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Lessons from joint development for cartilage repair in the clinic

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Abstract

More than 250 years ago William Hunter stated that when cartilage is destroyed it never recovers [1]. In the last 20 years, the understanding of the mechanisms that lead to joint formation and the knowledge that some of these mechanisms are reactivated in the homeostatic responses of cartilage to injury has offered an unprecedented therapeutic opportunity to achieve cartilage regeneration. Very large investments in ambitious clinical trials is finally revealing that, although we do not have perfect medicines yet, disease modification is a feasible possibility for human osteoarthritis.

Developmental morphogenesis and homeostatic responses to injury share basic mechanisms

During embryonic development, mesenchymal cells deriving from the lateral plate mesoderm (LPM), condense within the limb bud to form a cartilage anlage. The chondrocytes in the centre (diaphysis) of the skeletal elements undergo hypertrophic differentiation, expressing collagen type X, VEGF, MMP13 and alkaline phosphatase. Hypertrophic differentiation, in turn, triggers mineralization and ultimately replacement by bone [2,3]. Endochondral ossification proceeds from the diaphysis towards the epiphysis sparing the joint interzones, which separate the future skeletal elements and will give rise to the joints. The joint interzones are composed of a distinct population of chondrogenic precursors expressing GDF5, WNT9A, WNT16 and other markers. *Sox9*+ cells from the limb

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mesenchyme are recruited to the joint interzone where they transiently express *Gdf5* [7]. Cavitation through the middle of the joint interzone completes the separation of the skeletal elements. The first cells recruited to the *Gdf5*+ interzone lineage contribute largely to the epiphyses; cells recruited at later stages contribute to the articular cartilage and finally to the other soft tissues of the joints, including menisci, synovial membrane, ligaments and tendons [3–6]. WNT signaling is required and sufficient for joint formation. While the deletion of individual genes results in minor phenotypes – for instance, deletion of *Wnt9a* results in synovial chondromatosis [8], possibly because of compensation - complete suppression of WNT signalling resulted in joint fusion [9,10]. Misexpression of WNT9A was sufficient to induce interzone markers including *Gdf5*, but not the formation of ectopic joints [10]. *Gdf5*-null mice lack some distal skeletal elements in the autopod, and also have anomalies in the proximal joints: for instance, the knees are missing the menisci and the cruciate ligaments [6,11]. The more severe phenotype in the distal skeletal elements is possibly due to the proximo-distal expression gradient of *Gdf5* compared to other BMPs which may partially compensate in the proximal joints. Deletion of *c-Jun*, which is upstream of *Wnt9a* in the joint interzones, also resulted in lack of joint cavitation [12], however, morphologically, joint interzones were still present both after WNT blockade and *c-Jun* deletion [9,12]. Therefore, while substantial progress has been made in the understanding of joint morphogenesis and maturation, the prime mechanisms determining joint specification remain elusive.

As opposed to the epiphyseal cartilage, the articular cartilage is stable throughout life, resistant to hypertrophic differentiation and endochondral ossification. Once the skeleton is mature, in adult life, the articular cartilage enters a status of extremely low turnover [13]. However, shortly after injury the articular cartilage deploys an impressively rapid homeostatic response that, in many cases, restores integrity and function [14]. This repair response is triggered by the re-activation of several of those molecules which during development mark the joint interzone and are not normally expressed in quiescent uninjured adult cartilage [15]. These responses contribute to healing [16] and to re-establishing homeostasis [17,18]. Spontaneous healing is most likely to be successful for small isolated lesions in otherwise healthy and young patients. With age, joint instability and other comorbidities including obesity, the likelihood of successful repair decreases [14].

If repair is unsuccessful, or if injury persists, the excessive or prolonged molecular responses to injury may become a pathogenic factor leading to ectopic activation of hypertrophic differentiation and mineralization within the articular cartilage. This, in turn, drives further cartilage loss and development of osteoarthritis [19–21].

Osteoarthritis is the most common cause of disability for which we have no cure [22]. Targeting basic mechanisms shared by developmental morphogenesis and injury responses has allowed researchers to aim for cartilage regeneration for disease modification (Fig 1). One key example of this is ectopic chondrocyte hypertrophy: while hypertrophic differentiation is essential for normal development of the appendicular skeleton, adult articular chondrocytes should not undergo hypertrophy. When homeostasis is disrupted, a series of events requiring the transcription factor HIF-2α induce ectopic hypertrophy within the articular cartilage; the articular cartilage becomes mineralized and further cartilage loss

ensues [19–21] (Fig 2). Indeed, genetic deletion of *Hif-2α* in adult cartilage protected chondrocytes from ectopic hypertrophic differentiation and resulted in decreased cartilage degradation in murine models of osteoarthritis [20,21]. Additional mechanisms shared between developmental morphogenesis and homeostatic responses to injury are shown in Fig 1 and summarized in table 1.

Table 1. Processes with roles in both skeletal development and osteoarthritis.

Process	Function in development	Effect in osteoarthritis	Molecular player(s)	Key references
Chondrocyte hypertrophy and mineralization	Initiate endochondral bone formation	Cartilage mineralization and breakdown	WNTs, BMPs, PTH/PTHrP, HIF-2α, RUNX2	[19–21,23–25,25–29]
Apoptosis	Joint cavitation; replacement of cartilage by bone	Cartilage loss	BMPs, mTOR, inflammatory cytokines (e.g. CXCL12, GCP2)	[18,30–33,33–36]
Mobilization of stem cells	Growth and morphogenesis	Repair and homeostasis	HMGB1, BMPs; WNT16	[37–39]
Proliferation and extracellular matrix synthesis	Growth and morphogenesis	Repair and homeostasis	TGF-β, BMPs	[5,40]

At this point the main questions were: how can we utilize such mechanisms to support cartilage repair? What are the molecules that, in injured adult cartilage, become reactivated and regulate these processes? What initiates production of stable cartilage, which doesn't result in bone formation? A transcriptomic analysis of adult articular cartilage, 24 hours after injury, revealed the re-expression of morphogenetic signals that play important roles during joint development but are otherwise inactive in the adult cartilage including the WNT, TGF-β/BMP, Hedgehog, and FGF signalling pathways [15,16,41].

The re-activation of such molecules in the acute-injury phase have long-term consequences on the outcome of repair. The focus on early morphogenetic events is a complete change of perspective compared to the previous emphasis, in osteoarthritis research, which targets late, downstream events such as extracellular matrix production and degradation. Two examples are the re-activation of WNT16 and PTHR1/PTHrP in adult cartilage after injury.

The case of WNT16

Wnt16 is one of the earliest markers of the joint interzone [9,10], but is undetectable in adult cartilage [15,17]. We showed that 1 day after injury, *WNT16* became abundantly re-expressed in cartilage and canonical WNT signalling became activated [15]. The re-expression of WNT16 after injury was transient, however adult mice lacking *Wnt16* developed more severe cartilage loss and osteoarthritis 8 weeks following joint destabilization [17]. During its injury-induced expression, WNT16 supported the maintenance and activity of a *Prg4*+ stem cell population within the superficial layer of the articular cartilage [42,43], while at the same time preventing more potent WNT agonists from uncontrollably activating the β-catenin-dependent pathway, which would lead to ectopic cartilage hypertrophy and ultimately loss of cartilage [17]. Tong et al. showed that *Wnt16* overexpression protected cartilage in a model of osteoarthritis [44]. *Wnt16* delayed cartilage breakdown by activating JNK and the WNT planar cell polarity pathway, thereby ultimately upregulating *PTHrP* expression [44].

PTHrP/PTHR1 signalling

In the periarticular cartilage of the developing skeleton, activation of parathyroid hormone receptor 1 (PTHR1) by parathyroid hormone-related protein (PTHrP) increases proliferation and inhibits ectopic hypertrophic differentiation [25,26,29,45]. This mechanism allows elongation of the bones. PTHR1 is not normally expressed in adult articular cartilage. After chronic injury, such as in osteoarthritis, PTHR1 was re-expressed and, when stimulated by exogenous recombinant human parathyroid hormone, it protected cartilage from breakdown [46].

The pathway towards disease modification and clinical application

Manipulation of the molecules driving the homeostatic responses to cartilage injury have led to remarkable results in animal models, with a seemingly endless list of potential targets. In practice, however, several factors limit clinical applicability. Some molecules play important roles in several tissues and targeting these could possibly lead to undesired effects. For instance, this is the case of mTOR, which is a central regulator of metabolism. The mTOR inhibitor rapamycin improved the outcome of osteoarthritis in mice [47,48], but it has severe side effects on overall health. Accessibility is also a limiting factor. Intracellular molecules and transcription factors are often difficult to target in humans (RUNX2 [27] or HIF-2α [20,21]). The route of delivery and pharmacokinetics also present limitations: for instance, intra-articular injections may limit systemic toxicity, but they are painful and would not be tolerated by patients if required too frequently.

Hereafter we review, using three examples, the path that has led to clinical experimentation and, in some cases, successful clinical trials.

Blockade of WNT signalling

WNTs are a family of secreted morphogens originally discovered for their role in oncogenesis and subsequently well studied for their role in embryonic morphogenesis [49]. In the absence of WNTs, β-catenin is constitutively degraded. When so-called "canonical" WNTs such as WNT1 or WNT3A bind to frizzled (FZD) receptors and to their co-receptors LRP5 and LRP6, the molecular complex responsible for β-catenin degradation is disrupted. This causes β-catenin to accumulate in the cytoplasm, translocate to the nucleus where it binds to TCF/LEF transcription factors and contributes to the activation of target genes [49]. Other WNTs activate pathways collectively denominated "non-canonical", mediated by different co-receptors including ROR1 and ROR2 [50,51].

Early cell and developmental biology experiments showed that the canonical WNT pathway regulates skeletogenesis [24,52–54] and joint morphogenesis [9,10,24]. In broad terms, these studies suggested that activation of canonical WNT signalling inhibited chondrogenesis in progenitor cells and initiated hypertrophic differentiation in mature chondrocytes [24].

Wnt4, Wnt9A and *Wnt16* are the earliest markers of joint interzones [9,10]. Although WNT signalling is essential for joint formation [9,10,55–57], the specific functions of individual ligands and receptors are largely redundant. Their function in development and adulthood are summarized in table 2.

Table 2. Roles of WNTs in cartilage development and adulthood

Molecule	Development	Adult cartilage
WNT3A	Maintains mesenchymal stem cells in an immature state in the developing limb bud, in chicks induces expression of FGF8 in the apical ectodermal ridge, promoting its formation [58–60]	Dose-dependent activation of β-catenin pathway, increasing chondrocyte proliferation; and non-canonical Ca2+-CaMKII dependent pathways leading to dedifferentiation of chondrocytes [61].
WNT4	Expressed at site of future joint formation [10]. Promotes chondrocyte differentiation and hypertrophy [10], regulating the transition from pre-hypertrophy to hypertrophy [8], but is also not strictly required for normal joint formation [58].	Synergises with WNT9A in preventing ectopic chondrogenic differentiation of synovial MSCs [8]
WNT5A	Expressed in the perichondrium [62] and regulates limb outgrowth through the planar cell polarity pathway, by activating ROR2 and Vangl2 [63,64]. Inhibits chondrocyte transition from	Reduces *COL2A1* and *Aggrecan*, and induces MMP1 and MMP13 expression in osteoarthritic chondrocytes [67].

	a resting state to proliferative. It is required for the transition from proliferative to pre-hypertrophic state, but blocks hypertrophy. Knockout and overexpression of WNT5A show similar phenotypes *in vivo* [10,64–66].	
WNT8	Expressed in the perichondrium. Promotes chondrocyte hypertrophy and calcification [24].	
WNT9A	Expressed at site of future joint formation [10]. Blocks and can reverse chondrocyte differentiation [8–10]. Ectopic expression in chick limbs induces ectopic joint interzone formation [9,10] - but not required for joint formation [8].	Wnt9A deficient mice develop synovial chondromatosis [8]
WNT16	Early marker of joint interzone [9]. Prevents chondrocyte hypertrophy [44].	Buffers activation of β-catenin pathway by stronger canonical WNT ligands. Promotes cartilage homeostasis by maintaining Prg4+ expression [17], and prevents hypertrophy through regulating mTORC1-PTHrP [44].
Inactivation of β-catenin	Joint fusion and delayed endochondral ossification [9,24,55,68]. Reduced lubricin and COL2A1 expression [4].	Increased proteoglycan content and reduction in endochondral ossification *in-vitro* [24]. Increased chondrocyte apoptosis and articular cartilage destruction *in vivo* [57,69,70].
Over-activation of β-catenin signalling	Blocks and can reverse chondrocyte differentiation [4,8–10,71]. Ectopic expression of constitutively active β-catenin in chick limbs induced ectopic joint interzone formation [9,10] .	Promotes chondrocyte differentiation, hypertrophy and cartilage calcification [9,24,37,55–57,62]. ; Catabolism of cartilage extracellular matrix and chondrocyte apoptosis [54,57,72].

		Worse outcomes in rodent OA models with cartilage loss and osteophyte formation [54,73] .

In adult cartilage, both inhibition [70] and forced activation of β-catenin signalling [73] led to cartilage destruction in mice. This is because a population of cartilage-specific chondroprogenitors expressing *Prg4* [42] are dependent on β-catenin signalling [43] whereas excessive WNT activation inhibits chondrogenesis and drives hypertrophy in already differentiated chondrocytes [9,24,37,56].

Consequently, a "Goldilocks" theory took hold whereby WNT activation needs to be above a certain level in order to maintain progenitor cell populations, but below levels which drive hypertrophic differentiation in mature chondrocytes. Further complexity was added by the understanding that while a short burst of WNT activation supports articular cartilage formation and homeostasis [17,57], excessively prolonged activation resulted in cartilage breakdown. Interestingly, WNT16 is both required and sufficient for cartilage homeostasis following cartilage injury [17,44], and it is a partial activator of the canonical WNT pathway, maintaining "homeostatic levels" and preventing excessive activation from other more potent ligands [17,44].

The relevance of these findings to human osteoarthritis was confirmed by the association of allelic variants of WNT inhibitory molecules such as *FRZB* [74] and *DOT1L* [75] with osteoarthritis. These data were replicated in animal models [28,54].

These exciting data triggered the search for WNT inhibitors which could be used to treat osteoarthritis. Given the high level of redundancy of WNT ligands and receptors, it was unlikely that WNT inhibition outside or at the level of the cell membrane would be successful. Inhibition of WNT signalling through upregulating FRZB, by Verapamil [76], or with a small molecule XAV-939 [77] led to improved outcomes of osteoarthritis in animal models.

Deshmukh et al. identified a small compound (SM04690, now commercialized by SAMUMED as Lorecivivint) which, by inhibiting the intracellular kinases CLK2 and DYRK1A, inhibited WNT signalling downstream of β-catenin [78,79]. SM04690 proved to have a remarkable pharmacokinetic profile: after intra-articular administration in rats it could not be detected in plasma, it was detected just above therapeutic levels in bone, but it accumulated in cartilage for at least 180 days [79]. A single intra-articular administration of SM04690 improved structural outcomes in an instability-induced osteoarthritis model and improved structural outcomes, pain and weight-bearing in the monosodium iodoacetate model in rats [79]. In a phase I clinical trial, a single intra-articular injection of SM04690 proved to be safe after one year follow-up in patients with osteoarthritis (Kellgren-Lawrence score 2-3). Although the study was not designed for, and was vastly underpowered to test efficacy (61 patients in total followed up for one year), pain parameters were improved at all doses and joint space width was improved in the intermediate dose [80]. A phase II study, although it did not meet its primary endpoint, confirmed pain relief and some evidence of improvement of joint space width in patients with unilateral osteoarthritis at an intermediate dose [81].

Interestingly, and in keeping with the notion of a "Goldilocks zone" of WNT activation, the intermediate dose yielded the best results. Whether the long-term efficacy of this compound after a single injection is due to its long-term accumulation in cartilage [79] or that perhaps the initial delivery is sufficient to trigger a self-maintaining homeostatic cascade is unknown. Although these results are promising, phase III studies are required to demonstrate efficacy.

One important contribution of this paper was the improvement of the clinical trial technology in osteoarthritis. In particular, the finding that selecting patients with unilateral symptoms and without widespread pain allows a higher level of sensitivity, especially in terms of pain, will greatly facilitate future studies with this or other compounds.

TGF-β and bone morphogenetic proteins

In 1965 Urist and Daly published the seminal paper "Bone: formation by autoinduction" in which they described that ectopic implantation of demineralised bone matrix triggered ectopic endochondral bone formation, ultimately leading to the formation of an ossicle made by cells originating from the host organism [82]. They also correctly hypothesised that some substance contained in the acellular bone matrix would be responsible for initiating the morphogenetic events leading to bone formation. In the years that followed, several groups went on to identify a family of molecules, named Bone Morphogenetic Proteins (BMPs), which, when implanted in an appropriate matrix, resulted in ectopic cartilage and bone formation [83–85]. It became apparent that, far from being specific cartilage/bone growth factors, BMPs had a broad array of functions in different cells, stages of development and adult life. Regulation of the BMP pathway is essential for gastrulation, the formation of mesenchyme in early development, establishing the dorso/ventral patterning and the morphogenesis of virtually all organs [86,87].

In spite of relatively stereotypical, shared signalling mechanisms and similar *in vitro* effects on skeletal cells, the expression pattern and the biological activity of different BMPs *in vivo* varies enormously. This is exemplified by the elegant studies in the chick model in the Hurle laboratory in the mid 1990s [88]. Macias et al. showed that *BMP7* is expressed in the diaphyseal perichondrium, skipping the joint interzone, and when delivered next to a joint interzone, it inhibited joint formation. Conversely, *BMP2* was expressed in the joint interzone and its ectopic delivery resulted in ectopic joint-like structures [88].

Because of the capacity of BMPs to induce cartilage and bone formation, several products were generated and tested for the repair of critical size defects, non-unions and spinal fusions [89–92], however, over forty years after the discovery of BMPs, the results of clinical testing in osteoarthritis and chondral defects are underwhelming [93]. This could be due to several factors, but the stability of these molecules in inflammatory sites, their pleomorphic function in different settings and different cells, and especially their propensity to induce ectopic cartilage and bone represent serious issues [92,94]. Overexpression of BMP2 in adult joints resulted in ectopic cartilage and bone formation within the joint soft tissues [95] and additional cartilage degradation through upregulation of matrix metalloproteinases [96].

To circumvent this problem, investigators have tested the use of transforming growth factor (TGF)-β. TGF-β is a potent inducer of chondrocyte differentiation in a variety of stem cells, promoting *SOX9* expression, extracellular matrix production [97–99].

In humans, a genetic association of alleles of *TGF-β* and downstream molecules with osteoarthritis was identified [100]. In keeping with this, transgenic expression of a dominant negative Tgf-β receptor 2 (*Tgf-βr2)* led to formation of hypertrophic cartilage and an osteoarthritis-like phenotype [101]. However, *in vivo*, TGF-β overexpression exacerbated osteoarthritis, whereas its inhibition was beneficial in murine models [102]. This was true when using the TGF-β inhibitor halofuginone [103], an antibody against TGF-β1, systemic delivery of a TGF-βR1 inhibitor or by knocking out *Tgf-βr2* in nestin-positive MSCs [102].

The discrepancy between the requirement of TGF-β signalling in joint homeostasis and its negative effect when overexpressed in osteoarthritis remains enigmatic. One explanation was offered by Blaney-Davidson et al. who demonstrated that upregulation of ALK1 receptor switches TGF-β signalling from SMAD2/3 which inhibits chondrocyte hypertrophy to SMAD1/5/8 downstream, which induces chondrocyte hypertrophy [104,105].

The disappointing results of the use of TGF-β and BMPs in cartilage repair can be attributed to the fact that although BMP and TGF-β signalling are essential for cartilage morphogenesis and homeostasis, their function is tightly regulated both spatially and temporally, and inappropriate or excessive activation are detrimental.

FGF18

Fibroblast growth factor (FGF) signalling was one of the first pathways discovered to be activated by cartilage injury. It contributes to repair responses through release of FGF2 from the injured articular cartilage [106,107]. However, FGF2 activates both FGF receptor-1 (FGFR1) and FGFR3, the former associated with prevalently catabolic effects on articular cartilage and the latter promoting anabolic events [108,109].

Mutations of FGF receptors result in a variety of skeletal defects [110]. With few exceptions, mutations of *FGFR1* and *FGFR2* result in defects of skeletal elements that form through intramembranous bone formation (craniosynostoses and similar syndromes); whereas mutations in *FGFR3* result in dwarfisms caused by defects of the bones that form through endochondral bone formation. For all these reasons, FGFR3 signalling was considered a suitable target for articular cartilage homeostasis. Dominant activating mutations in *FGFR3* resulted in hypochondroplasia, achondroplasia [111] and thanatophoric dysplasia [112]. Recessive loss-of-function mutations of *FGFR3* resulted in camptodactyly, tall stature, scoliosis, and hearing loss syndrome (CATSHL syndrome) [113]. Mice lacking *Fgfr3* [114,115] had features resembling CATSHL syndrome, whereas mice with activating mutations of *Fgfr3* had features similar to achondroplasia [116]. The skeletal defects were due to a delay in chondrocyte hypertrophy and endochondral bone formation. Given the pathogenic role of ectopic chondrocyte hypertrophy in the adult articular cartilage, this property of FGFR3 signalling supported its activation to prevent osteoarthritis progression.

In addition to halting chondrocyte hypertrophy and endochondral bone formation, FGFR3 is essential for the differentiation of mesenchymal cells into chondrocytes, as limb bud mesenchymal cells from *Fgfr3* knockout mice failed to undergo chondrogenesis in 3D culture and to proliferate in monolayer [117]. Finally, the expression of *FGFR3* is associated with the capacity of adult articular chondrocytes to form stable articular-like cartilage *in vivo* [108] and to repair cartilage defects in goats [118] and humans [119,120].

Activating FGF receptors only in cartilage is difficult because FGFs are ubiquitous, pleiotropic and several FGF ligands can signal through multiple FGFRs. Fortunately, FGFR3 is mostly expressed in cartilage, its mutations result almost exclusively in skeletal phenotypes. FGF18, a selective ligand for FGFR3, promoted chondrocyte proliferation and differentiation [121] [117]. Alleles of *FGF18* were genetically associated with osteoarthritis in humans [100]. Therefore, recombinant FGF18 was tested as a therapeutic for osteoarthritis. Moore et al. showed that intra-articular injections of recombinant FGF18 induced chondrocyte proliferation and cartilage repair when delivered in a therapeutic regime in rats subjected to a severe model of instability-induced osteoarthritis [122].

Two large clinical trials provided evidence that treatment with intra-articular recombinant FGF18 (developed by Merck and Nordic Bioscience as Sprifermin) resulted in some degree of improvement of cartilage integrity in osteoarthritis [123,124]. However, symptomatic improvement which was suggested in the first trial (phase I) [124] was not replicated in the subsequent larger trial (phase II) [123]. Clearly, pain is a fundamental outcome for patients. We do not know whether the failure to detect pain improvement with FGF18 was due to trial design, for instance by not excluding patients with widespread chronic pain, or whether FGF18 treatment does not result in pain relief. Published pre-clinical data in animal models did not include pain measurements [122]. A corollary is that, in the absence of pain relief, it is difficult to judge the clinical relevance of the small degree of improvement in joint space narrowing reported by the authors.

Taken together, data on inhibition of WNT signalling and using FGF18 suggest that targeting homeostatic pathways has led us to turn the corner in developing pharmacological approaches for treating osteoarthritis. It is likely that improvements in clinical trial design and patient stratification will be key to measure efficacy.

From cell-based therapeutics to stem cell niches

In the mid-1990s Brittberg et al. successfully repaired full thickness cartilage defects by implanting autologous chondrocytes that had been briefly expanded *in vitro* [125]. Since then, autologous chondrocyte implantation (ACI) has been tested in multiple clinical trials and has resulted in good and persistent clinical and structural benefits [126,127]. Unfortunately, due to the autologous nature of the cells, these technologies are extremely laborious, costly, are not easily upscalable and therefore have not reached routine clinical application.

Transplantation of mesenchymal stem cells (MSCs) of various origins into cartilage defects have also shown some degree of efficacy, although the clinical trials so far have been much smaller than those with chondrocytes [128]. Stem cells are clonogenic cells that

have two remarkable features: the ability to differentiate into multiple lineages (multipotency) and the ability to simultaneously replenish the stem cell pool (self-renewal) [129]. Bone marrow mesenchymal cells, the first adult MSCs identified, were originally discovered as plastic adherent, non haematopoietic clonogenic cells that can differentiate into multiple skeletal lineages including adipogenic, osteogenic and chondrogenic [130–132].

The subsequent discovery that multiple stem cell populations persist within adult skeletal tissues [37,38,97,98,118,131] opened up the possibility of harnessing the regenerative machinery of the adult joint by recruiting local resident stem cells to the site of damage. Stem cells originating from different tissues - such as the synovial membrane [97], the periosteum [98] , the bone marrow [133] and the cartilage itself [42,118,134] have distinct "default" differentiation pathways [135–137]. The development of lineage tracking technologies enabled a much more sophisticated understanding of the nature and function of different progenitor lineages in development and in post-natal repair.

The pressing questions in order to pursue this opportunity are: why are there so many different stem cell types? Do they differ in their repair capacity? What are the molecules that govern their niches?

Progenitor lineages within the developing limb bud persist and contribute to healing in the adult skeleton

The development of the appendicular skeletal elements requires the proliferation and migration of mesenchymal cells from the LPM to form the limb bud [138–141]. The MSCs at the periphery of the limb bud are maintained in an undifferentiated state by molecular signals (specifically FGF8 and WNT3A) which are released from the apical ectodermal ridge (AER) and surrounding ectoderm respectively [58,142–146]. However, in the centre of the limb bud, away from the control of these signals, MSCs aggregate into the mesenchymal condensations to form the skeletal anlage. From the early stages of limb development, the anlage appears to be composed of distinct cell populations which give rise to specific cell lineages and sub-lineages, and ultimately to different skeletal structures (Fig 3) [2,60,140,147].

PRX1+ progenitors

Paired Mesoderm Homeobox 1 (*PRX1*) is one of the first progenitor cell markers that appears in the limb bud. *Prx1* is expressed as early as 9.5 days post coitum (dpc), stemming directly from the LPM, and by 10dpc it can be detected in almost all the skeletal mesenchyme in the limb, including the condensing mesenchyme, chondrocytes, periosteum and tendons [148–150]. By 15dpc however, the expression of *Prx1* becomes restricted to the periosteum and tendons [150]. The *Prx1*-expressing periosteal cells are maintained after birth and retain their capacity to differentiate into chondrocytes *in vitro*, suggesting that that these periosteum-*Prx1*+ cells are both chondro- and osteo-progenitor cells [91,150,151].

The generation of *Prx1-cre* mice and their crossing to suitable reporters have demonstrated that *Prx1*-lineage progenitor cells give rise to all the cell sub-lineages that contribute to the formation of the bony elements, the articular cartilage, tendons and ligaments [91,150]. *Prx1*+ cells also persist as MSCs postnatally within the periosteum where they contribute to callus formation after bone fracture [152].

The *Prx1* lineage arises from a still earlier mesenchymal lineage expressing platelet-derived growth factor receptor α (*Pdgfrα*) [153–155] which appear as early as 6.5 dpc. Interestingly, *Pdgfrα* is also a marker of progenitor cells which persist within the adult synovial membrane, and that contribute to cartilage repair [38].

Within the *Prx1*+ population, two distinct MSC populations emerge. *Osterix*+ cells give rise to calcified tissues including bone [156,157] and the calcified layer of the articular cartilage [42]; and *Gdf5*+ cells which give rise to the articular cartilage and other soft tissues of the joints including ligaments and tendons [4–6].

Osterix+ progenitors

During embryonic development *Osterix* (Osx) is expressed by both the epiphyseal chondrocytes where it promotes hypertrophic differentiation [157] and by an MSC population residing around the vessels of the perichondrium [156]. Subsequently, the *Osx*+ periosteal precursors enter the hypertrophic cartilage accompanying the ingrowing vessels and give rise to osteoblasts to form the bone replacing the hypertrophic cartilage [156].

Multiple separate cell lineages were detected within developing epiphyses including *Gremlin*+ [158], *Nestin*+ [159] and *LeptinR*+ [156,160,161] progenitor cells. Whereas their identity, marker profile, and differentiation potential has been well-studied in embryonic development, their persistence as distinct progenitor populations in adulthood and particularly their contribution to tissue repair is unclear. For instance, *Nestin*+ cells persist in the perivascular spaces of the periosteum [162] and in the adult synovial cells [38], however, in the absence of proper lineage tracking, their respective contribution to fracture and cartilage repair remains unconfirmed [38,162].

Scx+SOX9+ progenitors

Progenitor cells which contribute to the formation of tendons and their attachment to bones (entheses) express *Scleraxis* (*Scx*) and *Sox9* [163–166]. *Scx* was required for the formation of tendon-to-bone attachment sites [167]. TGF-β and BMP4 signalling were essential for the specification and differentiation of *Scx*+*Sox9*+ progenitors, respectively [167]. Further analysis was able to dissect another distinct subset of progenitors from this pool, those which are *Scx*+*Sox9*-; both *Scx*+*Sox9*+ and *Scx*+*Sox9*- progenitors can differentiate into tenocytes, however, those closest to the cartilaginous primordium are mostly derived from

the *Sox9*+ population [166]. The *Scx*+*Sox9*+ lineage persist in the adult enthesis and periosteum [164] and contribute to tendon and fracture healing [164,168–170].

Joint progenitors

The cells that form the adult articular cartilage derive from two main lineages: the superficial and intermediate zone are contributed from progenitor cells deriving from *Prg4*+ progenitors [4,42,43], whereas the deep layer, which is calcified, is contributed and renewed by *Osx*+ progenitors [42] and therefore can be considered a remnant of the epiphyseal growth plate.

GDF5+ progenitors

During embryonic development, *Gdf5/Cdmp1* is expressed within the portion of the cartilage anlage that forms the embryonic skeleton and is destined to give rise to the permanent articular cartilage. Lineage tracking experiments showed that all soft tissues (such as the articular cartilage, meniscus, synovial lining, and joint capsule) within the joint are composed of cells that, within the interzone, derive from the *Gdf5*+ progenitors [4–6]. Until recently, *Gdf5* lineage cells were thought to be determined early during embryonic development and represented a stable population. Challenging this hypothesis, Shwartz et al. elegantly demonstrated that there is a constant inflow of *Gdf5*-lineage cells recruited to the joint interzone throughout development from the surrounding *Sox9*+ mesenchyme and, depending on the time when such recruitment occurs, they contribute to different joint structures: the first cells to be recruited contribute to the epiphyses, then to the articular cartilage and the last ones to menisci and cruciate ligaments [6]. In addition, these studies demonstrated that far from being a “stable marker”, *Gdf5* expression in the interzone cells is transient.

Gdf5-lineage cells have recently been shown to persist in the adult joint in the perivascular spaces of the synovial membrane and to contribute to the repair of cartilage injuries [37,38].

LGR5+ progenitors

A subpopulation of *Gdf5* lineage progenitors acquire the expression of *Lgr5* and *Col22a1* and give rise specifically to cruciate ligaments, synovial membrane, and articular chondrocytes [171]. LGR5 is a receptor that amplifies the effect of β-catenin dependent WNT signalling through binding R-spondins [172], a signalling pathway that the progenitor cells of the superficial cartilage layer are strictly dependent on [43]. Implantation of embryonic *Lgr5*+ cells into an adult murine cartilage defect resulted in cartilage repair [171], however it is not known if *Lgr5*+ cells persist in the adult joint and, if so, whether they contribute to cartilage healing.

Interestingly, however, *Lgr5* expression is detected in the *Gdf5*-lineage population, prior to the expression of *Prg4* [171], an additional chondro-progenitor which largely resides in the surface of the articular cartilage.

Prg4+ progenitors

Prg4 (the gene encoding lubricin) is expressed in the mouse joint interzones starting from 15.5dpc [4]. With time, the expression of *Prg4* increases and *Gdf5* decreases [4]. PRG4 expression persists throughout adult life at the surface of the articular cartilage and within the synovium but is not detectable in the growth plate cartilage [173]. *Prg4*+ progenitor cells within the superficial layer of the articular cartilage contribute to the turnover of chondrocytes of the non-calcified cartilage layers throughout life [42] but it is likely that it is the synovium-residing *Prg4*+ progenitors that contribute to cartilage healing after injury [37]. By combining nucleoside labeling, *Prg4* lineage tracking and *Confetti* mice, Decker et al. showed that *Prg4*+ progenitors proliferated within the synovial membrane, migrated and contributed to repair of cartilage defects. *Prg4*+ cells residing within the cartilage adjacent to the defect sites did not proliferate or contribute to repair [37]. Interestingly, Seol et al showed that the transcription factor *Hmgb-1* released by dying chondrocytes functions as a chemoattractant for *Prg4*+ progenitor cells, thereby possibly supplying a mechanistic explanation of their recruitment to the site of damage [174].

The overlap between the *Gdf5*+ and the *Prg4*+ progenitor populations in mature cartilage is not definitively established, given the absence of dual lineage tracking, however, experiments from the Pacifici laboratory suggest that *Prg4* positivity is a feature of the differentiating *Gdf5*+ cells [4].

Summary

The identification of different stem cell lineages with unique roles during development, homeostasis or the repair of musculoskeletal tissues is a major step forward in regenerative medicine. The understanding of the molecular control of stem cell niches, their maintenance, proliferation, migration and differentiation is revealing molecular tools to trigger repair by mobilizing progenitor cells. This may enable timely morphogenesis of the repair tissues without the need for expensive and laborious cell manipulations outside of the body. These approaches will generate upscalable, affordable production of effective and safe off-the-shelf therapeutics for the treatment of cartilage defects or osteoarthritis.

Conclusions

As described in this review, several molecular targets are now available to treat cartilage degeneration. Clinical success will depend on our ability to understand the hierarchy of the homeostatic signals and the reason for repair failure in individual patients.

Molecular hierarchy. Interactions between the biological pathways and stem cells that contribute to skeletal homeostasis makes it hard to unpick the role of individual molecules, and the changing landscape of ligand/receptor/signalling molecule expression as cartilage degenerates must be carefully considered. The identification of key players that can initiate the repair cascade, from the recruitment of the stem cells from their niches to establishing morphogenesis of the repair tissues, without affecting the surrounding healthy tissues will be key to achieve affordable, effective and safe therapeutic interventions.

Patient stratification. Failure of repair/homeostasis in different patients may depend on different mechanisms. For instance some patients may fail to repair because of poor stem cell recruitment, while others will progress because of poor tissue patterning or failure of differentiation. The selection of the right treatment for the right patient can be achieved with the identification of downstream targets of the homeostatic signals which can be used to identify the failure mechanism and at the same time as surrogate efficacy markers.

Pharmacokinetics. The timing, duration, delivery method and dosing of the interventions within the appropriate tissues will be critical. For instance, a brief, well-dosed activation of WNT signalling is beneficial for cartilage health under certain conditions [57], but persistent WNT activation is detrimental and even a burst of activation in patients with already established osteoarthritis, where WNT signalling is already over-activated, may be detrimental. Understanding the pathological processes in individual patients, combined to the availability of controlled delivery systems will be key to success.

The understanding of the developmental mechanisms of joint morphogenesis has enabled the identification of individual targets and, we predict, will continue to be crucial to address some of these key issues.

References

1. Hunter W. VI. Of the structure and diseases of articu-lating cartilages. *Philos Trans R Soc Lond*. 1743;42(470):514-521. doi:10.1098/rstl.1742.0079
2. Decker RS. Articular cartilage and joint development from embryogenesis to adulthood. *Semin Cell Dev Biol*. 2017;62:50-56. doi:10.1016/j.semcdb.2016.10.005
3. Long F, Ornitz DM. Development of the Endochondral Skeleton. *Cold Spring Harb Perspect Biol*. 2013;5(1):a008334. doi:10.1101/cshperspect.a008334
4. Koyama E, Shibukawa Y, Nagayama M, et al. A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. *Dev Biol*. 2008;316(1):62-73. doi:10.1016/j.ydbio.2008.01.012
5. Rountree RB, Schoor M, Chen H, et al. BMP receptor signaling is required for postnatal maintenance of articular cartilage. *PLoS Biol*. 2004;2(11):e355. doi:10.1371/journal.pbio.0020355
6. Shwartz Y, Viukov S, Krief S, Zelzer E. Joint Development Involves a Continuous Influx of Gdf5-Positive Cells. *Cell Rep*. 2016;15(12):2577-2587. doi:10.1016/j.celrep.2016.05.055
7. Shwartz Y, Zelzer E. Nonradioactive In Situ Hybridization on Skeletal Tissue Sections. In: Hilton MJ, ed. *Skeletal Development and Repair*. Vol 1130. Humana Press; 2014:203-215. doi:10.1007/978-1-62703-989-5_15
8. Später D, Hill TP, O'sullivan RJ, Gruber M, Conner DA, Hartmann C. Wnt9a signaling is required for joint integrity and regulation of Ihh during chondrogenesis. *Dev Camb Engl*. 2006;133(15):3039-3049. doi:10.1242/dev.02471
9. Guo X, Day TF, Jiang X, Garrett-Beal L, Topol L, Yang Y. Wnt/beta-catenin signaling is sufficient and necessary for synovial joint formation. *Genes Dev*. 2004;18(19):2404-2417. doi:10.1101/gad.1230704
10. Hartmann C, Tabin CJ. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. *Cell*. 2001;104(3):341-351.
11. Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee SJ. Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. *Nature*. 1994;368(6472):639-643. doi:10.1038/368639a0
12. Kan A, Tabin CJ. c-Jun is required for the specification of joint cell fates. *Genes Dev*. 2013;27(5):514-524. doi:10.1101/gad.209239.112
13. Verzijl N, DeGroot J, Thorpe SR, et al. Effect of Collagen Turnover on the Accumulation of Advanced Glycation End Products. *J Biol Chem*. 2000;275(50):39027-39031. doi:10.1074/jbc.M006700200
14. Dell'accio F, Vincent TL. Joint surface defects: clinical course and cellular response in spontaneous and experimental lesions. *Eur Cell Mater*. 2010;20:210-217.
15. Dell'Accio F, De Bari C, Eltawil NM, Vanhummelen P, Pitzalis C. Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis. *Arthritis Rheum*. 2008;58(5):1410–1421.
16. Eltawil NM, De Bari C, Achan P, Pitzalis C, Dell'accio F. A novel in vivo murine model of cartilage regeneration. Age and strain-dependent outcome after joint surface injury. *Osteoarthr Cartil OARS Osteoarthr Res Soc*. 2009;17(6):695-704. doi:10.1016/j.joca.2008.11.003
17. Nalesso G, Thomas BL, Sherwood JC, et al. WNT16 antagonises excessive canonical WNT activation and protects cartilage in osteoarthritis. *Ann Rheum Dis*.

2017;76(1):218-226. doi:10.1136/annrheumdis-2015-208577
18. Sherwood J, Bertrand J, Nalesso G, et al. A homeostatic function of CXCR2 signalling in articular cartilage. *Ann Rheum Dis*. 2015;74(12):2207-2215. doi:10.1136/annrheumdis-2014-205546
19. Bertrand J, Fürst M, Rüther W, et al. Loss of NPP1 induces cartilage remodelling into bone and OA-like changes in mice. *Ann Rheum Dis*. 2011;70(Suppl 2):A18–A18.
20. Saito T, Fukai A, Mabuchi A, et al. Transcriptional regulation of endochondral ossification by HIF-2α during skeletal growth and osteoarthritis development. *Nat Med*. 2010;16(6):678-686. doi:10.1038/nm.2146
21. Yang S, Kim J, Ryu J-H, et al. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med*. 2010;16(6):687-693. doi:10.1038/nm.2153
22. Hiligsmann M, Cooper C, Arden N, et al. Health economics in the field of osteoarthritis: an expert's consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin Arthritis Rheum*. 2013;43(3):303-313. doi:10.1016/j.semarthrit.2013.07.003
23. Cornelis FMF, de Roover A, Storms L, Hens A, Lories RJ, Monteagudo S. Increased susceptibility to develop spontaneous and post-traumatic osteoarthritis in Dot1l-deficient mice. *Osteoarthritis Cartilage*. 2019;27(3):513-525. doi:10.1016/j.joca.2018.11.008
24. Enomoto-Iwamoto M, Kitagaki J, Koyama E, et al. The Wnt Antagonist Frzb-1 Regulates Chondrocyte Maturation and Long Bone Development during Limb Skeletogenesis. *Dev Biol*. 2002;251(1):142-156. doi:10.1006/dbio.2002.0802
25. Karaplis AC, Luz A, Glowacki J, et al. Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes Dev*. 1994;8(3):277-289. doi:10.1101/gad.8.3.277
26. Lanske B, Karaplis AC, Lee K, et al. PTH/PTHrP Receptor in Early Development and Indian Hedgehog--Regulated Bone Growth. *Science*. 1996;273(5275):663-666. doi:10.1126/science.273.5275.663
27. Liao L, Zhang S, Gu J, et al. Deletion of Runx2 in Articular Chondrocytes Decelerates the Progression of DMM-Induced Osteoarthritis in Adult Mice. *Sci Rep*. 2017;7(1):1-12. doi:10.1038/s41598-017-02490-w
28. Monteagudo S, Cornelis FMF, Aznar-Lopez C, et al. DOT1L safeguards cartilage homeostasis and protects against osteoarthritis. *Nat Commun*. 2017;8:15889. doi:10.1038/ncomms15889
29. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science*. 1996;273(5275):613-622. doi:10.1126/science.273.5275.613
30. Caramés B, Hasegawa A, Taniguchi N, Miyaki S, Blanco FJ, Lotz M. Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. *Ann Rheum Dis*. 2012;71(4):575–81. doi:10.1136/annrheumdis-2011-200557
31. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier J-P, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):33-42. doi:10.1038/nrrheum.2010.196
32. Lotz M, Hashimoto S, Kühn K. Mechanisms of chondrocyte apoptosis. *Osteoarthritis Cartilage*. 1999;7(4):389-391. doi:10.1053/joca.1998.0220
33. Lu W, Shi J, Zhang J, et al. CXCL12/CXCR4 Axis Regulates Aggrecanase Activation and Cartilage Degradation in a Post-Traumatic Osteoarthritis Rat Model. *Int J Mol Sci*.

2016;17(10). doi:10.3390/ijms17101522
34. Merino R, Gañán Y, Macias D, Rodríguez-León J, Hurle JM. Bone morphogenetic proteins regulate interdigital cell death in the avian embryo. *Ann N Y Acad Sci*. 1999;887:120-132. doi:10.1111/j.1749-6632.1999.tb07927.x
35. Ren G, Whittaker JL, Leonard C, et al. CCL22 is a biomarker of cartilage injury and plays a functional role in chondrocyte apoptosis. *Cytokine*. 2019;115:32-44. doi:10.1016/j.cyto.2018.11.030
36. Zhang Y, Vasheghani F, Li Y-H, et al. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. *Ann Rheum Dis*. 2015;74(7):1432-1440. doi:10.1136/annrheumdis-2013-204599
37. Decker RS, Um H-B, Dyment NA, et al. Cell origin, volume and arrangement are drivers of articular cartilage formation, morphogenesis and response to injury in mouse limbs. *Dev Biol*. 2017;426(1):56-68. doi:10.1016/j.ydbio.2017.04.006
38. Roelofs AJ, Zupan J, Riemen AHK, et al. Joint morphogenetic cells in the adult mammalian synovium. *Nat Commun*. 2017;8:ncomms15040. doi:10.1038/ncomms15040
39. Soul J, Dunn SL, Anand S, et al. Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage. *Ann Rheum Dis*. 2018;77(3):423. doi:10.1136/annrheumdis-2017-212603
40. Baffi MO, Slattery E, Sohn P, Moses HL, Chytil A, Serra R. Conditional deletion of the TGF-beta type II receptor in Col2a expressing cells results in defects in the axial skeleton without alterations in chondrocyte differentiation or embryonic development of long bones. *Dev Biol*. 2004;276(1):124–42. doi:10.1016/j.ydbio.2004.08.027
41. Dell'Accio F, De Bari C, El Tawil NM, et al. Activation of WNT and BMP signaling in adult human articular cartilage following mechanical injury. *Arthritis Res Ther*. 2006;8(5):R139.
42. Kozhemyakina E, Zhang M, Ionescu A, et al. Identification of a Prg4-Expressing Articular Cartilage Progenitor Cell Population in Mice. *Arthritis Rheumatol*. 2015;67(5):1261-1273. doi:10.1002/art.39030
43. Yasuhara R, Ohta Y, Yuasa T, et al. Roles of β-catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. *Lab Investig J Tech Methods Pathol*. 2011;91(12):1739-1752. doi:10.1038/labinvest.2011.144
44. Tong W, Zeng Y, Chow DHK, et al. Wnt16 attenuates osteoarthritis progression through a PCP/JNK-mTORC1-PTHrP cascade. *Ann Rheum Dis*. Published online February 11, 2019. doi:10.1136/annrheumdis-2018-214200
45. Schipani E, Lanske B, Hunzelman J, et al. Targeted expression of constitutively active receptors for parathyroid hormone and parathyroid hormone-related peptide delays endochondral bone formation and rescues mice that lack parathyroid hormone-related peptide. *Proc Natl Acad Sci*. 1997;94(25):13689-13694. doi:10.1073/pnas.94.25.13689
46. Sampson ER, Hilton MJ, Tian Y, et al. Teriparatide as a chondroregenerative therapy for injury-induced osteoarthritis. *Sci Transl Med*. 2011;3(101):101ra93. doi:10.1126/scitranslmed.3002214
47. Carames B, Taniguchi N, Seino D, Blanco FJ, D'Lima D, Lotz M. Mechanical injury suppresses autophagy regulators and its pharmacological activation results in chondroprotection. *Arthritis Rheum*. 2011;(1529-0131 (Electronic)).
48. Caramés B, Taniguchi N, Otsuki S, et al. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis.

Arthritis Rheum. 2010;62(1529-0131 (Electronic)):791–801. doi:10.1002/art.27305
49. Nusse R, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed: Three decades of Wnts: a personal perspective. *EMBO J*. 2012;31(12):2670-2684. doi:10.1038/emboj.2012.146
50. DeChiara TM, Kimble RB, Poueymirou WT, et al. Ror2, encoding a receptor-like tyrosine kinase, is required for cartilage and growth plate development. *Nat Genet*. 2000;24(3):271-274. doi:10.1038/73488
51. Grumolato L, Liu G, Mong P, et al. Canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors. *Genes Dev*. 2010;24(22):2517-2530. doi:10.1101/gad.1957710
52. Hoang B, Moos M, Vukicevic S, Luyten FP. Primary Structure and Tissue Distribution of FRZB, a Novel Protein Related to Drosophila Frizzled, Suggest a Role in Skeletal Morphogenesis. *J Biol Chem*. 1996;271(42):26131-26137. doi:10.1074/jbc.271.42.26131
53. Lin K, Wang S, Julius MA, Kitajewski J, Moos M, Luyten FP. The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of Wnt signaling. *Proc Natl Acad Sci U S A*. 1997;94(21):11196-11200. doi:10.1073/pnas.94.21.11196
54. Lories RJU, Peeters J, Bakker A, et al. Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. *Arthritis Rheum*. 2007;56(12):4095-4103. doi:10.1002/art.23137
55. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell*. 2005;8(5):739-750. doi:10.1016/j.devcel.2005.03.016
56. Dong Y-F, Soung DY, Schwarz EM, O'Keefe RJ, Drissi H. Wnt induction of chondrocyte hypertrophy through the Runx2 transcription factor. *J Cell Physiol*. 2006;208(1):77-86. doi:10.1002/jcp.20656
57. Yuasa T, Kondo N, Yasuhara R, et al. Transient activation of Wnt/{beta}-catenin signaling induces abnormal growth plate closure and articular cartilage thickening in postnatal mice. *Am J Pathol*. 2009;175(5):1993-2003. doi:10.2353/ajpath.2009.081173
58. Kawakami Y, Capdevila J, Büscher D, Itoh T, Rodríguez Esteban C, Izpisúa Belmonte JC. WNT signals control FGF-dependent limb initiation and AER induction in the chick embryo. *Cell*. 2001;104(6):891-900. doi:10.1016/s0092-8674(01)00285-9
59. Kengaku M, Capdevila J, Rodriguez-Esteban C, et al. Distinct WNT pathways regulating AER formation and dorsoventral polarity in the chick limb bud. *Science*. 1998;280(5367):1274-1277. doi:10.1126/science.280.5367.1274
60. Tickle C, Münsterberg A. Vertebrate limb development--the early stages in chick and mouse. *Curr Opin Genet Dev*. 2001;11(4):476-481. doi:10.1016/s0959-437x(00)00220-3
61. Nalesso G, Sherwood J, Bertrand J, et al. WNT-3A modulates articular chondrocyte phenotype by activating both canonical and noncanonical pathways. *J Cell Biol*. 2011;193(3):551-564. doi:10.1083/jcb.201011051
62. Hartmann C, Tabin CJ. Dual roles of Wnt signaling during chondrogenesis in the chicken limb. *Dev Camb Engl*. 2000;127(14):3141-3159.
63. Gao B, Song H, Bishop K, et al. Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. *Dev Cell*. 2011;20(2):163-176. doi:10.1016/j.devcel.2011.01.001
64. Kawakami Y, Wada N, Nishimatsu SI, Ishikawa T, Noji S, Nohno T. Involvement of

Wnt-5a in chondrogenic pattern formation in the chick limb bud. *Dev Growth Differ*. 1999;41(1):29-40. doi:10.1046/j.1440-169x.1999.00402.x
65. Bradley EW, Drissi MH. WNT5A regulates chondrocyte differentiation through differential use of the CaN/NFAT and IKK/NF-kappaB pathways. *Mol Endocrinol Baltim Md*. 2010;24(8):1581–93. doi:10.1210/me.2010-0037
66. Yang Y, Topol L, Lee H, Wu J. Wnt5a and Wnt5b exhibit distinct activities in coordinating chondrocyte proliferation and differentiation. *Dev Camb Engl*. 2003;130(5):1003-1015. doi:10.1242/dev.00324
67. Huang G, Chubinskaya S, Liao W, Loeser RF. Wnt5a induces catabolic signaling and matrix metalloproteinase production in human articular chondrocytes. *Osteoarthritis Cartilage*. 2017;25(9):1505-1515. doi:10.1016/j.joca.2017.05.018
68. Akiyama H, Lyons JP, Mori-Akiyama Y, et al. Interactions between Sox9 and β-catenin control chondrocyte differentiation. *Genes Dev*. 2004;18(9):1072–1087.
69. Chen LX, Lin L, Wang HJ, et al. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. *Osteoarthr Cartil OARS Osteoarthr Res Soc*. 2008;16(2):174–84. doi:10.1016/j.joca.2007.06.006
70. Zhu M, Chen M, Zuscik M, et al. Inhibition of beta-catenin signaling in articular chondrocytes results in articular cartilage destruction. *Arthritis Rheum*. 2008;58(7):2053-2064. doi:10.1002/art.23614
71. Ryu J-H, Kim S-J, Kim S-H, et al. Regulation of the chondrocyte phenotype by beta-catenin. *Dev Camb Engl*. 2002;129(23):5541-5550. doi:10.1242/dev.00110
72. Tamamura Y, Otani T, Kanatani N, et al. Developmental regulation of Wnt/beta-catenin signals is required for growth plate assembly, cartilage integrity, and endochondral ossification. *J Biol Chem*. 2005;280(19):19185-19195. doi:10.1074/jbc.M414275200
73. Zhu M, Tang D, Wu Q, et al. Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. *J Bone Miner Res Off J Am Soc Bone Miner Res*. 2009;24(1):12-21. doi:10.1359/jbmr.080901
74. Loughlin J, Dowling B, Chapman K, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *Proc Natl Acad Sci U S A*. 2004;101(26):9757-9762. doi:10.1073/pnas.0403456101
75. Castaño Betancourt MC, Cailotto F, Kerkhof HJ, et al. Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis. *Proc Natl Acad Sci U S A*. 2012;109(21):8218-8223. doi:10.1073/pnas.1119899109
76. Takamatsu A, Ohkawara B, Ito M, et al. Verapamil protects against cartilage degradation in osteoarthritis by inhibiting Wnt/β-catenin signaling. *PloS One*. 2014;9(3):e92699. doi:10.1371/journal.pone.0092699
77. Lietman C, Wu B, Lechner S, et al. Inhibition of Wnt/β-catenin signaling ameliorates osteoarthritis in a murine model of experimental osteoarthritis. *JCI Insight*. 2018;3(3). doi:10.1172/jci.insight.96308
78. Deshmukh V, O'Green AL, Bossard C, et al. Modulation of the Wnt pathway through inhibition of CLK2 and DYRK1A by lorecivivint as a novel, potentially disease-modifying approach for knee osteoarthritis treatment. *Osteoarthritis Cartilage*. 2019;27(9):1347-1360. doi:10.1016/j.joca.2019.05.006
79. Deshmukh V, Hu H, Barroga C, et al. A Small Molecule Inhibitor of the Wnt Pathway

(SM04690) As a Potential Disease Modifying Agent for the Treatment of Osteoarthritis of the Knee. *Osteoarthritis Cartilage*. Published online September 6, 2017. doi:10.1016/j.joca.2017.08.015

80. Yazici Y, McAlindon TE, Fleischmann R, et al. A novel Wnt pathway inhibitor, SM04690, for the treatment of moderate to severe osteoarthritis of the knee: results of a 24-week, randomized, controlled, phase 1 study. *Osteoarthritis Cartilage*. Published online July 13, 2017. doi:10.1016/j.joca.2017.07.006
81. Yazici Y, McAlindon TE, Gibofsky A, et al. Lorecivivint, a Novel Intra-articular CLK/DYRK1A Inhibitor and Wnt Pathway Modulator for Treatment of Knee Osteoarthritis: A Phase 2 Randomized Trial. *Arthritis Rheumatol*. 2020;n/a(n/a). doi:10.1002/art.41315
82. Urist MR. Bone: formation by autoinduction. *Science*. 1965;150(3698):893-899. doi:10.1126/science.150.3698.893
83. Wang EA, Rosen V, Cordes P, et al. Purification and characterization of other distinct bone-inducing factors. *Proc Natl Acad Sci U S A*. 1988;85(24):9484-9488. doi:10.1073/pnas.85.24.9484
84. Wozney JM. Overview of bone morphogenetic proteins. *Spine*. 2002;27(16 Suppl 1):S2-8. doi:10.1097/00007632-200208151-00002
85. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. *Science*. 1988;242(4885):1528-1534. doi:10.1126/science.3201241
86. Hogan BL. Bone morphogenetic proteins in development. *Curr Opin Genet Dev*. 1996;6(4):432-438. doi:10.1016/S0959-437X(96)80064-5
87. Jones CM, Dale L, Hogan BL, Wright CV, Smith JC. Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of Xenopus embryos. *Development*. 1996;122(5):1545-1554.
88. Macias D, Gañan Y, Sampath TK, Piedra ME, Ros MA, Hurle JM. Role of BMP-2 and OP-1 (BMP-7) in programmed cell death and skeletogenesis during chick limb development. *Dev Camb Engl*. 1997;124(6):1109-1117.
89. Ali IHA, Brazil DP. Bone morphogenetic proteins and their antagonists: current and emerging clinical uses. *Br J Pharmacol*. 2014;171(15):3620-3632. doi:10.1111/bph.12724
90. Gautschi OP, Frey SP, Zellweger R. Bone morphogenetic proteins in clinical applications. *ANZ J Surg*. 2007;77(8):626-631. doi:10.1111/j.1445-2197.2007.04175.x
91. Salazar VS, Gamer LW, Rosen V. BMP signalling in skeletal development, disease and repair. *Nat Rev Endocrinol*. 2016;12(4):203-221. doi:10.1038/nrendo.2016.12
92. Wozney JM, Seeherman HJ. Protein-based tissue engineering in bone and cartilage repair. *Curr Opin Biotechnol*. 2004;15(5):392-398. doi:10.1016/j.copbio.2004.08.001
93. Hunter DJ, Pike MC, Jonas BL, Kissin E, Krop J, McAlindon T. Phase 1 safety and tolerability study of BMP-7 in symptomatic knee osteoarthritis. *BMC Musculoskelet Disord*. 2010;11:232. doi:10.1186/1471-2474-11-232
94. van der Kraan PM, Blaney Davidson EN, van den Berg WB. Bone morphogenetic proteins and articular cartilage: To serve and protect or a wolf in sheep clothing's? *Osteoarthr Cartil OARS Osteoarthr Res Soc*. 2010;18(6):735-741. doi:10.1016/j.joca.2010.03.001
95. Blaney Davidson EN, Vitters EL, Bennink MB, et al. Inducible chondrocyte-specific overexpression of BMP2 in young mice results in severe aggravation of osteophyte formation in experimental OA without altering cartilage damage. *Ann Rheum Dis*.

2014;16(3):210. doi:10.1136/annrheumdis-2013-204528
96. Blaney Davidson EN, Vitters EL, van Lent PL, van de Loo FA, van Den Berg WB, van der Kraan PM. Elevated extracellular matrix production and degradation upon bone morphogenetic protein-2 (BMP-2) stimulation point toward a role for BMP-2 in cartilage repair and remodeling. *Arthritis ResTher*. 2007;9(1478-6362 (Electronic) LA-eng PT-Comparative Study PT-Journal Article PT-Research Support, Non-U.S. Gov't RN-0 (BMP2 protein, human) RN-0 (Bmp2 protein, mouse) RN-0 (Bone Morphogenetic Protein 2) RN-0 (Bone Morphogenetic Proteins) RN-0 ():R102.
97. De Bari C, Dell'Accio F, Neys J, Luyten F. Human synovial membrane-derived mesenchymal stem cells for skeletal muscle repair. In: *Arthritis and Rheumatism*. Vol 44. WILEY-LISS DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA; 2001:S101–S101.
98. De Bari C, Dell'Accio F, Luyten FP. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis Rheum*. 2001;44(1):85–95.
99. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng*. 1998;4(4):415-428. doi:10.1089/ten.1998.4.415
100. Tachmazidou I, Hatzikotoulas K, Southam L, et al. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. *Nat Genet*. 2019;51(2):230-236. doi:10.1038/s41588-018-0327-1
101. Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol*. 1997;139(2):541-552. doi:10.1083/jcb.139.2.541
102. Zhen G, Wen C, Jia X, et al. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. *Nat Med*. 2013;19(6):704-712. doi:10.1038/nm.3143
103. Cui Z, Crane J, Xie H, et al. Halofuginone attenuates osteoarthritis by inhibition of TGF-β activity and H-type vessel formation in subchondral bone. *Ann Rheum Dis*. 2016;75(9):1714-1721. doi:10.1136/annrheumdis-2015-207923
104. Blaney Davidson EN, Remst DFG, Vitters EL, et al. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol Baltim Md 1950*. 2009;182(12):7937–45. doi:10.4049/jimmunol.0803991
105. Zhao W, Wang T, Luo Q, et al. Cartilage degeneration and excessive subchondral bone formation in spontaneous osteoarthritis involves altered TGF-β signaling. *J Orthop Res Off Publ Orthop Res Soc*. 2016;34(5):763-770. doi:10.1002/jor.23079
106. Chia S-L, Sawaji Y, Burleigh A, et al. Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in murine osteoarthritis. *Arthritis Rheum*. 2009;60(7):2019-2027. doi:10.1002/art.24654
107. Vincent T, Hermansson M, Bolton M, Wait R, Saklatvala J. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. *Proc Natl Acad Sci U S A*. 2002;99(12):8259-8264. doi:10.1073/pnas.122033199
108. Dell'Accio F, De Bari C, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis Rheum*. 2001;44(7):1608-1619. doi:10.1002/1529-0131(200107)44:7<1608::AID-ART284>3.0.CO;2-T

