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Review

Fibroblast growth factors: properties, biosynthesis, biological functions, therapeutic applications and engineering

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Abstract

Fibroblast Growth Factors (FGFs) function as signaling molecules within various signaling pathways, regulating the proliferation, migration, and differentiation of soft connective tissues, nerves, epithelial tissue, and bone. The FGF family comprises 22 members, with acidic Fibroblast Growth Factor (aFGF/FGF-1) and basic Fibroblast Growth Factor (bFGF/FGF-2) being of primary significance. This article explores the biochemical and biological properties of different FGFs, elucidating their roles in various biological processes. Additionally, it delves into the interactions between FGFs and Receptor tyrosine kinases (RTKs), which activate several cell signaling cascades, such as the RAS/MAPK (Mitogen-activated Protein Kinase) pathway, PI3K (phosphoinositide 3-kinase)/AKT (v-akt murine thymoma viral oncogene homolog) pathway, PLC-γ (Phospholipase C-γ) pathway, and Signal Transducer and Activator of Transcription (STAT) pathway, to facilitate diverse cellular functions. The article also examines methods for engineering FGFs, including N-terminal truncation, point mutations, or combinations thereof, for therapeutic applications in tissue regeneration, angiogenesis, and repairing damaged tissues such as cartilage, bone, ligaments, and skin. Finally, it concludes with a discussion of the delivery systems for FGFs, encompassing scaffolds, hydrogels, as well as nano- and micro-particulate methods.

Keywords: Angiogenesis, engineered FGFs, fibroblast growth factor, RAS/MAP kinase pathway, tissue regeneration

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Growth factors are among the most studied and explored proteins of the human body. They have been in the limelight since the 1980s and have shown tremendous scope in therapeutics over the last few decades. They regulate cellular functions such as stem cell differentiation [1], cell proliferation [2], growth of cells, migration of cells, angiogenesis [3], adhesion in the epithelium, cartilage, bone, some soft connective tissues, nerve cells, and maintaining the stemness of stem cells [4]. They can also be modified genetically or structurally following their use in therapeutics and their commercial production. Growth factors are named based on their tissue of origin, such as the Cartilage-Derived Growth Factor (CDGF), Retina-Derived Growth Factor (RDGF), Astroglial Growth Factors (AGF), and Eye-Derived Growth Factor (EDGF); while some are named according to the tissue they stimulate, like endothelial cell growth factor (ECGF) [5], fibroblast growth factors (FGFs), and vascular endothelial growth factors (VEGFs). Among these, this review paper will emphasize Fibroblast growth factor and their therapeutic applications, structural modifications, and binding aspects of FGFs with FGFRs.

The first-ever discovered Fibroblast growth factor was bFGF. It was initially referred to as a polypeptide that acted

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like a cation and had a pI of 9.6. The bFGF was found in the pituitary gland and brain and could stimulate cell division in NIH/3T3 fibroblast cells [6]. Fibroblast growth factors have been observed in both vertebrates and invertebrates. Some vertebrates like zebrafish, Xenopus, chicken, mice, and humans have FGF genes in their genomes [7], while invertebrates like Drosophila and Caenorhabditis elegans also have FGF genes in their genomes [8]. Since this article is centered around the human Fibroblast Growth Factor, we won't go beyond these limits.

Biologically, FGFs are as important as other growth factors; they are key for cellular functions such as cell growth, repair, differentiation, proliferation, migration, and adhesion. The FGFs trigger receptor tyrosine kinases (RTKs), resulting in the initiation of various cellular signaling pathways, which leads to the transcription of genes into mRNAs, ultimately sequencing various cellular processes mentioned above [9]. FGFs have been implicated in imparting a series of biological functions, including the development, branching, and morphogenesis of limbs; patterning of the brain; helping the metabolism of Vitamin D and bile acid; and some cytoprotective functions [10]. Some of these functions are highlighted in this article.

The FGF family

The members of the FGF family are single polypeptide chain proteins that share some structural characteristics in common, most of them showing high avidity with heparin. Many of them are secreted into extracellular matrices where they bind to heparan sulfate (HS) or heparan-like glycosaminoglycans (HLGAGs). All the members of this growth factor family share a common 140-amino-acid-containing homologous core, which forms twelve folds of antiparallel β-sheets resulting in a barrel-shaped cylinder with variable amino acids and carboxy-terminal stretches covering it around. All the members of the FGF family are grouped because they are structurally similar. The initial FGF doesn't imply that they all stimulate fibroblast cells; for example, FGF7 doesn't stimulate fibroblast cells [11].

As shown in Figure 1, the human FGF family has 22 members so far, i.e., FGF1 (aFGF), FGF2 (bFGF), FGF3 (int-2), FGF4 (hst-1/kFGF), FGF5, FGF6 (hst-2), FGF7 (KGF), FGF8 (AIGF), FGF9 (GAF), FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF15/FGF19, FGF20 (XFGF-20), FGF21, FGF22, and FGF23, which are further subcategorized into 7 subfamilies, i.e., FGF1, FGF4, FGF7, FGF9, FGF8, FGF19, and FGF11 families, based on phylogenetic relations [12]. The FGFs contained in the FGF1, FGF4, FGF7, FGF8, and FGF9 subfamily act in a paracrine fashion and are referred to as canonical FGFs as they bind to the FGFRs with the help of Heparin/HS, which acts as a cofactor [13]. The FGF15/19 (human FGF19 is an ortholog of rodent Fgf15) subfamily members show low affinity to the FGFRs as well as the Heparan Sulfate and depend on the Klotho proteins to bring about their metabolic effects in the target tissues, while the FGF11 subfamily includes intracrine FGFs, which themselves act as a cofactor for voltage-gated sodium channels and other molecules [14].

