Pharmacological Research 107 (2016) 172–185

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

Blocking the FGF/FGFR system as a "two-compartment" antiangiogenic/antitumor approach in cancer therapy

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 15 March 2016
Accepted 16 March 2016
Available online 22 March 2016

Keywords:
Angiogenesis
Cancer
Cancer therapy
FGF
FGFR
Stroma
Therapeutic target

ABSTRACT

Fibroblast growth factors (FGFs) are a family of pleiotropic factors produced by stromal and parenchymal tumor cells. Even though FGFs have been firstly characterized as angiogenic factors, they exert autocrine and paracrine functions not only on endothelial cells but also on tumor cells and other stromal components. Thus, the FGF/FGF receptor (FGFR) pathway may represent a key player in tumor growth by regulating the complex cross-talk between stromal and tumor compartments.

The ligand dependent or independent activation of the FGF/FGFR system by gene upregulation, oncogenic mutation or amplification occurs in a variety of human tumors and is implicated in various key steps of tumor growth and progression. In addition, FGF/FGFR activation has been described as a mechanism of tumor escape in response to antiangiogenic/anti-VEGF therapies.

Experimental and clinical evidences provide a compelling biologic rationale for the development of anti-FGF/FGFR targeting agents in cancer therapy. However, the development of drugs specifically targeting the FGF/FGFR pathway proved to be difficult, also due to the high redundancy and pleiotropic effects of FGF and FGFR family members. On the other hand, the possibility to develop "two-compartment" targeting agents endowed with both antiangiogenic and antitumor activities remains promising.

Here we will review the preclinical and clinical approaches and potential therapeutics currently available to block the FGF/FGFR system in human cancer.

Contents

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http://dx.doi.org/10.1016/j.phrs.2016.03.024
1043-6618/

1. The FGF/FGFR system

1.1. Fibroblast growth factors

Fibroblast growth factors (FGFs) are secreted proteins that act as paracrine, autocrine or endocrine factors. The FGF family encompasses 22 members, grouped into seven subfamilies on the basis of phylogenetic analysis and sequence homology [1]. The subfamilies FGF1/2/5, FGF3/4/6, FGF7/10/22, FGF8/17/18 and FGF9/16/20 act as canonical FGFs; FGF11/12/13/14 are intracellular factors acting in an FGF receptor (FGFR) independent manner; FGF19/21/23 subfamily members function as hormones [2].

Canonical FGFs are paracrine factors that mediate their biological responses by binding to and activating tyrosine kinase (TK) FGFRs. The interaction with heparin/heparan sulfate (HS) proteoglycans (HSPGs) plays a pivotal role in mediating the biological activity of FGFs, leading to the formation of signalling FGF/FGFR/HSPG ternary complexes [3]. Moreover, HSPGs sequester FGF molecules near the site of action, providing a reservoir for the growth factor and allowing the formation of extracellular matrix (ECM)-associated FGF gradients [4].

Canonical FGFs mediate a plethora of functions during development. They are involved in patterning of germ cell layers, formation of body axes, induction of organogenesis and morphogenesis. Moreover, FGFs display homeostatic functions in the adult, being involved in tissue repair and remodelling processes. Finally, deregulation of FGF signalling can contribute to pathological diseases, including cancer. In this context, several alterations affecting the FGF/FGFR system have been reported in tumors, including gain- or loss- of function, altered gene expression or changes in binding specificity [2].

Intracellular FGFs act as intracellular signalling molecules in a FGFR-independent manner; they play a major role in neuronal functions at postnatal stages by interacting with intracellular domains of voltage-gate sodium channels and with the neuronal mitogen-activated protein kinase scaffold protein islet-brain-2 [5].

Hormone-like FGFs exhibit poor affinity for HSPGs, resulting in more diffusive properties through blood circulation [6]. These FGFs depend on Klotho co-receptors (see below) to activate intracellular signalling responses [7]. FGF19 (orthologue of murine FGF15) acts as a growth/differentiation factor in the heart and brain at embryonic stages and plays a crucial role in regulating hepatic bile acid production [8]. FGF21 is a metabolic regulator of lipolysis in the white adipose tissue [9] and FGF23 acts as a physiological regulator of phosphate and active vitamin-D blood levels [10].

