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Synovial Joints: from Development to Homeostasis

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Abstract

Synovial joint morphogenesis occurs through the condensation of mesenchymal cells into a non-cartilaginous region known as interzone, and the specification of progenitor cells that commit to the articular fate. Although several signaling molecules are expressed by the interzone, the mechanism is poorly understood. For treatments of cartilage injuries, it is critical to discover the presence of joint progenitor cells in adult tissues and their expression gene pattern. Potential stem cells niches have been found in different joint regions, such as the surface zone of articular cartilage, synovium and groove of *Ranvier*. Inherited joint malformation as well as joint degenerating conditions are often associated with other skeletal defects, and may be seen as the failure of morphogenic factors to establish the correct microenvironment in cartilage and bone. Therefore, exploring how joints form can help us understand how cartilage and bone are damaged and to develop drugs to reactivate this developing mechanism.

Keywords

synovial joint development; interzone; joint progenitor cells; articular cartilage; cytokines/chemokines; TGFβReceptor2; joint inherited diseases; joint acquired diseases; osteoarthritis

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INTRODUCTION

Formation and positioning of synovial joints are critically important during evolution allowing successful adaptation of vertebrate limbs to a variety of ecological environments. Because of the variety of tissues comprising the synovial joint (articular cartilage, synovial fluid, ligaments, joint capsule) disease or trauma affecting one tissue has wide-ranging implications on the health and function of the joint as a whole organ (1). Inherited joint malformations, although relatively rare, present with a wide range of forms and severity and are often associated with skeletal defects, suggesting a reciprocal cause/effect relationship (2, 3). On the other hand, degenerating conditions that affect joint function, such as osteoarthritis (OA) and inflammatory rheumatoid arthritis (RA), are the single largest cause of disability in the adult population (4, 5), and while our understanding of the pathology of these diseases has greatly improved, the only successful treatment for restoring joint function in end-stage diseases is total joint replacement (6). To some extent, this lack of effective medical treatments is due to the fact that the articular cartilage is avascular, and this limits its reparative capacity (7). Although physiologically and clinically significant there is limited information on the mechanisms and signaling molecules that lead to joint morphogenesis. A greater understanding of joint formation can provide critical insight on the pathogenesis of joint degeneration and to develop novel reparative strategies to restore long-term joint function following trauma and disease.

JOINT MORPHOGENESIS

During skeletogenesis, long bone formation initiates as uninterrupted mesenchyme condensations in the early limb buds, which undergo differentiation to chondrocytes (1, 6). Joint formation becomes morphologically evident with the appearance of a region called the interzone at the sites of future joint location where the resident cells flatten and form a clear separation of the previous uninterrupted cartilaginous skeletal anlagen (8–9). In most species, the interzone is a tripartite structure consisting of two outer layers adjacent to the epiphyseal end of the future bones and an intermediate zone, that adopt a nonchondrogenic phenotype, as indicated by the loss of chondrogenic markers such as *Sox-9* and *Collagen 2*, and the expression of new sets of genes including *Gdf5, Wnt14* and *Wnt4* (10–12). The pivotal role the interzone in joint formation is widely recognized, as its microsurgical removal results in joint ablation (8–9, 13). Ultrastructural analysis in developing rat embryos indicated that the outer interzone layers participate in initial lengthening of long bone anlagen, while articular chondrocytes largely derive from the intermediate layer (14). Despite wide recognition that the interzone region is essential for joint formation, there is limited information of the gene expression environment in which interzone cells emerge and distinguish themselves from adjacent growth plate chondrocytes (14–16). Numerous studies in recent years have reported several signaling molecules, growth factors, transcription factors, and other regulatory molecules expressed by the interzone: these include GDF-5, Wnt-14, Wnt-4, Wnt-16, Gli3, CD44; BMP antagonists Chordin and Noggin; fibroblast growth factor family member FGF-2, FGF-4 and FGF-13; transcription factors Cux1 and ERG (10, 12, 17–20). GDF-5 is expressed in the early interzone in mouse and chick embryo limb joints and lack of GDF-5 in the natural mouse mutant *brachypodism*, causes widespread joint defects and skeletal growth retardation (21). Subsequent studies established

