pain: Proteins and Signaling Pathways Affected in a Rat Model. J Proteome Res 2014;**13**:3957–3965.

[245] Sullivan CS, Ganem D. MicroRNAs and Viral Infection. Mol Cell 2005;**20**:3–7.

[246] Svensson I, Sjöström B, Haljamäe H. Assessment of pain experiences after elective surgery. J Pain Symptom Manage 2000;**20**:193–201.

[247] Tafuri E, Santovito D, De Nardis V, Marcantonio P, Paganelli C, Affaitati G, Bucci M, Mezzetti A, Giamberardino MA, Cipollone F. MicroRNA profiling in migraine without aura: Pilot study. Ann Med 2015;**47**:468–473.

[248] Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci 2006;**103**:12481–6.

[249] Takagi K, Okuda-Ashitaka E, Mabuchi T, Katano T, Ohnishi T, Matsumura S, Ohnaka M, Kaneko S, Abe T, Hirata T, Fujiwara S, Minami T, Ito S. Involvement of stem cell factor and its receptor tyrosine kinase c-kit in pain regulation. Neuroscience 2008;**153**:1278–1288.

[250] Tiffon C. The impact of nutrition and environmental epigenetics on human health and disease. Int J Mol Sci 2018;**19**.

[251] Ulitsky I, Shkumatava A, Jan CH, Sive H, Bartel DP. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell 2011;**147**:1537–50.

[252] Vaegter HB, Handberg G, Emmeluth C, Graven-Nielsen T. Preoperative Hypoalgesia After Cold Pressor Test and Aerobic Exercise is Associated With Pain Relief 6 Months After Total Knee Replacement. Clin J Pain 2017;**33**:475–84.

[253] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;**9**:654–9.

[254] Vassalli JD, Sappino AP, Belin D. The plasminogen activator/plasmin system. J Clin Invest 1991;**88**:1067–1072.

[255] Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-

density lipoproteins. Nat Cell Biol 2011;**13**:423–35.

[256] Voss P, Thomas ME, Cisneros-Franco JM, de Villers-Sidani É. Dynamic brains and the changing rules of neuroplasticity: Implications for learning and recovery. Front Psychol 2017;**8**:1–11.

[257] Voss TC, Hager GL. Dynamic regulation of transcriptional states by chromatin and transcription factors. Nat Rev Genet 2014;**15**:69–81.

[258] Wallenstein S, Heidrich d G, Kaiko R, Houde R. Clinical evaluation of mild analgesics: the measurement of clinical pain. Br J Clin Pharmacol 1980;**10**:319S-327S.

[259] Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. J Cell Physiol 2016;**231**:25–30.

[260] Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res 2010;**38**:7248–59.

[261] Wang L, Wu Q, Li Z, Sun S, Yuan J, Li J, Zhang Y, Yu D, Wang C, Sun S. Delta/notch-like epidermal growth factor-related receptor promotes stemness to facilitate breast cancer progression. Cell Signal 2019;**63**:109389.

[262] Wang L, Wu Q, Zhu S, Li Z, Yuan J, Yu D, Xu Z, Li J, Sun S, Wang C. Delta/notch-like epidermal growth factor-related receptor (DNER) orchestrates stemness and cancer progression in prostate cancer. Am J Transl Res 2017;**9**:5031–5039.

[263] Wang N, Liu W, Tan T, Dong C-Q, Lin D-Y, Zhao J, Yu C, Luo X-J. Notch signaling negatively regulates BMP9-induced osteogenic differentiation of mesenchymal progenitor cells by inhibiting JunB expression. Oncotarget 2017;**8**:109661–74.

[264] Wenham CYJ, Conaghan PG. The role of synovitis in osteoarthritis. n.d.

[265] Wilder-Smith CH, Hill L, Spargo K, Kalla A. Treatment of severe pain from osteoarthritis with slow-release tramadol or dihydrocodeine in combination with NSAID's: A randomised study comparing analgesia, antinociception and gastrointestinal effects. Pain 2001;**91**:23–31.

[266] Williams DE, de Vries P, Namen AE, Widmer MB, Lyman SD. The Steel factor. Dev Biol 1992;**151**:368–376.

[267] Wu CT, Morris JR. Genes, genetics, and epigenetics: A correspondence. Science (80-) 2001;**293**:1103–1105.

[268] Wu Q, Meng WY, Jie Y, Zhao H. LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/HMGB1 axis. J Cell Physiol 2018;**233**:6750–6757.

[269] Xia C, Braunstein Z, Toomey AC, Zhong J, Rao X. S100 proteins as an important regulator of macrophage inflammation. Front Immunol 2018;**8**:1.

[270] Xing D, Liang J qian, Li Y, Lu J, Jia H bo, Xu L yan, Ma X long. Identification of Long Noncoding RNA Associated with Osteoarthritis in Humans. Orthop Surg 2014;**6**:288–293.

[271] Xu H, Okamoto A, Ichikawa J, Ando T, Tasaka K, Masuyama K, Ogawa H, Yagita H, Okumura K, Nakao A. TWEAK/Fn14 interaction stimulates human bronchial epithelial cells to produce IL-8 and GM-CSF. Biochem Biophys Res Commun 2004;**318**:422–427.

