INVITED REVIEW

Advances in treatment of achondroplasia and osteoarthritis

Kendra A. Klag1,2 and William A. Horton1,2,*

1Research Center, Shriners Hospital for Children, Portland, OR, USA and 2Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA

*To whom correspondence should be addressed at: Research Center, Shriners Hospital for Children, 3101 SW Sam Jackson Park Road, Portland, OR 97239, USA. Tel: +1 503 221 1537; Fax: +1 503 221 3451; Email: wah@shcc.org

Abstract

Achondroplasia (ACH) is the prototype and most common of the human chondrodysplasias. It results from gain-of-function mutations that exaggerate the signal output of the fibroblast growth factor receptor 3 (FGFR3), a receptor tyrosine kinase that negatively regulates growth plate activity and linear bone growth. Several approaches to reduce FGFR3 signaling by blocking receptor activation or inhibiting downstream signals have been proposed. Five show promise in preclinical mouse studies. Two candidate therapies target the extracellular domain of FGFR3. The first is a decoy receptor that competes for activating ligands. The second is a synthetic blocking peptide that prevents ligands from binding and activating FGFR3. Two established drugs, statins and meclozine, improve growth of ACH mice. The strongest candidate therapy employs an analog of C-type natriuretic peptide (CNP), which antagonizes the mitogen-activated-protein (MAP) kinase pathway downstream of the FGFR3 receptor and may also act independently in the growth plate. Only the CNP analog has reached clinical trials. Preliminary results of Phase 2 studies show a substantial increase in growth rate of ACH children after six months of therapy with no serious adverse effects.

A challenge for drug therapy in ACH is targeting agents to the avascular growth plate. The application of gene therapy in osteoarthritis offers insights because it faces similar technical obstacles. Major advances in gene therapy include the emergence of recombinant adeno-associated virus as the vector of choice, capsid engineering to target vectors to specific tissues, and development of methods to direct vectors to articular chondrocytes.

Introduction

Human chondrodysplasias affect skeletal development in many ways. Primarily, they reflect disturbances involving endochondral ossification at the growth plate. The mutations responsible for various chondrodysplasias affect genes expressed in the cartilaginous growth plates that are responsible for creating and/or degrading the cartilage templates necessary for bone growth. In most cases, these mutations adversely affect both the rate and quality of skeletal growth.

Historically, it has been challenging to design nonsurgical therapies that normalize skeletal growth in these disorders owing to limitations in our understanding of how mutations affect the molecular mechanisms that stimulate and regulate bone growth. The delivery of potential therapeutic agents to the avascular growth plate also presents a barrier. Nevertheless, researchers have made considerable progress in understanding and treating achondroplasia (ACH) with strategies directed at FGFR3. Several approaches have shown promise in preclinical studies, and one strategy employing C-type natriuretic peptide (CNP) has produced encouraging results in Phase 2 clinical trials. Here, we will discuss the status of novel treatments for ACH along with their underlying rationale.

We will also examine recent advances in the treatment of osteoarthritis (OA), especially through gene therapy. While ACH and OA may appear unrelated, the two share considerable overlap, from development to regeneration. They share common genetic pathways and regulatory mechanisms that play important roles in endochondral bone growth and articular cartilage.
homeostasis. Interestingly, many chondrodysplasias have pro-
cocious OA. Surprisingly, individuals with ACH exhibit a lower
incidence of OA despite leg bowing and higher rates of obesity,
suggesting the ACH mutation may protect against OA. Thus, a
discussion of therapeutic strategies aimed at chondrodysplasias
is relevant to OA and vice versa. Specifically, ACH treatments
could benefit from gene therapy techniques intended to treat
OA, because they offer novel approaches for targeting therapeutic
agents to the growth plate. Given recent progress, we will focus
our attention for the chondrodysplasias on ACH.

**Achondroplasia**

ACH is by far the most common form of dwarfism in humans, oc-
curring in 1 out of every 10 000–30 000 live births (1). It results
from gain-of-function mutations of the transmembrane receptor
fibroblast growth factor receptor 3 (FGFR3), which is an important
negative regulator of growth plate activity and linear bone growth
(2,3). Virtually all patients with classical clinical features of ACH
harbor a heterozygous G380R mutation that maps to the trans-
membrane domain of the FGFR3 gene. As a receptor tyrosine ki-

Achondroplasia inspired the first attempts at FGFR3 blockade. Mutant ACH
FGFR3 behaves much like an oncogenic kinase at the molecular
level and oncogenic mutations of FGFR3 are common in bladder
and cervical cancer (12). Based on the success of chemical kinase
inhibitors in cancer therapy (13), a similar inhibitor of FGFR3 was
developed (14). This molecule showed selectivity for FGFR3 and
successfully stimulated normal growth in cultured limb bones
from a knock-in mouse model of ACH, but it was never validated
in vivo.