that *Gdf-5*-expressing cells give rise to most if not all joint tissues, including articular cartilage, ligaments and inner synovial lining (14). The current view is that GDF-5 has at least two roles in skeletogenesis. At early stages, it is expressed throughout the condensation and would stimulate recruitment and differentiation of chondrogenic cells. At later stages, when its expression becomes restricted to the interzone, GDF-5 would promote interzone cell function and joint development (22). Iwamoto et al. reported that there is a close spatiotemporal expression of GDF-5 with the transcriptional factor ERG (one of the *ets* gene family member) in developing mouse embryo joints which suggests that ERG acts downstream of GDF-5 leading chondrocytes to become joint forming cells (9).

Wnt14 and the BMP antagonist Noggin are anti-chondrogenic factors that are expressed early during interzone formation. Targeted misexpression of Wnt14 in developing chicken digit rays induced ectopic joints, with upregulation of Gdf5, Wnt4, CD44 and down-regulation of Col2, Sox9 (10) while ablation of Wnt-14 along with Wnt-4 impairs joint formation and causes some fusions (23). *In vitro* studies show that Wnt14 reversed chondrocyte differentiation in predifferentiated micromass culture, reflecting what happened in vivo(10, 24).

Experimental ablation of *Noggin* in mouse prevents limb joint formation and causes skeletal fusions (17). The role of Noggin is conserved between mouse and humans, and at least two human syndromes, which are characterized by multiple synostoses (absence of joints), are due to mutations in Noggin (25). The expression of another BMP antagonist, *Chordin*, is restricted to the joint interzone, emphasizing the role of BMP antagonism during joint development. The significant concept stemming from these studies is that joint formation involves the intervention of gene products with chondrogenic and anti-chondrogenic activities, the former critical for formation of fibro-cartilaginous joint structures and the latter for maintaining the initial mesenchymal character of cells present at joint sites (15). Thus, it is not surprising that the joint interzone appears to be also an essential regulator of skeletal development, and vice-versa (24). One example is shown by the conditional inactivation of Ext1 in developing joints in Gdf5-expressing cells. Ext1 encodes a subunit of the Ext1/Ext2 Golgi-associated protein complex responsible for heparan sulfate (HS) synthesis. Heparan sulfate (HS) is an essential component of cell surface and matrix-associated proteoglycans and mutations in HS-synthesizing and modifying enzymes cause several skeletal phenotypes, such as hereditary multiple exostoses (HME), described later in joint genetic disease chapter. Interestingly, mutation of Ext1 in Gdf5-expressing cells, led to lack of a distinct mesenchymal interzone with consequent joint fusion. They also found abnormal BMP and hedgehog activity and signaling leading to a delayed growth and lengthening of long bones indicating that defects in joint formation reverberate on, and delay, overall long bone growth (26).

Recent studies have identified an important role for HIF-1 and HIF-2 in the regulation of skeletal development as well as joint formation and homeostasis. In addition, overexpression of HIF-1 and HIF-2 has been clinically associated with osteoarthritis (27).

The critical role of Ihh and parathyroid Hormone-Related protein (PTHrP) during endochondral bone formation is well known, but also their importance in synovial joint

formation (28–30) has been reported. Ihh null have mice failed joint development (28, 31, 32) and have abnormal distribution and function of Gdf5-expressing interzone-associated cells (17, 29, 31, 33). These studies suggest that in the developing diaphysis, secreted Ihh could diffuse and reach the epiphyseal ends of the long bone analgen, where it would regulate interzone formation (1). Using a *PTHrP-Lac-Z* knockin mouse containing a β-gal reporter under endogenous PTHrP gene regulatory sequences, Chen et al. have confirmed two distinct PTHrP β-gal positive subpopulations: in the articular cartilage and in the proliferative chondrocytes, that are maintained through adulthood (34) (35). A role for PTHrP in articular cartilage maintenance has been recently proposed by Gdf5-Cre-targeted knock-down of PTHrP in mouse articular chondrocytes that led to accelerated development of OA in a DMM model (36). In addition, systemic administration of recombinant human PTH in mice inhibited cartilage degeneration and induced cartilage regeneration following meniscal/ligamentous injury (37).

