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Prg4-expressing cells: articular stem cells or differentiated progeny in the articular chondrocyte lineage?

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Articular cartilage protects opposing bone extremities in diarthrodial joints in the form of a highly resilient, shock-absorbing cushion lined with a well-lubricated surface. Built during development, it undergoes limited turnover in adulthood and responds poorly to the various environmental and genetic adverse conditions that it can be subjected to in a lifetime. Consequently, damage to the tissue is generally irreversible and becomes gradually worse, leading to common joint degenerative diseases collectively referred to as osteoarthritis (1). These diseases can cause severe joint pain, deformation, and incapacitation. Intense research efforts have therefore been deployed over the last decades to decipher mechanisms that underlie articular cartilage development, adult maintenance and degeneration, and to discover ways to prevent osteoarthritis and regenerate healthy joints (2–4). Recent findings have further stimulated these efforts by eliciting hope that articular stem cells (ASCs) may be present or inducible in adult joints and could thus be used to design efficient cell-based therapies (5–8). Some studies have pinpointed ASCs in the superficial layers of articular cartilage by exploiting cell quiescence (very slow cycling), a defining property of stem cells, and others have proposed the existence of ASCs in articular cartilage, synovium and intrapatellar fat pad surfaces based on the ability of cells to express mesenchymal markers in vivo and to undergo chondrocyte differentiation in vitro. Lineage-tracing experiments in the mouse using a *Gdf5^{Cre}* transgene along with a *Rosa26^{loxlacZ}* CRE recombinase reporter have demonstrated that articular chondrocytes and other joint cell types arise during development from a common pool of mesenchymal progenitors and that these progenitors are distinct from those for growth plate chondrocytes and endochondral bone osteoblasts (9,10). It is thus believed that some of these embryonic joint progenitors may persist as ASCs in adult articular tissues. However, since this *Gdf5^{Cre}/Rosa26^{loxlacZ}* genetic strategy activates *lacZ* expression in all embryonic joint precursor cells and transmits this property to all daughter cells for life, it has not allowed addressing a number of important questions, such as the following: do joint surface-lubricating cells and articular chondrocytes arise as independent lineages from joint progenitors or do joint surface-lubricating cells give rise to articular chondrocytes? An elegant study published in this issue of *Arthritis and Rheumatology* by the Harvard University groups of Andrew Lassar and Matthew Warman

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describes a novel lineage-tracing strategy that will greatly help answer such questions and that already starts tackling some of them (11).

Lubricin, which goes also by the names of proteoglycan 4 (PRG4), superficial zone protein (SZP), and camptodactyly-arthropathy-coxa vara-pericarditis syndrome protein (CACP), is a surface-active mucinous glycoprotein produced almost exclusively by the synovium and cartilage cells that line joint cavities (12). Most interest in lubricin has focused till today on its pivotal role in ensuring joint lubrication and in limiting synovium cell proliferation (13). In their new study, Kozhemyakina and colleagues specifically chose to tackle questions on the lineage relationships between lubricin-producing cells, ASCs and other articular chondrocytes. They knocked-in a *GFP-CreERT2* cassette into the translation initiation site of the endogenous *Prp4* gene. They show that their novel *Prp4^{GFP-CreERT2}* allele expresses the green fluorescent protein (GFP) and tamoxifen-activatable CRE recombinase (CreERT2) in all cells expressing *Prp4*, and although the modified allele no longer produces lubricin, heterozygous mice have no apparent disease. To trace the fate of *Prp4^{GFP-CreERT2}*-expressing cells and progeny, the authors created and analyzed *Prp4^{GFP-CreERT2}/Rosa26^{loxP-lacZ}* mice. When they gave tamoxifen to fetuses at the onset of *Prp4* expression (two days before birth), they found that newborn mice displayed few *lacZ*-positive cells and that most of these cells were located close to the incipient cartilage surface. Within two weeks, *lacZ*-positive cells became more numerous and populated all articular cartilage layers, i.e., from the most superficial *Prp4*-positive layers down to the middle and deep zones of the tissue. This cell distribution pattern persisted for at least one year. When tamoxifen was given to one-month-old pubertal mice, *lacZ*-positive cells were initially confined to the top layers of articular cartilage, accounting for up to 40% of all chondrocytes. By the time mice reached the advanced age of eighteen months, cells in the top half of the tissue were *lacZ*-positive, accounting then for about 70% of all chondrocytes. Based on all these data, Kozhemyakina and colleagues propose a model whereby articular chondrocytes derive from progenitors that are *Prp4*-positive in fetuses. Because *lacZ*-positive fetal cells appeared initially in the vicinity of the emerging tissue surface, the authors also propose that articular cartilage develops by appositional growth, i.e., surface cells give rise sequentially to each one of the cell layers that compose the adult tissue. The fact that cells induced to become *lacZ*-positive at one month of age did not give rise to deep-zone chondrocytes in adulthood is consistent with the virtual growth arrest of adult mice and with evidence that articular chondrocytes undergo little if any turnover once mice are adult.

