
The first international workshop on the epigenetics of osteoarthritis. 

Connective Tissue Research (2016)

DOI: http://dx.doi.org/10.3109/03008207.2016.1168409

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DOI link to article:

http://dx.doi.org/10.3109/03008207.2016.1168409

Date deposited:

03/05/2016

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To cite this article: Ingrid M. Meulenbelt, Nidhi Bhutani, Wouter den Hollander, Steffen Gay, Udo Oppermann, Louise N. Reynard, Andrew J. Skelton, David A. Young, Frank Beier & John Loughlin (2016): The first international workshop on the epigenetics of osteoarthritis, Connective Tissue Research, DOI: 10.3109/03008207.2016.1168409

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Accepted author version posted online: 30 Mar 2016.

Published online: 30 Mar 2016.

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The first international workshop on the epigenetics of osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a major clinical problem across the world, in part due to the lack of disease-modifying drugs resulting, to a significant degree, from our incomplete understanding of the underlying molecular mechanisms of the disease. Emerging evidence points to a role of epigenetics in the pathogenesis of OA, but research in this area is still in its early stages. In order to summarize current knowledge and to facilitate the potential coordination of future research activities, the first international workshop on the epigenetics of OA was held in Amsterdam in October 2015. Recent findings on DNA methylation and hydroxymethylation, histone modifications, noncoding RNAs, and other epigenetic mechanisms were presented and discussed. The workshop demonstrated the advantage of bringing together those working in this nascent field and highlights from the event are summarized in this report in the form of summaries from invited speakers and organizers.

Osteoarthritis

Osteoarthritis (OA) is a common, late-onset disease characterized by the loss of the articular cartilage of synovial joints. It principally affects the hands, the hips, and the knees (1). The disease is present throughout the world and demonstra...
gene-based studies and the hypothesis-free genomewide approach, testing hundreds of thousands of single-nucleotide polymorphisms (SNPs). So far up to 16 replicated OA susceptibility loci have been detected at or around the genome-wide significance threshold of \( p < 5 \times 10^{-8} \) (6,8). Functional studies using cell lines and patient tissues have revealed that effects on gene expression appear to be a common mechanism by which risk-conferring alleles mediate their OA susceptibility; clear examples are the OA-associated SNPs rs143383, rs225014, and rs3204689 and their correlation with expression changes at the genes GDF5, DIO2, and ALDH1A2, respectively (9–12).

### Epigenetics

Epigenetics refers to a heritable change in gene expression that occurs without a change to the underlying DNA sequence. There are three known mechanisms of epigenetic gene regulation: DNA methylation, histone modifications, and noncoding regulatory RNAs. The first two mechanisms regulate gene expression by modulating gene transcription, whereas regulatory RNAs act post-transcriptionally (13). Epigenetic patterns are both plastic, especially during development and cell differentiation when they undergo dynamic changes, and stable, allowing cellular identity to be maintained during mitotic cell division. Epigenetics provides the cell with a mechanism to respond to its environment at the cellular, tissue, and organismal level.

### Epigenetics and OA

During the last few years, there have been a number of studies undertaken into the role of epigenetics in OA etiology. These studies started with an investigation of the epigenetic regulation of the expression of genes coding for proteins that have a known central role in the homeostasis of joint tissue, and in particular cartilage (14). There have also been direct investigations of the differences in expression levels of regulatory RNAs between OA and non-OA cells, the aim being to identify key RNAs that could initiate or exacerbate disease development (15,16). The advent of high-throughput CpG DNA methylation arrays has enabled investigators to directly compare, at a genome-wide level, OA and non-OA methylomes and to compare methylation status between DNA from different sites within the joint and between different joints (17–22). These arrays have also been used to assess whether there are direct links between OA genetic risk loci and epigenetic status, and between gene expression and epigenetics (23,24).

