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MESENCHYMAL STEM CELL DYSREGULATION IN HEREDITARY OSTEOARTHRITIS

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The availability of families with precocious hereditary OA provides a unique opportunity to characterize altered mechanisms involved in pathways related to articular cartilage degeneration and to define the effect on these pathways of mutations in matrix components (namely, COL2A1). The Arg^{519}-Cys mutation in COL2A1 has been well characterized in humans[1-3] and is known to result in severe precocious generalized OA. Preliminary data in our laboratory demonstrate a defect in the differentiation of mesenchymal stem cells (MSC) into chondrocytes in these individuals. Studies of this phenomenon may provide insight into the pathogenesis of inherited degenerative disorders.

Our laboratory first described precocious hereditary OA in a Michigan family[4]. Subsequently, this familial autosomal dominant condition was found to be genetically linked to COL2A1[5] and the specific point mutation was identified[6]. Numerous investigations in our laboratory have described the Arg^{519}-Cys mutation in COL2A1.

There is evidence to suggest that MSC numbers, proliferation rate, population-doubling potential, and predisposition to differentiate along various cell lineages may be altered in OA[7-10]. Additional data suggest that severely osteoarthritic individuals[9] have MSC that differ from those of normal individuals with respect to their growth response to cytokines and their bone-forming potential in response to osteogenic stimuli. This suggests the possibility that changes thought to manifest themselves primarily at the site of the injury, i.e., articular cartilage, may have origins in cell populations not presently residing in the degenerating tissue. In studies of advanced OA by Murphy and colleagues[9], populations of MSC from OA patients were compared with those from controls with respect to yield, proliferation, and capacity for differentiation. Results demonstrated that the proliferative capacity of MSC derived from patients with OA was significantly reduced. In a more recent study, Alsalameh and coworkers[11] demonstrated that the numbers of multipotential mesenchymal progenitor cells present in adult human articular cartilage were increased in cartilage from osteoarthritic joints.

In a preliminary investigation, we employed bone marrow-derived MSC to study *in vitro* chondrogenesis in patients with the COL2A2 mutation. These initial experiments were designed to investigate the ability of such MSC to proliferate, differentiate into chondrocyte-containing pellets[12], and express and synthesize mutated type II collagen. Our goal was to develop a method for isolation and purification of heterotrimers of mutated and wild-type type II collagen molecules as a means of investigating altered synthetic mechanisms associated with the Arg^{519}-Cys defect.

In these analyses, MSC were derived from bone marrow obtained at the time of hip replacement surgery. Both mutated and non-mutated pellets synthesized type I collagen. The absence of reducible material suggested type III collagen was not present. Western blot analysis of the material, using antibody against type II collagen (C4F6), showed the presence of type II collagen in the non-mutated, but not in the mutated, samples. It was noteworthy that the pellets derived from patients with the mutation were uniformly smaller than their wild-type counterparts: 15 of 15 mutant pellets were < 1 mm and 15 of 15 wild-type pellets were > 1 mm after 14 days' growth. Attempts to direct Arg^{519}-Cys-related pellets into the chondrogenic pathway by addition of fibroblast growth factor (FGF) to primary cultures and of bone morphogenetic protein-2 to pellets did not result in evidence of type II collagen synthesis.

Studies of Perichondrial Mesenchyme (Ring of La Croix) in Relation to Pathophysiology of OA

In a collaborative study with Robinson, *et al*[13] at Tel Aviv University, it was demonstrated that MSC from the perichondrial mesenchyme (ring of La Croix) selectively migrated to the physeal region of the growth plate when implanted either in the ring of La Croix or in the synovial space[13].

In further studies related to the perichondrial mesenchyme, we assessed stem cell and growth factor responses in relationship to osteophyte formation in the rabbit partial meniscectomy model of OA[14]. Specimens from knees of rabbits that were developing OA after partial medial meniscectomy were stained with anti-fibroblast growth factor-receptor 3 (FGF-R3) antibodies. An increase in FGF-R3 receptor was observed at the site of osteophyte formation at the confluence of the perichondrial, periosteal, and synovial attachment corresponding to the ring of La Croix at the medial tibial plateau. In serial analyses, the concentration of FGF-R3 receptor diminished as osteophytes matured. These findings suggest a relationship of FGF to the formation of osteophytes in this experimental model of OA.

Our findings suggest inhibition of entry into the chondrogenic pathway by MSC derived from patients carrying the Arg^{519}-Cys mutation in COL2A1. Our expectation was that these cultures would produce a mixture of wild-type and mutated type II collagen. Failure of these cultures to produce any detectable type II collagen and failure of the mutated pellet cultures to accumulate cartilage-like proteoglycans and to attain dimensions equivalent to the control cultures suggest interruption of the pathway(s) leading to the chondrocyte phenotype. It seems clear that the point mutation in COL2A1 produces an effect on these cultures that is developmentally more profound than might be expected merely from production of mutated type II collagen. It is known that patients with this mutation synthesize, produce, and accumulate mutated type II collagen in their cartilage[1]. While the ratio of wild-type to mutated α-chains found in cartilage from these patients is greater than 1:1, clearly, the mutated collagen is expressed, suggesting that the MSC are under a different set of regulatory mechanisms than the chondrocytes in cartilage of mutated patients.

Studies on regulatory mechanisms investigating the expression of genes associated with the chondrogenic phenotype suggest several avenues of further investigation:
1. Is COL2A1 the only chondrocyte-lineage gene affected? Are genes for chondrocyte-associated matrix proteins and proteoglycan core proteins similarly affected?
2. When chondrogenic effectors are added to the MSC cultures, is the expression of central regulatory elements in mutated cultures altered, in comparison with that in control cultures?
3. Are regulatory growth factors and cytokines expressed in an altered pattern by mutated cultures?

In summary, data from our laboratory support a potential role for impairment of reparative mechanisms resulting from altered MSC function in the development of OA.

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HOW IMPORTANT ARE GENETIC FACTORS IN OSTEOARTHRITIS? CONTRIBUTIONS FROM FAMILY STUDIES

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A familial tendency to OA was first suggested over 120 years ago when it was observed that Heberden's nodes may cluster within families[1]. However, it was Stecher in the 1940s who provided convincing evidence for a strong genetic predisposition to Heberden's nodes by observing that nodes were twice as common in mothers, and 3 times as common in siblings, of affected probands as in the general population[1]. This finding was subsequently confirmed in family studies and was extended to include radiographic OA of joints other than the hand[1]. Within affected families the risk of OA of the hands, knees, and hips is significantly higher than that in the general population, with heritability estimates ranging from 40% to 70%, depending on the joint assessed[2]. Our report presents an overview of the epidemiological studies of familial clustering and/or heritability of OA and updates our presentation in the 2002 Indianapolis Workshop on Osteoarthritis Outcomes.