The study of Kozhemyakina and colleagues thus offers new insights into the mechanisms that may underlie articular cartilage formation and adult turnover. It also offers a new genetic tool that will be precious to further increase this knowledge as well as knowledge of joint disease mechanisms. A major advantage of this new tool is that it is inducible temporally from late gestation to late adulthood. It thus allows for timed recombination of *loxP*-flanked DNA sequences. It is thereby different from the joint-specific *Gdf5Cre* mouse line, which starts recombining DNA in embryonic presumptive joints and therefore does not easily allow studies on postnatal events. The unique spatial specificity of the *Prp4^{GFP-CreERT2}* allele distinguishes it from other available inducible transgenes, namely *Col2a1-CreER* and *Acan-CreER/Tet-on* mouse lines, which target articular and non-articular

chondrocytes (14–16). Other eminent features of *Prg4^{GFPCreERT2}*, which could be advantages or disadvantages depending on study goals, are that the allele is active in joints not only in cartilage-lining cells, but also in synovium-lining cells, and that it was unexpectedly found to become active in articular chondrocytes of aging mice. All in all, this new genetic tool, which is already available from the Jackson Laboratory, will undoubtedly valuably complement the currently limited collection of CRE transgenes/alleles available to target articular cell types in the mouse in a fairly specific manner.

By pushing the frontiers of knowledge further, the work of Kozhemyakina and colleagues also generates new questions. By demonstrating that adult articular chondrocytes derive from *Prg4^{GFPCreERT2}*-positive fetal cells, it raises a fundamental question on the actual identity of these progenitors. Are these cells ASCs? Are they differentiated articular surface-lining cells derived from ASCs and following a multi-step differentiation pathway that ultimately leads them to become middle- and deep-zone articular chondrocytes? Do they have dual identity and function, being both ASCs and effective producers of lubricin? Definitively answering these questions are valuable objectives for future studies. In the meantime, the new data and other knowledge can be used to put forward new ideas. For instance, it is conceivable that the population of *Prg4*-positive cells may include both multipotent ASCs and lubricin-producing cells. Multipotent stem cells typically co-express stem cell determinants and markers of the distinct lineages that they later give rise to. Specifically, presumptive joint cells are well known to co-express the undifferentiated marker *Gdf5* and the chondrogenic markers *Sox9* and *Col2a1*. As they engage into differentiation, all cells downregulate *Gdf5* expression. Progenitors that dedicate to the chondrocyte lineage upregulate *Sox9* and *Col2a1* expression, whereas those that commit to other lineages turn off these genes. Hence, in lineage-tracing experiments using a *Sox9^{Cre}* allele *Sox9*-positive joint progenitors gave rise to both chondrocytic and non-chondrocytic (synovium and tendon) articular cell lineages (17). Like *Sox9*, *Prg4* could be expressed in all embryonic joint progenitors. Later on, progenitors choosing the articular surface-lubricating lineage would upregulate *Prg4*, while those engaging in the articular chondrocyte and other cell lineages would turn off *Prg4*. This proposition contrasts with the fact that the *Prg4* RNA becomes detectable only when joint progenitors develop into articular surface cells in late fetuses and postnatally. The two notions can, however, be reconciled by speculating that the *Prg4* RNA may not only be expressed at a low level in joint progenitors, but may also be rapidly targeted by microRNAs and degraded. Since it lacks all coding and 3' untranslated exon sequences of the endogenous *Prg4* RNA, the *Prg4^{GFPCreERT2}* allele may escape the rapid degradation that normally prevents detection of *Prg4* mRNA in joint progenitor cells. Thus, Kozhemyakina and colleagues have revealed that the *Prg4* locus is active in the fetal progenitors of adult articular chondrocytes, but it remains to be determined whether these progenitors are ASCs and whether they give rise independently or sequentially to joint-surface lubricin-producing cells and to middle- and deep-zone articular chondrocytes. It is possible that the few *Prg4^{GFPCreERT2}/Rosa26^{flx lacZ}* cells that were *lacZ*-positive in newborn mice two days after tamoxifen injection were the first differentiating joint surface-lining cells, whereas the cells that were still at the progenitor stage were producing CreERT2 too weakly to recombine the *Rosa26^{lacZ}* locus and turn positive for *lacZ* within two days. The actual progenitors of articular chondrocytes may have been located at the prospective joint