### The first international workshop on the epigenetics of OA

The OA epigenetics field is however still quite small and, like many nascent research areas, lessons are still being learnt regarding data analysis and interpretation, and also the sample sizes needed to draw robust conclusions. In response to this ongoing epigenetics research and the likelihood that such research will play an increasing role in our understanding of this complex disease, a workshop was organized at the Dutch Royal Academy of Science in Amsterdam on the 20th and 21st of October 2015. The aims of the workshop were to discuss the current status of OA epigenetics research, to provide overviews of particular activities, to provide guidance on particular techniques (the analysis of DNA methylation arrays and of RNA sequencing data), and to offer a flavor of the status of epigenetics research in another arthritic disease, rheumatoid arthritis (RA). The workshop had 3 organizers (Frank Beier, John Loughlin, and Ingrid Meulenbelt), 8 invited speakers, and 12 oral abstract presentations selected from those who registered. In total, 60 scientists from 14 countries and 4 continents gathered to present and to discuss their OA epigenetics research. Reumafonds (http://www.reumafonds.nl/) and the Osteoarthritis Research Society International (http://oarsi.org/) provided financial support for the workshop.

This article provides summaries of the presentations from seven of the invited speakers (Nidhi Bhutani, Louise Reynard, Andrew Skelton, Udo Oppermann, David Young, Wouter den Hollander, and Steffen Gay) and from one of the organizers (Ingrid Meulenbelt), capturing their key points. Frank Beier also presents a discussion on higher-order nuclear organization and OA. The article ends with a look to the near future of how epigenetics will enhance our understanding of the causes of this most common of joint diseases.

### Stable cytosine modifications in the OA epigenome: new kids on the block

DNA methylation on the base cytosine [5-methylcytosine (5mC)] was long-thought to be a stable epigenetic mark leading to gene silencing (25). Recent studies have however revealed that DNA methylation is both dynamic and reversible and have identified the key enzymes and pathways mediating DNA demethylation (reviewed in 26). Nidhi Bhutani of Stanford University presented her groups’ investigations into DNA hydroxymethylation on the cytosine (5hmC), which has been
discovered to be a stable cytosine modification alongside 5mC and which is now known to be an independent epigenetic mark (26,27). The enzyme family that converts 5mC to 5hmC is the ten-eleven-translocation (TET) family of proteins consisting of three known members: TET1, 2, and 3 (27,28). These TET proteins are also capable of further oxidizing 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (29), intermediates that are acted upon by base excision repair enzymes like thymine DNA glycosylase, leading to replacement by an unmodified cytosine and hence active DNA demethylation (30,31). The precise mechanisms leading to a stable 5hmC generation or 5caC generation and subsequent demethylation are however not yet clear.

Over the years, evidence has been accumulating for a critical role for DNA methylation and demethylation in OA pathogenesis (32). Alterations in DNA methylation patterns have been observed in OA chondrocytes, particularly a loss of DNA methylation at the promoters and the concurrent “unsilencing” of various OA-associated genes including MMP3, 9, and 13, ADAMTS4, IL-1β, and iNOS (14,33,34). Recently, Professor Bhutani’s team reported a remarkable dysregulation of 5hmC homeostasis in patients with OA, with increases in global 5hmC levels observed in OA chondrocytes when compared to normal chondrocytes (35). 5hmC gain at specific sites in the promoters of key OA genes was associated with increased gene expression. Genome-wide 5hmC distribution in normal and OA chondrocytes identified a total of 70,591 differentially hydroxymethylated regions (DhMRs); 44,288 were increased in the OA chondrocytes as compared to the normal chondrocytes and 26,303 were decreased (36). Analysis of the genomic locations of these DhMRs revealed that the majority of 5hmC gain in OA occurs in gene bodies. Upon assessing the 5hmC distribution in the activated or repressed genes, it was observed that activated genes demonstrated elevated 5hmC in both the promoter regions and throughout the gene body in OA chondrocytes in comparison to normal chondrocytes, whereas the repressed genes exhibited no significant changes (36). In light of these observations, further studies are required on the distribution of these newly identified cytosine modifications and their effects on the OA epigenome and transcriptome.

