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Exploitation of phage display for the development of anti-cancer agents targeting fibroblast growth factor signaling pathways: New strategies to tackle an old challenge

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ABSTRACT

A tumor is defined as a group of cancer cells and 'surrounding' stromal bio-entities. Alongside the extracellular matrix (ECM) in the tumor microenvironment (TME), the stromal cells play key roles in cancer affliction and progression. Carcinoma-associated fibroblasts (CAFs) in the area of the tumor, whether activated or not, dictate the future of tumor cells. The CAFs and corresponding secreted growth factors (GFs), which mediate the crosstalk within the TME, can be targeted in therapies directed at the stroma. The impact of the fibroblast growth factor-fibroblast growth factor receptor (FGF-FGFR) signaling pathway in different kinds of tumors has been explored. Several tyrosine kinase inhibitors (TKIs), monoclonal antibodies (mAbs), and ligand traps targeting the formation of FGF-FGFR complex are in preclinical or early development phases. Moreover, there are numerous studies in the literature reporting the application of phage display technology for the development of peptides and proteins capable of functioning as FGF mimetics or traps, which are able to modulate FGF-related signaling pathways. In this review, prominent research in relation to phage display-assisted ligand identification for the FGF/FGFR system is discussed.

1. Introduction

A tumor is defined as a group of cancer cells and 'surrounding' stromal bio-entities. The surrounding setting is comprised of the extracellular matrix (ECM) and the cells (e.g., fibroblasts and immune system cells) with specific roles in the progression of the solid tumor. The crosstalk between cancer cells and the nearby elements has an important role in tumor development [1]. Cancer cells cannot differentiate to fully functional normal cells but instead can have defective growth regulatory systems and may become malignant [2]. Dvorak introduced the theory of "tumors: wounds that do not heal" [3]. In tumors of epithelial origin, the cancer cells and the cells in the vicinity of the tumor (so-called stromal cells) including fibroblast, vascular endothelial, and immune cells (e.g., macrophages and neutrophils) form a permissive milieu known as the tumor microenvironment (TME). The stromal cells have the potential to 'heal the wound' and fibroblasts appear to be the important stromal component for affecting this process. In the case of normal cells associated with a wound, once the fibroblasts perform their repairs, the signaling processes for wound healing decreases. However, in association with some cytokines and interleukins, the fibroblasts within the TME neither are subjected to apoptosis nor are they eliminated. This results in a continued course of healing in response to which cancer cells modify their environment toward survival and growth [4].

In normal tissues, fibroblasts play various functions such as regulation of tissue homeostasis, wound healing, aging processes, and manufacturing ECM [5]. Fibroblasts in the area of a tumor, whether activated or not, are called carcinoma-associated fibroblasts (CAFs) and along with other stromal cells and the ECM in the TME play key roles in cancer initiation and progression thereby helping to dictate the future of the tumor [6]; in other words they "orchestrate the composition of malignancy" [7]. The interaction of CAFs with cancer cells, ECM, and other stromal cells make them potential targets for preventing the proliferation and differentiation of cancer cells [8]. Growth factors (GFs), cytokines, and proteases secreted by CAFs stimulate tumor cell proliferation, induce cancer cell motility, regulate tumor metabolism,

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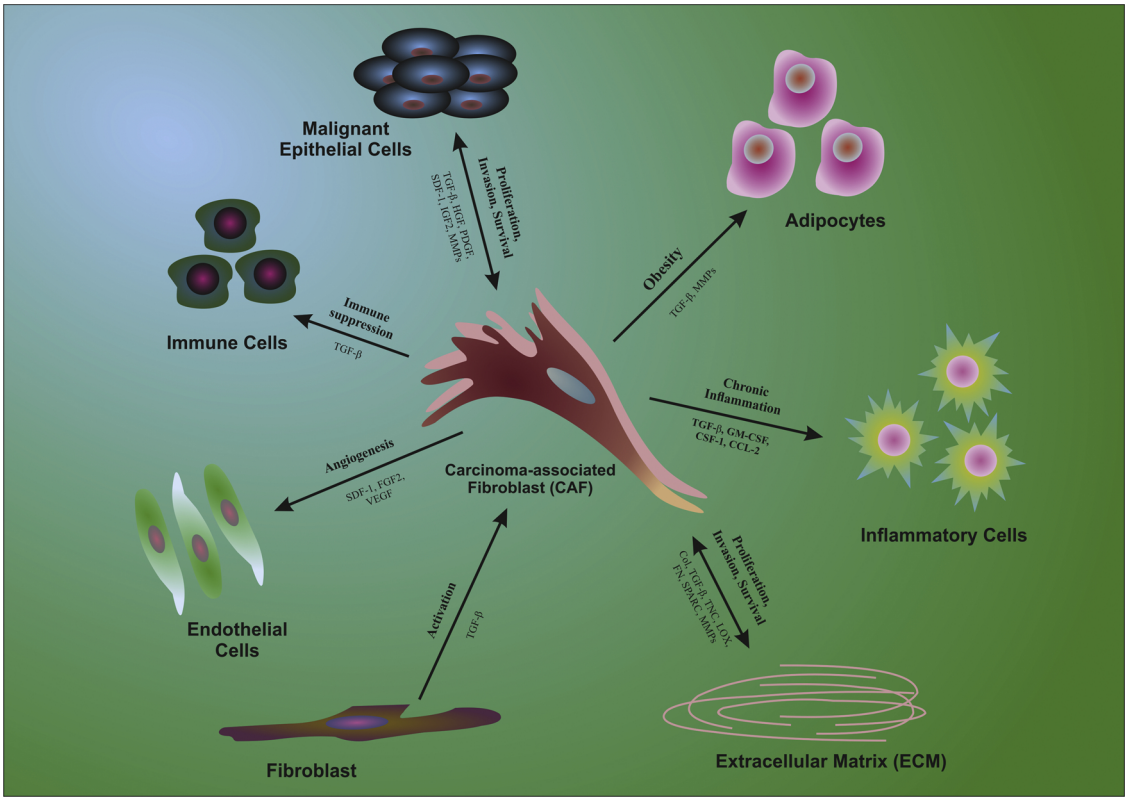

Fig. 1. Schematic representation of communications between CAFs and different cell types mediated via various growth factors and chemokines. The CAFs affect the function of ECM, endothelial cells, immune cells, malignant epithelial cells, adipocytes, and inflammatory cells in TME, which in turn promotes tumorigenesis by different means including proliferation, angiogenesis, invasion, immune suppression, and cellular survival by inhibiting the cell death. FGF2 or bFGF, basic fibroblast growth factor; CCL-2, chemokine (C-C motif) ligand 2; Col, collagen; FN, fibronectin; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IGF2, insulin-like growth factor 2; LOX, lysyl oxidase; SDF-1, stromal cell-derived factor 1; SPARC, secreted protein, acidic and rich in cysteine; TNC, tenascin-c. MMPs, Matrix metalloproteinases, PDGF, Platelet-derived growth factor, and TGFβ, Transforming growth factor beta.

and are also involved in ECM modifications [9]. Targeted mitigation of any one or more of these effects may help slow tumor progression.

The role of CAFs in angiogenesis, cancer progression, and migration has been documented. Angiogenesis is controlled by specific growth factors and chemokines secreted by CAFs (Fig. 1) [10]. Fig. 1 also shows GF- and chemokine-mediated communication between CAFs and other cell types.

CAFs are genetically more stable than cancer cells and since they help control cancer progression they may prove better drug targets than the cancer cells themselves. Additionally, the GFs secreted by CAFs, which mediate the crosstalk within TME, can be targeted as tumor stroma-directed therapies [9]. Within CAF-induced signaling pathways, the multi-targeting tyrosine kinase inhibitors (TKIs) of VEGFRs, PDGFRs, FGFRs, and EGFRs have efficient anti-tumor properties [11]. There are many examples of small molecule TKIs, such as sorafenib, sunitinib, and leflunomide that target these GFs and corresponding receptors [12,13].

The role of the FGF-FGFR signaling pathway in different kinds of tumors has been extensively investigated including in solid tumors such as prostate, lung, colorectal, renal, esophageal, and gastric cancers [14], where expression and secretion of autocrine and paracrine FGFs has been found to be irregular. The oncogenesis associated with FGFRs might be due to any one of several mechanisms including neoangiogenesis, activating mutations, and resistance to cancer therapy [15].

FGFs1, 2, 6, 8, 19 and 23 have been implicated in the progression of prostate cancer, the last two apparently resulting from an endocrine role [16–19]. The angiogenic effect of FGF2 is enhanced by an epithelial-mesenchymal transition (EMT) of the tumor cells in which the expression of receptors switches from FGFR2IIb to the IIIc isoform which has higher affinity for FGF2.

Moreover, there are several abnormalities associated with FGFRs that make them attractive therapeutic targets for a wide range of malignancies: Point mutations and translocations can produce overexpressed or fused proteins with intrinsic activity, or isoform switching can occur in metastatic processes such as EMT where the FGFR aberrations result in carcinogenesis [20].

2. FGFs

In 1971, tumor angiogenic factor (TAF) with a molecular weight of 10 kDa was isolated from rat Walker 256 carcinoma. TAF consists of 25% RNA, 10% protein, and 58% carbohydrate, and a possible lipid fraction [21]. The nature of the protein component was not characterized until the 1980s when the studies revealed that endothelial cell GFs had a remarkable affinity for heparin. This led to the identification, purification, and sequencing of two prototypic heparin-binding angiogenic GFs, FGF1 and FGF2. Subsequently, 21 additional structurally related members of FGF family have been identified [22].