surface or in deeper layers. In fact, the authors described a distribution pattern of *lacZ*-positive cells that is not inconsistent with an original location of *Prg4*^{GFP*CreERT2*}-positive progenitors of articular chondrocytes in both superficial and subjacent zones. The mechanism of appositional growth of articular cartilage proposed by the authors is thus an appealing model that like many brand new concepts will be worth further validation in complementary studies.

Many other questions are brought about by the Kozhemyakina study. For instance, are ASCs presumably present in adult articular cartilage *Prg4*/*Prg4*^{GFP*CreERT2*} positive? Could they be located in situ and sorted from the tissue based on this property? Is there an advantage for them to be *Prg4* positive? Can they regenerate damaged articular cartilage, including middle and deep zones? Since lubricin is a major lubricant, it may be critical for ASCs to be able to produce the proteoglycan in order to prevent damaging friction of cartilage surfaces in active joints. On the other end, since Rhee and colleagues (2005) showed that human patients and mice lacking lubricin develop non-inflammatory arthritis characterized by synovial cell hyper-proliferation, the proteoglycan may contribute to rarifying ASCs in adult joints and thus to lowering the capacity of articular cartilage to regenerate itself upon damage. The observation of the authors that articular chondrocytes become *Prg4*^{GFP*CreERT2*} positive in aging mice could be a new sign that the cells are reverting to a progenitor stage, a phenomenon well documented and often referred to as dedifferentiation. If confirmed, this abundant presence of *Prg4*^{GFP*CreERT2*}-positive progenitors in aging tissue would stress the importance of strengthening research efforts to find ways to re-differentiate chondrocytes in aging individuals in addition to pursuing the efforts currently deployed to locate and characterize ASCs. Last but not least, findings made in mice are significant only if relevant to humans. The Kozhemyakina study thus calls for translational studies to determine whether the same relationships exist in humans and mice between *PRG4*-positive cells, ASCs and articular chondrocytes. Since articular cartilage has more cell layers and must maintain itself for many more years in humans than in mice, differences might exist between the two species in the ability of articular surface cells to give rise to middle- and deep-zone chondrocytes.

In conclusion, the new findings from the Kozhemyakina study illuminate current understanding of mechanisms that underlie articular joint development and adult turnover and greatly motivate further research to more fully dissect these mechanisms and those involved in joint diseases, and in particular the lineage and functional relationships that exist between articular stem cells, *Prg4*-positive joint surface cells and underlying articular chondrocytes. The mouse genetic tool developed in this study will undoubtedly be very precious to help pursue these objectives.

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