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Fibroblast Growth Factor Receptor 3 (FGFR3) Signaling in Achondroplasia

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Achondroplasia is autosomal dominant genetic disease and fibroblast growth factor receptor 3 (FGFR3) is currently known to be the only gene that causes achondroplasia. Gain-of function mutation in fibroblast-growth-factor-receptor 3 (FGFR3) causes the disease and C-type natriuretic peptide (CNP) antagonizes FGFR3 downstream signaling by inhibiting the pathway of mitogen-activated protein kinase (MAPK). As FGFR3-related skeletal dysplasias are caused by growth attenuation of the cartilage, chondrocytes appear to be unique in their response to FGFR3 activation. However, the full spectrum of molecular events by which FGFR3 mediates its signaling is just beginning to emerge. This article summaries the mechanisms of FGFR3 function in skeletal dysplasias, the extraordinary cellular manifestations of FGFR3 signaling in chondrocytes, and finally, the progress toward therapy for ACH.

Keywords: Fibroblast-growth-factor-receptor 3, C-type natriuretic peptide, Mitogen-activated protein kinase, Achondroplasia

Introduction

Gain-of-function mutations in FGFR3 (MIM 134934) lead to achondroplasia (ACH [MIM 100800]), hypochondroplasia (HCH [MIM 146000]), and thanatophoric dysplasia (TD [MIM 187600])[1,2]. These conditions, all due to increased signaling of fibroblast-growth-factor-receptor 3 (FGFR3), are characterized by a disproportionate rhizomelic dwarfism and differ in severity, which ranges from mild (HCH) to severe (ACH) and lethal (TD)[3]. FGFR3 is expressed mainly by developing bones, brain, lung, and spinal cord[4,5], and FGFR3-deficient mice show enhanced endochondral bone growth, expansion of their growth plate, and increased chondrocyte proliferation[6,7]. This mouse phenotype suggests that FGFR3 is a negative regulator of endochondral bone formation, confirming that the mutations causing FGFR3 chondrodysplasias are gain-of-function mutations. FGFR3 is a key regulator of endochondral bone growth, which signals through several intracellular pathways including the signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase (MAPK)[8-10].

Mutations Activate FGFR3 by Different Mechanisms

Extracellular FGF ligands activate FGFR signaling via formation of FGFR dimers, which is assisted by heparin sulphate proteoglycans and mediated by bivalent ligand–receptor interactions[11]. All known mutations activate FGFR3 by facilitating its dimerization, although the exact mechanism varies depending on the location of given mutation. The G380R (ACH) substitution leads to ligand-independent stabilization of FGFR3 dimers via hydrogen bonds formed between the side-chains of the two arginine residues[12]. TDI-associated Y373C and R248C substitutions activate FGFR3 via forming covalently bound dimers, held together by a disulfide bond between the free cysteine residues introduced into the juxtamembrane domain (Y373C) or to the region linking two Ig domains in the extracellular part of FGFR3 (R248C)[13,14]. Finally, substitutions in the intracellular tyrosine kinase (TK)

domain, such as K650M (TDI) or K650E (TDII), activate FGFR3 by mimicking the conformational changes in the TK domain that are normally induced by ligand-mediated FGFR3 dimerization and autophosphorylation[12]. Both K650M and K650E-FGFR3 mutants show abnormal intracellular

localization, as they mature poorly after synthesis and accumulate in the endoplasmic reticulum (ER)[15], possibly via increased interaction with shisa protein within the ER[16]. In their ER-based signaling, K650M and K650E-FGFR3 appear to use noncanonical ways to activate the downstream signaling intermediates such as ERK MAP kinase[17]. This feature, however, does not appear to be essential for the development of skeletal dysplasias, since other FGFR3 mutants such as R248C and Y373C mature normally and signal from the cell membrane, yet cause a more severe skeletal phenotype[18].

FGFR3 Is a Physiological Negative Regulator of Bone Growth

Although FGFR3 is expressed in brain, lung, and spinal cord in addition to cartilage[4,5], its major domain of normal physiological function appears to be the regulation of cartilage growth. Fgfr3 null mice have long-bone overgrowth due to expanded zones of epiphyseal growth plate cartilage caused by increased chondrocyte proliferation[6,7]. Such data clearly identify FGFR3 as a physiological negative regulator of skeletal growth, which restricts the length of long bones via inhibition of chondrocyte proliferation. It is important to note, that the profound dwarfing phenotypes in individuals carrying gain-of-function mutations in FGFR3, as discussed in this review, are not due to novel FGFR3 functions in cartilage, but rather exaggeration of its physiological roles.

Molecular Mechanisms of FGFR3 Signaling in Cartilage

Aberrant activation of FGFR3 alters chondrocyte behavior by inducing premature growth arrest, loss of extracellular matrix, altered differentiation, and changes in cell shape. Together, these cellular phenotypes contribute to profound disruption of the growth plate cartilage resulting in skeletal dysplasia. At the molecular level, the growth arrest phenotype is mediated by induction of several inhibitors of the cell cycle, belonging to cip/kip family (p21) or INK4 family (p16, p18, p19)[19-22], whereas the loss of the extracellular matrix originates from both inhibition of production of major matrix components (collagen type II and aggrecan), and active matrix degradation, mediated by several mem-

bers of MMP family[9]. Expression of two important physiological regulators of chondrocyte differentiation, Indian hedgehog (Ihh) and parathyroid hormone-related protein (PTHrP), is inhibited by FGFR3 in cartilage, likely contributing to impaired chondrocyte hypertrophic differentiation[22]. ERK MAP kinase is a major pathway for growth arrest, extracellular matrix loss, and impaired chondrocyte differentiation[8,15]. FGFR3 causes prolonged activation of the ERK signaling module (RAS-RAF-MEK-ERK), mediated by adapter (GAB1,FRS2, and SHC) driven recruitment of SHP2-GRB2-SOS1 complexes to the cellmembrane, where they activate RAS[8]. In addition, SNAIL1 transcription factor is involved in regulation of FGFR3-mediated ERK activity, although the exact nature of this regulation is not presently clear[23].

The FGFR3-mediated activation of the ERK pathway is antagonized by CNP signaling, which inhibits ERK pathway by inactivation of RAF kinase,via inhibitory phosphorylation mediated by cGMP-activated protein kinase (PKG)[24]. Some FGFR3 mutants also activate STAT1, possibly via direct phosphorylation at Y701[17]. It is, however, currently unclear whether activated STAT1 or other STATs induce cell cycle inhibitor expression in cartilage, thereby contributing to the FGFR3-mediated growth arrest. Finally, chronic activation of FGFR3 leads to inhibition of canonical cytokine–STAT signaling in chondrocytes, via both induction of SOCS inhibitors of cytokine signaling and inhibition of expression of receptors for IL6 (IL6Rα) or LIF (LIFR)[25]. As the latter cytokines represent positive regulators of chondrocyte proliferation, their inhibition might contribute to the pathological effects of FGFR3[26].

Therapeutic Targeting of FGFR3

The mechanics of FGFR3 signaling offers targeting opportunities for different approaches, aimed at FGFR3 production and maturation, suppression of its activation, or activation of downstream signaling mediators[27]. To date, no FGFR3 targeting via locked nucleic acid (LNA) or adeno-associated virus (AAV) mediated expression of truncated, soluble FGFR3 variant have been reported, although these approaches have been used successfully for receptor tyrosine kinase (TK)[28], and thus represent a viable option to inhibit FGFR3 signaling in vivo.

Recently, FGFR3 was found to be a client of the heat-shock protein 90 (HSP90) molecular chaperone, and thus the disruption of FGFR3/HSP90 interaction may inhibit FGFR3 signaling in chondrocytes via interference with its proper folding and maturation[29]. With the original invention of protein TK inhibitors based on an oxindole core[30], it was possible to design a small chemi-

cal molecule that binds FGFR3 directly and inhibits its kinase activity, either via a competition with ATP for the ATP binding site, or through an ATP-independent mechanism. Today, several such inhibitors exist, including compounds named SU5402, PD173074, SU6668, PD161570, PKC412, CHIR-258, NF449, and AZD1480, which display variable inhibitory activity toward FGFRs and corresponding activity against FGFR signaling in experimental cell or animal models[31]. A central issue of chemical inhibitors is their target specificity, as known compounds typically inhibit other FGFR kinases in addition to FGFR3, as well as other kinases such as VEGFR, PDGFR, JAK, and IGF1R[32]. Although the slight lack of specificity of a given inhibitor might not represent a problem, or even be advantageous for anticancer therapy, it could certainly present an important problem when used for treatment in FGFR3-related skeletal dysplasias.In fact, PD173074[30], the one of the most specific FGFR3 inhibitors known to date, inhibits both FGFR3 and FGFR1 with the same efficiency, and thus might interfere with physiological functions of FGFR1 when used for treatment of ACH.

Another conceptually different approach to target FGFR3 directly is represented by an anti-FGFR3 antibody, specifically designed to bind the extracellular part of FGFR3 and interfere with ligand mediated FGFR3 dimerization and activation. To date, two such antibodies were developed, PRO-001 and R3Mab, both showing potent antitumor activity in mice xenograft models to FGFR3-driven MM and bladder carcinoma[33]. When used in skeletal dysplasias, however, FGFR3 targeting via antibody however carries an inherent risk of an antibody-dependent cell cytotoxic reaction in cartilage[33].

Compared to other means of FGFR3 inhibition, CNP is a physiological negative regulator of FGFR3 in cartilage. The predominant localization of the CNP pathway in cartilage, its relative straight forward manipulation, as well as an existing practical demonstration of CNP's potential to rescue pathological FGFR3 signaling in murine models makes CNP the foremost candidate for a therapy aimed at improving cartilage growth in FGFR3-related skeletal dysplasias. According to a recent public announcement, a stable CNP analogue developed by BioMarin Pharmaceuticals (http://www.bmrn.com) is progressing well toward treatment for ACH, with human clinical trials expected to begin in 2012.

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