Proper spatial positioning of synovial joints is crucial in skeletogenesis. Sohaskey et al. reported that mice carrying an insertional mutation in the *Jaws* gene (abnormal joint with splitting) die perinatally with striking skeletal defects, including ectopic interphalangeal joints (38). These ectopic joints develop along the longitudinal axis and persist at birth, suggesting that JAWS is uniquely required for the orientation and consequent positioning of interphalangeal joints within the endochondral skeleton.

Transgenic mice overexpressing fibroblast growth factor receptors (FGFR) 1 and 3 exhibit joint defects similar to the symphalangism that occurs in Apert syndrome, a rare congenital disorder characterized by craniosynostosis, midfacial malformation and symmetrical syndactyly. In this mice some synovial joints failed to develop and the presumptive joint space was replaced by cartilage. (39)

A recent study by Gao et al. reported a critical role of Osr1 and Osr2, the mammalian homologs of the odd-skipped family of zinc finger transcription factors required for Drosophila leg joint formation, in mouse synovial joint formation. They report that Osr1 and Osr2 and are both strongly expressed in the developing synovial joint cells and are required for Gdf5, Wnt4 and Wnt14 expressions (40).

Genetic manipulation of the TGF-β system has revealed critical roles in both joint development and skeletogenesis (41–43). In transgenic mice, overexpression of a dominant negative Tgfbr2 (DNIIR) resulted in OA(40). In humans, mutations of the Tgfbr2 have been associated with Loeys-Dietz syndrome, a Marfan-like syndrome that leads early onset of OA (OMIM#609192) (44,45). In previous studies, by generating a TGF-β type II receptor (TβRII) reporter mouse (β-Gal and GFP), we showed that TβRII is highly and specifically expressed in the developing joint (43). By generating a mouse in which the TβRII signaling is conditionally inactivated in mesenchyme limb buds starting at E9.5 ($Tgfbr2^{Prx1KO}$), we demonstrated that the lack of TβRII signaling led to complete absence of interphalangeal joints and was associated with down-regulation of key joint morphogenic factors (such as Noggin, GDF5 and the Notch ligand, Jagged 1). Interestingly, lack of joint was associated with up-regulation of many inflammatory cytokines/chemokines in the presumptive interzone, such as MCP-5 and Il 36 (43, 46, 47); blockade of MCP-5 receptor was able to

rescue the TβRII-mediated joint deficiency (47), suggesting that cytokines/chemokines may have a regulatory role during development, mediating proper interzone formation.

There is compelling evidence that Notch signaling is critical for progenitor cell survival and for their maintenance in an undifferentiated state, which sets the boundaries that segregates two distinct cell populations during development (48–52). Notch1 is a regulator of great importance for cell fate commitment during both early growth and in adult tissues and has been shown to play a critical role in the cell fate determinant in different stem cell niche structures such as the skin, teeth and nervous system (53). Hayes et al. reported that Notch 1 is expressed in the developing and post-natal articular surface (54). The Notch family members are also expressed in adult articular cartilage (55) and over 70% of chondrocytes on the surface zone of articular cartilage express Notch 1 receptor (16), suggesting a significant role of the Notch signaling in regulating articular cartilage homeostasis during adult life (55,56)

Despite important advances on multiple fronts, there are still fundamental aspects of joint formation that remain unclear and in particular, how joints acquire their diverse morphology and organization from embryonic life to adulthood. A better understanding of genes involved in patterning processes will be critical to link embryonic development with joint disease in adult, in both inherited and acquired debilitating diseases.

