REGENERATIVE MEDICINE

Cartilage stem cells identified, but can they heal?

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Regeneration of articular cartilage has been a long-standing challenge in the field of regenerative medicine. In the past 2 years, several studies have genetically identified the presence of stem cells in the surface of articular cartilage, but questions remain as to the healing properties of these cells.

Refers to Decker, R. S. *et al.* Cell origin, volume and arrangement are drivers of articular cartilage formation, morphogenesis and response to injury in mouse limbs. *Dev. Biol.* **426**, 56–68 (2017)

The existence of cartilage stem/progenitor cells (CSPCs) has long been postulated, but no specific markers were known that could be used for genetically tracing the CSPC lineage or for reliably characterizing these cells *in vivo*[1]. Now, however, Decker *et al.*[2] have added to a series of studies that, when put together, substantially improve our understanding of the cellular hierarchy and kinetics within articular cartilage, thereby providing clues for achieving regeneration of articular cartilage.

Employing novel and powerful genetic tools, over the past 2 years, three laboratories have independently demonstrated the presence of CSPCs in the superficial zone of articular cartilage in mice[2–4]. Initially, Kozhemyakina and co-workers[3] knocked the CreERT2-expressing cassette (a tamoxifen-inducible *Cre* allele) into *Prg4* (encoding proteoglycan 4 (PRG4), also known as lubricin), which is expressed by superficial cells at the surface of cartilage and by cells lining the synovial membrane. Employing this mouse strain for genetic tracing, Kozhemyakina *et al.* demonstrated that the progeny of PRG4$^+$ cells differentiate into articular chondrocytes[3].

Li *et al.*[4] extended this initial observation, showing that the progeny of PRG4$^+$ cells facilitated both appositional and interstitial growth of articular cartilage in juvenile animals and could entirely reconstitute adult articular cartilage. Furthermore, superficial PRG4$^+$ cells divided slowly, were self-renewing and expressed stem cell markers, thereby fulfilling the criteria for adult stem cells[4]. These findings have now been confirmed by Decker *et al.*[2] in two alternative, independently derived mouse strains (*Prg4*-CreERT2 mice and *Dkk3*-CreERT2 mice, the latter in which superficial cartilage cells and synovial cells are also labelled). Together, these three studies[2–4] demonstrate that superficial cells in cartilage are indeed CSPCs that reside at the cartilage surface during postnatal life.

The crucial question that remains to be answered concerns the healing potential of these CSPCs. Decker *et al.*[2] have shed the first light on this matter by demonstrating that within 7 days of injury, the progeny of PRG4$^+$ cells constitute ≤70% of the cells in an articular cartilage defect. Confirming these results, our own preliminary experiments have also revealed that the progeny of PRG4$^+$ cells can be found in cartilage defects (A.S.C., unpublished observations). Interestingly, Decker *et al.*[2] observed that the progeny of PRG4$^+$ cells in the synovial lining expand massively in response to injury, whereas in the cartilage superficial zone, neither PRG4$^+$ cells nor their progeny proliferate[2]. Furthermore, the contribution of PRG4-traced cells to the defect area is most pronounced when the hyperplastic synovial tissue is contiguous with the cells that fill the defect (Maurizio Pacifici, personal communication). These observations suggest that progeny of PRG4$^+$ cells located at the synovial membrane but not on the cartilage surface contribute to the healing site.

Excitingly, another study also published in 2017 addresses the same question of cartilage repair, although in this study[5], the authors genetically trace growth/differentiation factor 5 (GDF5)$^+$ cells in mice. During limb development and before joint cavitation, cells in the interzone area express GDF5; all future joint structures, including the articular cartilage, menisci, synovial membrane, tendons and ligaments are progeny of GDF5$^+$ cells[2,5,6]. Roelofs *et al.*[5] were the first to show that GDF5-traced cells can comprise ≤80% of the cells involved in the healing of certain defects in cartilage, a contribution comparable to that made by the descendants of PRG4$^+$ cells. Moreover, GDF5-traced cells in the synovial membrane expressed PRG4 (REF. 5), making GDF5$^+$ cells a likely progenitor of PRG4$^+$ cells, with the latter acting as long-term postnatal chondro-progenitor cells.

Interestingly, Roelofs *et al.*[5] observed large-scale expansion of GDF5-traced cells in the synovial membrane in response to acute cartilage defects, but little response from the superficial cells adjacent to the defect, similar to observations made by Decker *et al.* for PRG4$^+$ cells[2]. Since both PRG4$^+$ cells and GDF5$^+$ cells are present in the synovial membrane and in the superficial zone of cartilage, the question as to where exactly CSPCs are located remains. Both studies[2,5] suggest that the cells that contribute to cartilage repair originate in the synovial membrane rather than in the superficial zone, mainly because of the hyperplasia that takes place in the synovial membrane in response to injury. Additionally, inactivation of transcriptional co-activator YAP abrogates this synovial hyperplasia and substantially reduces the proportion of descendants of GDF5$^+$ cells that are present at the site of injury[5]. By contrast, superficial zone cells are known to migrate to the site of injury in cartilage explants[7]. Since such migration cannot be easily detected in genetic tracing experiments, contribution from the superficial zone cannot be fully excluded.