FGFs are classified into various subfamilies based on phylogenetic analysis (Fig. 2). The site of action is also used to divide FGF subfamilies into autocrine, paracrine, and endocrine classes (Fig. 2) [23,24]. Most FGFs are secreted (also known as canonical FGFs), including FGF1, FGF4, FGF7, FGF8, and FGF9 subfamilies, and perform their action in the vicinity of the secreting cells. Even though FGF1 and FGF9 subfamilies lack a classical cleavable N-terminal signal peptide, they are, nevertheless, transported across the cell membrane [25]. The next class is comprised of three endocrine FGFs including FGF15/19, FGF21, and FGF23 (FGF15/19 subfamily) with unique properties distinguishable from canonical FGFs. The endocrine FGFs mediate their action on FGFRs through the Klotho family of proteins instead of heparin sulfate.

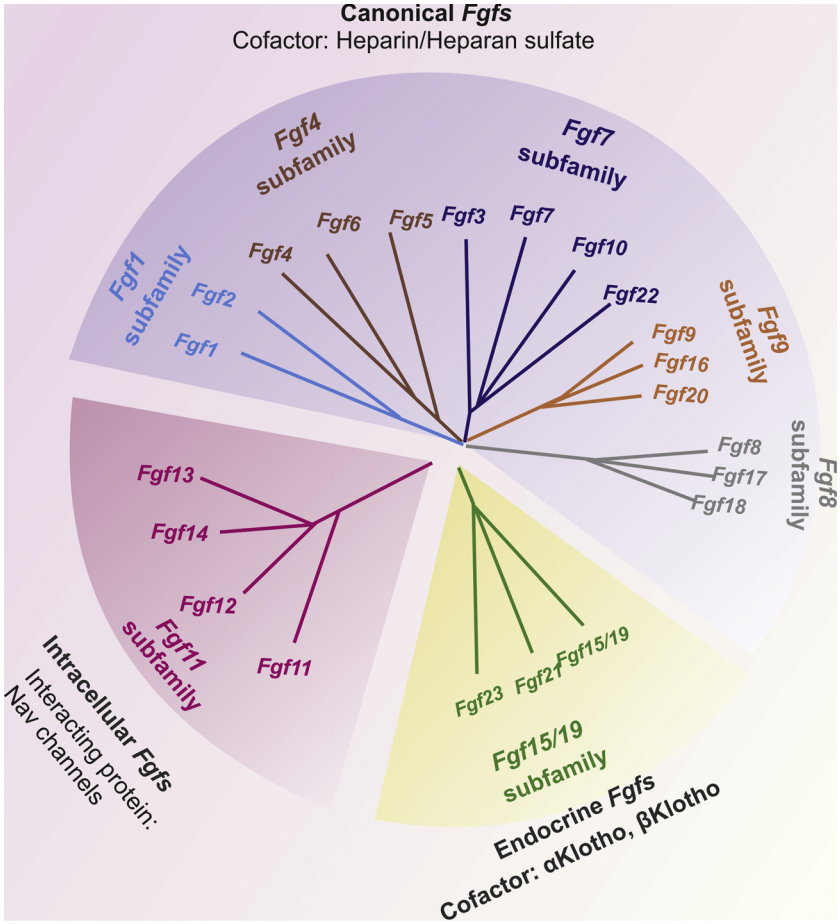

Fig. 2. Classification of Fgf genes into seven subfamilies using phylogenetic analysis. FGFs 1, 4, 7, 8, and 9 subfamilies are known as "secreted" or "canonical" FGFs where heparin or heparin sulfate work as the cofactor for the activation of FGFRs. The FGF15/19 subfamily is comprised of FGFs with endocrine activity via binding and activation of corresponding FGFRs with Klotho family proteins as cofactors. The FGF11 subfamily genes (intracellular FGFs) encode specific proteins which act as cofactors for voltage gated sodium channels (Nav channels) and other molecules.

The FGF11 subfamily, also known as the intracellular FGFs (iFGFs), consists of FGF11, FGF12, FGF13, and FGF14. These autocrine FGFs exert their biological activity in their original cells via interaction with cytoplasmic domains of ion-gated sodium channels [26].

3. FGFRs

The paracrine and endocrine FGFs produce their biological actions by signaling through fibroblast growth factor receptors (FGFRs). These transmembrane tyrosine kinase receptors (TKRs) are comprised of three extracellular immunoglobulin (Ig)-like domains, a transmembrane domain, and an intracellular tyrosine kinase domain [27]. Diversity regarding the effects of FGFs includes diversity at the level of receptors. Splice variants can produce different isoforms of FGFRs, but in particular the specificity and affinity of FGFs with their receptors is derived largely from splicing in Ig-like domain III. In contrast to other family members, FGFR4 has only one transmembrane isoform [28,29]. Fig. 3a shows the different domains for FGFRs 1-4, including an example of alternative splicing in Ig-like domain III. Fig. 3b shows the domain structure for FGFR5 (also known as FGFRL1), which lacks the intracellular tyrosine kinase domains. It is believed that FGFRL1 acts as a ligand trap for FGFs or makes a dimer with other FGFRs in order to inhibit their autophosphorylation [30].

4. Cofactors and associated signaling pathways of FGF

Heparin and heparan sulfate proteoglycans (HSPG) act as essential cofactors for the binding of FGF subfamilies 1, 4, 7, 8, and 9 and subsequent FGFR signaling (Fig. 2). For the endocrine FGFs (subfamily 15/19), initiation of signalling requires the formation of a dimeric ternary complex of FGF-FGFR-HSPG/Klotho cofactors [31]. Klotho cofactors include αKlotho, βKlotho, and Klotho/lactase-phlorizin hydrolase-related (KLPH) [32]. For example, regulation of phosphate uptake and calcium ion absorption is mediated by FGF23 by signal transduction through formation of FGF23-αKlotho-FGFR complexes [33]. βKlotho is a cofactor for FGF15/19 and FGF21 in which an equivalent complex is formed in order to initiate the signal cascades [34]. Although KLPH interacts with a number of FGFRs and results in signalling to and phosphorylation of extracellular signal-regulated kinase (ERK), its function remains unclear [26].

Fig. 4 shows the signaling cascade for FGF-FGFR pathways. The signal transduction for canonical FGFs is initiated by the formation of FGF-HSPG-FGFR ternary complex, followed by trans-autophosphorylation of tyrosine residues of FGFR-TK domain in the following order: 653, 583, 463, 766, 585, and 654 (the residue numbering is based on FGFR1 amino acid sequence). This results in a 500–1000 fold increase in tyrosine kinase activity [35]. The activated TK domains phosphorylate proteins specific to four distinct pathways: RAS-MAPK [36], PI3K-AKT [37], PLCγ [38] and STAT [39]. To trigger the RAS-MAPK and PI3K-AKT pathways, FGFR substrate 2α (FRS2α) has to be phosphorylated, and this requires phosphorylation of residue Y463 on the receptor and involvement of protein CRKL [40,41]. In turn, the activated FRS2α binds to growth factor receptor-bound 2 (GRB2) and tyrosine phosphatase SHP2 proteins [42,43]. Subsequently, GRB2 recruits SOS and GAB1 to activate the RAS-MAPK and PI3K-AKT pathways, respectively [44]. The activated MAPK pathway activates various regulatory proteins including E26 transformation-specific (ETS) transcription factor family members (i.e., Etv4, and Etv5) and inhibitory controllers such as SHP2, CBL, SPRY, SEF, and DUSP6 [45,46]. Eventually, the phosphorylated ETS proteins regulate the expression of different target genes such as scleraxis which is required for maturation of tissue domains for proper integration of the musculoskeletal system