A major caveat in widely prevalent techniques to assess DNA methylation, such as bisulfite conversion, is the failure to distinguish between 5mC and 5hmC (37). In light of the recent observations regarding a genome-wide role for 5hmC alterations in OA chondrocytes, it is imperative to utilize recently developed chemical tools that can distinguish between 5mC and 5hmC as well as between the less stable 5fC, 5caC, and unmethylated cytosines (38). These tools consist of (a) antibody-based enrichment using specific anti-5hmC, -5mC, -5fC, and 5caC antibodies and (b) affinity-based enrichment after specific modifications of 5hmC (39,40), followed by high-throughput sequencing. In addition, methods have been developed that can be used in conjunction with bisulfite sequencing for a base resolution mapping of 5hmC (TET-assisted bisulfite sequencing (41)), 5fC (5fC chemical-modification assisted bisulfite sequencing (41)), and 5caC (5caC chemical-modification-assisted bisulfite sequencing (42)). Utilizing these tools, it is possible to study precise genome-wide distribution of these cytosine modifications in OA joint cells in order to understand how these stable cytosine modifications and DNA methylation and demethylation dynamics influence the gene expression changes observed in OA and other rheumatic diseases.

Candidate gene analysis of DNA methylation in OA

The application of measuring DNA methylation content of CpGs sites at targeted genes was covered by Ingrid Meulenbelt of the Leiden University Medical Center. Targeted methylation of CpG sites has been studied in the context of OA susceptibility genes, mainly to explore whether the risk-conferring alleles that were found to influence in cis gene expression act via modulation of DNA methylation of transcriptionally active CpG sites that are close to or that are themselves SNPs (43,44). Such targeted analysis of methylation levels at positional CpG sites is typically performed by subjecting bisulfite converted DNA to MALDI-TOF mass spectrometry (Epityper, Sequenom; (45)) or to pyrosequencing (46). Epityper is suitable for high-throughput analysis, but cannot always capture every CpG of interest, whereas pyrosequencing is not suitable for high-throughput analysis but quantification of methylation at any CpG site is possible.

By applying Epityper analyses of 20 CpG sites that mapped across the DIO2 locus, it was shown that methylation at a particular CpG site 2 kb upstream of the promoter enhanced DIO2 expression, particularly in carriers of the OA risk C allele of the rs225014 T>C SNP (43). Since this CpG maps within an active CCCTC-transcription factor (CTCF) binding site, methylation-dependent binding of this CTCF is suggested to act as a positional isolator of DIO2 expression (43). Additionally, by applying pyrosequencing analyses of methylation at a CpG site located in the 5’ untranslated region of GDF5, it was shown that the OA risk T
allele of the rs143383 C>T SNP, which had previously been shown to correlate with GDF5 expression in articular cartilage (10), disrupts a functional CpG dinucleotide (44). Furthermore, the reported allele-specific expression (ASE) of GDF5 in heterozygous carriers marked by the rs143383 alleles is affected by the extent of methylation of the respective CpG (44). Together, these studies demonstrated OA risk alleles that confer susceptibility to OA via changing the epigenetically modulated gene expression. Nonetheless, genome-wide analysis of the transcriptome and methylome of articular cartilage revealed that only 24% of the differentially methylated CpG sites correlated to proximal in cis gene expression and as such could be considered transcriptionally active in articular cartilage (19,24). Moreover, it was shown that for 31 and 26 OA cartilage-relevant genes, methylation and expression, respectively, are additionally affected by genetic variation proximal to these genes, which may further modify the OA pathophysiology (19,24).