Thus, the genetic tracing of GDF5$^+$ cells and PRG4$^+$ cells has revealed that the progeny of these cells contribute to the healing of cartilage damage in mice; however, the functional importance of this contribution remains to be determined. Decker and co-workers[2] examined only acute cellular responses, not the

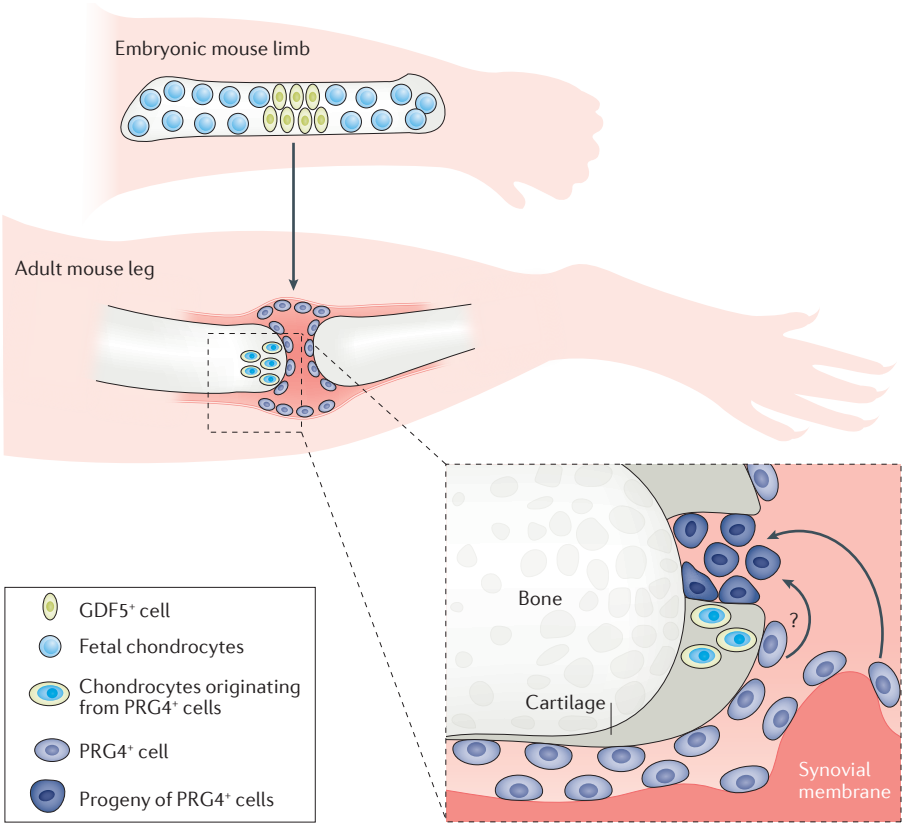

Figure 1 | **Developmental origin and healing capacity of PRG4⁺ stem cells.** Growth/differentiation factor 5 (GDF5)⁺ cells are likely to give rise to proteoglycan 4 (PRG4)⁺ cells during development. Descendants of PRG4⁺ cells form the articular cartilage in juveniles and can contribute to the healing of cartilage defects in adult mice. Whether this contribution comes from PRG4⁺ cells residing in the synovial membrane or in the superficial zone of cartilage remains to be clarified.

healing of cartilage, but Roelofs *et al.*[5] noted that cartilage defects that are repaired effectively contain a high proportion of GDF5-traced cells, even though substantial healing is observed in the presence of low numbers of these cells. Furthermore, upon ablation of YAP in cells of the GDF5-lineage, the contribution of GDF5-traced cells to the healing of cartilage defects was sometimes totally absent[5]. At the same time, repopulation of the cartilage defect with unlabelled cells was observed[5], indicating the existence of additional sources of CSPCs.

Overall, it can be safely said that at least one type of CSPC (PRG4⁺ cells) has now been identified genetically in mice[2–4], and that these cells are likely to be descendants of GDF5⁺ cells[5,6]. These PRG4⁺ CSPCs are present both at the cartilage surface and in the synovial membrane; PRG4⁺ CSPCs at the cartilage surface generate chondrocytes postnatally to form the entire adult articular cartilage[4]. The descendants of PRG4⁺ CSPCs can migrate to cartilage defects, but the origin of these cells remains to be elucidated, as well as their healing potential (FIG. 1). Although the importance of these observations is unequivocal, their relevance to human physiology still remains to be demonstrated.

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Competing interests statement
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