Structure of FGF proteins

The FGF family members are closely related to each other, both structurally and to some extent functionally. Their molecular weight ranges from 17 to 34 kDa, and the amino acid sequence length ranges between 126 and 268 aa, with a core region of 120 to 140 amino acids. Additionally, a sequence of 28 amino acids from this core region is conserved in all members [15]. Out of all these FGFs, aFGF and bFGF are the most extensively discussed and studied. The secondary and tertiary structures of human FGF1, which has a molecular weight of around 15,900 Da, have eight tyrosine residues exposed to solvent and only a single tryptophan, while the secondary structure of native human aFGF comprises 52% β-sheet, 28% turns, and 11% α-helices. The remaining 9% of the structure is disordered [16, 17]. On the other hand, bFGF is entirely composed of β-sheets, in which each antiparallel β-strand is bound to the adjacent β-strand in its primary sequence through hydrogen bonding. The continuous turns or β-meanders give it the shape of a barrel, which is closed by the amino and carboxyl-terminal strands. The core of bFGF consists of hydrophobic and aromatic amino acid side chains, while the charged amino acids, particularly arginine and lysine, make up the surface of the molecule [18]. The tertiary structure of bFGF comprises three copies of β-meander motifs, where the first copy consists of residues 18–59, the second one involves residues 60–100, and the third one consists of residues 101–143. Each one has four β-strands, while the first seventeen amino-terminal residues, which are rich in serine, glycine, and proline residues, appear to be disordered. There are four cysteine residues in bFGF, i.e., Cys25, Cys69, Cys87, and Cys92, out of which Cys25 and Cys92 are conserved in the FGF family. Out of those four cysteine residues, Cys69 and Cys87 are supposed to be involved in the dimerization of bFGF molecules. Residues 106–115 provide a binding site for FGFRs where Tyr-114 and Trp115 determine the binding strength between bFGF and FGFRs [19].

Biosynthesis of FGFs

The FGFs are primarily expressed in embryonic or adult tissue. Although they are expressed in various cells like neurons, smooth muscle cells, fibroblast cells, cartilage, and bone cells, the reason behind their expression is unclear. Some features of FGF biosynthesis have been understood to some extent; for instance, signal sequences of some FGFs help in their secretion, the mRNA sequences from 5′ to the starting AUG codon may play a significant role in FGF biosynthesis, and upstream and in-frame CUG triplets can behave like an alternative translation initiation site in bFGF and INT-2 mRNAs. However, it is well established that stress related to the liver and heart prompts the production of FGF21 [20].

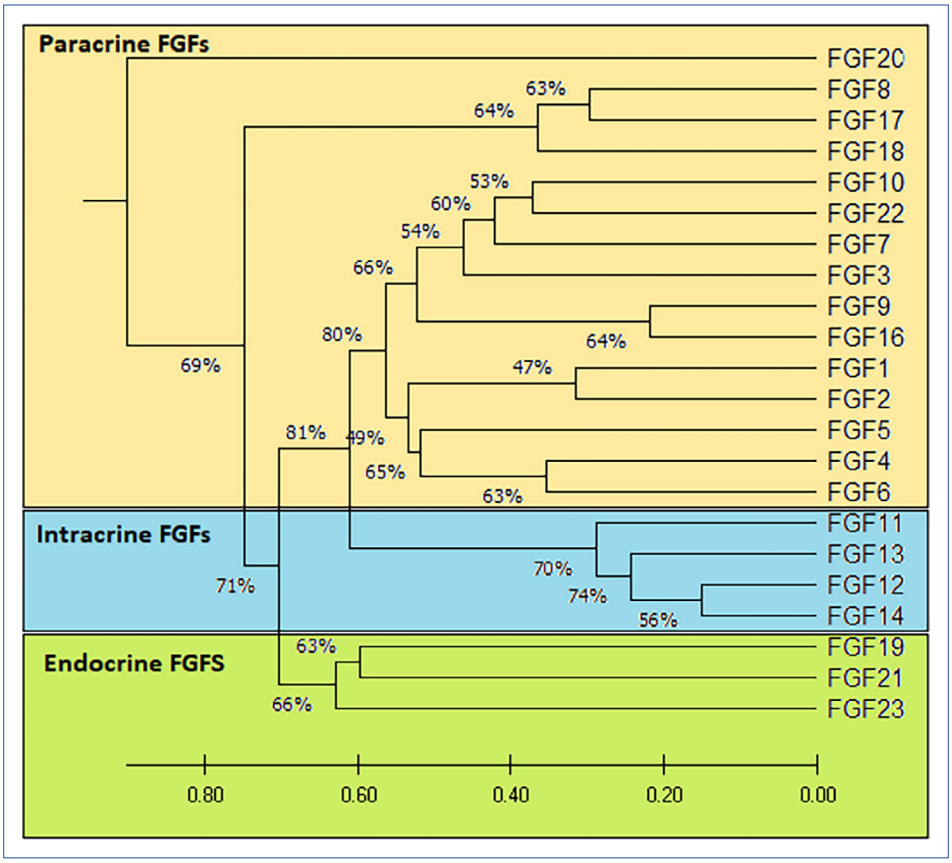

Figure 1. The phylogenetic tree of the FGF family based on the evolutionary relationships of FGFs [Phylogenetic and molecular evolutionary analyses were conducted using MEGA version X [83].

