Understanding Developmental Mechanisms in the Context of Osteoarthritis

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Abstract Osteoarthritis (OA) is a joint disease that is highly related to aging. However, as OA development is the consequence of interplay between external stimuli, such as mechanical loading and the structure and physiology of the joint, it can be anticipated that variation in developmental processes early in life will affect OA development later in life. Genes involved in patterning processes, such as the Hox genes, but also genes that encode transcription factors, growth factors and cytokines and their respective receptors and those that encode molecules involved in formation of the extracellular matrix, will influence embryonic skeletal development and OA incidence and severity in the adult. The function of genes involved in patterning processes can be partly be understood by close analysis of inborn diseases that result in musculoskeletal syndromes, but a deeper understanding will be the result of specific gene knockouts or overexpression in transgenic mouse models.

Keywords Osteoarthritis · Developmental mechanisms · Homeobox genes · Genetics · Skeletogenesis · Cartilage · Joint development · Congenital aberrations

Introduction

Limbs, including synovial joints, are the hallmarks of the vertebrate body plan. The development of the skeletal system in vertebrates is a highly regulated and complex process, involving numerous elements of control, which have been comprehensively reviewed in an article by Lefebvre and Bhattaram [1]. The skeletal system has gone through series of evolutionary steps to become what we now see in modern vertebrates, such as mammals. The evolutionarily oldest tissue that is a major contributor to the formation of the skeletal system in vertebrates is cartilage. However, cartilaginous tissues are also present in invertebrates, such as mollusks, arthropods and polychaetes, indicating that cartilage itself is not a newly invented tissue forming the vertebrate skeleton [2]. Nevertheless, type II collagen appears to be specific to vertebrate cartilage, and the evolutionary relationship between vertebrate and invertebrate cartilage is obscure [3]. Bone is only found in vertebrates and has not been detected in any invertebrate.

Synovial or diarthrodial joints are an efficient mechanism to make movement possible. The evolutionarily oldest synovial joints are found in the jaw of the lungfish [4]. The other joints of the lungfish are lined with cartilage. It is clear that formation of a fluid-filled cavity in the articulating joints make them more bendable and improves their function. The formation of a capsule is needed to keep the lubricating fluid inside the joint. This is an evolutionarily hypothetical sequence of events, but intermediate joint forms can be found in the fossil record.

During evolution, the skeletal system and synovial joints were optimized to support survival and reproduction. Adaptations that were advantageous during early life and boosted reproduction were maintained in the population, even when those adaptations were deleterious at old, post-reproductive ages. Therefore, mutations that are positively selected at a relatively young age could lead to OA when people age. As an example, GDF5 variants are related to shorter stature and increased risk of OA incidence with aging. Apparently, in east Asian populations, selective pressure on shorter stature has led to increased frequency of the OA-associated GDF5 variant as a by-stander effect [5].

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Since the skeletal system is formed during development, variations in development and in the skeletal end product will affect skeletal function and the later development of OA. This can clearly be seen in the variable incidence of OA in different breeds of dogs [6]. Differences in developmental patterning and the final body plan may lead to different risks of OA incidence. In certain breeds, this is the case not only in overall OA occurrence but also in incidence per specific joint. These findings clearly show that embryonic development and the final skeletal make-up are highly related to OA development.

Skeletogenesis and Cartilage Formation

The fetal skeleton is formed by mesenchymal cells derived from the neural crest and the mesoderm [7]. Cells from these tissues differentiate into chondrocytes, osteoblasts, and other cells that form the joints. In addition, a portion of these cells remains in the body as mesenchymal stem cells during adult life. A vertebrate skeleton starts as a cartilaginous mold that is replaced by bone during fetal and adolescent maturation. It is still unclear whether all chondrocytes die and are replaced by invading cells or if a fraction of the chondrocytes transdifferentiate to osteoblast [8]. In zebrafish, two different populations of osteoblasts have been identified. One population is directly derived from chondrocytes and the other is formed outside the cartilaginous structures [9].

The formation of cartilage can be separated into a number of stages. In the initiation phase, mesenchymal cells condense and begin to produce abundant cartilage-specific extracellular matrix. In the case of articular cartilage, this tissue is permanent, but most of the chondrocytes in the embryonic and post-natal or adolescent growth plates undergo terminal differentiation and hypertrophy, following which the cartilage matrix is substituted by bone in the so-called process of endochondral bone formation.

Joint Development

Osteoarthritis is a disease of the synovial joints. The exact process of joint formation is not yet elucidated. For example, it is not clear whether joint formation occurs within continuous cartilage structures or if cartilage structures develop as discrete, discontinuous anlagen, separated by predetermined joint locations [10]. In general, synovial joint formation is divided into two stages: (1) joint-specific interzone formation, and (2) the subsequent formation of the joint cavity. The interzone forms as precursor cells condense under the control of a growth factor cascade involving GDF5, Wnt14 (Wnt9a), and the type II TGF-β receptor [1]. It has been suggested that cell death is the major driving force of cavity formation [11]. However, an alternative opinion is that the cells do not die but instead a space forms due to the separation of the cell layers by the abundant production of hyaluronic acid [12].