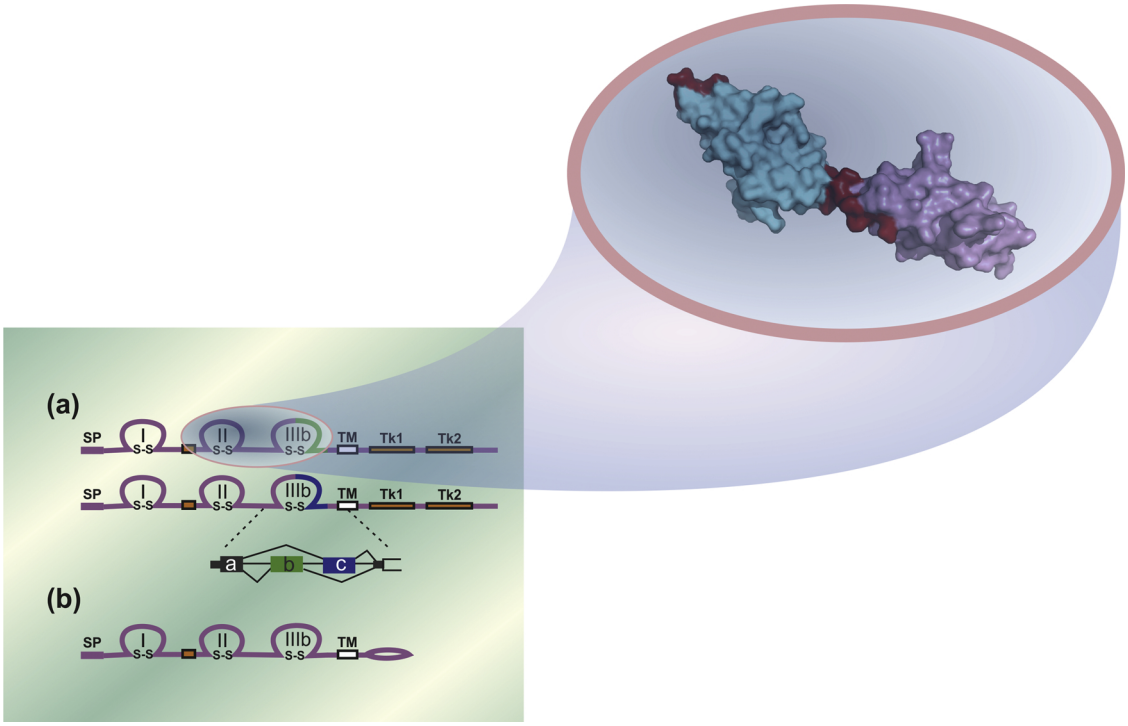

Fig. 3. Structural properties of FGFRs. Panel a shows an example of the alternative splicing products of Ig-like domains and the corresponding gene splicing routes are shown. The two represented FGFRs differ in their b and c regions depicted by green and blue colors, respectively. In addition to the extracellular domains, the receptors have transmembrane (TM), and tyrosine kinase (TK) domains (e.g., Tk1 and 2). Panel b shows FGFRL1 (FGFR5), which lacks TK domains and acts as a ligand trap to inhibit the intracellular signaling cascade of other FGFRs. The experimentally derived protein structure on the right shows Ig-like domains II and IIIb for human FGFR2 (PDB ID 1EV2-chain E) in cyan and purple. SP indicates a cleavable secreted signal sequence.
Adopted from [26].

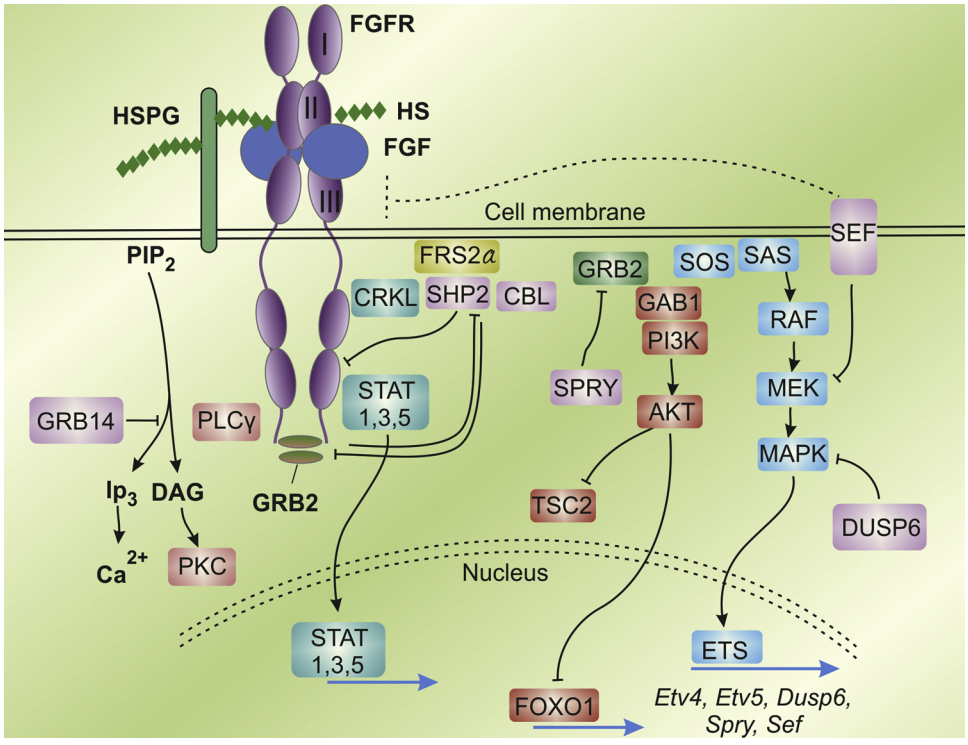

Fig. 4. Schematic representation of FGF-FGFR signaling pathways. The formation of FGF-HSPG-FGFR ternary complex initiates trans-autophosphorylation of tyrosine residues of FGFR-TK domains at specific positions and in the following order: 653, 583, 463, 766, 585, and 654. Four distinct pathways, RAS-MAPK, PI3K-AKT, PLCγ, and STAT, are activated by phosphorylated TK domains. The phosphorylated FGFR substrate 2γ (FRS2γ) initiates the RAS-MAPK and PI3K-AKT pathways by binding to growth factor receptor-bound 2 (GRB2) and tyrosine phosphatase SHP2 proteins. Subsequently, GRB2 recruits SOS and GAB1 to activate the RAS-MAPK and PI3K-AKT pathways, respectively. The activated MAPK pathway stimulates the expression of various regulatory proteins including E26 transformation-specific (ETS) transcription factor family members (i.e., Etv4, and Etv5) and inhibitory controllers such as SHP2, CBL, SPRY, SEF, and DUSP6. In the PI3K-AKT pathway, AKT inactivates the forkhead box class transcription factor (FOXO1) and the cytosolic tuberous sclerosis complex 2 (TSC2) which leads to the stimulation of cell survival as well as cell growth and proliferation through activation of mTOR complex 1, respectively. The phospholipase Cγ (PLCγ) pathway is initiated by the phosphorylation of Y766 on the receptor, which is regulated by GRB14 protein. The activated PLCγ enzyme catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to generate inositol triphosphate (IP3) and diacylglycerol (DAG). TIP3 elevates the intracellular calcium ion concentration while DAG stimulates protein kinase C (PKC). The STAT pathway is triggered by Y677 phosphorylation. The activated STAT1, 3, and 5 proteins control the expression of various genes involved in cell migration, differentiation, proliferation, apoptosis and wound healing.

[47].

Downstream of PI3K-AKT pathway, the forkhead box class transcription factor (FOXO1) and the cytosolic tuberous sclerosis complex 2 (TSC2) are inactivated [48], leading to the stimulation of cell survival, growth, and proliferation through activation of mTOR complex 1 [49].

The phosphorylation of Y766 is linked to the initiation of the phospholipase Cγ (PLCγ) pathway [50]. Activated PLCγ catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to generate inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 production result in elevation of the level of intracellular calcium ion while DAG stimulates protein kinase C (PKC). A feedback mechanism operates in which GRB14 protein inhibits the activation of PLCγ pathway by interacting with pY766 [51].

STAT pathway is triggered by Y677 phosphorylation. Activated STAT1, 3, and 5 proteins control the expression of various gene involved in cell migration, differentiation, proliferation, apoptosis, and wound healing [52].

5. Phage display

In recent years, the power of research and diagnosis has been boosted through advances in hybridoma technology resulting in the discovery of therapeutically valuable monoclonal antibodies. In addition to this traditional method, phage display offers in vitro generation and identification of antibodies and this approach has revolutionized antibody-related research [53]. In 1985, Smith invented phage display as a method to express peptides and proteins on the surface of lysogenic filamentous bacteriophages [54,55]. There are many applications of phage display including the study of protein-protein interactions, defining epitopes and mimotopes, identifying enzyme-specific inhibitors, screening antibody libraries, and identifying agonists and antagonists to probe the receptor structure and function [56–58].

Fig. 5 is the schematic representation of M13 filamentous bacteriophage. M13 infects *Escherichia coli* and is the most commonly used approach to display proteins and peptides of different size. It consists of 11 genes in a single-stranded DNA (ssDNA), and these are classified into three groups according to their function: Capsid proteins (pIII, pVI, pVII, pVIII,and pIX), replication proteins (pII, pV and pX), and assembly proteins (pI, pIV and pXI) [56]. The pIII and pVIII coat proteins are the most commonly used for display. pVIII is expressed at high copy number (2700 molecules) on the phage surface (capsid) but is almost exclusively suitable to the display of short peptides (up to 20 amino acids in length). On the other hand, pIII is expressed at low copy number (3–5 copies) on the capsid but can be used to display peptides and even proteins up to several tens of kilodaltons [55,59].

In the process of antibody phage display, combinatorial fragments of antibodies are used to build a library which consists of the coding sequences for the variable domains of heavy (VH) and light chain (VL). The VH and VL fragments generally contain antigen-binding sites of three complementary determining regions (CDRs) to increase the diversity of the library and generally take the form of antigen-binding fragments (Fabs) or single-chain variable fragment (scFvs), in which VH and VL are attached by a linking sequence [60]. The DNA components of the library are ligated into the coding sequence of the pIII or pVIII coat proteins of a phage display vector such as phagemid. Where phagemid is used, a helper phage is utilized to enable the packaging of the phagemid DNA and assembly of the relevant coat proteins in the capsid. Ultimately the ligated DNA sequence becomes displayed as a peptide/protein on the surface of the phage [61]. This phage library can now be screened against an antigen and the clones which bind can be subjected to purification, sequencing and identification, and further affinity analysis [62] (Fig. 5).

Advantages of using phage display-derived antibodies include the speed of production and the bypassing of animal immunization, both of which are time-consuming and error-prone [62]. The FGFR signaling pathway is a promising target for phage screening, and is reviewed in the following next sections.