FGFs: Fibroblast growth factors.

Molecular interactions of FGF

Heparan Sulfate

Heparan Sulfate acts as a necessary cofactor for all paracrine FGFs, all of which show moderate to high affinity toward Heparan Sulfate. The binding of HS to FGF1 protects it from thermal denaturation, and the binding to FGF2 protects it from both thermal denaturation and proteolysis. The binding of FGFs to Heparan sulfate proteoglycans limits their diffusion and secretion into the interstitial space, therefore aiding them in exerting their effect at the site of their production. Cells that are unable to synthesize HS on their surfaces require heparin for effective and strong binding between FGFs and FGFRs. Studies have also shown that heparin/HS increases the half-life of the FGF/FGFR complex [21].

HS consists of heterogeneously sulfated linear polymers of repeating disaccharide subunits of hexuronic acid (iduronic or glucuronic acid) and amino acetylglucosamine, linked through α-1,4-glycosidic bonds. HS is sulfated at the 2-O position of glucuronic acid and amino acids and at the 6-O position of N-acetylglucosamine. HS shows covalent relationships with selective serine residues of proteoglycans, such as membrane-bound syndecans and glypicans and extracellular matrix (ECM) perlecan, which means they are present within the extracellular matrix of tissues and are also located on the surface of the cells [22].

HS binds to the β1-β2 loop and β10-β12 region of FGFs, which are composed of solvent-exposed basic amino acids and backbone atoms of FGFs. The HS-FGF binding affinity depends on the primary variable sequence of the HS binding region of different FGF molecules. Consequently, to some extent, this differential affinity between HS and different FGFs results in distinct biological functions of FGFs [23].

FGF-receptors

Many cell types that express FGFs also bear FGF receptors (FGFRs) on their surfaces. These FGFRs are tyrosine kinase receptor proteins with a molecular weight of 125–160 kDa, which transduce the FGF signals via phosphorylation of tyrosine residues of FGFR polypeptides, elicited by the binding of FGFs to FGFRs [24]. The first FGF receptor, FGF receptor 1 (FGFR1), was isolated from membrane fractions of chicken embryos by tagging it with crosslinked FGF2 and I^{125}. It showed a resemblance with a partial human cDNA clone called FLG (Fms-like gene). Subsequently, three more FGFRs were discovered, along with their isoforms. These FGFRs are characterized as transmembrane