109. Ellman MB, Yan D, Ahmadinia K, Chen D, An HS, Im HJ. Fibroblast growth factor control of cartilage homeostasis. *J Cell Biochem*. 2013;114(4):735-742. doi:10.1002/jcb.24418
110. Passos-Bueno MR, Wilcox WR, Jabs EW, Sertié AL, Alonso LG, Kitoh H. Clinical spectrum of fibroblast growth factor receptor mutations. *Hum Mutat*. 1999;14(2):115-125. doi:10.1002/(SICI)1098-1004(1999)14:2<115::AID-HUMU3>3.0.CO;2-2
111. Shiang R, Thompson LM, Zhu YZ, et al. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*. 1994;78(2):335-342. doi:10.1016/0092-8674(94)90302-6
112. Tavormina PL, Shiang R, Thompson LM, et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nat Genet*. 1995;9(3):321-328. doi:10.1038/ng0395-321
113. Toydemir RM, Brassington AE, Bayrak-Toydemir P, et al. A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet*. 2006;79(5):935-941. doi:10.1086/508433
114. Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nat Genet*. 1996;12(4):390-397. doi:10.1038/ng0496-390
115. Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. *Cell*. 1996;84(6):911-921. doi:10.1016/s0092-8674(00)81069-7
116. Li C, Chen L, Iwata T, Kitagawa M, Fu XY, Deng CX. A Lys644Glu substitution in fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors. *Hum Mol Genet*. 1999;8(1):35-44. doi:10.1093/hmg/8.1.35
117. Davidson D, Blanc A, Filion D, et al. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. *J Biol Chem*. 2005;280(21):20509-20515. doi:10.1074/jbc.M410148200
118. Dell'Accio F, Vanlauwe J, Bellemans J, Neys J, De Bari C, Luyten FP. Expanded phenotypically stable chondrocytes persist in the repair tissue and contribute to cartilage matrix formation and structural integration in a goat model of autologous chondrocyte implantation. *J Orthop Res*. 2003;21(1):123–131.
119. Saris DBF, Vanlauwe J, Victor J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. *Am J Sports Med*. 2009;37 Suppl 1:10S-19S. doi:10.1177/0363546509350694
120. Vanlauwe J, Saris DBF, Victor J, et al. Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. *Am J Sports Med*. 2011;39(12):2566-2574. doi:10.1177/0363546511422220
121. Ohbayashi N, Shibayama M, Kurotaki Y, et al. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. *Genes Dev*. 2002;16(7):870-879. doi:10.1101/gad.965702
122. Moore EE, Bendele AM, Thompson DL, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage*. 2005;13(7):623-631. doi:10.1016/j.joca.2005.03.003
123. Hochberg MC, Guermazi A, Guehring H, et al. Effect of Intra-Articular Sprifermin vs Placebo on Femorotibial Joint Cartilage Thickness in Patients With Osteoarthritis: The

FORWARD Randomized Clinical Trial. *JAMA*. 2019;322(14):1360-1370. doi:10.1001/jama.2019.14735
124. Lohmander LS, Hellot S, Dreher D, et al. Intraarticular Sprifermin (Recombinant Human Fibroblast Growth Factor 18) in Knee Osteoarthritis: A Randomized, Double-Blind, Placebo-Controlled Trial. *Arthritis Rheumatol*. 2014;66(7):1820-1831. doi:10.1002/art.38614
125. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *NEnglJ Med*. 1994;331(0028-4793 LA-eng PT-Journal Article SB-AIM SB-IM):889–895.
126. Ogura T, Mosier BA, Bryant T, Minas T. A 20-Year Follow-up After First-Generation Autologous Chondrocyte Implantation. *Am J Sports Med*. 2017;45(12):2751-2761. doi:10.1177/0363546517716631
127. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous Chondrocyte Implantation: A Long-term Follow-up. *Am J Sports Med*. Published online February 24, 2010. doi:10.1177/0363546509357915
128. Kim SH, Ha C-W, Park Y-B, Nam E, Lee J-E, Lee H-J. Intra-articular injection of mesenchymal stem cells for clinical outcomes and cartilage repair in osteoarthritis of the knee: a meta-analysis of randomized controlled trials. *Arch Orthop Trauma Surg*. 2019;139(7):971-980. doi:10.1007/s00402-019-03140-8
129. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science*. 2000;287(5457):1442-1446. doi:10.1126/science.287.5457.1442
130. Caplan AI. Mesenchymal stem cells. *J Orthop Res Off Publ Orthop Res Soc*. 1991;9(5):641-650. doi:10.1002/jor.1100090504
131. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A Quadripotential Mesenchymal Progenitor Cell Isolated from the Marrow of an Adult Mouse. *J Bone Miner Res*. 1999;14(5):700-709. doi:10.1359/jbmr.1999.14.5.700
132. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966;16(3):381-390.
133. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*. 2001;105(3):369-377. doi:10.1016/s0092-8674(01)00328-2
134. Dowthwaite GP, Bishop JC, Redman SN, et al. The surface of articular cartilage contains a progenitor cell population. *J Cell Sci*. 2004;117(Pt 6):889-897. doi:10.1242/jcs.00912
135. De Bari C, Dell'Accio F, Karystinou A, et al. A biomarker-based mathematical model to predict bone-forming potency of human synovial and periosteal mesenchymal stem cells. *Arthritis Rheum*. 2008;58(1):240–250.
136. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum*. 2005;52(8):2521-2529. doi:10.1002/art.21212
137. Shirasawa S, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells. *J Cell Biochem*. 2006;97(1):84-97. doi:10.1002/jcb.20546
138. Jin L, Wu J, Bellusci S, Zhang J-S. Fibroblast Growth Factor 10 and Vertebrate Limb Development. *Front Genet*. 2019;9. doi:10.3389/fgene.2018.00705

139. Ohuchi H, Nakagawa T, Yamamoto A, et al. The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. *Dev Camb Engl*. 1997;124(11):2235-2244.
140. Shimizu H, Yokoyama S, Asahara H. Growth and differentiation of the developing limb bud from the perspective of chondrogenesis. *Dev Growth Differ*. 2007;49(6):449-454. doi:10.1111/j.1440-169X.2007.00945.x
141. Vogel A, Rodriguez C, Izpisua-Belmonte JC. Involvement of FGF-8 in initiation, outgrowth and patterning of the vertebrate limb. *Development*. 1996;122(6):1737-1750.
142. Hall BK, Miyake T. Divide, accumulate, differentiate: cell condensation in skeletal development revisited. *Int J Dev Biol*. 1995;39(6):881-893.
143. Kumar D, Lassar AB. Fibroblast growth factor maintains chondrogenic potential of limb bud mesenchymal cells by modulating DNMT3A recruitment. *Cell Rep*. 2014;8(5):1419-1431. doi:10.1016/j.celrep.2014.07.038
144. Marín-Llera JC, Garciadiego-Cázares D, Chimal-Monroy J. Understanding the Cellular and Molecular Mechanisms That Control Early Cell Fate Decisions During Appendicular Skeletogenesis. *Front Genet*. 2019;10. doi:10.3389/fgene.2019.00977
145. Tabin C, Wolpert L. Rethinking the proximodistal axis of the vertebrate limb in the molecular era. *Genes Dev*. 2007;21(12):1433-1442. doi:10.1101/gad.1547407
146. ten Berge D, Brugmann SA, Helms JA, Nusse R. Wnt and FGF signals interact to coordinate growth with cell fate specification during limb development. *Dev Camb Engl*. 2008;135(19):3247-3257. doi:10.1242/dev.023176
147. Zeller R, López-Ríos J, Zuniga A. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nat Rev Genet*. 2009;10(12):845-858. doi:10.1038/nrg2681
148. Cserjesi P, Lilly B, Bryson L, Wang Y, Sassoon DA, Olson EN. MHox: a mesodermally restricted homeodomain protein that binds an essential site in the muscle creatine kinase enhancer. *Dev Camb Engl*. 1992;115(4):1087-1101.
149. Jägle U, Gasser JA, Müller M, Kinzel B. Conditional transgene expression mediated by the mouse β-actin locus. *genesis*. 2007;45(11):659-666. doi:10.1002/dvg.20342
150. Logan M, Martin JF, Nagy A, Lobe C, Olson EN, Tabin CJ. Expression of Cre recombinase in the developing mouse limb bud driven by a Prxl enhancer. *genesis*. 2002;33(2):77-80. doi:10.1002/gene.10092
151. Kawanami A, Matsushita T, Chan YY, Murakami S. Mice expressing GFP and CreER in osteochondro progenitor cells in the periosteum. *Biochem Biophys Res Commun*. 2009;386(3):477-482. doi:10.1016/j.bbrc.2009.06.059
152. Murao H, Yamamoto K, Matsuda S, Akiyama H. Periosteal cells are a major source of soft callus in bone fracture. *J Bone Miner Metab*. 2013;31(4):390-398. doi:10.1007/s00774-013-0429-x
153. Miwa H, Era T. Tracing the destiny of mesenchymal stem cells from embryo to adult bone marrow and white adipose tissue via Pdgfrα expression. *Development*. 2018;145(2):dev155879. doi:10.1242/dev.155879
154. Reinhardt R, Gullotta F, Nusspaumer G, et al. Molecular signatures identify immature mesenchymal progenitors in early mouse limb buds that respond differentially to morphogen signaling. *Development*. 2019;146(10):dev173328. doi:10.1242/dev.173328
155. Takakura N, Yoshida H, Ogura Y, Kataoka H, Nishikawa S, Nishikawa S-I. PDGFRα Expression During Mouse Embryogenesis: Immunolocalization Analyzed by Whole-

mount Immunohistostaining Using the Monoclonal Anti-mouse PDGFRα Antibody APA5. *J Histochem Cytochem*. 1997;45(6):883-893. doi:10.1177/002215549704500613
156. Maes C, Kobayashi T, Selig MK, et al. Osteoblast Precursors, but Not Mature Osteoblasts, Move into Developing and Fractured Bones along with Invading Blood Vessels. *Dev Cell*. 2010;19(2):329-344. doi:10.1016/j.devcel.2010.07.010
157. Nakashima K, Zhou X, Kunkel G, et al. The Novel Zinc Finger-Containing Transcription Factor Osterix Is Required for Osteoblast Differentiation and Bone Formation. *Cell*. 2002;108(1):17-29. doi:10.1016/S0092-8674(01)00622-5
158. Worthley DL, Churchill M, Compton JT, et al. Gremlin 1 Identifies a Skeletal Stem Cell with Bone, Cartilage, and Reticular Stromal Potential. *Cell*. 2015;160(1-2):269-284. doi:10.1016/j.cell.2014.11.042
159. Méndez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010;466(7308):829-834. doi:10.1038/nature09262
160. Liu Y, Strecker S, Wang L, et al. Osterix-Cre Labeled Progenitor Cells Contribute to the Formation and Maintenance of the Bone Marrow Stroma. Rameshwar P, ed. *PLoS ONE*. 2013;8(8):e71318. doi:10.1371/journal.pone.0071318
161. Mizoguchi T, Pinho S, Ahmed J, et al. Osterix Marks Distinct Waves of Primitive and Definitive Stromal Progenitors during Bone Marrow Development. *Dev Cell*. 2014;29(3):340-349. doi:10.1016/j.devcel.2014.03.013
162. Tournaire G, Stegen S, Giacomini G, et al. Nestin-GFP transgene labels skeletal progenitors in the periosteum. *Bone*. 2020;133:115259. doi:10.1016/j.bone.2020.115259
163. Cserjesi P, Brown D, Ligon KL, et al. Scleraxis: a basic helix-loop-helix protein that prefigures skeletal formation during mouse embryogenesis. *Dev Camb Engl*. 1995;121(4):1099-1110.
164. Sakabe T, Sakai K, Maeda T, et al. Transcription factor scleraxis vitally contributes to progenitor lineage direction in wound healing of adult tendon in mice. *J Biol Chem*. 2018;293(16):5766-5780. doi:10.1074/jbc.RA118.001987
165. Schweitzer R, Chyung JH, Murtaugh LC, et al. Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. *Development*. 2001;128(19):3855-3866.
166. Sugimoto Y, Takimoto A, Akiyama H, et al. Scx+/Sox9+ progenitors contribute to the establishment of the junction between cartilage and tendon/ligament. *Development*. 2013;140(11):2280-2288. doi:10.1242/dev.096354
167. Blitz E, Sharir A, Akiyama H, Zelzer E. Tendon-bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. *Development*. 2013;140(13):2680-2690. doi:10.1242/dev.093906
168. Best KT, Loiselle AE. Scleraxis lineage cells contribute to organized bridging tissue during tendon healing and identify a subpopulation of resident tendon cells. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2019;33(7):8578-8587. doi:10.1096/fj.201900130RR
169. McKenzie JA, Buettmann E, Abraham AC, Gardner MJ, Silva MJ, Killian ML. Loss of scleraxis in mice leads to geometric and structural changes in cortical bone, as well as asymmetry in fracture healing. *FASEB J*. 2017;31(3):882-892. doi:10.1096/fj.201600969R
170. Tan C, Lui PPY, Lee YW, Wong YM. Scx-Transduced Tendon-Derived Stem Cells (TDSCs) Promoted Better Tendon Repair Compared to Mock-Transduced Cells in a

Rat Patellar Tendon Window Injury Model. Awad HA, ed. *PLoS ONE*. 2014;9(5):e97453. doi:10.1371/journal.pone.0097453
171. Feng C, Chan WCW, Lam Y, et al. Lgr5 and Col22a1 Mark Progenitor Cells in the Lineage toward Juvenile Articular Chondrocytes. *Stem Cell Rep*. 2019;13(4):713-729. doi:10.1016/j.stemcr.2019.08.006
172. de Lau W, Barker N, Low TY, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*. 2011;476(7360):293-297. doi:10.1038/nature10337
173. Rhee DK, Marcelino J, Al-Mayouf S, et al. Consequences of Disease-causing Mutations on Lubricin Protein Synthesis, Secretion, and Post-translational Processing. *J Biol Chem*. 2005;280(35):31325-31332. doi:10.1074/jbc.M505401200
174. Seol D, McCabe DJ, Choe H, et al. Chondrogenic progenitor cells respond to cartilage injury. *Arthritis Rheum*. 2012;64(11):3626-3637. doi:10.1002/art.34613

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Conflicts of interest

Professor Dell'Accio has obtained consultancy fees from Samumed and UCB Pharma.

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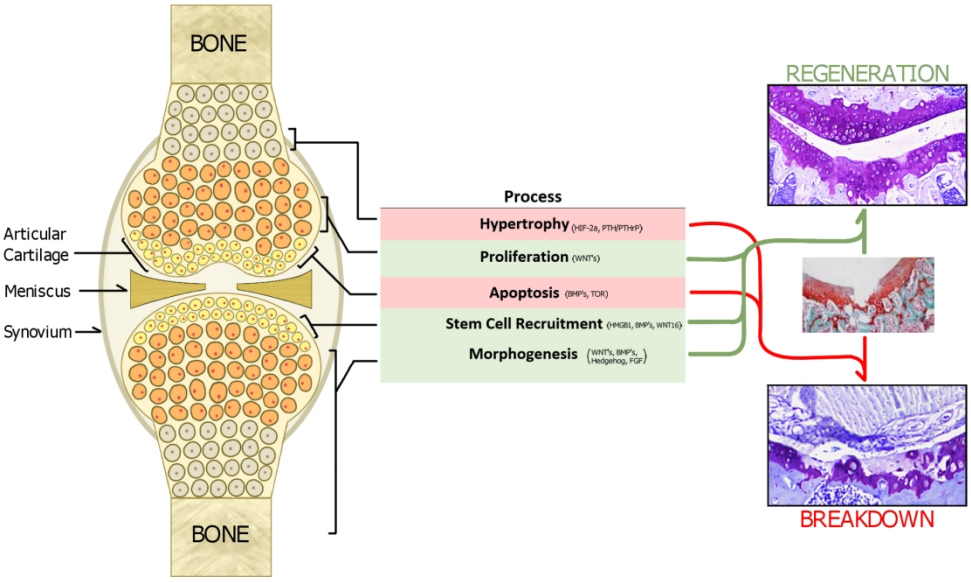

Figure 1. Embryonic morphogenesis and skeletal repair in adulthood share basic processes. Proliferation, hypertrophy, apoptosis, stem cell recruitment and morphogenesis are processes which are initiated during development to create the joint. These processes are re-activated in injured joint tissues. The spatial and temporal regulation of these processes determines the outcome of the injury response, either successful regeneration or breakdown.

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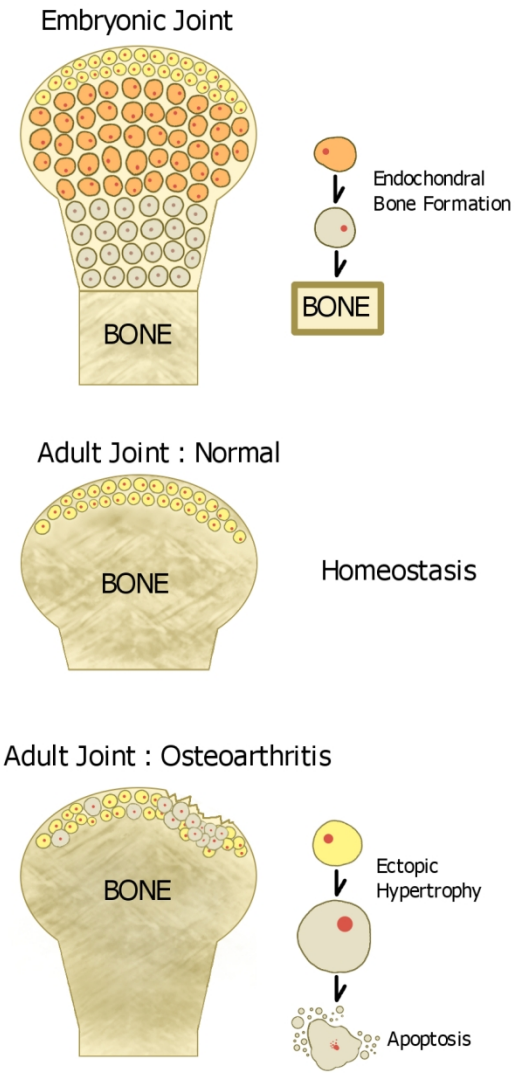

Figure 2. Chondrocyte hypertrophy is required for endochondral bone formation during development but is a pathogenic event in the adult osteoarthritic joint.
During development, epiphyseal chondrocytes undergo hypertrophy and ultimately endochondral bone formation. In a healthy adult joint, chondrocytes remain at the joint surface and reside in the permanent articular cartilage. Throughout the progression of osteoarthritis, however, these articular chondrocytes begin to undergo 'ectopic hypertrophy', resulting in the mineralisation and ultimately, breakdown of the articular cartilage.

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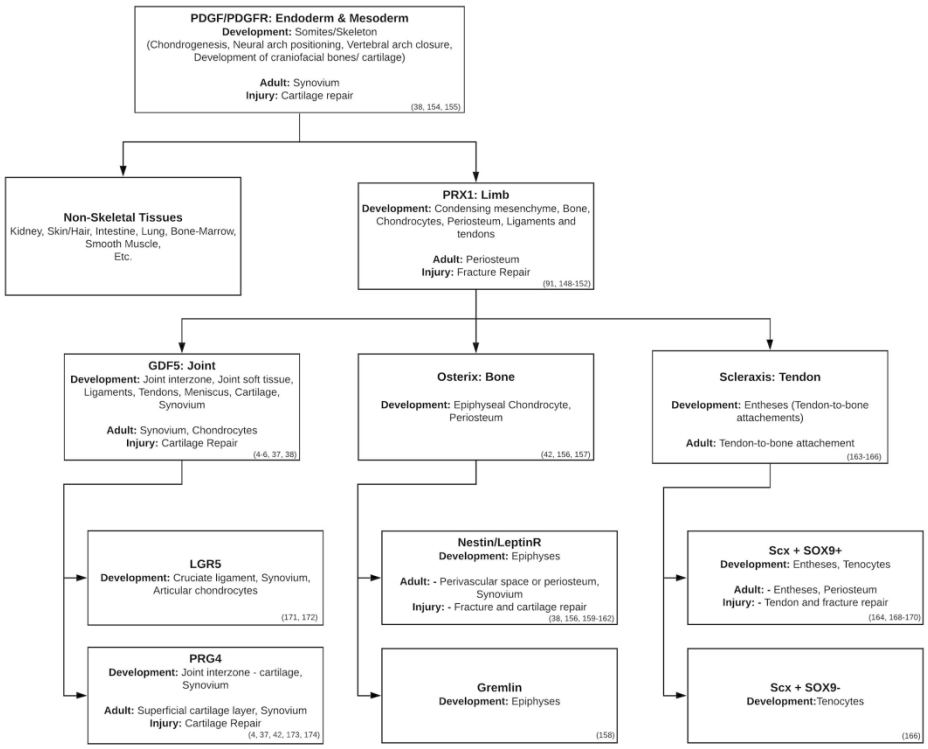

Figure 3. Musculoskeletal stem cell lineage tree.
Condensed mesenchymal stem cells expressing PDGFRα differentiate to express PRX1 in the early limb bud. Subsequent differentiation of the PRX1 expressing stem cells recruits them to GDF5, Osterix or the Scleraxis lineage, which are responsible for the development of the tissues in the joint, bone and tendons respectively.

203x161mm (300 x 300 DPI)