We have also shown in a recent twin study that the rate of progression of knee OA has a strong heritable basis.
Reports of significant associations for candidate genes for common forms of OA of the knee and hip now include over 50 genes – and over a dozen have now been replicated independently. These include VDR, ERG, CILP, Col2A1, AACT, BMP-2, FRZB, ADAM12, IL-1, IL-1-RA, ASPN, LRCH1, matrillin3, COMP, and OPG. Linkage studies using families and affected sib pairs have to date shown a number of significant replicated loci, especially areas on chromosome 2q and Chromosome 19.
OA is best studied genetically by dividing the phenotype into its constituent parts and by studying intermediate phenotypes, which may operate independently or together in clusters determined by pleiotropic genes. For example Cartilage loss at the fingers or knee medial compartment or serum COMP or bone turnover marker levels have all been shown to be heritable.
In conclusion, OA is a strongly genetic disease, which is likely to be a complex polygenic disorder that may differ genetically by gender site and race. We now have more than a dozen replicated genes and are close to being able to use these clinically for both diagnosis and clinically to predict progression and prognosis. Understanding how the individual genes influence the many intermediate processes is likely to be a fruitful avenue to provide insight into disease pathways and potential new drug targets.

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TISSUE ENGINEERING BASED ON MUSCLE-DERIVED STEM CELLS: POTENTIAL APPLICATIONS FOR TISSUE REGENERATION

Johnny Huard

Summary: Members of my laboratory have isolated various populations of myogenic cells from the postnatal skeletal muscle of normal mice on the basis of the cells' adhesion characteristics, proliferation behavior, and myogenic and stem cell marker expression profiles. Although most of these cell populations have displayed characteristics similar to those of satellite cells, we also have identified a unique population of muscle-derived stem cells (MDSCs). MDSCs exhibit long-term proliferation and high self-renewal rates and can differentiate toward various lineages, both in vitro and in vivo. The transplantation of MDSCs, in contrast to that of other myogenic cells, has improved the efficiency of dystrophic muscle regeneration and the delivery of dystrophin to dystrophic muscle. The ability of MDSCs to proliferate in vivo for an extended period of time, combined with their capacity to exhibit self-renewal, multipotent differentiation, and transplantation. Recent studies performed by members of my laboratory have shown that transplantation of female MDSCs (F-FMSCs) rather than male MDSCs (M-MDSCs) significantly improves skeletal muscle regeneration despite the similar myogenic and stem cell marker expression by both cell types. I will explain the increased muscle regeneration efficiency exhibited by F-MDSCs. My presentation will also address the influence of environmental cues within dystrophic or injured skeletal muscle on the differentiation of MDSCs into fibrotic cells. I will discuss potential strategies by which to prevent scar tissue formation within injured muscle by blocking TGF-β1 activity. I then will discuss the use of MDSCs in gene therapy and tissue engineering applications designed to improve bone and articular cartilage healing through the genetic modification of MDSCs to express osteogenic proteins (BMP2 and -4) and the angiogenic factor VEGF. I will also outline in my presentation new results obtained with human muscle derived stem cells, which we believe will open new avenues by which researchers could use muscle stem cell-based gene therapy and tissue engineering to improve tissue regeneration.

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NEW THERAPEUTIC STRATEGIES AND AGENTS WITH STRUCTURE MODIFYING POTENTIAL IN OSTEOARTHRITIS