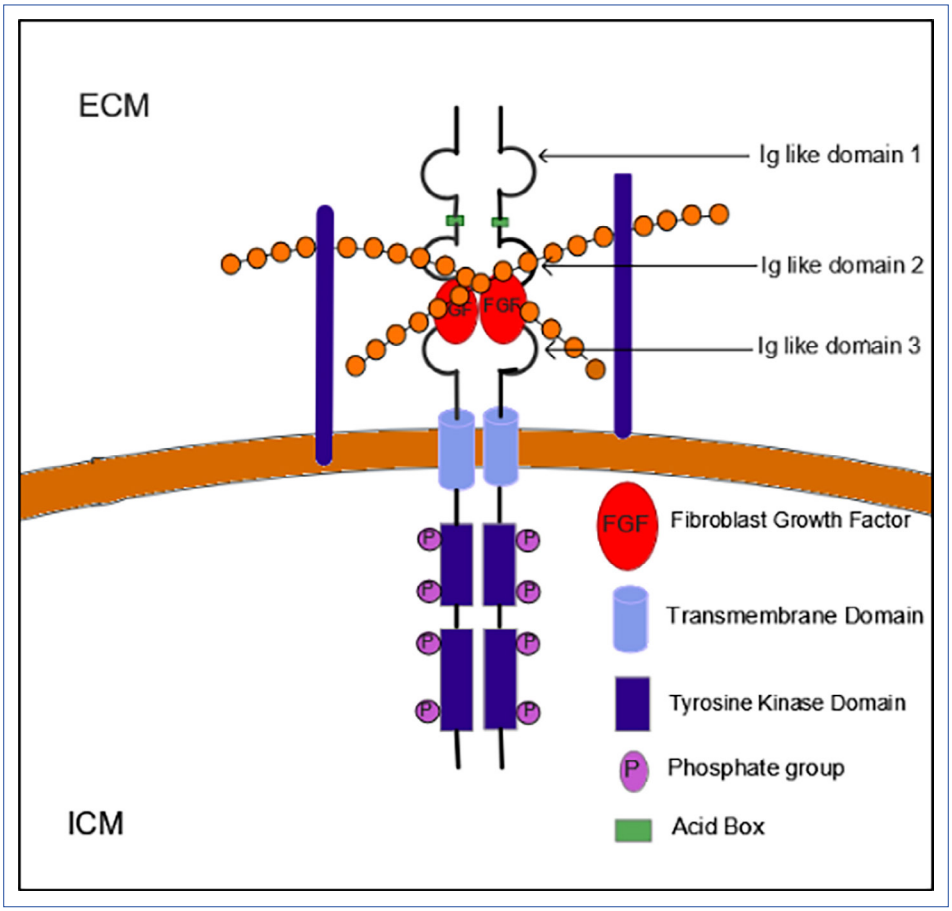

Figure 2. The schematic shows an Auto phosphorylated transmembrane FGFR dimer bound to the FGF molecules. Each FGFR molecule has one extracellular domain with three Ig-like subdomains, one transmembrane domain and one split type Tyrosine kinase domain. The alternative splicing of exon 8 and exon 9 of gene encoding Ig like domain III of FGFR 1, FGFR2, and FGFR3 results in Ig-IIIb (green) and Ig-IIIc (orange) isoforms of these FGFRs [25].

ECM: Extracellular matrix; ICM: Inner cell mass; FGF: Fibroblast growth factor; FGFR: Fibroblast growth factor receptor.

proteins consisting of three domains: an N-terminal extracellular domain with three immunoglobulin-like subdomains (IgI, IgII, and IgIII), a transmembrane domain that is a single α-helix, and an intracellular single split-type tyrosine kinase domain (Fig. 2) [25]. These FGFRs can be placed under the Ig superfamily of receptors, along with other tyrosine kinase receptors like the platelet-derived growth factor-α receptor (PDGFαR), PDGFβR, and interleukin receptor-1 (IL-1R). These FGFRs form dimers after binding to the FGFs. The FGFs bind to the Ig-like domain II in the presence of HSPG, and these altogether form a dimer with a similar complex [26].

FGF-activated cell signaling pathways

As all Tyrosine kinase receptors transduce extracellular signals to cytoplasmic transduction signal pathways by phosphorylating tyrosine residues, similarly, FGF receptors also transduce the extracellular signals to cytoplasmic transduction signal pathways by auto-transphosphorylation of tyrosine residues. The dimerization of FGFRs is necessary for their activation and signal transduction. The lateral dimerization of FGFRs in the plasma membrane brings the two tyrosine domains nearby, resulting in the trans-autophosphorylation of the tyrosine residues present in their activation loops, which results in the activation of kinase domains. These activated kinase domains interact with adapter proteins and other cytoplasmic substrates, finally triggering the cascade process of signaling pathways that control cellular functions like differentiation, proliferation, regeneration, repair, growth, etc. [27].

When FGFs act as signaling molecules for the activation of four major cellular pathways, they bind to the FGF tyrosine kinase receptors to induce dimerization and auto-trans-autophosphorylation in their kinase domains. The phosphorylation of the tyrosine kinase domain of FGFR1 is completed in three phases: in phase one, Y653 residue is phosphorylated, resulting in a 50- to 100-times increased activity of the kinase domain; the second phase phospho-