A second strategy utilized a humanized antibody to block ac-
tivation of FGFR3. This approach originated with the effective
treatment of breast cancer using trastuzumab (Herceptin) (15).
Herceptin is a humanized antibody raised against the transmem-
brate receptor kinase HER2/neu (ErbB2) expressed on the surface
of certain types of breast cancer cells. Rauchenberger et al. de-
scribed a comparable antibody against FGFR3 that effectively
blocks ligand-induced FGFR3 activation (16). However, there
have been no subsequent reports describing its capacity to stimu-
late bone growth in vivo.

**Fgfr3 targeted therapy—second generation**

Decoy receptor. This strategy aims to mitigate excess ACH FGFR3
signaling using a soluble isoform of the receptor (sFGFR3) to com-
pete for the physiologic ligands (i.e. FGFs 9, 18) that activate en-
dogenous FGFR3 receptors on chondrocytes (10,17–19). The
sFGFR3 lacks the transmembrane domain of the receptor needed
to anchor it within the cell membrane so it is unable to transmit
signals across the membrane (20). A 3-week course of subcutane-
ous sFGFR3 injections administered twice per week restored nor-
mal limb length and decreased mortality of transgenic ACH
(Fgfr3<sup>ach/+</sup>) mouse pups compared with controls in a dose-
dependent manner and with no obvious toxicity (19). Concerns
remain about how well the relatively large sFGFR3 molecule
will penetrate the avascular growth plate and surrounding the
possible adverse effects of sFGFR3-mediated sequestration of
FGF ligands in other tissues (21). If higher plasma levels of
sFGFR3 are required to overcome the diffusion barrier of cartilage,
then complications may increase in other normally vascularized
tissues that respond to these same ligands.

P3 blocking peptide. This strategy resembles the 'blocking antibody'
approach discussed previously (14). Using the phage display
technique with FGFR3 as bait, Jin et al. generated the 12 amino
acid peptide P3, which binds with high affinity to the FGFR3 extrac-

![Figure 1. FGFR3 signal transduction and therapeutic strategies. FGF and heparin
binding to the extracellular domain of FGFR3 induce kinase activation leading
to activation of downstream signaling pathways such as STAT and MAP kinase
cascades. CNP binding to NPR-B induces the generation of the second
messenger cGMP, which activates PKG leading to attenuation of the MEK
pathway via RAF. Peptide P3 blocks ligand binding whereas meclozine is
proposed to antagonize MEK activation of ERK. Modified with permission from
cartilage-like nodules that displayed hallmarks of TDI cartilage: reduced proliferation and diminished cartilage gene expression. Using these nodules as a TDI experimental model, the investigators screened compounds previously reported to influence FGFR3 signaling or chondrocyte differentiation for the ability to correct the defect and found that statins promoted chondrogenesis. They proposed that statins act by reducing the signaling lifetime of mutant FGFR3 receptors, but their data were minimal and they did not pursue further experiments. \( \text{Fgf3}^{ach/-} \) mouse pups were treated with IP injections of rosvastatin (Crestor) 6 days a week from postnatal Day 3 to Day 15. They observed a modest but statistically significantly greater length measurements for femur, tibia and bones of the cranial base in treated versus vehicle control pups. This was a very short-term study, so further investigation is needed to evaluate the safety and efficacy of this strategy.

Meclozine. Meclozine, an antihistamine long used for motion sickness, was found to stimulate proliferation of rat chondrosarcoma (RCS) cells in a screen of FDA-approved drugs (25). RCS cells are commonly used as a model of chondrogenesis. After documenting that meclozine reduced ERK signaling downstream of FGFR3 in RCS cells and stimulated growth of cultured embryonic mouse tibia, it was administered orally to \( \text{Fgf3}^{ach/-} \) mouse pups from 3 to 6 weeks of age (26). They detected a modest but statistically significant increase in limb and cranium length compared with controls. They proposed meclozine acted downstream of FGFR3 signaling as a block between mitogen-activated protein kinase (MAPK) kinase pathway, but did not address a specific mechanism. The advantage of meclozine over the other candidate therapies is its oral administration route. However, as with the other novel approaches, this was a relatively cursory investigation, so further studies are needed to demonstrate long-term safety and efficacy.