PROGENITOR STEM CELL NICHE

The view of articular cartilage as a non-regenerative organ has been challenged in recent years. The superficial zone of articular cartilage demonstrates a distinct pattern regarding stem cell markers (Notch-1, Stro-1, and vascular cell adhesion-1) (57) and has been hypothesized to harbor stem cells (58). Studies regarding cartilage development have shed light on the question of whether any of the joint tissues harbors potential joint progenitor cells. Through the use of BrdU (bromo-deoxyuridine) injections to localize slow-cycling cells, a trait characteristic of progenitor cells, a few potential joint stem cell niches have been determined. A Notch-1 positive cell population has been isolated from the surface zone of articular cartilage (59); these cells possess high colony-forming efficiency that was abolished when Notch signaling was blocked (16).

Karystinou et al reported the isolation and characterization of multipotent MSCs from adult human synovium, that could be expanded for up to 30 population doubling, with limited senescence and maintenance of multilineage differentiation capacity toward chondrogenesis, osteogenesis, adipogenesis and skeletal myogenesis (60). In response to injury of various types, including trauma, the synovial membrane rapidly becomes hyperplastic, which seems to be sustained mainly by stromal synovial fibroblasts (61–63). The biologic function of synovial cell proliferation is believed to have a pivotal role in joint homeostasis and deregulation of this process is thought to contribute to the formation of pannus, which in RA causes destruction of cartilage and bone (63). Although very little is known about the identity of the synovial cells that proliferate following injury, this study provides the first evidence of the existence *in vivo*, within postnatal knee synovium, of non-hematopoietic, non-endothelial stromal cells with MSC phenotype (64–66) which proliferate following

articular cartilage injury and, under specific experimental conditions, differentiated into chondrocytes in area of cartilage metaplasia within the synovium (67).

An area of potential interest with regard to joint progenitor cells is the perichondrial groove of *Ranvier*. This area is located at the periphery of the epiphyseal growth plate and has been demonstrated to contain proliferating cells (68). Studies by Karlsson et al. in the knee of sexually mature rabbits have proved the existence of different subpopulations of progenitor cells, in the articular cartilage, in the groove of *Ranvie*r, as well as the epiphyseal plate near the groove of *Ranvier*; however, whereas the progenitor cells in the surface layer of the articular cartilage seem to lose their progenitor properties and become dispersed throughout the articular cartilage as they grow older, the progenitor cells in the groove of *Ranvier* were positive for several marker associated with stem cell niche (including Stro-1, Jagged-1 and BMPr1a) and maintain their progenitor properties and localization over time (58).

We have addressed in the previous section the critical role of the TβRII in joint-element development. Our finding that mice lacking of TβRII signaling in developing limbs failed interzone formation and lacked interphalangeal joints, led us to investigate the expression pattern of TβRII-expressing cells and their characterization as joint progenitors(69) By using a Tgfbr2-β-Gal-GFP-BAC mouse previously described, we have recently characterized the TβRII-expressing cells as joint progenitors(69), clustering in a contiguous niches that comprises the groove of *Ranvier* and the synovio-entheseal complex, including part of the perichondrium, the synovium, the articular cartilage superficial layer and the tendon's enthuses(69) Developmental-stage studies showed that TβRII expression was in synchrony with expression of joint-morphogenic genes such as Noggin, Gdf-5, Notch1 and Jagged1. Pre-natal and post-natal BrdU-incorporation studies showed that within this synovio-entheseal articular cartilage niche most of the TβRII-expressing cells exhibit stem cell traits. In addition, TβRII-expressing cells isolated from embryonic limb mesenchyme expressed joint progenitor markers in a time- and TGF-β-dependent manner. TβRII-expressing cells were maintained in their specific niches from embryonic life throughout early adulthood, although characterized by a progressive age-dependent reduction in number.

