

Meeting report

Report on the Molecular Approaches to Osteoarthritis Symposium, Imperial College London, UK, 18–20 April 2004

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The Kennedy Institute of Rheumatology Symposium 'Molecular Approaches to Osteoarthritis' was held at Imperial College London on 18–20 April 2004. It encompassed current research into the molecular processes in cartilage underlying the initiation and progression of osteoarthritis (OA). The symposium also examined the potential impact on the field of new methodologies such as transcriptional profiling and proteomics, and the scope for gaining insights from genetic analysis of OA and from studying mutations that affect cartilage structure.

The meeting opened with presentations on the clinical problem. Stefan Lohmander (Lund, Sweden) stressed the heterogeneity of the disease and the difficulty of predicting its progression. There is often disparity between the X-ray appearance and symptoms, and a lack of satisfactory markers for monitoring progression or responses to therapy. Age, obesity, malalignment and previous trauma to the joint are all risk factors and interact additively. Marc Hochberg (Baltimore, MD, USA), after emphasising the huge socioeconomic burden of OA, focused on risk and progression factors in more detail. The body mass index is more strongly associated with knee OA than with hip OA, and a large case-controlled study of hip OA revealed that pain was a strong indicator of progression. Therapy for OA is symptomatic and supportive, and badly damaged joints are replaced with prostheses. Cartilage loss is thought to be due, at least in part, to an excess of proteolytic enzymes destroying the collagen and proteoglycan.

A novel therapeutic approach was described by Ken Brandt (Indianapolis, IN, USA), who found that the antibiotic doxycycline, an inhibitor of the matrix metalloproteinases (MMPs), slowed joint space narrowing in

knees with established OA – although, puzzlingly, it did not stop the onset of the disease in the contralateral knees that were used as controls. Steve Abramson (New York, USA) has investigated changes in gene expression in OA by transcriptional profiling. This showed upregulation of inflammatory cytokines and chemokines in cartilage, synovium and peripheral blood mononuclear leukocytes of OA patients. Abramson suggested this was an 'IL-1 signature' and that the leukocytes may become activated by trafficking through affected joints.

The second session of the meeting was concerned with chondrogenesis and the control of chondrocyte metabolism and death. Benoit de Crombrughe (Houston, TX, USA) described his elegant work on the role of SOX transcription factors in chondrocyte differentiation from mesenchymal precursors. SOX9 is needed for mesenchymal condensation and for expression of SOX5 and SOX6, which is then followed by differentiation to chondrocytes. Overexpression of SOX9 profoundly inhibited chondrocyte proliferation, resulting in dwarfism and achondroplasia. SOX9 binds β -catenin, preventing its interaction with ternary complex factor. The inhibition of proliferation is probably due to inhibition of cyclin D₁ expression. SOX9 expression is needed for maintenance of the phenotype of mature chondrocytes and its expression is inhibited by inflammatory cytokines IL-1 and tumour necrosis factor (TNF) alpha.

IL-1 and TNF alpha are well known to inhibit synthesis of type II collagen and proteoglycan by chondrocytes. Mary Goldring (Boston, MA, USA) has shown that the inhibition of expression of type II collagen occurs at the transcriptional level. The mechanism is complex; it involves IL-1 induction of Egr-1, which prevents binding of Sp1 transcription factor to a site in the proximal promoter, and

induction of an ETS transcription factor ESE-1, which acts as a repressor by blocking protein–protein interactions needed for transcriptional activation. Chondrocyte 'dedifferentiation' by IL-1 is also caused by downregulation of SOX expression. Nuclear factor κ B inhibits SOX9 promoter and destabilises SOX9 mRNA.

Further studies on the regulation of chondrocytes by inflammatory cytokines were described by Linda Sandell (St Louis, MO, USA). She has found that bone morphogenic protein 2, an anabolic factor, is expressed in osteoarthritic cartilage in response to IL-1 or an IL-1-like factor. IL-1 is generally thought of as catabolic, but Sandell suggests that there are situations where IL-1 could be anabolic and could be driving repair processes.

For the final talk of this session, Martin Lotz (La Jolla, CA, USA) discussed chondrocyte death and apoptosis. Apoptosis is a feature of osteoarthritic cartilage in humans and in animal models, and apoptotic bodies promote matrix degradation. Cytoprotective agents could be chondroprotective. A caspase inhibitor was protective in a cruciate ligament section model of OA in rabbits. Chondrocyte apoptosis is induced by Fas, reactive oxygen species, anoikis and mechanical stress. The latter, which has attracted attention as a potentially important mechanism in OA, is caspase dependent and may actually be Fas dependent.

There then followed a session on structural proteins of the cartilage matrix. Dick Heinegard (Lund, Sweden) emphasised the interconnectivity of the major cartilage matrix proteins that establish complex networks. Metabolic studies of OA cartilage show that certain proteins increase in synthesis. One of these is cartilage oligomeric protein, a catalyst that speeds up fibrillogenesis of collagen without binding to the mature fibres. Perhaps overproduction inhibits fibrillogenesis in OA. Changes in the catabolism of matrix proteins are also important. IL-1, well known as a catabolic cytokine, induced an MMP-13-dependent cleavage of fibromodulin.

David Eyre (Seattle, WA, USA) discussed the mechanisms of assembly and decay of the collagen fibre network in cartilage. The fibres are predominantly collagen type II, with small amounts of type IX and type XI. Collagen type IX is thought to decorate the surface, and type IX–IX crosslinks may link fibrils. Collagen fibrils grow in diameter during life, implying remodelling of the type IX network. Destruction of the collagen network in OA may involve telopeptide cleavage as well as the specific cleavage by classical collagenases. The importance of collagen type IX for mechanical properties of the collagen network is shown by two amino acid polymorphisms in the protein being linked to lumbar disc degeneration.

Cartilage contains several small leucine-rich proteoglycans as well as large proteoglycans such as aggrecan and perlecan. The small proteoglycans are biglycan, decorin, fibromodulin and lurnican. Their multiple roles in skeletal tissues were discussed by Laurent Ameye (Lausanne, Switzerland). They bind to a variety of connective tissue components including collagens and are involved in collagen fibrillogenesis and mineralisation. Single and double knockout mice have an array of musculoskeletal abnormalities. The biglycan and fibromodulin knockouts showed OA-like cartilage degeneration. Double knockouts of fibromodulin and biglycan, or lumican, had more severe phenotypes. The first had joint instability, abnormal gait and ectopic ossification in fibrocartilage. The second had Ehlers Dahnlos-like joint hyperextensibility and OA. The complex phenotypes suggest that the OA could have arisen from abnormalities in the surrounding articular tissues.

Proteinases are involved in pathological and physiological turnover of the extracellular matrix. There is evidence in OA of an increase in both synthesis and degradation of matrix molecules. The turnover of aggrecan in cartilage is thought to be mediated by MMPs and aggrecanases. Bruce Caterson (Cardiff, UK) showed that ADAMTS-4 is upregulated by inflammatory cytokines such as IL-1 and causes aggrecan breakdown. Intriguingly, the induction by IL-1 of ADAMTS-4 together with a variety of other inflammatory response gene mRNAs is inhibited when the chondrocytes are cultured in a medium enriched for omega-3 fatty acids. Patients undergoing surgery for OA whose diet had been enriched with purified omega-3 fatty acids showed differences in proteolytic activity in their cartilage compared with a control group receiving a normal diet. That the diet could change the lipid composition of cells so as to inhibit inflammatory responses or signalling has intriguing therapeutic implications.

Tim Cawston (Newcastle, UK) has found that MMP expression and cartilage degradation are enhanced if IL-1 acts together with an IL-6-like cytokine such as oncostatin M. This synergy has now been shown *in vivo* with adenoviral gene transfer. Proteomic analysis is being used to find other synergistically regulated genes and one candidate is gp39, a chitinase-like molecule implicated in remodelling of extracellular matrices.

Hideaki Nagase (London, UK) further developed the aggrecanase theme. He described the different domains of ADAMTS-4, showing how they affected both its catalytic activity and its ability to bind and recognise substrates. He also showed that TIMP-3, which of the four TIMPs is the only one that inhibits ADAM family members, blocks IL-1-induced cartilage proteoglycan degradation in explant culture.

Gillian Murphy (Cambridge, UK) has used mutational analysis to change the specificity of the different TIMPs. Her work suggests it may be possible to make TIMPs more specific for individual proteinases. Murphy also questioned whether, for therapy, it would be preferable to have highly selective TIMPs or to have TIMPs with broad specificity.

The final session of the second day was devoted to pathology and animal models. Robin Poole (Montreal, Canada) emphasised the need to measure both synthesis and degradation of the cartilage extracellular matrix in OA. He showed that cartilage from different locations apparently responds differently to disease. For example, cartilage from early OA lesions in the ankle showed a greater increase in synthetic activity than from similar lesions in the knee, suggesting that ankle cartilage may be more resistant to OA. Using the IL-1 receptor antagonist protein and TNF blockade, Poole also showed that IL-1 and TNF may be inhibiting synthesis in diseased cartilage.