A fully developed synovial joint does not only consist of articular cartilage separated by a cavity but it also includes fibroblasts, macrophages, fat cells, and tenocytes in the construct of an adequately functioning joint. The processes that lie beneath the integration of these cells within a synovial joint are unclear, but it is obvious that developmental disturbances in any of the joint structures will influence joint biology and succeeding OA development.

Variations in these processes will not only lead to alterations in joint structure but also to different interactions among the different joint cells and with the body as a whole. It has been shown that joint shape is related to OA incidence [13•, 14–16]. Variations in structure will lead to variations in loading and the occurrence of peak stresses on articular cartilage. However, not only will loading be influenced by the state of the tissues formed during joint development but it can also be anticipated that variations in biochemical composition of articular cartilage and tendons will affect joint health and OA development. For instance, patients with Ehlers–Danlos syndrome , showing mutations in type I or type III collagens or collagen cross-linking enzymes, often demonstrate early OA [17]. Also, cellular interactions and the specific cellular composition of a joint will determine the integral reaction of a joint to internal and external stimuli, and whether this joint can maintain its long-term homeostasis. Therefore, congenital differences between joints will strongly determine the development and progression of OA at a later age.

Congenital Aberrations and OA

Congenital variations in skeletal development during fetal life can be a consequence of not only genetic factors but also external stimuli that affect embryonic development. A well-known environmental factor strongly affecting skeletal development is thalidomide, but other compounds may be expected to have more subtle effects on intra-uterine development [18]. Comparative studies in animal development, for instance comparing front paw development in mice and bats, have shown that small changes in gene expression can have profound effects on morphology [19]. Similar, but much smaller, variations in gene expression will determine skeletal and joint development and subsequent susceptibility to develop OA in later life.

Severe skeletal disorders occur in about 1/4,000 births [20]. In the classification of skeletal genetic disorders, 456 separate conditions clustered in 40 separate groups are described [21]. Of these conditions, 316 are associated with

226 different genes, some of which have relatively common mutations and others occur in single individuals [21]. The different groups that are recognized are partly based on known gene mutations, characterized, among others, by mutations in genes encoding growth factors or growth factor receptors [e.g., fibroblast growth factor receptor 3 (FGFR3), GDF5], matrix molecules or enzymes involved in the synthesis of matrix molecules (e.g., type collagen, aggrecan, sulphate transporters), and transcription factors (e.g., Runx2, Sox9). Classification of other groups is based on radiographic criteria or the clinical picture. Many of these disorders have accelerated OA development as a symptom. However, although many known mutations are correlated with obvious OA phenotypes, it can be anticipated that additional mutations or single nucleotide polymorphisms in the same genes are associated with variations in development that are associated with milder forms of OA.

Genes Known to be Involved in Skeletal Patterning or Joint Formation

The number of genes and proteins that potentially play a role in development of the skeletal system and, consequently, in the development of OA in later life is nearly endless. Therefore. only a few examples can be illustrated, and this list is certainly not all-embracing.

Homeobox or Hox genes are known to be important in body plan patterning, but are thought to be down-regulated during cellular differentiation. However, it has been shown that several Hox genes can regulate chondrocyte differentiation [22•, 23•]. Mutations in Hox genes, such as HOXA11 and HOXD13, are related to disorders of skeletal development [21]. The synpolydactyly (spd) mouse, a mutant mouse that is caused by the expansion of a polyalanine encoding repeat in the 5' region of the Hoxd13 gene, shows abnormal joint formation [24]. Moreover, knockout of Hoxd13 also results in skeletal defects and the absence of joint formation, demonstrating the importance of Hox genes in this process [25].

A number of transcription factors are very important in cartilage and bone formation and will therefore affect the skeletal structure. The exact function of specific transcription factors in joint formation are not yet clear, but a lot of information is available on their roles in chondrocyte differentiation, as reviewed recently by Nishimura et al. [26].

The well-known SOX trio, consisting of SOX5 and 6 and its main component SOX9, is essential for cartilage formation [27]. In addition, expression of the SOX trio decreases during osteoarthritis [28]. Deficiency of SOX9 in humans leads to campomelic dysplasia characterized by a severe skeletal phenotype and sex reversal in males [29]. Mice deficient in Sox5 and Sox6 show disturbed joint formation, in which joint cell differentiation is unsuccessful and no cavitation occurs [30•].

The transcription factor MEF2C is essential for endochondral bone development. Both deficiency of Mef2C and overactive MEF2C disturb chondrocyte hypertrophy, revealing that an adequate balance of MEF2C is crucial for proper chondrocyte differentiation [31]. Moreover, there appears to be a subtle balance between MEF2C and histone deacetylase 4 (HDAC4) in the process of endochondral ossification. Deficiency of MEF2C can be rescued by a Hdac4 mutation and vice versa [31]. Reduced expression of HDAC4 increases Runx2 expression and it has been shown that SIK3 anchors HDAC4 in the cytoplasm, in this way liberating MEF2C from inhibition by HDAC4 [32, 33]. Deficiency of SIK3 leads to inhibition of chondrocyte hypertrophy, while HDAC4 mutations result in brachydactyly [21, 32].

Absence of Runx2 leads to the absence of bone formation in mice, and mutations in this transcription factor lead to cleidocranial dysplasia in humans [21, 34]. Related family members, such as Runx1 and 3, are also involved in chondrocyte differentiation, and it appears that the factors have different but overlapping functions in chondrocyte differentiation [35, 36].

Differences in expression of factors that govern intercellular communication, such as cytokines and growth factors and their respective receptors, will also influence skeletal and joint development. A well-known example is the relationship between mutations in the FGFR3 and the most common form of dwarfism, achondroplasia [37]. It is postulated that increased activity of the mutated FGFR3 is the basis of the observed skeletal defect. Also, mutations in other FGF receptors show a skeletal phenotype. Mutations in other growth factors, such as Sonic hedgehog, Indian hedgehog, Wnt3, Wnt7A, WISP3, transforming growth factor (TGF) β1, GDF5, GDF6, and bone morphogenetic protein (BMP) 2, or growth factor receptors like TGFβR1 and BMPR1B, have been shown to result in skeletal abnormalities. Moreover, mutations in the cytokine receptor leukemia inhibitory factor (LIF) receptor result in the Stüve–Wiedemann syndrome, characterized by distinctive shortening and bending of the long bones and reduced bone volume [21, 38].

A striking example of the effect of a mutation in a growth factor receptor of the TGFβ superfamily is fibrosis ossificans progressive (FOP). Patients with FOP have an activating mutation in the BMP type I receptor ACVR1 and show mostly ectopic calcification in soft tissues that are mainly induced after injury [21, 39]. Although direct effects of mutations that cause FOP on joint formation and function have not been reported, it can be anticipated that similar subtle activating or inactivating mutations can have effects on joint formation in development and subsequent OA incidence or severity.

It is not unexpected that mutations in structural matrix proteins, such as collagens, or enzymes involved in the proper synthesis and cross-linking of matrix molecules, may also result in skeletal phenotypes. Mutations in numerous collagen genes, including, for example, those encoding type I, II, IX, and X collagens, are known to be related to skeletal malformations. Also, mutations in enzymes involved in the synthesis of aggrecan, including xylosylprotein 4-beta-galactosyltransferase and aggrecan sulfation-related enzymes, play a role in the incorrect formation of the skeleton [21].

Mutations in a factor important for joint lubrication, lubricin encoded by PRG4, result in camptodactyly-arthropathy-coxa vara-pericarditis syndrome. A major feature of this syndrome is early joint failure [40]. Lubricin is expressed in the joints after cavitation and these mice develop joints without overt malformations. However, when these mice age, the cartilage deteriorates and OA develops [41]. Although obvious joint abnormalities are not clear at birth, subtle changes may exist due to abnormalities during development and later result in OA development.

Conclusions

Mutations that lead to easily recognizable syndromes usually exhibit very severe phenotypes that are clear at birth or young age. However, genes with known mutations resulting in less severe phenotypes may lead to more subtle changes in skeletal and joint structure. These subtle variations in joint structure can make a joint more vulnerable to OA development later in life. The occurrence of OA in a specific joint is not only dependent on the way the joint is built but also on the stimuli that act on this joint throughout life. As a result, OA development will be a combination of the construction of the joint and the environmental stress that this joint experiences during childhood, maturity, and old age. Theoretically, it can be predicted that variations in all genes and proteins that play a crucial role in joint formation will affect OA development when individuals age.

Since the joint is complex organ made of various cell types and tissues, all with their own roles in joint formation and maintenance, early alterations in all these cell types and tissues can contribute to the occurrence and severity of OA in a specific joint. A relatively large amount is known about cartilage and bone formation during embryogenesis, whereas less is known about the formation of other essential joint structures such as tendons. Therefore, in addition to considering how defects in cartilage and bone formation might influence OA development, it is also necessary to consider how subtle differences in the development of tendons and other joint structures may affect postnatal joint stability and resistance to strain.

The involvement of so many genes and molecules in skeletal development, potentially subtly influencing the development of the skeletal system, does not make OA research an easy task. Most likely, changes in function or expression any of the genes involved in joint formation, which can be observed in transgenic animals, can potentially affect OA development in later life. However, this does not imply that these genes are crucial in the development of OA in the majority of patients. Therefore, studies using transgenic mice that overexpress or are deficient in specific genes during embryogenesis should be extrapolated to human OA with caution. The anticipated relationship between embryonic skeletal development in general, or joint development specifically, and OA development in postnatal and adult life, makes the use of permanent transgenic mice a delicate business that warrants additional experiments that can refute or confirm the reported observations and disease mechanisms [42, 43]. To put it more simply, knockout or overexpression of genes can cause OA in an animal model, but this does not necessary implies that these genes are involved in human OA.

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