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Review

Altered FGF signalling in congenital craniofacial and skeletal disorders

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ABSTRACT

The fibroblast growth factor (FGF) signalling pathway has been the focus of intense genetic and functional research for several decades. The emerging data implicate FGF signalling in diverse regulatory processes, both in the developing embryo as well as in the adult organism. Alterations in this tightly regulated pathway can lead to a number of pathological conditions, ranging from well-recognized congenital disorders to cancer. In order to mediate their cellular processes, FGFs signal through a subfamily of tyrosine kinase receptors, called FGF receptors (FGFRs). In humans, four FGFRs are described, and, to date, mutations in *FGFR1*, *FGFR2*, and *FGFR3* have been shown to underlie human developmental disorders. FGFs/FGFRs are known to be key players in both endochondral and intramembranous bone development. In this review, we focus on the major developmental craniofacial and skeletal disorders which result from altered FGF signalling.

Contents

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1. Introduction

The mammalian fibroblast growth factor (FGF) family comprises a group of 18 structurally related proteins, which play important roles in the regulation of a variety of cellular processes [1–3]. FGFs are highly conserved and have been identified in many species, ranging from nematodes to humans. The first member of the FGF family was described in 1973 and named for its ability to induce proliferation in 3T3 fibroblasts [4]. To date, FGF signalling has been shown to be important for a growing list of fundamental processes in embryonic development and organ formation, as well as in adult tissue homeostasis [5]. FGFs play a critical role in embryonic morphogenesis by regulating cell proliferation, differentiation, senescence and migration. In the adult organism, FGFs are crucial for example for angiogenesis, tissue repair, wound healing, cholesterol metabolism, and serum phosphate regulation [6,7]. Furthermore, altered FGF/FGFR signalling has been linked to the aetiology of several cancers [8].

FGFs mediate their cellular processes through a subfamily of tyrosine kinase receptors, named FGF receptors (FGFRs). In humans, four FGFRs are described and congenital disease-associated mutations have been identified in *FGFR1*, *FGFR2*, and *FGFR3.* Although mutations in several of the FGF genes have also been linked to congenital syndromes, the focus of this review will be on the FGFR-related groups of skeletal dysplasias and craniosynostosis syndromes.

Linking dysregulated FGF signalling to disease has opened up avenues for further research into therapeutic options that act directly or indirectly on this pathway and/or its downstream targets. Promising first evidence exists for the use of compounds targeting the FGF/FGFR pathway in the development of therapies for achondroplasia and certain cancer types. A brief overview of the current therapeutic options for the group of FGFR3 skeletal dysplasias is provided at the end of this review.

2. General aspects of FGF/FGFR signalling

The many important cellular functions and processes controlled by FGF signalling infer that the growth factor signalling pathway is highly complex. In mammals, the FGF family comprises 18 ligands acting through FGFRs, their high-affinity binding partners. FGFs interact with heparan sulphate proteoglycans. While paracrine FGFs have a high affinity for heparan sulphate and require heparan sulphate for signalling [9–13], endocrine FGFs, demonstrate a lower affinity for heparan sulphate and rely on Klotho co-receptors to elicit their metabolic effects [14–18].

Proteins of the FGFR family share a highly conserved structure with an extracellular domain that contains three immunoglobulin (Ig)-like domains (designated D1, D2, and D3), a single transmembrane domain and a split cytoplasmic tyrosine kinase domain [19,20]. FGF binding occurs at the second and third Ig-like domains (D2 and D3) and the linker between these domains. A stretch of negatively charged amino acids in the linker connecting the D1 and D2 domains is termed the acid box. Furthermore, a conserved positively charged region in the D2 domain serves as the binding site for heparan sulphate or heparin. The D3 domain is encoded by two separate exons: exon IIIa encodes the N-terminal part of the D3 domain, while the C-terminal part is encoded by either exon IIIb or IIIc [21,22]. *FGFR1-3* undergo alternative splicing in their D3 domains, thereby generating receptor isoforms with different affinities and specificities for the different FGF ligands [23]. It has been shown that these isoforms are expressed in a tissue-specific manner. The so-called "c" isoform of the receptors is predominantly expressed in skeletal tissues.

FGFs can bind to the extracellular domain of the inactive FGFR monomer and, in the presence of heparan sulphate, induce FGFR dimerization [24,25]. The receptor dimerization causes the two intracellular kinase domains of the two FGFRs to phosphorylate each other on specific tyrosine residues (see Fig. 1). This process of FGFR activation then initiates a complex cascade of further intracellular signalling through several downstream pathways [7,26]. The most well-studied of these pathways include (i) the phospholipase Cγ (PLCγ) pathway, (ii) the RAS-MAP kinase pathway, which includes ERK1/2, p38 and JNK kinases, and (iii) the PI3 kinase/AKT pathway (see Fig. 2). The activities of these signal transduction pathways vary depending on the cell type, although the activation of the RAS-MAP kinase pathway has been observed in all cell types. Key components of the FGF signalling are the docking protein FGF-receptor substrate 2 (FRS2) and the signalling enzyme PLCγ. Most of these phosphorylation transduction pathways target transcription factors within the nucleus, thereby affecting gene expression, cell proliferation, differentiation and survival (see Fig. 2).

The complexity and diverse functions of the FGF/FGFR pathway further explain the wide range of congenital disorders observed when the pathway is disturbed. Germline mutations in the *FGF/FGFR* genes result in at least 24 distinct human congenital disorders. Prominent amongst them are groups of syndromes with skeletal, cranial and limb abnormalities. Disorders associated with deafness, endocrine abnormalities, renal abnormalities and neurological disease have also been described (listed in Table 1). The skeletal effects are primarily a consequence of altered endochondral ossification, resulting in short-limbed dwarfism, while the craniosynostosis effects are thought to be a consequence of altered intramembranous ossification, leading to premature fusion of one or several cranial sutures.

3. FGF/FGFR signalling in cranial suture development

The human skull consists of five bones: paired frontal bones, paired parietal bones, and the occipital bone, in addition to lateral contributions from the squamous part of the temporal bone and the greater wing of the sphenoid bone (see Fig. 3). These bones form by intramembranous ossification. During embryonic development, the bones grow and expand, but do not fuse. The unossified junctions between the calvarial bones are known as sutures. Before skull bones fuse later in postnatal development, the intervening sutures ensure the growth potential of the skull to be able to accommodate the underlying growing and developing brain. Growth at the sites of the sutures involves the maintenance of a population of proliferating osteoprogenitor cells. These osteoprogenitor cells differentiate into osteoblasts, which express type I collagen, bone sialoprotein, and osteocalcin, and synthesize and secrete bone matrix. Therefore, sutures are a major site of intramembranous bone growth and function as growth centres for the continuously expanding skull.

FGF signalling is crucial for normal morphogenesis, development and growth of the craniofacial skeleton [27–30]. It is well understood that the expression of the FGFs and their receptors is temporally and spatially regulated during development. From studying mouse development, we know that *Fgf2*, *Fgf4* and *Fgf9* are important players during embryonic cranial vault development

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