**Methylation microarrays: where to start**

Genome-wide studies of the human methylome have been assisted by microarray technology for a number of years, with the principal array provider being Illumina with their HumanMethylation series of BeadChips. The array platform has grown from 27,578 CpG dinucleotide probes (the 27K chip) covering over 14,000 genes to 485,000 CpG probes (the 450K chip) covering 99% of the Reference Sequence genes. It is very important to consider the experimental design before conducting a methylation array, and in this regard Louise Reynard and Andrew Skelton of Newcastle University offered guidance in their presentation. There are biases between chips and there are positional effects within a chip, and so even before data have been generated, samples should be randomized within and across chips to allow for correction of technical variation. A phenotype table should also be created, which includes all relevant sample information (such as gender, cell type, and any treatment regimen); this table will aid downstream data analysis. A flow chart listing the main steps in processing data generated using the Illumina methylation arrays is shown in Figure 1. The raw data generated by the Illumina scanners come in a standard binary format called IDAT files, and there are many packages available for analyzing these files in Bioconductor (http://www.bioconductor.org/), an open-source repository of bioinformatics software packages that includes Minfi, CHAMP, WateRmelon, and methylumi (http://doi.org/10.1093/bioinformatics/btu049, http://doi.org/10.1093/bioinformatics/btt684, http://bioconductor.org/packages/release/bioc/html/wateRmelon.html, http://bioconductor.org/packages/2.8/bioc/html/methylumi.html). Once the IDAT file is inputted, all packages will recommend a normalization procedure; this is a mathematical process to make samples comparable to one another and is crucial. Probe filtering is an essential step prior to downstream data analysis. Each probe on the array has a detection p value based on how confident the intensity call is, and probes with p values greater than 0.01 should be considered for removal. Additional probe filtering, for example, removal of sex chromosome probes or those containing SNPs, should be considered based on the experimental design. Leaving in probes containing SNPs with a high minor allele frequency can lead to data biases, whilst gender is often an overwhelming source of variation in methylation studies. Quality control (QC) should be carried out on all samples to ensure that the data generated are as expected; the Minfi QC Report tool, or the MethylAid (http://bioinformatics.oxfordjournals.org/content/30/23/3435) package, will allow visualization of potential biases in the experiment, such as bisulphite conversion and chip hybridization. Additional tools, such as principle component analysis, are very good at identifying known technical variation. If samples are removed for QC reasons, then the normalization and probe filtering steps must be repeated.

There are two types of metric in methylation microarray data, beta values, and M values. Beta values are between 0 and 1, and are equated to percentage methylation. M values are the log ratio of methylation against unmethylation intensities. Whilst beta values are useful for graphical representation and interpretation of the
Histone modifications are critical elements of the epigenomic landscape: using chemical tools to interrogate epigenetic biology

The importance of local chromatin structure for controlling gene expression was the focus of the presentation by Udo Oppermann of the University of Oxford. Accessible chromatin, such as that found in euchromatin, provides the environment for gene-regulatory proteins such as transcription factors or remodeling complexes to interact with their cognate binding sites within the regulatory regions of genes, such as proximal promoters, enhancers, or silencers (48). For example, trimethylated lysine 9 of histone 3 (H3K9me3) indicates heterochromatic or repetitive regions, whereas H3K4me3 marks regulatory elements associated with active promoters or transcription start sites and H3K27me3 marks those for developmentally repressed genes. Modification systems (so-called writers and erasers of chromatin marks) that covalently alter specific residues of chromatin proteins play a pivotal role in this process and generate a so-called “histone code” (49) that recruits specifically recognition domains and components (readers) of transcriptional complexes, which thus serve as the effectors of the modification. In this complex and interdependent manner, chromatin-modifying systems exert control of global and local gene activation. Importantly, histone modifications and DNA methylation act in concert with respect to gene regulation because both activities are functionally linked (50).

At present, several classes of histone modifications and their respective enzymatic modification systems have been identified (51–53). Amongst their epigenetic substrate marks, lysine and arginine modifications are probably the best studied: acetylation and methylation of lysine residues, as well as methylation of arginine. Whereas acetylation of histone tails is correlated with gene activation, the influence of histone methylation on regulating gene transcription depends on the exact residue methylated and the number of added methyl groups, both for arginine and lysine residues. The involvement of histone modifications in the regulation of key aspects of the musculoskeletal system, for example, in inflammation or differentiation, has recently been described (54). The best understood systems of histone modifications that potentially allow transmission of stable heritable marks through cell divisions comprise methylation of H3K9 (HP1, heterochromatin establishment), and of H3K27 and H3K4 (repression and activation of genes through polycomb and trithorax complexes, respectively).

A chemical probe is a reagent that perturbs specifically a protein’s function and allows the user to ask mechanistic and phenotypic questions about its molecular target in biochemical, cell-based or animal studies (55). Chemical probes are impactful and complementary to genetic approaches, such as CRISPR/Cas and RNA interference (RNAi). They can rapidly and reversibly inhibit a protein function in cells or animals. When coupled with RNAi, they can distinguish between effects due to scaffolding and effects due to inhibition of catalytic or protein-interaction activity. These features of chemical probes are relevant for translational studies and target validation efforts. Such chemical tools are currently being developed for epigenetic biology by Professor Oppermann and his colleagues at the Structural Genomics Consortium (http://www.thesgc.org/scientists/groups/oxford), an international public–private partnership that promotes open-access drug discovery and target validation.