5.1. FGF signaling pathway as a target in cancer therapy

The advances in the field of drug development for FGF-FGFR signaling pathway have led to the introduction of novel drug candidates, which are currently at different stages of clinical trial. These potential therapeutics are divided into three categories: TKIs, mAbs, and FGF ligand traps [63]. The methodologies employed include the application of phage display technology for the development of peptides and proteins capable of functioning as FGF mimetics or traps, both of which have the potential to modulate FGF-related signaling activity (Table 1). In this section, we present several prominent examples of these three categories and phage display-assisted ligand identification for the FGF/FGFR system (Table 2).

5.2. TKIs

TKIs have the ability to target the ATP-binding site of the TK domain of FGFRs and are in two different classes according to their selectivity. Table 1 lists selective and nonselective FGFR TKIs, which are in various clinical trial phases. Nonselective agents are capable of inhibiting FGFRs and other tyrosine kinase domains of receptors such as VEGFRs and PDGFRs.

As a potent multi-kinase inhibitor, Dovitinib® (CHIR258, TKI258) has high affinity for VEGFRs, PDGFR, FGFRs and many other kinase receptors with an IC_{50} value of around 20 nM. This molecule shows antitumor and anti-angiogenic activities when administered to patients with advanced solid tumors. In addition, Dovitinib was evaluated in patients with HER2-negative breast, bladder, and endometrial cancers (trials NCT01528345, NCT00790426, and NCT01379534, respectively). Several side effects have been reported for Dovitinib including gastrointestinal and cardiovascular effects [64]. Dovitinib, due to its anti-VEGFR kinase inhibitory effect, has been also evaluated as the second- and third-line treatment in phase II and III clinical trials in patients with renal cell carcinoma (NCT01223027).

E-3810, a potent anti-angiogenic TK inhibitor of FGFRs and VEGFRs, was evaluated in phase I clinical trials in patients with advanced solid tumors and was well-tolerated in oral doses. E-3810 can be effective in cases nonresponsive to Sunitinib, a multi-kinase inhibitor [65]. Nintedanib® (BIBF1120) is an indolinone triple angiokinase inhibitor, which targets FGFR2 and VEGFR1-3 as well as PDGFR. Nintedanib was also tested in a combination therapy for advanced ovarian cancer treatment in a randomized phase III clinical trial (NCT01015118). Similar to Dovitinib, Nintedanib showed adverse gastrointestinal (GI) effects when used in patients with non-small cell lung cancer (NSCLC) [66]. Ponatinib® (AP24534) is another multi-kinase and a pan-FGFR inhibitor that is currently being investigated against BCR-ABL-positive acute lymphoblastic leukemia and chronic myelogenous leukemia (CML) (NCT01207440, NCT01641107) [67].

Drug resistance is one of the main problems in cancer therapy. Imatinib- and Sunitinib-resistant gastrointestinal stromal tumors (GISTs), which usually arise in the stomach and small intestine, have been treated with multi-targeting TKIs [68]. In this regard, the inhibition of the FGFR signaling pathway appears to be a promising approach: Dovitinib has been evaluated in patients with Imatinib- and Sunitinib-resistant GISTs (NCT01478373 and NCT01440959, respectively). With a disease control rate (DCR) of 52.6%, Dovitinib is favorably comparable with that of other second-line treatments. Moreover, the effectiveness of Dovitinib and Brivanib in patients with endocrine-resistant HER2-negative/HR-positive breast cancer (NCT01528345) and *KRAS*-wild-type colorectal cancer (NCT00640471), respectively, has been studied. The consistency of safety profile for Dovitinib plus Fulvestrant with that of single-agent Dovitinib was shown. Moreover, the combination therapy of Dovitinib with Fulvestrant showed promising clinical activity in the FGF

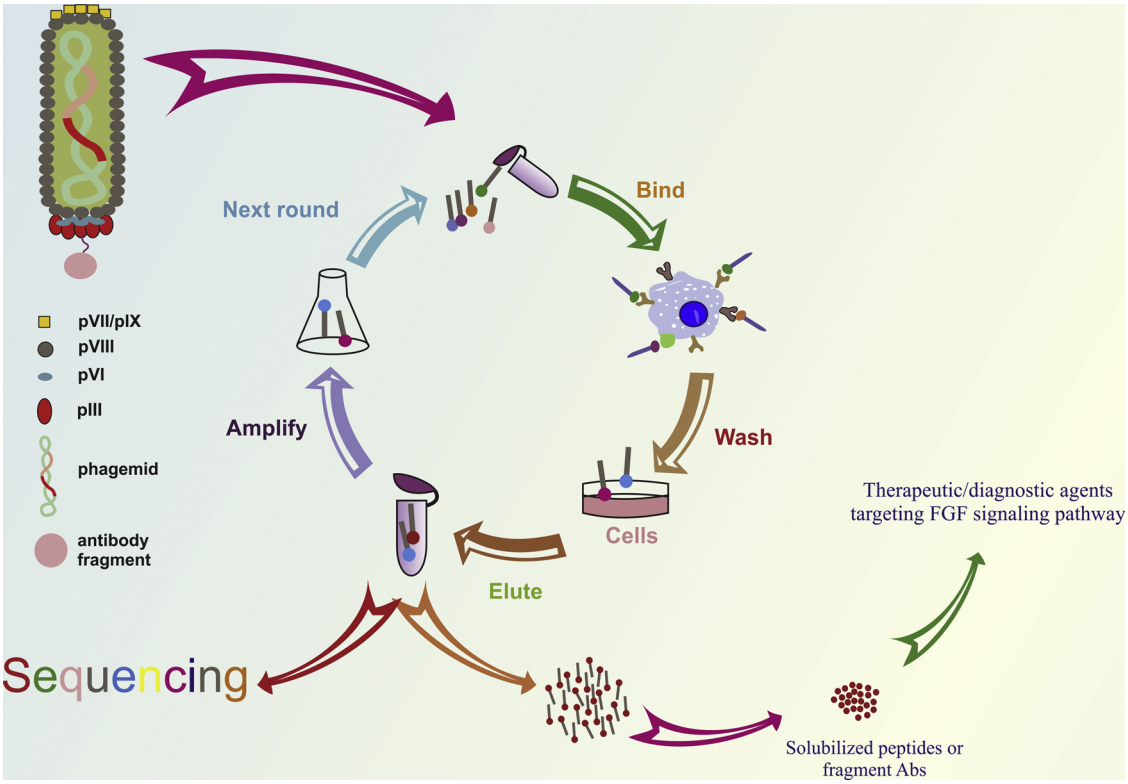

Fig. 5. Schematic representation of M13 bacteriophage and the phage display screening procedure. The M13 bacteriophage is comprised of 11 genes in a single stranded DNA (ssDNA) format, which encodes three categories of proteins classified by function. The fragment Abs can be fused to the pIII or pVIII coat proteins to be displayed on the phage surface. After the construction of the phage library, the phage particles are screened against the target(s) of interest. After several washing steps, the eluted phage particles are amplified and used in the next round of the panning. Following phage enrichment during these biopanning steps, the phage particles can be used to transform bacteria for the production of the solubilized form of the displayed fragment Ab/peptides and can also be used to identify the DNA sequence of fragment Ab/peptides.

pathway-amplified subgroup [69]. In the case of Brivanib, despite positive effects on progression-free survival (PFS) and objective response, the combination therapy of Cetuximab and Brivanib increased toxicity and did not significantly improve overall survival in patients with metastatic, chemotherapy-refractory, *KRAS*-wild-type colorectal cancer [70].

In addition to the above-mentioned multi-targeting TKIs, several other inhibitors such as Brivanib [71], Lenvatinib [72], Orantinib [73], and ENMD-2076 [74] have been reported to be active as anti-FGFR and VEGFR agents, and are now in phase I/II studies.

Recently, *selective* FGFR TKIs have been studied in cancer therapy. AZD4547 has been proposed as a highly selective pan inhibitor of FGFRs and has a 120-fold higher activity against FGFRs compared to VEGFR2. Orally administered AZD4547 was evaluated in a mouse tumor xenograft model and the results indicated the high potency of this agent [75]. A phase I clinical trial (NCT00979134) is being run to evaluate the effectiveness of AZD4547 against cancers with *FGFR1*- and/or *FGFR2*-amplified genes. Moreover, other AZD4547-based mono- and multi-therapies have been assessed in ER-positive breast cancer and gastroesophageal junction cancers (NCT01202591, NCT01457846). Another pan inhibitor of FGFRs is BGJ398, which is undergoing phase I/II clinical trial studies after successful testing in RT112 bladder cancer xenograft models [76].

Another selective TKI with inhibitory activity against FGFR1-4 is LY287445, which has 6–9 fold higher potency toward FGFRs compared to VEGFRs. Moreover, when orally administered to patients with solid-organ cancers it has been well tolerated [77]. Currently, LY287445 is being used in a phase I clinical trial against advanced cancers (NCT01212107).

Selective FGFR TKIs show less adverse effects compared to the nonselective ones due to their lower affinities toward VEGFRs. Multi-targeting agents produce cardiovascular side effects, proteinuria, skin reactions, and GI disorders [78]. Other common adverse effects reported for FGFR TKIs include hyperphosphatemia (resulting from FGF23 signal blocking), fatigue, and GI events including diarrhea and nausea. Hyperphosphatemia, which leads to tissue calcification, can be managed by phosphate binders and diuretics [14]. The toxicity management for potent selective TKIs should be considered an important issue when combination therapy is used for cancer treatment.