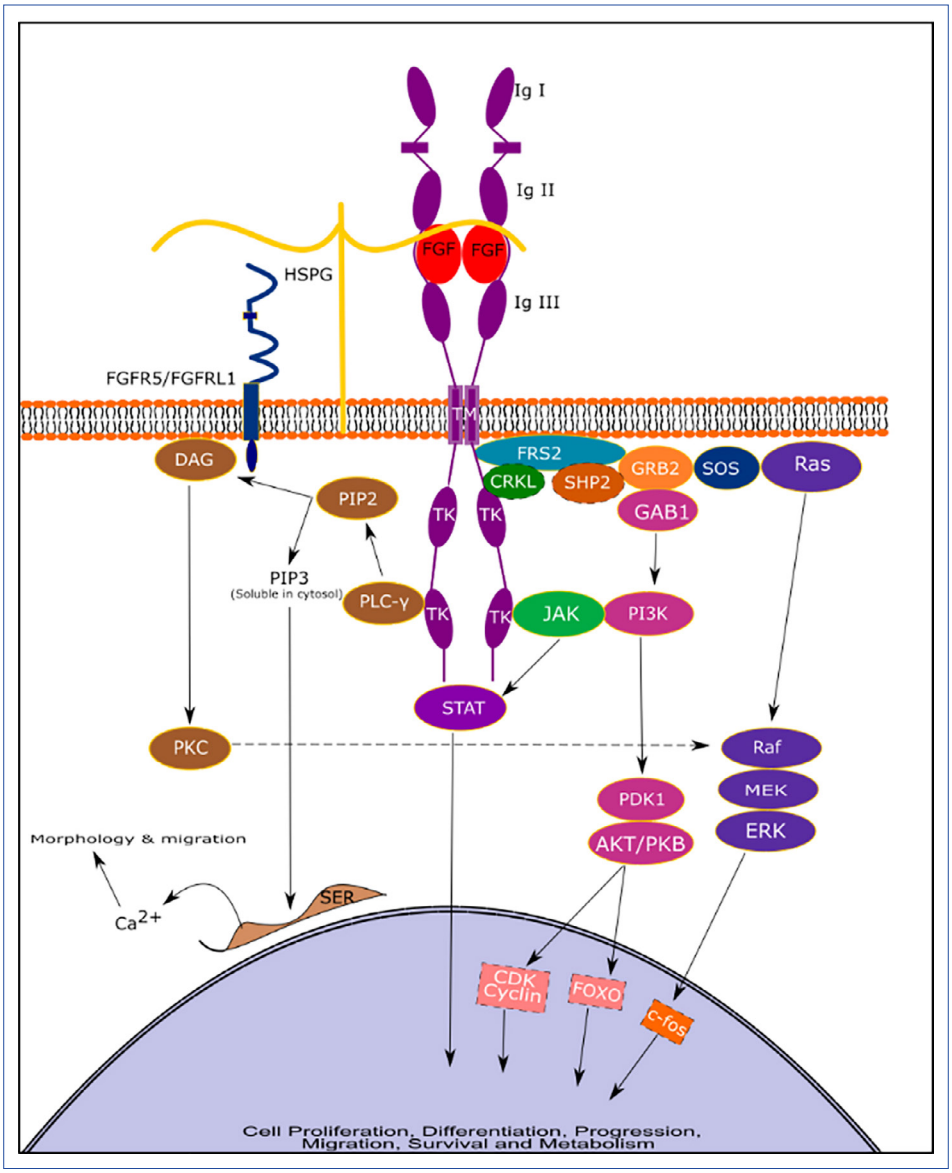

Figure 3. The FGF binding to the FGFRs triggers the dimerization of FGFRs which in turn leads to the trans-autophosphorylation of the Tyrosine kinase domains of FGFR dimer which ultimately leads to the initiation of cellular signaling cascades like RAS-MAPK, PI3K-AKT, PLCγ, and (STAT). Now, these cascades induce the activation of several genes in the nucleus leading to the transcription of respective mRNAs which are transferred out to the cytosol for the respective protein synthesis [84].

FGF: Fibroblast growth factor; FGFR: Fibroblast growth factor receptor; Ig I, II, III: Immunoglobulin-like domain I, II, III respectively; HSPG: Heparan sulfate proteoglycan; FGFRL1: Fibroblast Growth Factor Like 1; SER: Smooth endoplasmic reticulum; JAK: Janus kinase; CRK: CT10 regulator of a tyrosine kinase; CRKL: Crk-like protein; CDK: Cyclin-dependent kinase; FOXO - forkhead box transcription factor.

rylation of Y583, Y463, Y766, and Y585 does not result in any increment in kinase activity; whereas, during the third and final stage, Y654 phosphorylation leads to a further 10-times increase in the activity of the kinase domain [28]. This phosphorylation of six tyrosine residues of the tyrosine domain of FGFR1 completely activates it and increases the overall activity of the tyrosine kinase by 500–1000 folds. The activated FGFRs trigger the chain reactions of various cellular cascades including Ras/Raf/MAPK, PI3K/AKT, PLCγ, and STAT (Fig. 3). The binding of STAT3 and PLCγ requires the phosphorylation of two additional tyrosine residues, i.e., Y677 and Y766 [29].

RAS/RAF/MAP kinase pathway

The RAS/MAP Kinase Pathway is involved in important cellular functions like cell growth, proliferation, and migration. The phosphorylation of FRS2α by the tyrosine kinase domain of FGFR activates the RAS/MAP Kinase Pathway. The phos-

phorylation of FRS2α is dependent on the phosphorylation of Y463 residue of the intracellular domain of FGFR and the presence of CRKL at the docking site of the FGFR tyrosine kinase domain. A complex comprising GRB2 and the tyrosine phosphatase SHP2 is recruited by this activated FRS2α. Further, GRB2 recruits the son of sevenless (SOS), which activates the Ras molecule by exchanging one molecule of GTP for GDP, which leads to the activation of the Ras/MAP Kinase Pathway. Activation of this pathway by FGF1 relates it with the protection of the heart, development of nerve tissue, tumor invasion, biosynthesis of cholesterol, differentiation of adipocytes, and metastatic cancer [30].

According to studies, the negative feedback loop mediated by MAP-Kinase is induced by FGF signaling which phosphorylates the threonine of FRS2α, resulting in decreased engagement of GRB2 to FRS2α due to hampered phosphorylation of tyrosine residues of FRS2α. This ultimately results in the attenuation of the Ras/MAP Kinase Pathway [31].

PI3K/AKT pathway

Indeed, in addition to the Ras/Raf/MAP Kinase Pathway, the PI3 Kinase/AKT Pathway is also activated by FRS2α. Following the formation of the FRS2α, GRB2, and SHP2 complex, GRB2 recruits GAB1, subsequently triggering PDK1 and AKT to initiate the PI3K pathway. The PI3K/AKT pathway is implicated in cell survival, determination of cell fate, cell growth, cell proliferation, and cell migration. Regarding the FGF1-related PI3 Kinase/AKT Pathway, it plays a role in various physiological processes, including angiogenesis, lung development, maintenance of neuronal phenotype, preservation of neuronal structure and/or function, and ApoE-HDL (Apolipoprotein E-high-density lipoprotein) release [32].

PLCγ pathway

In this pathway, the activated FGFR tyrosine kinase domain phosphorylates PLCγ, which is activated by the formation of IP3 and DAG from the breakdown of PIP2 (Fig. 3). This membrane-bound DAG activates Protein kinase C, and the soluble IP3 stimulates the smooth endoplasmic reticulum (SER) to release high amounts of calcium ions, affecting cellular morphology and migration. Activated PKC likely expresses the gene for adhesion purposes [33]. Here, GRB14 acts as an inhibitor of PLCγ by inhibiting the tyrosine phosphorylation by binding to activated FGFR1 at pY766 [34].