The functional characterization of adult joint progenitors, together with the identification of embryonic markers that are preserved in postnatal joints, opens prospects for potential ways to reactivate the joint-forming ability of such cells, to implement their survival during aging and to evaluate their role in joint genetic disease as well as joint degenerative pathologies, such as arthritis.

JOINT GENETIC DISEASES

Genetic constitution is a factor in the development of most diseases, including those of bones and joints. Evidence for the influence of genes in most disease development is expressed in two ways. In polygenic disorders, the genetic influence is indirect, based only on the higher rate of recurrence in some families compared to the general population. The second group follows Mendelian inheritance, where a specific gene is directly responsible for the disease and the gene is passed on to subsequent generations. These diseases may also appear spontaneously as new mutations. There are over 5000 documented Mendelian disorders, and over 500 of these affect bones and joints (70). Some of these single gene

disorders affect many tissues, and the skeletal system, including joints, is one of many organs involved (71), others are directly related to joint malformation. It is beyond the scope of this review to give a detailed list of all the joint genetic diseases; however, we will provide some examples in Table 1. There are very few genes that are known to be necessary and/or sufficient to initiate the joint formation process, among them *Noggin, Gdf5*, *Tgfbr2* and *Wnt14* (10,17,22,41). The factor(s) that induce the expression of these joint morphogenic molecules are undefined. Gong et al. (25) demonstrated that five dominant mutations in the *Noggin* gene in unrelated families segregate with proximal symphalangism (SYM1A, OMIM#185800), an autosomal dominant disorder characterized by a complete bony fusion between the proximal and middle phalanges of the digit. Another form of proximal symphalangism (SYM1B) is caused by a heterozygous missense mutation in the *Gdf5* (OMIM #615298).

We reported that TβRII functions as a master regulator for the expression of key joint marker genes, such as *Gdf5* and *Noggin*, and is necessary for proper joint development (43). The joint phenotype observed in mice lacking the *Tgfbr2* gene in limbs is similar to that in patients with SYM1. In humans, heterozygous mutations of the *Tgfbr2* gene have been associated with Loeys-Dietz syndrome (OMIM #610168), a Marfan-like syndrome characterized by several defects including joint hypermotility and/or contractures that lead to early onset of OA (44,45). It has been hypothesized that in Loeys-Dietz syndrome, an excess of TGF-β signaling accounts for the pathological manifestations (72).

Several lines of evidence have demonstrated the importance of Notch signaling in joint morphogenesis and genetic inactivation of the Notch signaling in *Drosophila*, resulting in loss of joints (73). A derangement of Notch signaling has been reported in articular synoviocytes from patients with RA, characterized by aberrant synoviocyte proliferation (74). Heterozygous mutation in the *Notch2* gene causes the Hajdu-Cheney syndrome (HJCYS, OMIM #102500), a rare autosomal dominant disorder that, in addition to severe skeletal abnormalities including acroosteolysis, is characterized by joint laxity. Mutations and deletions in *Jagged-1* (Notch ligand) have been found in patients with Alagille syndrome characterized by intrahepatic cholestasis, eye and cardiac abnormalities, as well as skeletal defects in particular in the fingers (75, 76).

Mouse studies have indicated that embryonic long bone development is altered by mutations in *Ext* genes members (Ext1, Ext2 and Ext3) of the exostosin protein family and responsible for the synthesis of heparan sulphate (HS)(77). Recent data have shown that loss of Ext-1 expression postnatally is not only affecting the growth and organization of long bones, but is incompatible with normal joint structure, resembling human OA (78, 79). In humans, HS deficiency due to mutation in the genes encoding exostosin-1, -2, -3, causes multiple hereditary exostoses (EXT, OMIM #133700), an autosomal dominant disorder characterized by multiple projections of bone capped by cartilage, most numerous in the metaphyses of long bones, but also occurring on the diaphyses of long bones. Deformity of the legs, forearms (resembling Madelung deformity), and hands is frequent (80).