Higher-order nuclear organization and OA

Another emerging area in epigenetic regulation of gene expression is the three-dimensional organization of chromatin in the nucleus, which was described by Frank Beier of the University of Western Ontario. It is now well accepted that chromosomes and chromatin are not randomly distributed in the nucleus, but...
organized in a highly sophisticated manner that is closely linked to transcriptional activity (56). Such higher-order organization includes the folding of large genomic regions, chromatin looping that connects enhancers and promoters, and interactions of chromosomes with the nuclear periphery (e.g., the lamina). These interactions result in the formation of a number of domains such as active chromatin hubs, topologically associating domains (TADs), and lamin-associated domains (56,57). A number of key proteins mediating these interactions have been identified, most notably the previously mentioned factor CTCF and the cohesin complex (58). The function of these proteins in cartilage, other joint tissues, or OA has not been addressed, although the association of a CTCF binding site with the before-mentioned DIO2 SNP (43) points toward important roles.

Moreover, studies on developmental defects in humans and mice suggest essential functions of CTCF and its partners in the skeleton. For example, limb-specific knockout mice for Ctf completely lack forelimbs while their hindlimbs are severely shortened (59). Similarly, disruption of CTCF-associated TADs in mice and in human cells has been linked to limb malformations (60). Finally, mutations in genes encoding cohesin components give rise to limb malformations (in addition to other defects) in both human patients and animal models (61). Given the close links between skeletal development and OA pathogenesis (62), it is therefore likely that CTCF and cohesin proteins are also essential for normal cartilage and joint homeostasis in the adult. Nevertheless, specific functions of these proteins and three-dimensional chromatin organization in the adult joint and in OA have not been reported. Similarly, specific approaches that have been developed to examine three-dimensional chromatin interactions, such as chromatin conformation capture (3C) and its derivatives (4C, 5C, HiC, etc.) as well as ChIA-PET (63), have yet to be applied to adult cartilage and joint tissues.

Regulatory RNAs and OA

The completion of the human genome project revealed a surprising dearth of protein-coding genes, circa 20,000. However, with the advent of massively parallel sequencing technologies the complexity of the transcriptome made by the genome is becoming apparent. The transcriptome now includes 2,588 microRNAs (small, 22–23 nucleotide long, post-transcriptional regulators of gene expression), an abundance of other small RNAs (SnoRNAs, Y RNAs, and Pi-RNAs), and long noncoding RNAs (lncRNAs), for which over 200,000 isoforms are predicted to exist (64). The role of such regulatory RNAs in OA was the focus of the presentation by David Young of Newcastle University.

In terms of microRNAs, miR-140 still represents the most abundant, cartilage-specific, small RNA. Deletion of miR-140 in mice causes a shorter skeleton and early-onset OA (65). MicroRNAs are transcribed as a stem-loop transcript with a -5p and -3p microRNA, either or both of which can be functional after processing (13). Because the miR-140 null mouse lacks both miR-140-5p and miR-140-3p, it is unclear which is most active in cartilage. Both are highly induced in a model of mesenchymal stem cell (MSC) differentiation to chondrocytes; however, analysis of the regulated transcriptome leads to the prediction that miR-140-5p is the dominant microRNA since the transcripts that are most downregulated are significantly enriched for predicted miR-140-5p targets (66). In fact, other genes that are also downregulated, but less robustly so, are actually predicted targets of a previously undefined variant, or isomer, of miR-140-3p. This leads to the hypothesis that miR140-5p is a major factor in chondrogenesis, while the isomer of miR-140-3p fine-tunes the process.

For lncRNAs, RNA sequencing (RNA-seq) analysis of cartilage and MSC chondrogenesis has identified several thousand transcripts, many novel and/or differentially expressed between normal and OA cartilage. One such novel, cartilage-specific, noncoding RNA, termed SOX9nc2, is located upstream in the genome of the important chondrocyte transcription factor SOX9. Depletion of the SOX9nc2 transcript, by RNAi, prevents chondrogenesis and concomitant induction of SOX9 expression. Stem cell chondrogenesis models often require TGFβ and SOX9nc2 appears to regulate the activity of TGFβ, possibly through regulating the levels of TGFβ-receptor expression. There are numerous mechanisms as to how lncRNAs exert their affects (67,68) and still this requires exploring for SOX9nc2.