5.3. Monoclonal antibodies in clinical trials

Several mAbs targeting the formation of FGF-FGFR complex are also in preclinical or early phases of development (Table 1). These antibodies are aimed to inhibit a specific FGFR or its dimerization and hence hamper the ligand interaction. GP369 antibody has been developed to restrain the proliferation of cancer cells through FGFR2-specific inhibition [79]. Recently, FPA144, a glycol-engineered humanized mAb, which is an FGFR2-IIIb blocking agent with potential anti-neoplastic activity, has entered phase I trials (NCT02318329). The effectiveness of BAY1187982 as an anti-FGFR2-drug conjugate (ADC) in tumor growth inhibition and regression has been observed in some cancer xenograft models with overexpressed FGFR2 [80]. scFv-IgG1 is in phase I clinical development for advanced solid tumor treatment (NCT01881217). BAY1179470 interacts with FGFR2 and inhibits its phosphorylation, leading to suppression of FGFR2-mediated signal transduction and repression of cell proliferation [81]. Some studies have demonstrated that the FGFR3 inhibition by target-specific mAbs

Table 1
FGFR signaling pathway targeting agents in clinical trials.

Compound	Alternative name	Application	ID	CCT	Status	Company
Multi-targeted TKIs						
dovitinib	TKI258	Metastatic Breast Cancer	NCT01528345	phase II	Terminated	Novartis
		Urothelial Cancer	NCT00790426	phase II	Completed	
		Advanced Endometrial Cancer	NCT01379534	phase II	Completed	
		Metastatic Renal Cell Carcinoma	NCT01223027	phase III	Completed	
		GIST	NCT01478373	phase II	Completed	
		GIST	NCT01440959	phase II	Completed	
		CRC	NCT00640471	phase III	Completed	
Lucitanib	E-3810	Advanced Solid Tumors	NCT03117101	phase I	Completed	Ethical Oncology of Science
		Solid Tumors	NCT01283945	phase I/II	Completed	
Nintedanib	BIBF1120	Ovarian cancer	NCT01015118	phase III	Completed	
		NSCLC	NCT01948141	phase II	Completed	
Ponatinib	AP24534	CML	NCT01207440	phase II	Active, not recruiting	Ariad
		BCR-ABL Positive ALL	NCT01641107	phase II	Active, not recruiting	
lenvatinib	E7080	Advanced Solid Tumors	NCT02578316	phase I	Completed	Eisai
orantinib	SU6668	Solid Tumor	NCT00024206	phase I	Completed	Taiho Pharmaceutical
brivanib	BMS-582664	HCC	NCT03516071	phase II	Recruiting	Bristol-Myers Squibb
ENMD-2076		OCCC	NCT01914510	phase II	Completed	Entremed
Selective TKIs						
AZD4547	–	Advanced Solid Malignancies	NCT00979134	phase I	Completed	AstraZeneca
		ER + Breast Cancer	NCT01202591	phase I/II	Completed	
		Gastric Cancer	NCT01457846	phase II	Terminated	
BGJ398	–	Advanced Cholangiocarcinoma	NCT02150967	phase II	Recruiting	Novartis
LY287445	–	Advanced Cancer	NCT01212107	phase I	Completed	Eli Lilly
Monoclonal antibodies						
Bemarituzumab	FPA144	Advanced Solid Tumors	NCT02318329	phase I	Active, not recruiting	Five Prime Therapeutics
BAY1179470	–	Neoplasms	NCT01881217	phase I	Completed	Bayer Healthcare
MGFR1877S	–	Solid Tumor	NCT01363024	phase I	Completed	Genentech
FGF traps						
FP-1039	GSK3052230	Neoplasms	NCT01868022	phase I	Completed	GlaxoSmithKline
		advanced cancer	NCT00687505	phase I	Completed	
		Endometrial Cancers	NCT01244438	phase II	Withdrawn	

ID: clinical trial identifier; **CCT**: current clinical trials; **TKIs**: tyrosine kinase inhibitors; **GIST**: gastrointestinal stromal tumor; **CRC**: colorectal cancer; **NSCLC**: non-small cell lung cancer; **CML**: chronic myeloid leukemia; **ALL**: acute lymphoblastic leukemia; **HCC**: Hepatocellular carcinoma; **OCCC**: Ovarian clear cell carcinoma.

Table 2
Phage display-mediated FGF/FGFR system targeting peptides and fragment Abs.

Target	Peptide/Ab	Name	Sequence	Origin	Functionality/Application	Ref.
FGF1	peptide	–	PSPVSELSSGRMAYG	15-peptide	antagonist	[110]
	peptide	AP8	AGNWTPI	7-mer peptide	antagonist	[111]
FGF2	peptide	P-7	PLLQATL	7-mer peptide	antagonist	[113,114]
	peptide	P-8	PLLQATAGGGS-NH2	7-mer peptide	antagonist	[115]
	Fragment Ab	IgG1-1A2	–	scFv	antagonist	[112]
FGF3	peptide	FP16	VLWLKNR	7-mer peptide	antagonist	[116]
FGF7	peptide	–	KGHSLMP	7-mer peptide	agonist	[120]
	peptide	–	IPTLPSS	7-mer peptide	agonist	[120]
	Fragment Ab	sdAb	–	sdAb	antagonist	[119]
FGF8b	peptide	P12	HSQAAVP	7-mer peptide	antagonist	[122]
FGF23	peptide	23-b6	SSPPKSP	7-mer peptide	antagonist	[126]
FGFR1	peptide	#106	LEERPV	6-mer peptide	antagonist	[104]
	peptide	C19	AESGDDYCVLVFTD SAWTKICDWSHFRN	26-residue peptide	agonist	[100]
	peptide	–	MQLPLAT	7-mer peptide	Drug Delivery	[101]
	peptide	–	VYMSPF	6-mer peptide	antagonist	[98]
	peptide	P9	LSPPRYP	7-mer peptide	antagonist	[99]
	Fragment Ab	–	–	scFv	Large scale analysis of protein function in cells	[105]
	Fragment Ab	scFvD2-Fc	–	scFv	Drug Delivery	[103]
	Fragment Ab	RR-C2	–	scFv	antagonist	[96]
	Fragment Ab	–	–	VHH	antagonist	[97]
FGFR2	peptide	–	SRRPASFRTARE	12-mer peptide	Imaging in tissue biopsy or early detection of EAC	[106]
	Fragment Ab	BAY1179470	–	scFv- IgG1	antagonist	[81]
FGFR3	Fragment Ab	3C	–	scFv	antagonist	[83]
	Fragment Ab	7D	–	scFv	antagonist	[83]
	Fragment Ab	R3Mab	–	Fab fragments	antagonist	[82,107]

VHH: a VHH antibody or nanobody is the antigen binding fragment of llama heavy chain only antibodies.

significantly reduces bladder and t(4;14)-positive multiple myeloma cancer cell proliferation [82,83]. There are also unsuccessful examples where attempts to find new isoform-specific mAbs against FGFR1-IIIc (i.e., IMCA1) have led to severe adverse effects in animal studies [84].

5.4. Phage display-derived fragment antibodies and peptides

Peptide and antibody libraries are 'gold mines' to identify and develop new therapeutics against various disorders. Several phage

display-derived therapeutic agents are at different stages of clinical trial or have been approved by FDA or EMA for a number of autoimmune diseases and cancers [85,86]. The first mAb developed from phage display technology is Adalimumab, which acts as a ligand trap by efficiently binding to tumor necrosis factor (TNF) and inhibits the initiation of TNF-associated inflammatory signaling cascade [87]. There are also mAbs developed from phage display for autoimmune diseases such as systemic lupus erythematosus (SLE) [88], rheumatoid arthritis [89], psoriasis [90], and multiple sclerosis (MS) [91]. Moreover, the extracellular domains (ECD) of epidermal growth factor receptor (EGFR) [92], vascular endothelial growth factor (VEGF) [93], vascular endothelial growth factor receptor (VEGFR) [94], and fibroblast growth factor receptor (FGFR) [95] have been developed as cancer therapeutics based on phage. The following sections provide information regarding some of the FGFR-targeting fragment Abs and peptides, which have been evaluated in solid tumor therapy.

5.5. FGFR1: the predominantly targeted receptor

The ETH2-Gold human antibody phage display library has been screened against the ECD of FGFR1IIIc isoform and RR-C2 scFv was identified as binding [96]. Further biological and biochemical analysis indicated binding affinity sufficient to block the formation of the ternary complex of FGF-FGFR-HSPG and hampering the FGF2-mediated mitogenic activity in endothelial cells of human, bovine, and murine origin [96]. Furthermore, Veggiani et al. characterized VHH nanobodies (Nbs) from a phage display llama naïve library against FGFR1. Competitive elution with FGF2 led to the isolation of specific Nbs, which recognize regions overlapping with the FGF2 binding site on FGFR1. These competitive strategies and follow-up studies are suitable for identifying antibodies and antibody fragments which bind receptor and are also involved in physiological and pathological processes at the same time [97].

In an attempt to design FGF antagonists, Fan et al. identified 6-mer peptides that can bind to FGFR1 on the surface of Sf9 insect cells. The synthesized peptide (i.e., VYMSPF) can constrain the mitogenic activity of FGF1 and thus has potential therapeutic activity [98]. In another study, the identified FGFR1 binding P9 peptide (LSPPRYP) was evaluated for its antitumor activity in vitro and in vivo. The results demonstrated the reversal of FGF2-mediated effects on proliferation, cell cycle progression, and Erk1/Erk2 activation of B16-F10 melanoma cells, and inhibition of tumor growth in C57BL/6 mice [99].