CNP antagonism of FGFR3 signals. This therapeutic strategy has been investigated in much more depth and those just mentioned. It was developed by Nakao and colleagues over the past two decades. To summarize, they found that CNP knockout mice exhibit severe growth deficiency, and that crossing this line with transgenic mice overexpressing CNP in cartilage restores normal growth (27,28). Similarly, crossing CNP overexpressing transgenic mice with the ACH mouse model (\( \text{Fgf3}^{ach/-} \)) rescues their short limb phenotype (29,30). Furthermore, ACH mice exposed to continuous high levels of CNP due to transgene expression or intravenous infusion of CNP displayed increased linear bone growth establishing a foundation for clinical studies (31,32).

In contrast to other natriuretic peptides, which act systemically as endocrine factors, CNP functions primarily in a paracrine or autocrine fashion in a number of tissues including the growth plate (33,34). Through interaction with its cognate receptor natriuretic peptide receptor-B (NPR-B), CNP induces accumulation of intracellular cGMP. Importantly, homozygous mutations of NPR-B are responsible for acomosemic dysplasia, type Maroteaux (OMIM 602875) (35). Also of interest is the report of a 14-year-old girl with relatively tall stature, a Marfanoid habitus and a de novo balanced t(2;7)(q37.1;q21.3) translocation (36). The CNP gene maps near the translocation breakpoint on chromosome 2. The authors observed a doubling of plasma CNP levels of this gene in cultured fibroblasts; they speculated the elevated CNP caused the tall stature.

The growth plate expresses both CNP and NPR-B, creating a potential autocrine or paracrine regulatory circuit (37). CNP also induces expression of NPR-C in the growth plate and other tissues (38). NPR-C is a clearance receptor for natriuretic peptides that limits the cellular response to these signaling molecules.

There is still debate as to how CNP promotes growth plate activity. Several reports suggest that CNP initiates signals downstream of NPR-B that antagonize the MAP kinase pathways downstream of FGFR3, particularly the ERK arm of this pathway at the level of RAF (Fig. 1) (39–42). However, evidence also suggests that CNP acts independent of FGFR3 to stimulate growth and that these two pathways reciprocally antagonize one another (38,43,44). In fact, children with ACH have elevated plasma levels of CNP and its amino-terminal propeptide, suggesting that FGFR3 activation induces CNP resistance (45). In practice, it may not matter if CNP antagonizes FGFR3-MAPK-ERK signals or overcomes FGFR3-induced CNP resistance as long as exogenous CNP overrides the growth inhibitory effects of mutant FGFR3.

Despite the strong case for CNP as a treatment for ACH, one major drawback remains—its rapid clearance from the plasma (estimated half-life of 2.6 min) (32,46). To overcome this obstacle, investigators at BioMarin Pharmaceuticals developed a CNP analog (BMN 111), which retains the biologic properties of native CNP but has an extended half-life due to its resistance to neutral-endopeptidase digestion, allowing for once daily subcutaneous administration. BioMarin has documented BMN 111 stimulates bone growth in two mouse models of ACH (\( \text{Fgf3}^{ach/-}, \text{Fgf3}^{ach/-}/\)) wild-type mice and cynomolgus monkeys (21,47). It should be noted that candidate therapies that effectively reduce FGFR3 signaling in ACH models typically stimulate bone growth in wild-type animals used as controls and in larger animal models used to document preclinical efficacy and safety.

Theoretical complications of BMN 111 relate primarily to increased activation of NPR-B or possibly aberrant activation of NPR-A in tissues other than the growth plate. As noted above, CNP normally acts as a local paracrine or autocrine factor. Therefore, introducing BMN 111 systemically in sufficient amounts to produce therapeutic levels in the avascular growth plate will expose other cells to unusually high concentrations of CNP. In principle, induction and activation of the clearance receptor NPR-C should protect against excess CNP. Further study is needed to determine if these concerns have clinical relevance.