Overall, though chondrocytes as a cell type have largely been overlooked by large sequencing consortia, data show they express a wealth of noncoding transcripts (69), many of which appear novel. Future work will be needed to investigate the function of many of these in vitro and in vivo.

RNA sequencing

Gene expression microarrays have been utilized in OA research for a number of years, having yielded valuable information on transcriptome wide gene expression measurements of OA affected tissues such as articular cartilage (70–74), synovium (75–77), and subchondral...
bone (78, 79). The accompanying bioinformatic challenges hereof have now been addressed and multiple easy-to-use software solutions are currently apparent to aid in silico experiments for research groups that have traditionally no background in computational biology (80, 81). Presently, transcriptome-wide gene expression measurements are increasingly being performed using sequencing efforts, specifically RNA-seq, as opposed to microarray-based experiments. RNA-seq was therefore the focus of the presentation by Wouter den Hollander of Leiden University Medical Center. RNA-seq offers increased sensitivity (82), as well as more comprehensive analyses of mechanisms such as alternative splicing and ASE (83); however, as such it also requires less convenient bioinformatic tools and might thus be less accessible for researchers without the computational know-how. Library preparation, sequencing protocol and depth, read alignment, and the statistical estimation of gene expression levels substantially influence the outcome of the respective experiment. While most bioinformatic practices that involve handling sequencing data are compared against one another somewhat regularly (84–86), there is no golden rule on when to implement what solution. Consequently, proper QC is key to understanding whether a methodological approach serves the scientific question posed. A frequently used QC tool for sequencing experiments is FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/), which aims to provide an easily accessible method to inspect raw, but also processed sequencing data. It generates, in a modular way, web reports for a number of statistics such as, but not limited to, per base sequence quality, nucleotide content, sequence length distribution, and k-mer content. Generally, upon visual inspection of FastQC reports, sequencing data need to be processed so that unique sample adapters and low-quality base calls among reads are removed prior to alignment. Again, multiple tools are available that achieve these objectives (e.g., cutadapt and sickle) and it should thus ideally be tested what tool performs best in a given experimental design (e.g., single-end sequencing compared to paired-end sequencing). When one is certain that the reads are of ample quality to continue, an aligner must be chosen to explore what regions of the genome are found to what extent in the mRNA pool, reflecting gene expression levels. As opposed to aligning DNA sequencing data that can theoretically cover regions across the entire genome, RNA-seq data inherently only consist of exon reads. As a consequence of that, read aligners must be aware of the fact that a substantial number of reads from an RNA-seq experiment might consist of multiple exons, which are by definition not directly adjacent to one another on the genome. As RNA-seq is becoming more common in transcriptome studies, currently multiple aligners support alignment of so-called split reads (e.g., BWA (87), TopHat2 (88), GSNAP (89), and Bowtie2 (90)), but differ in the underlying method. Aside from the aligner used, it is important that a proper reference genome to align against must be chosen. Whereas general reference genomes like hg19 or hg38 serve their purpose in RNA-seq experiments to estimate gene expression levels, when one is interested in alternative splicing and/or ASE it should be noted that genetic variation, and likely RNA editing even more so, considerably influences whether reads will be aligned properly and must thus be addressed (91).

**Epigenetics of RA**

Based on the previous observation from his laboratory that synovial fibroblasts (SFs) from patients with RA can invade into cartilage without the stimulating influence of immune cells and proinflammatory cytokines (92), Steffen Gay of University Hospital Zurich tasked his group with searching for the causes of this aggressive behavior.

Since his group and others could demonstrate that synovial fibroblasts in RA (RASF) are not only effector cells responding to pro-inflammatory cytokines, but act endogenously when activated, they searched for the epigenetic modifications leading to the activated phenotype of these RASF; this search could also identify novel targets (93). As early as the year 2000, they had discovered that RASF are hypomethylated and thereby endogenously activated through the upregulation of matrix-degrading enzymes and potent chemokines (94). This led his laboratory to focus on epigenetics (95) including histone acetylation, methylation, sumoylation, and noncoding RNAs. This research was and still is supported by a number of European Commission grants with closely collaborating centers throughout Europe, including the consortia Autocure, Masterswitch, Osteoimmune, TEAM, and BeTheCure.