Mutations in the *Jaws* gene (joints abnormal with splitting), also known as *Impad1* (inositol monophosphatase domain-containing protein 1) cause the GPAPP type of chondrodysplasia

with joint contractures, an autosomal recessive disorder characterized by short stature, chondrodysplasia with brachydactyly, congenital joint dislocations, micrognathia, cleft palate, and facial dysmorphism (OMIM #614078).

Alkaptonuria (AKU, OMIM #203500) is an autosomal recessive metabolic disorder caused by homozygous or compound heterozygous mutations in the homogentisate 1,2-dioxygenase gene (HGD) and characterized by accumulation of homogentisic acid, leading to darkened urine, ochronotic pigmentation of fibrous tissues including cartilage, tendons, ligaments, chronic joint pain, and destruction of the cardiac valves. Ultimately, patients develop severe secondary OA.

Current knowledge of joint biology and pathologies has not been translated, however, into effective therapies to treat joint conditions (103, 104). Knowing how joints form during fetal life, express certain genes that become established in early post-natal life and are maintained in adult life would shed light on the homeostasis of the whole joint and could lead to novel treatment for many joint disorders.

CAN WE UNDERSTAND BETTER OA FROM JOINT DEVELOPMENT?

OA is a complex disease caused by the interaction of multiple genetic and environmental factors and the major cause of disability in the adult population. Although articular cartilage degeneration is the primary concern in OA, it is now the prevailing paradigm that OA is a disease of the entire joint and homeostasis and integrity of articular cartilage depends also on the biochemical interplay with subchondral bone; thus, it not surprising that mutation in genes that are needed during skeletogenesis may lead to OA later in life.

We have addressed the critical role of the TGF-β system in both joint development and skeletogenesis (41–43). The scope of this section is to provide an example of how genes that are so critical in joint morphogenesis may also have key roles in joint degenerating disease, such as OA. TGF-βs bind to TGF-β type II receptor (TβRII) leading to TβRII-TβRI complex formation, which then activates the signaling cascade through R-Smad dependent (Smad-2, -3,-4) and Smad-independent pathways (81, 82).

In human and mouse embryonic cartilage, TGF-βs are expressed in the endochondral template (83–86). Using laser capturing microdissection (LCM) we have obtained RNA samples from E13.5 progenitor joint-forming interzone cells and from the adjacent growth plate chondrocytes. Each population-related mRNA was subjected to microarray analysis. We have found that *Tgfbr2* expression was 12.3 times higher, while *Tgfbr1* and *Tgfbr3* were slightly lower in the interzone cells. In mice, targeted manipulation of the TGF-β system genes have revealed their critical, but still undefined, function in skeletogenesis (41, 42, 87, 88). R-Smad gene targeting in mice has led to variable phenotypes ranging from early lethality (Smad2 and Smad4 ablation) to a normal phenotype at birth with progressive OA in adulthood (Smad3 ablation) (89–92). In transgenic mice, overexpression of a dominant negative *Tgfbr2* (DNIIR) can result in OA in adults (42); in chick embryos, however, implanted beads releasing TGF-β induce extra digit formation (93). In humans, mutations of the *Tgfbr2* have been associated with Loeys-Dietz syndrome (44, 45, 72).

In previous studies, we demonstrated that lack of TβRII signaling in developing limbs was associated with complete absence of interphalangeal joints as well as with impaired growth plate development (43).

By combining LCM with microarray analysis we were also able to show that interzone cells express a gene pattern that is distinct from adjacent growth plate chondrocytes and such gene regulation is impaired in mutant limbs lacking TβRII. We performed a pathway analysis using the PANTHER (protein analysis through evolutionary relationships) Classification System (Celera) to classify genes into canonical pathways. This enabled us to identify functional categories in the joint-forming interzone cells of wild type mice that were different from mutants. We determined that in the interzone cells the "*inflammation mediated by chemokines and cytokines*" was the most down-regulated pathway in the wild type interzone compared to mutant. Specifically, we found that monocyte chemoattractant protein-5 (MCP-5) and Interleukin 36-α (IL-36α) were among the most down-regulated genes in this pathway (46, 47). It has been reported that MCP5, MCP-1 and their sole common receptor CCR2, as well as IL-36α are increased in the inflamed joints of patients with different forms of arthritis and in rodent models for arthritis (94–97). While the role of inflammatory cytokines in the arthritic process appears indisputable, their role in joint development has never been evaluated. A finding of cytokine involvement in joint formation would determine a shift in our current view of joint cells as passive victims of the disruptive force of cytokines but as active participants in maintaining control of the cytokine effects and therefore joint integrity. It has been shown that in healthy cartilage, a balance between cytokines and anabolic growth factors is critical to maintain proper tissue homeostasis (98, 99). Supported by our previously published data and other current evidence, in Figure 1 we propose a model for the role of TβRII signaling in regulating cell commitment to joint-forming cells or chondrocytes within the joints (47). Specifically, TβRII signaling, by blocking critical inflammatory cytokines expression in the interzone, halts Collagen 2 expression and induces interzone specific markers (i.e. Gdf5, Noggin). In mutant mice, lack of the TβRII expression in developing limbs, leads to disregulated levels of cytokine/chemokines in the interzone (such as MCP-5 and IL-36α) which, in turn, block expression of interzone specific markers while increasing Collagen 2-expressing cells, with consequent accumulation of chondrocytes within the joint region and impaired of joint formation.

The activation of the inflammatory cytokine/chemokine cascade is associated with the more common forms of arthritis (100). The generally accepted hypothesis is that during the OA/RA process, the increased levels of cytokines and chemokines are the last step of a long lasting damage process that sees the joint as a passive target. To determine the role of TβRII signaling in joint homeostasis we generate a genetically modified mouse to tamoxifen induces, a Cre-conditional inactivation of floxed Tbfbr2 in osteochondro progenitors starting at postnatal day 3 of life (46). We found that postnatal TβRII signaling inactivation was associated with high expression of IL36α and MCP-5 (unpublished data) in the articular cartilage and synovium and progressive OA development(46). Blockage of IL36α signaling led to rescue of the OA phenotype(46). In a mouse model of post-traumatic OA, namely caused by a destabilization of the medial meniscus (DMM) surgically-induced after medial meniscotibial ligament transection, we found that an intense early cellular response of TβRII

positive cells that gradually decreased with OA progression and was associated with an increase of MCP-5 (101). Blockage of the MCP-5 signaling as well as implant of TβRII positive cells improved OA lesions and decreased subchondral bone reaction (101, 102). These findings suggest that TβRII signaling and cellular response is critical in joint development and it is an active mechanism to preserve joint homeostasis in adulthood. In this respect we provide a novel perspective to evaluate the OA process that results from the failure of an active cell joint population, expressing TβRII, to maintain a controlled cytokine environment rather than from the damage induced by a systemic influx of inflammatory cells and cytokines against a passive target (the joint).

CONCLUSIONS

Joint biology has been the subject of extensive research activity, and much has been learned about the structure and composition of articular cartilage, ligaments, synovium, and joint capsule, and the specific roles each of these tissues plays in joint function in developing and adult organisms Much is also known about susceptibility of joint tissues to damage and malfunction during natural aging and congenital or acquired skeletal conditions (20). Diseases that target and disrupt structure and function in the articular cartilage, synovium, meniscus and subchondral bone result in painful, debilitating and costly conditions for patients and society as a whole. The articular cartilage is avascular, and this presents limitations on its reparative capacity and for drug delivery. Joint homeostasis is maintained preserving the articular cells to proceed down the differentiation pathway that leads to chondrocyte hypertrophy and bone replacement such as it occurs in the growth plate cartilage. Many joint degenerating conditions may be seen as the failure of morphogenic factors to establish the correct microenvironment in cartilage and bone. Therefore, exploring how joints form can lead us to understand how cartilage and bone are damaged and develop drugs that are able to reactivate this developing mechanism. This information would have major biomedical relevance, as it could lead to novel repair strategies to restore joint function following trauma, as well as providing insight on the pathogenesis of acquired and inherited joint diseases.