Jean-Pierre Pelletier

Over the last decade, there have been several interesting advances in the treatment of osteoarthritis (OA). A clearer understanding of the pathophysiology of this disease has facilitated the development of new approaches for treatments aimed at specifically and effectively retarding the progress of the disease. Several new classes of molecules that inhibit one or more OA pathophysiological processes are under evaluation for their potential to alter the degenerative process.
Osteoarthritis can be described primarily as the degradation and loss of articular cartilage accompanied by subchondral bone remodeling, osteophyte formation, and synovial membrane inflammation. The most attractive recent findings are the data pointing to an association between inflammation and disease appearance and progression. There is significant interest in new agents that have the potential to reduce or stop the progression of structural changes observed in OA. Such agents offer great promise and are likely to lead to very significant changes in therapeutic approaches in the near future.
The use of NSAIDs or COX-2 selective inhibitors has shown that PGE_2 inhibition alone has not proven to delay the natural history of progressive OA. Other lipid mediators, including leukotrienes (LT), could play a major role in the development and persistence of the inflammatory process, and it is now clear that PG and LT have complementary effects. Recent studies have also revealed that LTB_4 potently stimulated the release of pro-inflammatory cytokines, such as IL-1β and TNF-α in human OA synovial membrane. Thus, the failure of NSAIDs to impact OA progression in these tissues could be due to the fact that inhibiting only the COX pathways leads to a shunt to LT production. Based on this concept, it is hypothesized that blocking production of both LT and PGE_2 could have a synergistic effect in achieving an optimal or a wider spectrum of anti-inflammatory activity.
Pro-inflammatory cytokines are likely responsible for some of the signs and symptoms as well as structural changes present in OA patients. There exists a number of ways by which the production or activity of cytokines could be reduced. These will be reviewed in brief. The action of cytokines can be reduced at the cell membrane level by decreasing the membrane receptor level or by the use of receptor antagonists or soluble receptors. Blocking the intracellular signaling pathways is another way to reduce the action of the cytokine.
The natural IL-1 receptor antagonist (IL-1Ra) is capable of reducing several cartilage catabolic processes that are IL-1β dependent. In vivo studies in animal models have demonstrated that the intra-articular injection of IL-1Ra could block the action of IL-1β and reduce disease progression. Data from early phase clinical trials in knee OA patients have also shown interesting symptomatic effects of IL-1Ra.
Another interesting target for controlling the activity of the IL-1 system is the IL-1 converting enzyme (ICE)/caspase-1, an enzyme which is responsible for the conversion of the proform of IL-1β into its active (mature) form. Usage of ICE inhibitor also represents another interesting potential target for the treatment of OA.
The role of proteases in the degradation of the extracellular matrix of cartilage in OA has been well documented, and metalloproteases (MMPs) are believed to play a major role in this process. Inhibition of the synthesis/activity of these enzymes as a treatment for OA has been the focus of intensive research. To date, the most promising strategy is still the use of chemical molecules that can block the activity of MMPs. A number of these compounds that have a broad range of MMP activities have already been tested in clinical trials. From these trials, we

have learnt that MMP inhibitors could produce side effects and have not yet demonstrated a major reduction in the progression of the disease. Certain MMPs, such as MMP-13 and ADAMTS proteases, such as ADAMTS5, have been selected as being the most attractive targets for the treatment of OA. The main reason(s) for choosing selective inhibition, instead of a broad inhibition, is based on the hypothesis that by doing so a certain number of side effects that could potentially be related to a broad MMP inhibition can be avoided.

Another possible way to inhibit the effect of certain catabolic factors lies in the inhibition of certain intracellular signaling pathways such as the protein kinase cascades. The kinases, which constitute the most appealing targets, include p38, ERK 1/2, and SAPK/JNK. A recent study showed that an orally active ERK 1/2 selective inhibitors could very significantly and effectively reduce the progression of lesions in the rabbit OA model by inhibiting the synthesis of catabolic factors, such as MMPs. Another emerging field of research focuses on the development of molecules that can inhibit the binding of the transcription factors at the DNA level. The major transcription factors targeted for the inhibition of cytokines and MMP synthesis are NF-κB and AP-1.

On the subject of cell signaling, another interesting target are the ligands to a group of nuclear transcription factors, the peroxisome proliferator-activated receptor gamma (PPARγ), which act as anti-inflammatory agents. In vivo, synthetic PPARγ ligands were found to protect against structural damages in the collagen-induced arthritis model and in OA models.

OA structural changes also involve modifications in the morphology of the surrounding bone. Subchondral bone remodeling is a well-recognized manifestation of OA. New data underline the concept that abnormal subchondral bone cell functions may contribute to the onset/progression of OA. Therapeutic effects of drugs that prevent the abnormal metabolism of subchondral osteoblasts on the progression of OA lesions is currently of major interest in the context of future DMOAD development.

Several new pharmacologic DMOAD agents are under investigation. Preclinical results are promising and demonstrate the possibility of retarding or inhibiting the progression of joint tissue structural changes. The tools to study such effects in the human population remain unsatisfactory. Existing radiologic methods are useful but this methodology is imperfect and time consuming. Faster and more accurate methods are needed to improve the investigation of new drugs that have the potential to modify the progression of OA.

In conclusion, several interesting new approaches for the treatment of this disease are now being explored. New classes of molecules that inhibit one or more of the disease processes of OA are under evaluation for their potential to alter the degenerative process

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BIOREACTORS FOR CARTILAGE TISSUE ENGINEERING

Ivan Martin

Introduction: Recent animal experiments have demonstrated that engineered cartilage tissues generated by culturing chondrocytes into 3D scaffolds provide functional templates for the orderly repair of critically sized osteochondral lesions. In order to reproducibly generate functional cartilage tissues starting from adult human cells, efforts have to be directed not only to the identification of stimulatory biochemical factors, but also to the development and use of controlled bioreactor systems, applying defined regimes of physical forces. In this work, we present some examples on the use of bioreactors for processes that are key for engineering of 3D cartilage tissues based on cells and scaffolds, namely the chondrocyte seeding into porous scaffolds, their efficient nutrition, and the pysical conditioning of the developing tissues.

Chondrocyte seeding and culture under perfusion: In the cell seeding process, cells must be utilized with maximum efficiency to minimize the biopsy size needed and/or the extent of cell expansion, and must be dispersed uniformly throughout the scaffold volume to form the basis for uniform tissue formation. To overcome limitations associated with the most commonly employed seeding techniques, we developed a bioreactor for the automated cell seeding of three-dimensional scaffolds by continuous perfusion of a cell suspension through the scaffold pores in oscillating directions. Perfusion seeding of chondrocytes into Polyactive foams (IsoTis OrthoBiologics, NL) or Hyaff$^{?}$-11 non-woven meshes (Fidia Advanced Biopolymers, IT) resulted in the highest fraction of viable cells within the foam pores, the greatest efficiency of seeding and the highest uniformity of cell distribution in comparison to the typically used static and spinner flask methods [1].

Constructs uniformly seeded by perfusion and then cultured statically for 2 weeks were highly heterogeneous in structure, consisting of a layer of cells and matrix at the periphery and an essentially void interior region. Instead, constructs cultured under prolonged perfusion were remarkably homogeneous, containing a uniform distribution of both cells and matrix throughout the cross-section [2].

Physical conditioning of cartilagel constructs: Application of dynamic compression to cell-polymer constructs could potentially improve the development of cartilaginous tissue *in vitro*. We exposed human articular chondrocytes-based cartilaginous constructs at different stages of maturation, as defined by the glycosaminoglycan (GAG) content, to intermittent compressive deformation for 3 days. Compression-induced changes in GAG synthesis and accumulation were positively correlated to the GAG content prior to loading, such that compression was stimulatory only for the most developed constructs. Therefore, under our experimental conditions, cyclic loading appears to be applicable for the enhancement of cartilaginous tissue development only in the late phases of tissue regeneration [3]. Our results also point out the possible use of bioreactors applying defined regimes of physical forces as a quality control tool for engineered cartilage, with the goal of defining when the tissues are sufficiently developed for immediate load bearing after implantation.

Conclusion: The reviewed studies indicate that bioreactors enable generation of cartilaginous tissue constructs and may contribute to understand the function of specific chemico-physical culture parameters on cartilage tissue development. In the future, bioreactors are expected to efficiently translate laboratory- to inductrial-scale cartilage tissue engineering, possibly providing an economically viable approach to the automated manufacture of functional cartilage grafts for broad clinical use [4].

References

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PAIN IN OSTEOARTHRITIS

Hans-Georg Schaible

Purpose: The most dominant subjective problem of the patient suffering from osteoarthritis is pain. In most cases pain is localized to the joint with OA but it can be referred to other joints. OA pain varies in intensity and is usually worsened by exercise