His group’s most recent research has focused on the analysis of methylation of novel transcription factor genes. Thereby they have shown that the promoter of TBX5 is hypomethylated in RASF and induces the activation of these cells to produce proinflammatory cytokines and chemokines including IL8, CXCL12, and CCL20 (96). His colleague Michel Neidhart has further analyzed polyamine metabolism in RASF and developed a novel therapeutic strategy to inhibit one of the key enzymes regulating methylation (97). Since RASF are modified in their state of acetylation through the expression of specific bromodomains
(acetyl lysine binding domains), another colleague of Professor Gay, Kerstin Klein, has studied these bromo-domains, which are involved in chromatin remodeling and transcriptional activation. She has discovered that BRD2-4 could be inhibited with BET-inhibitors, which are currently of interest to a number of global pharmaceutical companies (98).

A detailed analysis by two further colleagues of Professor Gay, Caroline Ospelt and Mojca Frank-Bertoncelj, of microRNAs and lncRNAs revealed differences in the transcriptome of SFs that is dictated by joint-specific positional differences in the origin of the cells. This could be a contributing factor to RA and was presented as a plenary lecture at the 2014 annual meeting of the American College of Rheumatology (99). A complete analysis of microRNA profiling in RASF versus normal SFs has been undertaken by his group and the role of specific microRNAs further identified. For example, miR-155 is expressed at a higher level in monocytes than in RASF (100) and has a pivotal role in collagen-induced arthritis (101). Moreover, his group demonstrated that miR-203 is regulating IL-6 (102) and that miR-18a enhances not only the IL-6-mediated production of the acute-phase proteins fibrinogen and haptoglobin in human hepatocytes (103), but also, when induced by TNFa, activates RASF through a feedback loop in NFKB signaling (104). His group has also shown that the targeting of microRNAs by antago-miRs can be highly effective (105) with, for example, miR-20a inhibited by an antago-miR for the treatment of pulmonary hypertension (106). Last but not least, his group has also shown that miR-323 regulates TIMP3 and thereby inhibits the activation of the highly potent proinflammatory cytokines TNFa and IL-6, providing a novel strategy to inhibit both the cytokine-dependent and the cytokine-independent pathways of RA.

Conclusions and perspectives

As shown in numerous talks at the workshop, there is very strong evidence for a key role of epigenetic mechanisms in the pathogenesis of OA. This raises hopes that drugs targeted at specific epigenetic regulators (many of which are currently under development for the treatment of cancer and other diseases) might be useful in the treatment of OA. However, research in this area is still at an early stage, with mechanistic links between specific epigenetic changes and disease progression still unclear in many cases. As was observed in the analysis of OA genetics, the stratification of OA phenotypes by, for example, severity of disease, site of disease, and the involvement of other clinical parameters may aid the identification of epigenetic markers. An additional lesson from the genetic studies is the need to study large sample sizes, although the tens of thousands needed in GWAS are unlikely to be necessary for epigenetic studies, with several hundred well-phenotyped samples being a more realistic target number at this stage. Although the majority of OA epigenetics research has been cartilage-centric, other relevant joint tissues are being investigated (107) and this joint-wide approach will be of great value in elucidating both tissue-specific and joint-wide epigenetic effects. With regard to pain, which is the number one complaint of OA patients, epigenetic changes in the nervous system should also be considered, especially since the chronic pain in these patients suggests an epigenetic component.

Whilst a tremendous amount therefore remains to be done, the workshop did highlight a number of advances and was a great success in bringing scientists and trainees with interests in this area together to discuss future plans. The requirement to share resources, knowledge, and expertise was a strikingly clear conclusion and has already led to the very tangible outcome of the OA methylome database that was discussed earlier. It is hoped that this OA epigenics workshop will be the first of many; a desire for future workshops will be a mark of advancement in this important OA research field.

Acknowledgments

We would like to thank all of those who made this workshop possible, in particular the abstract presenters and all other delegates who ensured that we had a scientifically stimulating meeting.

Declaration of interest

We thank Reumafonds (http://www.reumafonds.nl/) and the Osteoarthritis Research Society International (http://oarsi.org/) for their generous encouragement and their financial support of speaker expenses. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References


81. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy-