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Research Article

IFT80 is essential for chondrocyte differentiation by regulating Hedgehog and Wnt signaling pathways

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ABSTRACT

Partial mutation of intraflagellar transport 80 (IFT80) in humans causes Jeune asphyxiating thoracic dystrophy (JATD) and short-rib polydactyly (SRP) syndrome type III. These diseases are autosomal recessive chondrodysplasias that share clinical similarities, including shortened long bones and constricted thoracic cage. However, the role and mechanism of IFT80 in the regulation of chondrocyte differentiation and function remain largely unknown. We hypothesize that IFT80 is required for the formation and function of cilia and plays a critical role in chondrogenic differentiation by regulating Hedgehog (Hh) and Wingless (Wnt) signaling pathways. To test this hypothesis, we first analyzed the IFT80 expression pattern and found that IFT80 was predominantly expressed in growth plate chondrocytes and during chondrogenic differentiation. Silencing IFT80 impaired cilia formation and chondrogenic differentiation in mouse bone marrow derived stromal cells (BMSCs), and decreased the expression of chondrocyte marker genes—collagen II and aggrecan. Additionally, silencing IFT80 down-regulated Hh signaling activity whereas up-regulated Wnt signaling activity. The overexpression of Gli2 in IFT80-silenced cells promoted chondrogenesis and recovered the chondrogenic deficiency from IFT80 silencing. Overall, our results demonstrate that IFT80 is essential for chondrocyte differentiation by regulating the Hh and Wnt signaling pathways.

Introduction

Cilia are highly conserved microtubule-based organelles that project from the cell surface into the extracellular environment and play essential roles in vertebrate development, signaling, cellular motility, sensory transduction, and homeostasis [1,2]. There are both motile cilia (9+2 microtubular pattern) and nonmotile, or primary cilia (9+0 microtubular pattern) [3,4]. Construction of the axoneme of cilia requires effective intraflagellar transport (IFT), a bidirectional transport system run by IFT protein complexes. The role of IFT in the assembly and maintenance of cilia has been intensively studied [2,5]. Accumulating

Abbreviations: JATD, Jeune asphyxiating thoracic dystrophy; SRP, Short-rib polydactyly; OA, Osteoarthritis; IFT, Intraflagellar transport; Ptch1, Patched 1; Smo, Smoothened; Hh, Hedgehog; Shh, Sonic Hh; shRNA, Small hairpin RNA; BMSC, Bone marrow-derived stromal cells; PBS, Phosphate-buffered saline; DAPI, Propidium iodide (PI) 6-diamidino-2-phenylindole; SDS, Sodium dodecyl sulfate; PVDF, Polyvinylidene difluoride; RNAi, RNA interference; IL-1, Interleukin-1.; BSA, Bovine serum albumin

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evidence has demonstrated that defects in some IFT proteins, such as IFT88 [6] and IFT172 [7] results in impaired cilia formation, which contributes to ciliopathies in mouse models [8,9] and/or humans [10]. Interestingly, defective other IFT proteins such as IFT25 and IFT27 do not affect cilia formation, but cause severe developmental and functional abnormalities in mouse models [11–13]. These results indicate that different IFT proteins probably have their distinct functions in cell process. So far, it remains largely unknown how IFT proteins contribute to chondrocyte differentiation and function during bone development and remodeling.

Primary cilia are present on almost every vertebrate cell, and their existence on chondrocytes was first reported about 40 years ago [14,15]. Later Poole and Jensen groups further analyzed the ultrastructure and function of primary cilia in chondrocytes [16,17]. Their and other studies suggested that primary cilia might serve as mechanical or chemical sensors in chondrocytes to mediate normal cell-extracellular matrix communication, which is required to maintain normal cellular orientation, growth and differentiation [18–21]. Additionally, osteoarthritis (OA) [22] is a disease associated with chondrocyte differentiation and function. Some studies demonstrated that primary cilia are present in normal cartilage as well as in mild and severe OA tissues [23]. Compared to normal cartilage, the proportion and length of the cilia in OA tissues are increased with OA severity at the eroding articulating surface [23]. Inflammatory cytokines such as interluekin-1 (IL-1), which are highly expressed in OA, are able to induce the elongation of the cilia [24]. Cultured chondrocytes from the patients with SRP syndrome showed morphologically abnormal, shortened cilia and abnormal cytoskeletal microtubule architecture [25]. In mouse models, deletion of IFT88 or Kif3a during chondrocyte development using the Col2α1-Cre promoter, causes a progressive loss of the cartilaginous growth plate and postnatal dwarfism that resembles the phenotype of mice with a conditional deletion of India Hedgehog (Ihh) induced in postnatal cartilage [26–28]. IFT88 deletion also results in symptoms of early OA due to reduced Hedgehog (Hh) signal repression by Gli3 [29]. Collectively, these findings highlight that IFT/cilia proteins play critical role in the differentiation and function of chondrocytes and chondrocyte-related diseases.