Acknowledgments

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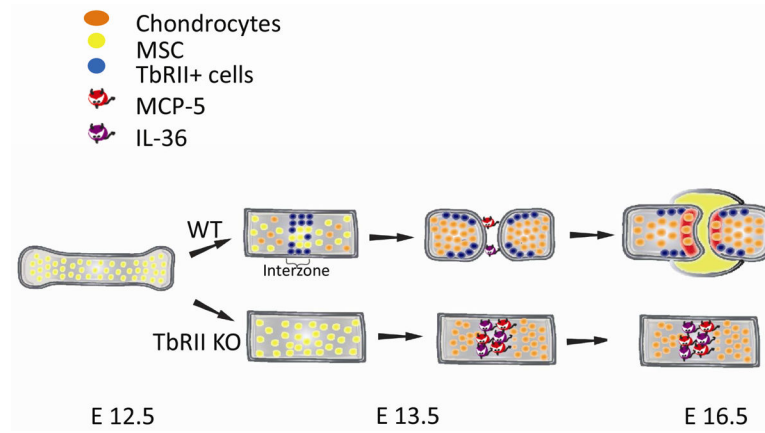
Figure 1. A model for the TβRII-mediated regulation of joint development
TβRII down-regulates MCP-5/IL-36α expressions in the interzone, allowing expression of key joint markers (such as *Gdf5*) while inhibiting *Collagen2* expression. Lack of the TβRII signaling leads to up-regulation of interzone MCP-5/IL-36α with consequent lack of joint formation.

Table 1

Joint Genetic Diseases

Location	Gene locus	Inheritance	Phenotype	Clinical Synopsis (skeletal)
17q22	Noggin	Autosomal dominant	Proximal symphalangism 1A	Joint fusion Proximal interphalangeal joint synostoses Distal interphalangeal joint synostoses (occasional) Carpal and tarsal bone fusion
20q11.22	Gdf5, CDMP1, SYNS2, OS5,BDA1C, SYM1b	Autosomal dominant	Proximal symphalangism 1B	Joint fusion Distal interphalangeal joint fusions Flat feet Absence of the cuboid bone Bone fusion (proximal/middle phalanges)
3p24.1	Tgfbr2		Loeys-Dietz syndrome type 2 (formerly, Marfan syndrome type 2)	Joint laxity Osteoporosis (some patients) Low-impact fractures Arachnodactyly Camptodactyly Brachydactyly Contractures
1p13-p11	Notch2	Autosomal dominant	Hajdu-Cheney syndrome	Joint laxity Short stature Osteopenia Osteoporosis Pathologic fractures Acroosteolysis
8q12.1	Jaws/Impad1	Autosomal recessive	Chondrodysplasia with joint dislocations, GRAPP type	Joint dislocations Shortening and deformity of the limbs Brachydactyly Longitudinal splitting of the proximal phalanx of forefinger Splitting of the first metacarpal in two parts Short stature
8q24.11	Ext1	Autosomal dominant	Multiple cartilaginous exostoses, type 1	Cartilaginous protuberances at ends of long bones Short metacarpal Exostoses in juxtaepiphyseal regions of long bones Genu valgum Madelung-like forearm deformities Bilateral overriding of single toes
3q13.33	Hgd	Autosomal recessive	Alkaptonuria (AKU)	Ochronotic pigmentation of connective tissue Ochronotic arthritis Ochronotic arthropathy Chronic joint pain Degeneration of intervertebral disks Fusion of vertebral bodies