Biological functions of FGFs

The FGFs exert their effect on cells by binding to the tyrosine kinase receptors and phosphorylating them. As a result of this phosphorylation, many cellular signaling cascades are activated, which result in cellular functions through gene transcription and then protein synthesis. Thus, some of the cellular functions are regulated directly or indirectly by FGFs, which are summarized in Table 1.

Cell proliferation

Cell proliferation implies the division of cells at a war scale, meaning repetitive division of cells. This is mostly observed at the developmental stage and to some extent in adults during tissue regeneration or repair, and in cancerous conditions. The essentiality of FGFs in cell proliferation begins with the proliferation of the inner cell mass (ICM) in mice [35]. Whereas FGF4 is expressed in epiblast cells [36] and FGF1 is involved in the proliferation of human preadipocytes, promoting adipogenesis in humans [37]. Similarly, FGF10 also facilitates the proliferation of epithelial cells in prostate cancer [38]. It has been established that FGF2 promotes cell proliferation by activating the Ras and RAF cascade process. FGF2 binds to the FGFRs which leads to the dimerization of FGFR, and this leads to the autophosphorylation of the tyrosine domain of the receptor. This phosphorylated domain acts as the binding site for some of the intracellular signal transducers like Grb2, which makes a complex with Ras guanine nucleotide-releasing factor Sos. This Grb2-SOS complex recruits the Ras oncogene in the plasma membrane. This recruited Ras is further activated by SOS by exchanging GDP for GTP. Further, this fully activated Ras activates the Raf-mediated MAP kinase signaling cascade, which finally results in cell proliferation [39]. This clearly shows the involvement of FGFs in cell proliferation.

Cell differentiation

Developmental biology defines cell differentiation as the process by which more specialized cells develop from less specialized ones, as observed in the development of the human zygote. The zygote develops from a single cell into an embryo and then into an adult human being. The process of differentiation can be observed in early life during development and in adults during normal cell turnover and tissue repair. Various FGFs play significant roles in the process of cellular differentiation during both the developmental and adult stages [40].

Many FGFs have been identified as crucial for cellular differentiation. FGF2, for example, is used in the divergence of neural stem cells into fully-fledged neurons and glial cells. Similarly, the morphogenesis and differentiation of suprabasal keratinocytes without FGF7 are unimaginable. Additionally, the differentiation of monkey stem cells into dopamine-synthesizing neurons would not be possible without in vitro treatment with exogenous FGF20 [41].

Cell migration

During intrauterine development, wound healing, tissue repair, and immunological responses, chemotactic movement of cells or cell migration are key processes involving many FGFs at some stages. FGF7 stimulates the migration of human keratinocytes and also regulates plasminogen activity in these cells. Similarly, FGF2 and FGF8 act as chemoattractants in the migration of mesencephalic neural crest cells [42].

Table 1. Biological functions of FGFs and their target cells/Tissues

Function	FGF involved in the function	Target cell/Tissue/ Organ	Reference
Cell proliferation	FGF1, FGF2	Preadipocyte, Endothelial cell, epithelial cell, fibroblast cell, neural stem cell	[8]
	FGF2	hematopoietic stem cells, embryonic stem cells, dental pulp stem cells and periodontal ligament stem cells	[62]
	FGF4	Trophoblast stem cell	[8]
	FGF7, FGF10	Epithelial cell	[8]
	FGF18	Osteoblast, chondrocytes, osteoclast	[63]
Cell migration	FGF2	Vascular endothelium	[64]
	FGF4	mouse Embryonic Skeletal Muscle cells	[65]
	FGF7	Human Pancreatic Duct Epithelial cells	[66]
	FGF8	Neural crest cell	[67]
Cell differentiation	FGF1, FGF2	Neuroepithelial	[68]
	FGF4	Embryonic stem cell	[27]
	FGF2	mesenchymal stem cells	[62]
		rabbit bone marrow stromal cells	[69]
	FGF7	mouse Progenitor cells	[70]
	FGF20	Monkey embryonic stem cell	[55]
Angiogenesis	FGF1, FGF2	Endothelial cell	[8]
	FGF9	Mouse Bone tissue	[71]
Metabolism	FGF21	Adipocytes and β-cells of the pancreas	[44]
	FGF23	Parathyroid gland and Kidney (Phosphate and vitamin D metabolism)	[72]
	FGF15/19	Hepatocytes (glucose metabolism)	

FGFs: Fibroblast growth factors.

Angiogenesis

During intrauterine life development, wound healing, tumor development, and tissue repair, angiogenesis is a central process. It refers to the development of new vessels from pre-existing vessels. FGFs are established to play a crucial role in inducing angiogenesis. FGF1, FGF2, and FGF4 have prominently defined angiogenic properties. These FGFs have been reported to upregulate urokinase-type plasminogen activator (uPA) and metalloproteinases (MMPs) in endothelial cells, consequently resulting in the proliferation of endothelial cells and the organization of endothelial cells into tube-like structures [43].