In addition to the identification of inhibitors, phage library screening can be designed to find FGFR1 agonists in order to mimic the function of FGF2 [100], delivery of a specific gene to the tumeric area [101–103], fractionating of FGFR1 polyclonal antibodies (pAb) [104], and undertaking large-scale analysis of protein functions in cells [105].

5.6. FGFR2 and FGFR3: specific targets for specific diseases

Overexpression of FGFR2 on the cell surface is one of the early events in the progression of Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC). Recently, a peptide identified using phage display was exploited for early detection of EAC. The Ph.D.-12 phage library was panned against FGFR2-ECD and the identified 12-mer peptide SRRPASFRTARE was synthesized, labeled with Cy5.5, and used for binding studies. The results demonstrated high affinity and rapid binding of the synthesized peptide to QhTERT cells in vitro (apparent dissociation constant of $k_d = 68$ nM and apparent association time constant $k = 0.16\ min^{-1}$). Binding studies in human esophageal specimens indicated the higher affinity of the peptide to high-grade dysokasia (HGD) versus normal cells. This peptide offers a possible approach for clinical imaging in tissue biopsy and/or early detection of EAC and other cancers associated with epithelium [106].

FGFR3 is a potential therapeutic target for different kinds of neoplasia and carcinoma. The Tomlinson I + J human scFv libraries were panned against FGFR3 and a collection of scFv antibodies specific for FGFR3α IIIc isoform was developed. The cell proliferation inhibitory effect of selected scFvs was assessed on FGFR3-expressing RT112 cells. While 3C and 7D scFvs significantly prevented FGFR3-mediated proliferation, the inhibitory effect of 2D was small and 3B had no inhibitory effect [83]. In another study, the importance of FGFR3 inhibition in human bladder carcinoma cells, as well as t(4;14)-positive multiple myeloma, was studied in vivo. In addition, an R3Mab antibody was developed using phage display to inhibit the function of diverse mutants of FGFR3 as well as wild-type FGFR3 [82]. Crystallography disclosed that R3Mab has the capability of blocking ligand binding, and hence inhibiting receptor dimerization and important conformational changes in the receptor [82]. More recently, R3Mab was tailored through structure-guided phage display to inhibit FGFR2 and FGFR3 without the inhibition of FGFR1 and FGFR4: The modified R3Mab initially had binding capacity toward FGFR2, FGFR3, and FGFR4, and was subsequently further engineered to decrease FGFR4 binding affinity in order to reduce the safety risks as a result of crucial metabolic functions fulfilled by FGFR4 [107].

5.7. Ligand traps

Ligand trapping also hinders FGFs binding to their corresponding receptors. For example, FP-1039, a fusion protein of FGFR1-IIIc extracellular domain linked with IgG1 Fc domain, binds and traps FGF1, FGF2, and FGF4 preventing them binding to FGFR1 (Table 1) [108]. A phase I clinical trial was designed to evaluate the effectiveness of FP-1039 in combination with chemotherapy in patients with lung cancer (NCT01868022) [14], and a phase I trial has been undertaken in patients with advanced cancer (NCT00687505).

5.8. Phage display to find ligands traps

5.8.1. The most trapped ligands: aFGF and bFGF

Overexpression of FGF1 is implicated in the pathogenesis of human diseases such as arthritis, atherosclerosis, and tumor angiogenesis as a result of binding to FGFR1 [109]. To date, some anti-FGF1 peptides have been identified by means of phage display as potential therapeutic agents. Fan et al. identified a 15-mer peptide based on an innovative strategy: Subtractive screening of the phage library against heparin-coated wells before moving to the FGF1 panning procedure [110]. The heparin panning process removes peptides with a potential affinity toward the heparin-binding site of FGF1. This facilitates the identification of peptides capable of attaching to the receptor-binding region of FGF1. The synthesized 15-mer peptide inhibited the mitogenic activity of FGF1 through blocking the FGF1-FGFR1 interaction [110]. Recently, a specific anti-FGF1 heptapeptide, AP8 (i.e., AGNWTPI) was identified that impeded the cell cycle at the G0/G1 phase by enhancing the activity of proliferation-associated protein 2G4 (PA2G4) and suppressing the activity of Cyclin D1 and proliferating cell nuclear antigen (PCNA) [111]. Furthermore, AP8 suppressed FGF1-stimulated proliferation in MDA-MB-231, MCF-7, and HUVEC cells thereby inhibiting Erk1/2 and Akt kinase activity [110].

FGF2 also plays a pivotal role in cancer progression and angiogenesis and several peptides and antibodies have been identified by phage display which block the activity of FGF2 in tumorigenesis. Human scFvs have been isolated from a phage display library, which show significant binding toward FGF2. One of the selected scFvs, 1A2, was cloned into the pIgG1 vector in order to produce the full-length IgG1-1A2 antibody. The engineered antibody appeared to have a high affinity for recombinant human FGF2 and inhibited its biological activities [112]. In another study, the Ph.D.-7™ phage display peptide library was screened against FGF2 and a high-affinity FGF2-binding peptide named P7 (i.e., PLLQATL) was isolated. The synthesized P7 peptide inhibited the mitogenic activity of FGF2 in BALB/c 3T3 cells and FGF2-induced angiogenesis in chick embryo chorioallantoic membrane (CAM) [113].

The same group then studied the sensitizing effect of P7 in patients with colorectal cancer (CRC) taking irinotecan hydrochloride (CPT-11). The results indicated that P7 counteracted the anti-apoptotic effects of FGF2 on CPT-11-treated HT-29 cells and had benefits in combination with chemotherapeutic agents in CRC treatment [114]. Fan et al. further developed P8 (PLLQATAGGGS-NH2) based on phage display technology and alanine scanning through structural modification of P7 peptide because of its potent anti-proliferative activity [115]. P8 was found to have multi-target antagonistic effects on diverse proteins involved in the FGF2-FGFR signaling pathway and hence was proposed as a potent agent in related tumor treatment [115].

5.8.2. Canonical FGFs as trapped ligands

FGF3 regulates brain patterning, branching morphogenesis, and limb development [23], and is involved in tumorigenesis through mechanisms involving cellular proliferation, differentiation, and invasion [23]. Peptides binding to FGF3 have been identified by panning the Ph.D.-7™ phage library against FGF3-coated wells. The heptapeptide FP16 (i.e., VLWLKNR), which has four amino acids identical to FGF3's binding site to FGFR2, has the ability to inhibit the biological activity of FGF3 [116]. Further analysis revealed that FP16 has the ability to arrest cell progression from G1 to S phase and counteracts cell proliferation by reducing the expression of cyclin D1 and PCNA and inhibiting the activation of MAPK and Akt pathways [116].

FGF7, or keratinocyte growth factor (KGF), which is secreted by fibroblasts and microvascular endothelium, exerts its effects on epithelial cells in a paracrine manner. KGF is involved in differentiation and division morphogenesis processes, epithelial wound repair, embryonic development, immunity, and keratinocytes migration [117]. Furthermore, KGF has regenerative and protective effects on the skin, bladder, lung, and gastrointestinal tract [118]. Recently, antibodies against FGF7 were found using phage display and the human single domain antibody library (sdAb). The sdAb library was screened against purified FGF7 and the affinity of the selected phage particles displaying sdAbs was assessed by ELISA [119]. Although such ligand trapping is one of the most efficient ways to inhibit cancer progression and angiogenesis, in some cases developing ligand mimetics has also been sought. In this regard, Li et al. identified two peptides lacking the instability associated with Palifermin (Kepivance™: Recombinant human KGF), and used them in the treatment of oral mucositis (OM) [120].

Finally, four isoforms of FGF8 including FGF8a, FGF8b, FGF8e, and FGF8f have been identified resulting from alternative splicing of the human FGF8 gene and among which FGF8b has angiogenic and mitogenic activities [121]. FGF8b, which binds to FGFR3c with high affinity, is highly expressed in prostate cancers. Hence, it was targeted as a potential tumorigenic agent in prostate cancer treatment. Wang et al. (2013) identified a heptapeptide, P12 (i.e., HSQAAVP), with significant affinity and specificity toward FGF8b and which has a strong inhibitory activity on PC-3 cell proliferation stimulated by FGF8b [122].

5.8.3. The special case: FGF23

The FGF19 subfamily is comprised of FGF19, FGF21, and FGF23, which appear to have metabolic regulatory and hormonal functions instead of cell proliferation, differentiation, and migration activities associated with other FGFs [123,124]. FGF23 is the crucial regulator of phosphate homeostasis through its ability to inhibit phosphate uptake in renal proximal tubule epithelium [125]. It is believed that FGF23 is the pathogenic factor in many phosphate-wasting disorders such as autosomal dominant hypophosphatemic rickets (ADHR), tumor-induced osteomalacia (TIO), X-linked hypophosphatemic rickets (XLH), and fibrous dysplasia (FD) [126]. Residues 180–203 of FGF23 are required for it to bind FGFR1c. Hence, the Ph.D.-7™ phage library was panned against the $FGF23_{180\text{-}203}$ fragment instead of the full-length FGF23. Peptide 23-b6 (i.e., SSPPKSP) neutralized phosphate uptake inhibition by FGF23 in opossum kidney cells (OK cells). Moreover, the suppressing effect of 23-b6 prevented FGF23-induced Erk1/2 phosphorylation and reversed downregulation of type II sodium-coupled phosphate a and c cotransporter (NaPi-2a and NaPi-2c) expression normally mediated by FGF23 in OK cells. Collectively, the FGF23-binding peptide 23-b6 appears to be an efficient FGF23 antagonist that inhibits the phosphaturic action of FGF23 in hypophosphatemic disorders [126].