As BMN 111 progressed along the preclinical pipeline, there were also concerns about the known hemodynamic effects of natriuretic peptides (21,33). Initial dosing studies in mice showed a modest drop in the mean arterial blood pressure and a modest increase in heart rate following BMN 111 administration. Subsequent acute and long-term (6 month) studies in cynomolgus monkeys suggested these effects were transient and well tolerated at doses likely to be therapeutic. Clinical chemistry and hematologic parameters remained normal and unchanged for the study duration except for increased alkaline phosphatase, presumably related to increased bone formation (21).

**Clinical trials in ACH**

**BMN 111 (vosoritide).** To date BMN 111, recently named vosoritide, is the only candidate therapy for ACH to have entered clinical trials. A Phase 1 trial completed in 2012 evaluated safety, tolerability and pharmacokinetics in 48 healthy adult males (48). The drug was generally well tolerated with no dose-limiting toxicities identified other than mild, transient and self-limited hypotension that was usually orthostatic and asymptomatic.

The initial Phase 2 trial labeled Clinical Trial 1 was an open-label, sequential cohort dose-escalation study in which children with ACH received one of three doses (2.5, 7.5 or 15 \( \text{mg/kg} \)) as a daily subcutaneous injection for 6 months (49). Twenty-six
children with ACH, diagnosed clinically and confirmed by genetic testing, enrolled in the trial, aged 5–14 years (average age 7.8 years). Each patient had at least 6 months of pretreatment growth measurements and no evidence of a medical condition known to interfere with skeletal growth, other than ACH.

Recently released data from the first 6 months of treatment with BMN 111 demonstrated a favorable safety profile and efficacy at the 15 µg/kg/day dose regimen (50). Ten children at this dose showed a 50% increase (P-value = 0.01) in annualized growth velocity compared with their pretreatment annualized growth rate. They did not display any changes in body proportions and patients reported no serious adverse side effects, although asymptomatic hypotension occurred in some patients. Most adverse events were mild such as reactions at the injection site, headache, back pain and cough. Consistent with the mode of CNP action, they observed a dose-related increase in urinary excreted cGMP. Given these results, all children in this trial have switched to the high dose (15 µg/kg/day) regimen for the duration of the 18-month extension study.

Considering these encouraging results, BioMarin will likely move forward with additional clinical trials in pursuit of approval from USA, European and Japanese Health Authorities for BMN 111 treatment of ACH.

Next steps

Targeting delivery to the affected growth plate remains a major challenge in implementing new therapies for chondrodysplasias. The therapeutic strategies discussed thus far are systemic. While many tissues such as brain, gut, pancreas and adrenal glands express FGFR3, and FGFR3 is mutated globally in ACH, drug development work has focused on the growth plate. Thus, systemic administration of therapeutic agents may cause adverse effects in these other tissues. These risks increase when we consider that high blood levels of agents may be necessary to achieve therapeutic levels in the avascular cartilage growth plate (1). To minimize the risk of adverse effects in other tissues, the logical solution is to directly target therapeutic agents to the growth plate or at least to cartilage (51). However, no such targeting strategy exists for growth plate cartilage.

Delivering therapeutic agents to articular cartilage in OA faces many of the same challenges. In this case however, advances have been made using gene therapy. Below we briefly review the pertinent progress in OA gene therapy and consider how it may influence future treatments for chondrodysplasias.

**Osteoarthritis**

OA is an extremely common disease of adulthood affecting mostly weight bearing joints. Its pathology is dominated by progressive loss of articular cartilage mediated by proteolytic enzymes directed against the major matrix macromolecules, as well as inflammation and metabolic changes that reduce the ability of cells to cope with mechanical and oxidative stresses (52–54). The primary goals of OA therapy are to prevent or minimize cartilage loss and/or promote repair of the damaged cartilage.

**Gene therapy**

The first excursions into OA gene therapy employed ex vivo strategies that involved harvesting synovial fibroblasts, transducing them with transgenes encoding therapeutic agents in vitro and returning the modified cells into the joint space to colonize the synovial surface. Despite early successes, direct injection of vectors into the joint space offered advantages over the more cumbersome ex vivo approach. Concurrently, studies extensively tested many different viral vectors as vehicles for in vivo gene delivery. Recombinant adeno-associated virus (rAAV) emerged as the vector of choice due to its ability to transduce non-dividing cells, long-term transgene expression without need for viral integration, lack of known disease association and low immunogenicity (55–57). Its relatively small size improves tissue penetration, but limits its cargo capacity.