The wide-ranging biological roles of FGFs, the variety of activated signalling pathways and the complex and dynamic expression of FGF ligands and receptors implies that the FGF/FGFR system must be tightly regulated.

1.2. Fibroblast growth factor receptors

In mammals, FGFRs are encoded by four distinct genes (*FGFR1-4*). FGFRs consist of three extracellular immunoglobulin-like (Ig) domains (D1-3), a single transmembrane helix domain and an intracellular TK domain [11]. D2 and D3 domains are responsible for FGF binding. FGFRs show diverse specificities for FGF ligands. In addition, alternative splicing of the D3 domain that may occur in FGFR1, 2 and 3, but not in FGFR4, generates "IIIb" and "IIIc" isoforms with additional ligand-binding properties. For instance, FGFR2IIIb binds FGF7 and FGF10, but not FGF2, whereas the FGFR2IIIc isoform binds FGF2 and FGF18, but not FGF7 and FGF10 (see Ref. [12] for further details about the ligand-binding properties of the different FGFRs). Interestingly, FGFR1-3IIIb and FGFR1-3IIIc isoforms often display differential expression in epithelial and mesenchymal tissue, respectively [13].

FGFRs interact with HSPGs via the D2 domain. The formation of a 2:2:2HSPG/FGF/FGFR ternary complex [14] causes receptor dimerization with conformational shift in receptor structure that leads to *trans*-phosphorylation of multiple residues in the intracellular TK domain. Receptor phosphorylation activates multiple signal transduction pathways that generate distinct cellular responses. As summarized in Fig. 1, major substrates of FGFR TK are the intracellular specific adaptor protein FGFR-substrate-2 (FRS2) and phospholipase-Cγ (PLCγ) [15]. Activated FRS2 allows the recruitment of the adaptor protein GRB2 that in turn recruits SOS or GAB1 to the signal complex. The recruitment of SOS activates RAS and the downstream RAF/mitogen-activated protein kinase (MAPK) pathway. The downstream effect of this pathway is mainly cell proliferation, even though cell differentiation or cell cycle arrest can also be induced depending on the different cellular context. The recruitment of GAB1 causes the PI3K–mediated activation of the AKT antiapoptotic pathway. PLCγ leads to the activation of protein kinase C (PKC) that sustains MAPK and AKT pathways and plays a role in cell migration. Other pathways may be activated in different cell subtypes, including p38 MAPK, JAK-STAT and RSK2 [16].

Several extracellular and intracellular mechanisms have been described able to regulate/attenuate FGFR signalling at different levels. FGFRs are internalized upon receptor activation [17], inducing receptor degradation or recycling. Relevant to this point, N-glycosylation of the receptor affects the assembly of the FGF/FGFR1/HSPG complex [18] and internalization of FGFR2 [19]. At intracellular level, MAPK signalling may phosphorylate threonine residues on FRS2, inhibiting the recruitment of GRB2 [20]. Sprouty proteins are negative modulators that compete for GRB2 binding by preventing RAS activation or directly binding RAF and disrupting MAPK signalling [21]. In addition, FGFs can induce the activation of phosphatases, including SEF and MAPK-phosphatase 3 (MKP3). SEF interacts directly with FGFRs, thus preventing their activation, whereas both enzymes can dephosphorylate and inactivate $ERK_{1/2}$ [22].

Different molecules can act as cell surface co-receptors for FGFs (Fig. 2). As already mentioned, HSPGs are required for a productive FGF/FGFR interaction that enables FGFR signalling [23]. For this reason, structural modifications of the HS chains deeply affect FGFR signalling and can be responsible for its fine-tuning. As an exception, hormone-like FGFs have reduced affinity for HSPGs and their activity depends on the presence of Klotho proteins as co-receptors. Cell surface β-Klotho and α-Klotho are co-factors for FGF19/21 and FGF23, respectively, and convert FGFRs into high affinity receptors for endocrine FGFs, limiting nonspecific/off-target signalling. The cell membrane ganglioside GM1 acts as a FGF co-receptor by interacting with FGF2 and promoting its biological activity in endothelial cells [24]. In addition, αvβ3 integrin promotes FGF-mediated endothelial cell proliferation, motility, and FGFR1 recruitment [25], thus contributing to the cross-talk between FGFR and integrin signalling [26]. Neural cell adhesion molecule (N-CAM), neuronal cadherin (N-cadherin) and L1 can activate FGFR1-2