6. Concluding remarks

Stromal bio-entities of TME such as CAFs and the associated cytokines as well as GFs are important targets in cancer therapy. Phage display technology has been used to find novel drug candidates interfering with the FGF signaling pathway. The phage screening procedure can be designed in various ways to efficiently find peptides/fragment Abs with agonistic or antagonistic activities. FGF1, 2, and 3 are mostly used as a target to identify peptides with antagonistic properties. In the case of FGF7, the selected peptides and fragment Abs were used for mimicking as well as inhibiting the target antigen. It has also been reported that using phage displaying peptide/fragment Abs targeting FGFRs, it is possible to direct the drug payloads to the area of the tumor. In some cases, the selected phage particles were used to monitor the function of specific pathways or to detect specific types of cancer. Overall, phage display technology has proved to be a flexible and versatile approach in the pipeline for developing therapeutic/diagnostic drugs targeting FGF-related signaling pathways.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] F. Teng, et al., Cancer-associated fibroblasts promote the progression of endometrial cancer via the SDF-1/CXCR4 axis, J. Hematol. Oncol. 9 (2016) 8.
[2] H.F. Dvorak, Tumors: wounds that do not heal-redux, Cancer Immunol. Res. 3 (1) (2015) 1–11.
[3] H.F. Dvorak, Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing, N. Engl. J. Med. 315 (26) (1986) 1650–1659.
[4] P. Micke, A. Ostman, Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy? Lung Cancer 45 (Suppl. 2) (2004) S163–75.
[5] S. Madar, I. Goldstein, V. Rotter, 'Cancer associated fibroblasts'–more than meets the eye, Trends Mol. Med. 19 (8) (2013) 447–453.
[6] S. Togo, et al., Carcinoma-associated fibroblasts are a promising therapeutic target, Cancers (Basel) 5 (1) (2013) 149–169.
[7] P. Gascard, T.D. Tlsty, Carcinoma-associated fibroblasts: orchestrating the composition of malignancy, Genes Dev. 30 (9) (2016) 1002–1019.
[8] M. Shimoda, K.T. Mellody, A. Orimo, Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression, Semin. Cell Dev. Biol. 21 (1) (2010) 19–25.
[9] P. Cirri, P. Chiarugi, Cancer associated fibroblasts: the dark side of the coin, Am. J. Cancer Res. 1 (4) (2011) 482–497.
[10] K. Shiga, et al., Cancer-associated fibroblasts: their characteristics and their roles in tumor growth, Cancers (Basel) 7 (4) (2015) 2443–2458.

[11] S. Kakarla, X.-T. Song, S. Gottschalk, Cancer-associated fibroblasts as targets for immunotherapy, Immunotherapy 4 (11) (2012) 1129–1138.
[12] N. Steeghs, J.W. Nortier, H. Gelderblom, Small molecule tyrosine kinase inhibitors in the treatment of solid tumors: an update of recent developments, Ann. Surg. Oncol. 14 (2) (2007) 942–953.
[13] B. Escudier, et al., Sorafenib in advanced clear-cell renal-cell carcinoma, N. Engl. J. Med. 356 (2) (2007) 125–134.
[14] Y.K. Chae, et al., Inhibition of the fibroblast growth factor receptor (FGFR) pathway: the current landscape and barriers to clinical application, Oncotarget 8 (9) (2017) 16052–16074.
[15] I. Ahmad, T. Iwata, H.Y. Leung, Mechanisms of FGFR-mediated carcinogenesis, Biochim. Biophys. Acta 1823 (4) (2012) 850–860.
[16] H. Nagamatsu, et al., FGF19 promotes progression of prostate cancer, Prostate 75 (10) (2015) 1092–1101.
[17] E.K. Lee, et al., FGF23: mediator of poor prognosis in a sizeable subgroup of patients with castration-resistant prostate cancer presenting with severe hypophosphatemia? Med. Hypotheses 83 (4) (2014) 482–487.
[18] S. Feng, et al., FGF23 promotes prostate cancer progression, Oncotarget 6 (19) (2015) 17291–17301.
[19] S. Feng, et al., Endocrine fibroblast growth factor FGF19 promotes prostate cancer progression, Cancer Res. 73 (8) (2013) 2551–2562.
[20] R. Dienstmann, et al., Genomic aberrations in the FGFR pathway: opportunities for targeted therapies in solid tumors, Ann. Oncol. 25 (3) (2014) 552–563.
[21] J. Folkman, et al., Isolation of a tumor factor responsible for angiogenesis, J. Exp. Med. 133 (2) (1971) 275–288.
[22] M. Presta, et al., Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis, Cytokine Growth Factor Rev. 16 (2) (2005) 159–178.
[23] A. Beenken, M. Mohammadi, The FGF family: biology, pathophysiology and therapy, Nat. Rev. Drug Discov. 8 (3) (2009) 235–253.
[24] D.M. Ornitz, N. Itoh, Fibroblast growth factors, Genome Biol. 2 (3) (2001) 1–12.
[25] N. Itoh, D.M. Ornitz, Evolution of the Fgf and Fgfr gene families, Trends Genet. 20 (11) (2004) 563–569.
[26] D.M. Ornitz, N. Itoh, The fibroblast growth factor signaling pathway, Wiley Interdiscip. Rev. Dev. Biol. 4 (3) (2015) 215–266.
[27] C.J. Powers, S.W. McLeskey, A. Wellstein, Fibroblast growth factors, their receptors and signaling, Endocr. Relat. Cancer 7 (3) (2000) 165–197.
[28] A.T. Chellaiah, et al., Fibroblast growth factor receptor (FGFR) 3. Alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1, J. Biol. Chem. 269 (15) (1994) 11620–11627.
[29] S.G. Gong, Isoforms of receptors of fibroblast growth factors, J. Cell. Physiol. 229 (12) (2014) 1887–1895.
[30] B. Trueb, Biology of FGFRL1, the fifth fibroblast growth factor receptor, Cell. Mol. Life Sci. 68 (6) (2011) 951–964.
[31] M.T. Huhtala, O.T. Pentikainen, M.S. Johnson, A dimeric ternary complex of FGFR [correction of FGFR1], heparin and FGF-1 leads to an' electrostatic sandwich' model for heparin binding, Structure 7 (6) (1999) 699–709.
[32] H. Kurosu, M. Kuro-o, The Klotho gene family and the endocrine fibroblast growth factors, Curr. Opin. Nephrol. Hypertens. 17 (4) (2008) 368–372.
[33] H. Kurosu, et al., Regulation of fibroblast growth factor-23 signaling by klotho, J. Biol. Chem. 281 (10) (2006) 6120–6123.
[34] D. Guan, et al., Regulation of fibroblast growth factor 15/19 and 21 on metabolism: in the fed or fasted state, J. Transl. Med. 14 (2016) 63.
[35] C.M. Furdui, et al., Autophosphorylation of FGFR1 kinase is mediated by a sequential and precisely ordered reaction, Mol. Cell 21 (5) (2006) 711–717.
[36] M.J. Harding, A.V. Nechiporuk, Fgfr-Ras-MAPK signaling is required for apical constriction via apical positioning of Rho-associated kinase during mechanosensory organ formation, Development 139 (17) (2012) 3130–3135.
[37] K.M. Hardy, et al., FGF signalling through RAS/MAPK and PI3K pathways regulates cell movement and gene expression in the chicken primitive streak without affecting E-cadherin expression, BMC Dev. Biol. 11 (2011) 20.
[38] N. Turner, R. Grose, Fibroblast growth factor signalling: from development to cancer, Nat. Rev. Cancer 10 (2) (2010) 116–129.
[39] D.D. Deo, et al., Phosphorylation of STAT-3 in response to basic fibroblast growth factor occurs through a mechanism involving platelet-activating factor, JAK-2, and Src in human umbilical vein endothelial cells. Evidence for a dual kinase mechanism, J. Biol. Chem. 277 (24) (2002) 21237–21245.
[40] A.M. Moon, et al., Crkl deficiency disrupts Fgf8 signaling in a mouse model of 22q11 deletion syndromes, Dev. Cell 10 (1) (2006) 71–80.
[41] J. Zhang, et al., Frs2α-deficiency in cardiac progenitors disrupts a subset of FGF signals required for outflow tract morphogenesis, Development (Cambridge, England) 135 (21) (2008) 3611–3622.
[42] H. Kouhara, et al., A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway, Cell 89 (5) (1997) 693–702.
[43] Y.M. Agazie, et al., The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3, Oncogene 22 (44) (2003) 6909–6918.
[44] B. Lamothe, et al., The docking protein Gab1 is an essential component of an indirect mechanism for fibroblast growth factor stimulation of the phosphatidylinositol 3-kinase/Akt antiapoptotic pathway, Mol. Cell. Biol. 24 (13) (2004) 5657–5666.
[45] A.E. Brent, C.J. Tabin, FGF acts directly on the somitic tendon progenitors through the Ets transcription factors Pea3 and Erm to regulate scleraxis expression, Development 131 (16) (2004) 3885–3896.
[46] N. Firnberg, A. Neubuser, FGF signaling regulates expression of Tbx2, Erm, Pea3, and Pax3 in the early nasal region, Dev. Biol. 247 (2) (2002) 237–250.
[47] Y. Yoshimoto, et al., Scleraxis is required for maturation of tissue domains for proper integration of the musculoskeletal system, Sci. Rep. 7 (2017) 45010.
[48] R. Forough, et al., Role of AKT/PKB signaling in fibroblast growth factor-1 (FGF-1)-induced angiogenesis in the chicken chorioallantoic membrane (CAM), J. Cell. Biochem. 94 (1) (2005) 109–116.
[49] B.D. Manning, A. Toker, AKT/PKB signaling: navigating the network, Cell 169 (3) (2017) 381–405.
[50] A.A. Dudka, S.M. Sweet, J.K. Heath, Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification, Cancer Res. 70 (8) (2010) 3391–3401.
[51] E. Browaeys-Poly, et al., Grb14 inhibits FGF receptor signaling through the regulation of PLCγ recruitment and activation, FEBS Lett. 584 (21) (2010) 4383–4388.
[52] A.A. Dudka, S.M.M. Sweet, J.K. Heath, STAT3 binding to the FGF receptor is activated by receptor amplification, Cancer Res. 70 (8) (2010) 3391–3401.
[53] A.R.M. Bradbury, et al., Beyond natural antibodies: the power of in vitro display technologies, Nat. Biotechnol. 29 (3) (2011) 245–254.
[54] G. Smith, Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface, Science 228 (4705) (1985) 1315–1317.
[55] J. Bazan, I. Calkosinski, A. Gamian, Phage display–a powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications, Hum. Vaccin. Immunother. 8 (12) (2012) 1817–1828.
[56] M. Hamzeh-Mivehroud, et al., Phage display as a technology delivering on the promise of peptide drug discovery, Drug Discov. Today 18 (2013) 23–24 1144–57.
[57] J. Pande, M.M. Szewczyk, A.K. Grover, Phage display: concept, innovations, applications and future, Biotechnol. Adv. 28 (6) (2010) 849–858.
[58] M. Paschke, Phage display systems and their applications, Appl. Microbiol. Biotechnol. 70 (1) (2006) 2–11.
[59] N.V. Tikunova, V.V. Morozova, Phage display on the base of filamentous bacteriophages: application for recombinant antibodies selection, Acta Naturae 1 (3) (2009) 20–28.
[60] A. Mesgari-Shadi, M.H. Sarrafzadeh, Osmotic conditions could promote scFv antibody production in the Escherichia coli HB2151, Bioimpacts 7 (3) (2017) 199–206.
[61] C.M. Hammers, J.R. Stanley, Antibody phage display: technique and applications, J. Invest. Dermatol. 134 (2) (2014) e17–e17.
[62] J. Rakonjac, et al., Filamentous bacteriophage: biology, phage display and nanotechnology applications, Curr. Issues Mol. Biol. 13 (2) (2011) 51–76.
[63] M.V. Dieci, et al., Fibroblast growth factor receptor inhibitors as a cancer treatment: from a biologic rationale to medical perspectives, Cancer Discov. 3 (3) (2013) 264–279.
[64] C. Porta, et al., Dovitinib (CHIR258, TKI258): structure, development and preclinical and clinical activity, Future Oncol. 11 (1) (2015) 39–50.
[65] E. Bello, et al., E-3810 is a potent dual inhibitor of VEGFR and FGFR that exerts antitumor activity in multiple preclinical models, Cancer Res. 71 (4) (2011) 1396–1405.
[66] C. Rolfo, et al., BIBF 1120/ nintedanib : a new triple angiokinase inhibitor-directed therapy in patients with non-small cell lung cancer, Expert Opin. Invest. Drugs 22 (8) (2013) 1081–1088.
[67] S.M. Hoy, Ponatinib: a review of its use in adults with chronic myeloid leukaemia or Philadelphia chromosome-positive acute lymphoblastic leukaemia, Drugs 74 (7) (2014) 793–806.
[68] D. Kee, J.R. Zalcberg, Current and emerging strategies for the management of imatinib-refractory advanced gastrointestinal stromal tumors, Ther. Adv. Med. Oncol. 4 (5) (2012) 255–270.
[69] A. Musolino, et al., Phase II, randomized, placebo-controlled study of dovitinib in combination with fulvestrant in postmenopausal patients with HR(+), HER2(−) breast cancer that had progressed during or after prior endocrine therapy, Breast Cancer Res. 19 (1) (2017) 18.
[70] L.L. Siu, et al., Phase III randomized, placebo-controlled study of cetuximab plus brivanib alaninate versus cetuximab plus placebo in patients with metastatic, chemotherapy-refractory, wild-type K-RAS colorectal carcinoma: the NCIC Clinical Trials Group and AGITG CO.20 Trial, J. Clin. Oncol. 31 (19) (2013) 2477–2484.
[71] J.K. Chan, et al., A phase II evaluation of brivanib in the treatment of persistent or recurrent carcinoma of the cervix: an NRG Oncology/Gynecologic Oncology Group study, Gynecol. Oncol. 146 (3) (2017) 554–559.
[72] M. Schlumberger, et al., Lenvatinib versus placebo in radioiodine-refractory thyroid cancer, N. Engl. J. Med. 372 (7) (2015) 621–630.
[73] M. Kudo, et al., Orantinib versus placebo combined with transcatheter arterial chemoembolisation in patients with unresectable hepatocellular carcinoma (ORIENTAL): a randomised, double-blind, placebo-controlled, multicentre, phase 3 study, Lancet Gastroenterol. Hepatol. 3 (1) (2018) 37–46.
[74] G.C. Fletcher, et al., ENMD-2076 is an orally active kinase inhibitor with antiangiogenic and antiproliferative mechanisms of action, Mol. Cancer Ther. 10 (1) (2011) 126–137.
[75] J. Jang, et al., Antitumor effect of AZD4547 in a fibroblast growth factor receptor 2–amplified gastric cancer patient–derived cell model, Transl. Oncol. 10 (4) (2017) 469–475.