Over the last decade, researchers have made numerous advances in rAAV vector design. Most have involved engineering capsid proteins that enable better targeting to specific tissues and cells types (58). Examples include altering the epitopes responsible for neutralizing antibodies found in many individuals, modifying amino acids that trigger proteasomal degradation and immune processing of viral particles and incorporating high-affinity ‘targeting’ ligands into the viral capsid (59–62).

To date OA gene therapy has not needed to take advantage of the advances in vector design that enable tissue and cell targeting because current strategies inject the vector directly into the synovial space with the intent of transducing synovial and other cells that border the joint space (Fig. 2) (53,56,57). The transduced cells secrete the therapeutic agent into the synovial fluid where it then diffuses into relevant cells (63). Using this method, agents tested in OA animal models include anti-inflammatory proteins, and cartilage growth factors (53,56,57). Clinical trials for OA have used TGF-β1 and the interleukin antagonist IL-1RA.

A drawback of the synovial cell secretion strategy is that production of therapeutic agents is lost over time due to the limited life span of synovial cells (63,64). Chondrocytes appear an ideal target cell because of their long life span and direct participation in OA (53). Early in vivo injection studies utilizing relatively large retroviral vectors suggested that the cartilage matrix is too dense for vector penetration. However, subsequent investigations have shown that the much smaller rAAV can transduce chondrocytes within articular cartilage, although studies in a horse model observed local variability (64,65). Related studies suggested that rAAV serotypes vary in their chondrocyte transduction efficiency (66). If rAAV-mediated targeting of articular chondrocytes gains acceptance, then advances in rAAV vector design will likely enhance targeting chondrocytes accessible by joint space injection, over other cell types in the area.

Despite considerable progress in gene therapy vectors and delivery strategies, progress in the clinical arena has been slow. The
Thoughts for the Future

Could gene therapy ever be used effectively in the treatment of chondrodysplasias? Conceivably yes, but there are many hurdles. For instance, even if agents such as CNP could be targeted to growth plate chondrocytes, how would one achieve uniform transduction from bone to bone or from one region to another within a single growth plate? How could production of the therapeutic factors be regulated? Would therapists be able to fine tune expression to modulate chondrocyte proliferation or differentiation? Similarly, what mechanisms could be put in place to discontinue therapy if problems arose or after cessation of growth at puberty?

Could knowledge derived from chondrodysplasias be applied to OA in the context of gene therapy? Potentially so. Take the example of ACH in which OA is not a feature despite severe bowing of the knees and a higher incidence of obesity (1). Studies of ACH in which OA is a feature might involve injecting a rAAV vector encoding FGF18, the major ligand for FGFR3, into OA joints as a possible OA therapy. FGFR3-mediated inhibition in the knockout mice allows for accelerated bone growth plate, increased FGFR3 activity characteristic of ACH. FGFR3 knockout mice show they exhibit severe OA, suggesting that the excessive FGFR3 signaling in ACH may protect ACH from OA (68). The most likely explanation involves the inhibitory effect FGFR3 has on chondrocyte hypertrophy. In the growth plate, increased FGFR3 activity characteristic of ACH slows endochondral ossification by reducing chondrocyte hypertrophy. However, chondrocyte hypertrophy is an important mechanism in the progression of OA (54,69). It is associated with reduced expression of cartilage matrix protein genes, production of the matrix degrading enzymes and metabolic changes characteristic of OA chondrocytes. Not surprisingly, loss of this FGFR3-mediated inhibition in the knockout mice allows for accelerated hypertrophy in articular cartilage and OA (68). A potential early-stage OA therapy might involve injecting a rAAV vector encoding FGF18, the major ligand for FGFR3, into OA joints as a strategy to reduce or even prevent the sequelae of chondrocyte hypertrophy in OA.

At first glance ACH and OA only seem connected by the anatomical proximity of their affected tissue. But, from that proximity stems a common developmental origin. Studying the pathways of development in the context of ACH and OA holds the potential to inform efforts in regenerative medicine that seek to reverse these disease processes.

Conflict of Interest statement. W.A.H. has received a fee from BioMarin Pharmaceuticals Inc for advising on medical complications of achondroplasia.

Funding

This work was supported by a Research Grant (85600-POR-15) from Shriners Hospitals for Children (wah).

References


47. Lorget, F., Kaci, N., Peng, J., Benoist-Lasselin, C., Mugnier, E., Oppeneer, T., Wendt, D.J., Bell, S.M., Bullens, S., Bunting, S.