[272] Y. Benjamini and Y. Hochberg. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing. J R Stat Soc 1995;**57**:289–300.

[273] Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, Yasunaga Y, Asahara H, Ochi M. Expression of microRNA-146a in osteoarthritis cartilage. Arthritis Rheum 2009;**60**:1035–41.

[274] Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, Quan Y, Dong S. Effect of microRNA-145 on IL-1β-induced cartilage degradation in human chondrocytes. FEBS Lett 2014;**588**:2344–52.

[275] Yang J-H, Li J-H, Shao P, Zhou H, Chen Y-Q, Qu L-H. starBase: a database for exploring microRNA-mRNA interaction maps from Argonaute CLIP-Seq and Degradome-Seq data. Nucleic Acids Res 2011;**39**:D202-9.

[276] Ye E-A, Steinle JJ. miR-146a Attenuates Inflammatory Pathways Mediated by TLR4/NF-κB and TNFα to Protect Primary Human Retinal Microvascular Endothelial Cells Grown in High Glucose. Mediators Inflamm 2016;**2016**:1–9.

[277] Yu J, Wu Y, Yang P. High glucose-induced oxidative stress represses sirtuin deacetylase expression and increases histone acetylation leading to neural tube defects. J Neurochem 2016;**137**:371–383.

[278] Yu W, Zhao G, Cao R, Zhu Z, Li K. LncRNA NONRATT021972 Was Associated with Neuropathic Pain Scoring in Patients with Type 2 Diabetes. 2017;**2017**.

[279] Yuan F, Xu ZM, Lu LY, Nie H, Ding J, Ying WH, Tian HL. SIRT2 inhibition

exacerbates neuroinflammation and blood-brain barrier disruption in experimental traumatic brain injury by enhancing NF-κB p65 acetylation and activation. J Neurochem 2016;**136**:581–593.

[280] Zeng L, Fagotto F, Zhang T, Hsu W, Vasicek TJ, Perry WL, Lee JJ, Tilghman SM, Gumbiner BM, Costantini F. The Mouse FusedLocus Encodes Axin, an Inhibitor of the Wnt Signaling Pathway That Regulates Embryonic Axis Formation. Cell 1997;**90**:181–192.

[281] Zentner GE, Henikoff S. Regulation of nucleosome dynamics by histone modifications. Nat Struct Mol Biol 2013;**20**:259–266.

[282] Zhang Q, Zhang B, Sun L, Yan Q, Zhang Y, Zhang Z, Su Y, Wang C. MicroRNA-130b targets PTEN to induce resistance to cisplatin in lung cancer cells by activating Wnt/β-catenin pathway. Cell Biochem Funct 2018;**36**:194–202.

[283] Zhang X, Wang C, Zhao J, Xu J, Geng Y, Dai L, Huang Y, Fu SC, Dai K, Zhang X. MiR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. Cell Death Dis 2017;**8**:e2734.

[284] Zhang Y, Chi D. Overexpression of SIRT2 Alleviates Neuropathic Pain and Neuroinflammation Through Deacetylation of Transcription Factor Nuclear Factor-Kappa B. Inflammation 2018;**41**:569–578.

[285] Zhang Y, Wang F, Chen G, He R, Yang L. LncRNA MALAT1 promotes osteoarthritis by modulating miR-150-5p/AKT3 axis. Cell Biosci 2019;**9**:54.

[286] Zhao J, Lee M-C, Momin A, Cendan C-M, Shepherd ST, Baker MD, Asante C, Bee L, Bethry A, Perkins JR, Nassar MA, Abrahamsen B, Dickenson A, Cobb BS, Merkenschlager M, Wood JN. Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. J Neurosci 2010;**30**:10860–71.

[287] Zhao X, Tang Z, Zhang H, Atianjoh FE, Zhao J-Y, Liang L, Wang W, Guan X, Kao S-C, Tiwari V, Gao Y-J, Hoffman PN, Cui H, Li M, Dong X, Tao Y-X. A long noncoding RNA contributes to neuropathic pain by silencing Kcna2 in primary afferent neurons. Nat Neurosci 2013;**16**:1024–1031.

[288] Zheng X, Chen F, Zhang Q, Liu Y, You P, Sun S, Lin J, Chen N. Salivary exosomal PSMA7: a promising biomarker of inflammatory bowel disease. Protein Cell 2017;**8**:686–695.

[289] Zhou Q, Tang X, Tian X, Tian J, Zhang Y, Ma J, Xu H, Wang S. LncRNA MALAT1 negatively regulates MDSCs in patients with lung cancer. J Cancer 2018;**9**:2436–2442.

[290] Zhou Y, Shu B, Xie R, Huang J, Zheng L, Zhou X, Xiao G, Zhao L, Chen D. Deletion of Axin1 in condylar chondrocytes leads to osteoarthritis-like phenotype in temporomandibular joint via activation of β-catenin and FGF signaling. J Cell Physiol 2018;**234**:1720–1729.

[291] Zorina-Lichtenwalter K, Meloto CB, Khoury S, Diatchenko L. Genetic predictors of human chronic pain conditions. Neuroscience 2016;**338**:36–62.

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