Metabolism

Many FGFs also play important roles in metabolism. They bind to their receptors on cells and induce effects related to metabolism. For example, FGF21 is primarily expressed by the liver to reduce hepatic glucose output. It also increases the uptake of glucose by adipocytes and improves or preserves the functions of β-cells in the pancreas [44]. The FGF19 subfamily regulates many mainstream metabolic pathways, including those involving carbohydrates, lipids, and bile acids, as well as vitamin D and phosphate homeostasis [45].

Engineered fibroblast growth factor: The new hope

The natural FGFs are strongly mitogenic and also have a very short half-life, making it difficult to control their mitogenicity and maintain their viability at the site of administration. Thus, the use of structurally modified/engineered FGFs for therapeutics or tissue engineering is becoming increasingly popular among biotechnologists.

As discussed earlier, FGFs have been very effective in the repair and regeneration of tissue. They stimulate FGFRs by acting as signaling molecules, which results in the initiation of a cascade of several cellular signaling pathways, such as RAS/MAP kinase, PLCγ, SNT-1/FRS2, Crk-mediated signaling, and PI3 kinase/AKT pathway. These pathways ultimately result in cell differentiation, proliferation, migration, and angiogenesis. Due to this broad range of biological activities, FGFs have attracted significant interest for their application in therapeutics and tissue regeneration and repair. However, due to their mitogenic nature and the challenges in delivering them to the target tissue, their use has been limited. To combat these issues, researchers have engineered FGFs both structurally and genetically and have developed several delivery systems for administering FGFs to the target tissue.

Table 2. Different types of FGF medication and their resulting recombinant FGF examples.

Type of Modification	Mutation/ modification	Example	References
Point mutation	Point Mutation (such as amino acid substitutions, deletions, additions, or combinations) of at least one amino acid residue	Q40P rFGF1, S47I rFGF1 and H93G rFGF1 respectively	[50]
Chain truncation	Deletion of continuous amino acids from one end or both	FGF1ΔNT; K25 to D155 and FGF1ΔNT2; L29 to D155	[73]
Combination of point mutation and chain truncation	FGFs modified both with point mutations and chain truncation altogether	FGF1 ΔNT1 (1-140 αα) M 1	[73]
Polypeptide chain extension	The addition of a few more amino acids to the natural polypeptide chain of FGFs	H6-FGF2 (rFGF2)	[62]
Chimeric proteins	Combination of specific sequences of one FGF to another mutated FGF.	FGF19/21-1	[68]
Extra group addition	Addition extra functional group or chemical compound	PEGylated FGF2	[74]
FGF-mAb fusion	The fusion of monoclonal human Ig fragments with FGFs	Fc-FGF21	[75]
rFGF-VLP conjugate	A recombinant FGF is conjugated with a virus-like particle (HBsAg in this case)	(Trx-FGF2)-HBcAg	[76]
SUMO ubiquitination	Small ubiquitin-related modifier (SUMO) chaperone protein attached to recombinant human FGF21	srhFGF-21	[77]

Table 3. Use of recombinant FGFs in Tissue regeneration

FGF	rFGF variant	Target tissue	Treatment	Reference
FGF1	TTHX1114	Corneal epithelial cells	Short-term Corneal Nitrogen Mustard Injury (In rabbit)	[78]
FGF2	-	Alveolar epithelium	COPD and Emphysema	[79]
	rhFGF-2	Osteocytes	Tibial Shaft Fractures	[80]
FGF18	rhFGF18	Cartilage	Repair of Articular Cartilage	[81]
FGF19	M70	Biliary and hepatic cells	Liver injury (in mice)	[82]

COPD: Chronic obstructive pulmonary disease; rFGF: Recombinant Fibroblast Growth Factor.

Engineered/recombinant FGFs represent a new hope for use in therapeutics. Recent clinical trials and concept studies are exploring the use of recombinant FGFs for tissue engineering and other applications. There are various methods for modifying FGFs, including point mutations targeting specific amino acids and the formation of chimeric proteins by combining sequences of two different FGFs [46, 47].

FGFs can be modified both at the genetic and protein levels, and various recombinants of FGFs have been developed so far. Mutated FGF proteins retain their ability to bind with FGFRs with the same specificity but without altering their properties that trigger cell growth, proliferation, survival, etc. [48]. Based on modifications done at the genetic level of FGFs or in the protein structure of FGFs, modifications can be classified into various categories (Table 2). Table 2 illustrates almost all modifications/mutations performed to date on the native forms of FGFs (with examples) to improve their activity—such as reducing their mitogenic properties while maintaining other desired properties intact and also obtaining desired characteristics like structural stability and longer half-life.

Engineered FGFs in tissue regeneration

The regeneration of damaged and injured tissues remains a significant challenge for humanity. It has been a major focus for researchers and biotechnologists for decades. The tissue regeneration capability in mammals, including humans, is very limited. In the case of tissue injury, cells from adjacent tissue and progenitor cells recruited from the bone marrow migrate and proliferate at the site of injury. Both the repair and regeneration of tissue are controlled by various cytokines and growth factors, with FGFs being among them. For example, FGF9 promotes long bone repair, and a combination of FGF7 and FGF10 promotes wound re-epithelialization in mice [49]. However, the challenge with FGFs lies in their almost uncontrollable mitogenesis and their controlled administration to specific sites.

Low stability and susceptibility to degradation by proteolytic enzymes also hinder the therapeutic use of FGFs. To overcome these problems, researchers have introduced mutations and alterations in the native sequences of FGFs, finding it feasible to some extent. For instance, the Q40P/S47I/H93G variants of

recombinant/mutated FGF1 have shown 10-fold higher activity in DNA synthesis [50]. In recent years, the use of mutated FGFs in tissue regeneration has become increasingly popular. Table 3 shows the mutated FGFs used in tissue regeneration or repair over the past few years.

Engineered FGFs in angiogenesis

The process of forming new blood vessels or capillaries from pre-existing ones during embryonic wound healing, embryonic development, or the menstrual cycle in females is known as angiogenesis. FGFs regulate the stimulation of endothelial cells to secrete proteases and plasminogen activator, which in turn degrade the basement membrane. This degradation allows new cells to migrate to the area, where they proliferate and differentiate into new vessels. bFGF was the first-ever FGF identified as an angiogenesis-stimulating growth factor [51]. Several trials, such as FIRST (FGF Initiating Revascularization Trial), AGENT (Angiogenic GENe Therapy), and TRAFFIC (Therapeutic Angiogenesis with recombinant Fibroblast Growth Factor for Intermittent Claudication), have been conducted to treat ischemic diseases with recombinant/engineered FGFs. However, none of these trials have yielded significant results [52], although the clinical trial TALISMAN (Therapeutic Angiogenesis with Intramuscular NV1FGF) did reduce the mortality rate in patients with Critical Limb Ischemia, though it was not found to be significant [53]. The hopes do not end here, as there are expectations that recombinant and improved FGFs will soon be used for revascularization/angiogenesis processes.

Methods of FGF administration

As mentioned above, the low stability and susceptibility to degradation by proteolytic enzymes also hinder the therapeutic use of FGFs. Various strategies have been employed to increase the stability, reachability, and efficacy of FGFs. FGFs have been directly used for healing wounds and administered in vivo to induce the regeneration of various tissues such as nerve, bone, cartilage, skin, endothelial tissue, and dental tissue. However, studies have shown that FGFs either become functionally degraded by enzymes or suffer diffusional loss [54]. To combat this situation, FGFs are either engineered or delivered to the site using some delivery systems listed below. The following are examples of different drug delivery systems used to administer FGF to the site of action [55, 56].

Scaffolds

FGFs or other growth factors are immobilized on matrices made of materials like collagen, beta-tricalcium phosphate (β-TCP), polyhydroxy ethyl methacrylate, polyurethane, etc. These are then molded into scaffolds using several techniques such as particulate leaching, solvent casting, freeze-drying, phase separation, melt molding, in situ polymerization, gas foaming, and phase emulsion. An excellent example of this is the delivery of FGF2 immobilized into a polyhydroxy ethyl methacrylate scaffold for bone regeneration [57]. Due to the specific biochemical interactions between natural polymers of scaffolds and FGFs, these structures are better suited for long-term delivery and stability of FGFs [58]. Scaffolds need to be implanted at the site of tissue defects.

Hydrogels

Similar to scaffolds, hydrogels also impregnate growth factors onto gels such as a carboxymethylcellulose-based topical gel, hydroxypropyl cellulose (HPC) gel, and fibrin gel. These impregnated growth factors in hydrogels are then injected into the tissue defect site. A notable example of FGF delivery using this technique is the delivery of FGF2 via a 3% hydroxypropyl cellulose gel for the regeneration of periodontal tissue [59].

Nano- and micro-particulates

The delivery of FGFs using nano- and micro-particulate techniques is even easier than the scaffold and hydrogel methods. In this growth factor delivery system, many natural and synthetic polymers are designed to be spheres a few micrometers to a few nanometers in size, and these spheres incorporate the growth factors. These FGF-containing polymers are administered either via the bloodstream or orally. The particulate method of growth factor delivery is easily manipulated to safely deliver FGFs to the defect site, and this method also ensures the full utilization of the roles and functions of FGFs. An example of this particulate method of delivery is the physical and chemical conjugation of FGF to magnetic iron oxide nanoparticles for targeting the nasal olfactory mucosa [60, 61].

Conclusion

FGFs are crucial growth factors for various physiological processes, including cell proliferation, growth, metabolism, angiogenesis, cell survival, and migration. They play an active role in embryonic growth and other physiological processes like tissue regeneration. FGFs, along with their receptors, have been associated with various pathophysiological conditions, including cancers. Their mitogenic activity has been linked to several types of cancer, including lung, breast, and prostate cancer. Due to their broad range of activities, they are a focal point for therapeutic use. Several recombinant and engineered FGFs have been shown to be effective in conditions such as bone injuries, COPD, and emphysema. Research on metabolic disorders like diabetes is ongoing to determine the therapeutic use of FGFs in these conditions. Since the half-life of FGFs is very short, various methods of site-specific delivery of FGFs have also been developed, including hydrogel, scaffold, and nano- and micro-particulate methods. These methods enhance the application of FGFs and improve their therapeutic use. In conclusion, FGFs could potentially offer solutions for various human health issues, including cancers, some metabolic and cardiac disorders,

and bone injuries. Several studies have been conducted, and others are ongoing, to determine the therapeutic effects of FGFs on various disorders; however, the number of studies, especially in the context of cancer, is still limited. Although many studies have established the role of FGFs in tumorigenesis and cancer progression, only a few have explored their therapeutic roles in cancer biology.

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