There is little scope for analytical studies of molecular mechanisms in humans. Mouse models of OA are attractive since the effects of genetic modifications upon pathogenesis can be studied. Mandy Fosang (Melbourne, Australia) is breeding mice in which the main cleavage sites in aggrecan for either aggrecanase or MMP-3 are mutated to a noncleavable form. These cleavage-resistant mutations do not affect the normal physiological turnover of aggrecan: whether or not they delay the onset of experimental OA remains to be seen.

One spontaneous model of OA is the STR/Ort mouse. These animals develop OA of the medial tibial plateau: 85% of males show lesions by 35 weeks. Roger Mason (London, UK) has found that they have complex changes in proteinase and cytokine expression. A change preceding overt pathology is low expression of extracellular superoxide dismutase, suggesting that the extracellular matrix may be susceptible to oxidative damage. Mason has also investigated the susceptibility to surgically induced OA of mice in which putative mediators have been knocked out. Paradoxically, mice lacking IL-1 β , IL-1 β convertase, MMP-3 or inducible nitric oxide synthase showed accelerated progression of disease rather than protection.

The final day opened with a session on mechanical stress. Mechanical stimulation is important for chondrocyte function. Off-loading cartilage causes thinning, while excessive or injurious loading leads to degeneration. Alan Grodzinsky (Cambridge, MA, USA) stressed how the nature of the load alters the cartilage response. Dynamic compressive loads first downregulate, then upregulate, anabolic genes, whereas static compressive loads do the opposite. Rapid compressive loads, in which there is little

fluid flow, are injurious and induce a quite different pattern of gene expression.

Tonia Vincent (London, UK) has found that articular cartilage contains a pool of basic fibroblast growth factor (bFGF) that is sequestered in the extracellular matrix, possibly by perlecan. Damaging the tissue by simple cutting releases the growth factor very rapidly and activates the chondrocytes. bFGF upregulates synthesis of a number of proteins, but particularly TIMP-1, suggesting that it is an anabolic factor. Cyclical loading of articular cartilage also activates the chondrocytes in a bFGF-dependent manner, implying that bFGF may be a transducer of mechanical stimuli as well as being involved in responses to injury.

While these first two speakers had studied effects of mechanical stimuli on cells in their extracellular matrix, Donald Salter (Edinburgh, UK) has investigated the responses of chondrocytes in monolayer culture. Twenty minutes of mechanical stimulation at 0.33 Hz caused membrane hyperpolarisation, an increase in aggrecan, but a decrease in MMP-3 mRNA. The response was via signalling through the integrin $\alpha_5\beta_1$, and involved production of IL-4, and possibly substance P. IL-4 and substance P were thus thought to be autocrine. OA chondrocytes did not undergo hyperpolarisation in response to strain, but instead underwent an integrin-dependent depolarisation, which might be IL-1-induced.

The second session was devoted to cartilage repair and tissue engineering. Hari Reddi (Sacramento, CA, USA) outlined the discovery and importance of the bone morphogenetic proteins that induce and maintain the chondrocyte phenotype. The cell source and synthetic scaffold will both be crucial for successful tissue engineering. Tim Hardingham (Manchester, UK) has expanded osteoarthritic chondrocyte populations, and has countered their tendency to dedifferentiate by retroviral transfer of SOX9. This and the presence of insulin-like growth factor-I and transforming growth factor β increased collagen type II mRNA 5000-fold.

Chris Murphy (London, UK) discussed the merits and demerits of stem cells and chondrocytes for tissue engineering. Stem cells, whether embryonic or mesenchymal in origin, produce chondrocytes that become hypertrophic (like cells in the growth plate) and eventually undergo apoptosis. Articular chondrocytes, when expanded, dedifferentiate. Dedifferentiation can be reversed by culturing the expanded population in alginate beads in the presence of low oxygen tension.

Rocky Tuan (Bethesda, MD, USA) drew attention to the importance of N-cadherin and signalling in developmental and regenerative chondrogenesis. Tuan also showed

remarkable tissue constructs generated from mesenchymal stem cells *in vitro* grown on biodegradable scaffolds with growth and differentiation factors. Three-dimensional structures comprising discontinuous bone-like tissues and cartilage-like tissues have been grown.

The first session of the final afternoon was devoted to the genetics of OA. Identification of genes predisposing to OA could open up avenues for therapy or even prevention. OA is such a common disorder, however, that genetic factors are likely to be diverse. One difficulty is defining OA phenotypes for genetic studies. Hand OA, which is common in females, is often familial. However, this is less clear in the case of hip OA, which is common with advancing age, and in knee OA, which often follows trauma in younger patients and is associated with obesity. Tim Spector (London, UK) described the contribution of twin studies. These suggest that OA could be 60% heritable. However, although there is some association of hand OA with knee OA, there is no association with hip OA and neither are hip OA and knee OA linked. This is surprising because if articular cartilage is prone to OA due to genetic factors, then OA in different joints should cluster in the same individuals. The different OA 'syndromes' behave like different diseases. Structural mutations in collagen type II and type IX cause severe chondrodysplasias and the cartilage degenerates. However, such severe defects may not contain any lessons for our understanding of OA.