Much evidence revealed that there are connections between the Hh signal pathway and the primary cilium as well as IFT [9,30,31]. For example, conditional deletion of Kif3a causes abnormal Hh signaling topography, growth plate dysfunction, and excessive bone and cartilage formation during mouse skeletogenesis [32], which is similiar to the phenotype in Ihh-deficient mouse [33]. Gli transcription factors (Gli1, Gli2 and Gli3) are key effectors of the Hh signaling pathway [34]. Recent studies demonstrated that Gli transcription factors as well as several other Hh pathway components, including Smoothen (Smo) and Hh receptor Patched (Ptch), are accumulated in primary cilia either under resting conditions or during pathway activation [35]. Gli1 is dispensable for embryonic development and encodes a secondary mediator of Hh signaling [36]. Gli2 are the major transcriptional activator whereas Gli3 is the repressor of the mammalian Hh pathway [34,37]. These findings suggest the relationship between IFT/cilia and Hh/Gli and their roles in chondrocyte differentiation and function. In addition, Wnt signaling has been implicated in a wide variety of developmental processes from cell proliferation to cell fate determination and differentiation [38,39]. Wnt/β-catenin signals developmentally regulate chondrocyte differentiation, growth plate assembly and cartilage integrity [40]. Mice with transient activation of Wnt/β-catenin induced premature closure of the growth plate in postnatal mice [41]. Loss of primary cilia has been shown an increase in response to the canonical Wnt signaling pathway [42,43], suggesting that primary cilia/IFT proteins, at least some of them have a role in restraining Wnt/β-catenin signaling.

IFT80 protein is a recently-defined component of IFT complex B [44]. Loss of IFT80 in zebrafish results in cystic kidneys and a reduced number of cilia, whereas silencing IFT80 in *Tetrahymena thermophila* produces shortened or absent cilia [44,45]. Furthermore, IFT80 mutations in humans cause JATD [44] and SRP syndrome type III [46]. Those phenotypes suggest that mutation of IFT80 possibly affects chondrocyte differentiation and function. However, the precise role and mechanism of IFT80 in regulating chondrogenic differentiation remain unknown. Hence, in this study, our aim is to elucidate the role of IFT80 in cilia formation and chondrocyte differentiation and to identify the pathways involved in this progress. We first tested the expression pattern of IFT80 in growth plate and during chondrocyte differentiation. Furthermore, we used lentivirus-mediated small hairpin RNA (shRNA) to silence IFT80 in mouse BMSCs and characterized the effect of IFT80 silencing in cilia formation and chondrocyte differentiation in vitro. Additionally, we analyzed the mechanism by which IFT80 regulates Hh signaling and Wnt signaling pathways. Our results demonstrated that IFT80 plays an essential role in chondrocyte differentiation likely through regulating Hh/Gli and Wnt/β-catenin signal pathways.

Materials and methods

Cells and cell culture

Mouse BMSCs were isolated from a 6-week-old C57BL/J mouse and cultured in our laboratory as described previously [47]. All animal procedures were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University at Buffalo (UB). Briefly, the mouse was sacrificed by CO_2, and the femurs were dissected free from the surrounding soft tissue in a sterile hood. BMSCs were collected by flushing the diaphysis with phosphate-buffered saline (PBS) after resetting the metaphysis from both ends. The cells were maintained in modified Eagle's medium alpha (a-MEM) containing 10% fetal bovine serum. The cells were cultured in 100-mm culture dishes in a humidified, mixed environment of 37 °C and 5% CO_2. The basic media consisted of a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium containing 5% fetal bovine serum.

Lentiviral gene transfer

To identify the function of the IFT80 gene in the chondrogenic differentiation and chondrocyte signaling pathways, we used lentivirus-mediated mouse pLKO.1-IFT80 shRNA plasmids and control pLB-scrambled shRNA (Open Biosystems, Lafayette, CO) to package IFT80 recombinant lentivirus according to the manufacturer's instructions. Briefly, five individual vector pLKO.1-IFT80 shRNAs (I1, I2, I3, I4, and I5) and pLKO.1-scramble shRNA (pLB Control) were respectively co-transfected with the packaging