John Loughlin (Oxford, UK) discussed results of a genome-wide scan in families containing siblings with severe large-joint OA coming to joint replacement surgery. There were six regions linked to susceptibility to female hip arthritis. Investigation of these has identified FRZB, COL9A, bone morphogenic protein 5 and IL-4R as encoding for OA susceptibility. Five IL-4R single nucleotide polymorphisms showed evidence of association (twofold increased risk). No bone morphogenic protein 5 single nucleotide polymorphisms correlated with OA but there was some correlation between differential allelic expression and age at joint replacement.

Maripat Corr (San Diego, CA, USA) reported the existence of two variants of the secreted frizzled-related protein 3 (sFRP3 or FRZB): R200W and R324G. R324G was associated with OA in a series of 558 female cases and 399 age-matched controls, and possession of the W-G haplotype, which contains the minor allele of both single nucleotide polymorphisms and results in substitution of both arginines, was a particular risk factor with an odds ratio of 4:1. The double mutation reduced the ability of sFRP3 to antagonise Wnt signalling.

Michael Briggs (Manchester, UK) described insights into connective tissue protein function obtained from studying

the chondrodysplasias. Pseudoachondroplasia results from mutations in cartilage oligomeric protein, and multiple epiphyseal dysplasias result from mutations in cartilage oligomeric protein, collagen type IX and matrilin-3. Mutations in matrilin-3 have also been associated with hand OA in a Finnish study. Mutations in different regions of matrilin-3 cause different pathologies. Those in the A domain, which are associated with multiple epiphyseal dysplasia, delay protein folding and prevent secretion. Mutations in the epidermal growth factor repeats are associated with short-limbed dwarfism (spondylo-epimetaphyseal dysplasias) and hand OA.

The advent of transcriptional profiling has promised new insights into OA. Thomas Aigner (Erlangen, Germany) described the application of this technology to OA, pointing out the difficulties and pitfalls, and illustrated the ways in which such data can be analysed. The final presentation from Jeremy Saklatvala (London, UK) was on the proteomic analysis of cartilage. Normal adult cartilage makes very little type II collagen, while most OA samples produce striking amounts. A search for regulatory molecules had identified activin A and connective tissue growth factor. Activin A (a relative of transforming growth factor beta) regulates TIMP-1 expression and is downstream of bFGF, which looks like an important anabolic factor in cartilage transducing mechanical load and activating cells on tissue injury. Physical injury to articular cartilage also activates inflammatory signalling pathways in the chondrocytes and induces intracellular IL-1.

The meeting was strongly oriented towards cartilage, but covered many current OA research topics investigated under diverse scientific disciplines. Molecular approaches have identified numerous key factors involved in chondrogenesis, and in anabolic and catabolic processes of adult cartilage. Biomechanical investigations have started to unravel how cartilage responds to physical stress. Epidemiological studies indicate heritability of OA, and genetic linkage analysis has started to map susceptibility loci. However, it is still not known whether OA, which is a histopathological diagnosis, is a single disease initiated by the same fundamental process in the different sites or is the common sequel of several different fundamental disturbances.

Do the molecular approaches provide clues for new ideas for developing treatments for OA? If early diagnosis were possible then therapy could be aimed at slowing progression of disease, although whether we should be inhibiting catabolism or stimulating anabolism, or doing both, is unclear. The only definite molecular targets so far have been the tissue proteinases. Perhaps IL-1 is a target, but the role of inflammatory cytokines as primary drivers of the process is unproven, as is the role of apoptosis. Joint replacement is likely to remain the mainstay therapy for

late disease for the foreseeable future: cartilage tissue engineering is a huge challenge.

Chronic, slowly progressive diseases that take years or decades to develop, such as OA and Alzheimer's disease, perhaps require new therapeutic concepts. A complete shutdown of a particular pathway, a traditional therapeutic approach in inflammation, may not be beneficial to degenerating tissue. For example, generally considered to be a catabolic factor, IL-1 might also be anabolic (as discussed by Sandell). Newer approaches could involve integrative rather than reductionist approaches because the progression of OA involves an intricate interplay of transcription factors, extracellular matrix synthesis, assembly and degradation, all of which may be affected by the genetic background and environment. Understanding the role of individual molecules in cartilage metabolism is an essential step towards such integrative approaches. The meeting provided a mass of information for future research into the causes and treatment of this common disabling disease.

Competing interests

None declared.