[76] V. Guagnano, et al., Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase, J. Med. Chem. 54 (20) (2011) 7066–7083.
[77] M. Michael, et al., A phase 1 study of LY2874455, an oral selective pan-FGFR inhibitor, in patients with advanced cancer, Target. Oncol. 12 (4) (2017) 463–474.
[78] F. Broekman, E. Giovannetti, G.J. Peters, Tyrosine kinase inhibitors: multi-targeted or single-targeted? World J. Clin. Oncol. 2 (2) (2011) 80–93.
[79] A. Bai, et al., GP369, an FGFR2-IIIb-specific antibody, exhibits potent antitumor activity against human cancers driven by activated FGFR2 signaling, Cancer Res. 70 (19) (2010) 7630–7639.
[80] A. Sommer, et al., Abstract 1684: preclinical anti-tumor efficacy of FGFR2-ADC BAY 1187982 in patient-derived gastric, breast and ovarian cancer models, Cancer Res. 75 (15 Supplement) (2015) 1684–1684.
[81] W.M. Zhao, et al., Monoclonal antibodies to fibroblast growth factor receptor 2 effectively inhibit growth of gastric tumor xenografts, Clin. Cancer Res. 16 (23) (2010) 5750–5758.
[82] J. Qing, et al., Antibody-based targeting of FGFR3 in bladder carcinoma and t (4;14)-positive multiple myeloma in mice, J. Clin. Invest. 119 (5) (2009) 1216–1229.
[83] J. Martinez-Torrecuadrada, et al., Targeting the extracellular domain of fibroblast growth factor receptor 3 with human single-chain Fv antibodies inhibits bladder carcinoma cell line proliferation, Clin. Cancer Res. 11 (17) (2005) 6280–6290.
[84] H.D. Sun, et al., Monoclonal antibody antagonists of hypothalamic FGFR1 cause potent but reversible hypophagia and weight loss in rodents and monkeys, Am. J. Physiol. Endocrinol. Metab. 292 (3) (2007) E964–76.
[85] C.-H. Wu, et al., Advancement and applications of peptide phage display technology in biomedical science, J. Biomed. Sci. 23 (1) (2016) 8.
[86] A. Frenzel, T. Schirrmann, M. Hust, Phage display-derived human antibodies in clinical development and therapy, mAbs 8 (7) (2016) 1177–1194.
[87] Z. Kaymakcalan, et al., Comparisons of affinities, avidities, and complement activation of adalimumab, infliximab, and etanercept in binding to soluble and membrane tumor necrosis factor, Clin. Immunol. 131 (2) (2009) 308–316.
[88] A. Vilas-Boas, S.A. Morais, D.A. Isenberg, Belimumab in systemic lupus erythematosus, RMD Open 1 (1) (2015) e000011.
[89] T.W. Huizinga, et al., Phase 1b randomized, double-blind study of namilumab, an anti-granulocyte macrophage colony-stimulating factor monoclonal antibody, in mild-to-moderate rheumatoid arthritis, Arthritis Res. Ther. 19 (1) (2017) 53.
[90] S.V. Navarra, et al., Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial, Lancet 377 (9767) (2011) 721–731.
[91] F. Behrens, et al., MOR103, a human monoclonal antibody to granulocyte–macrophage colony-stimulating factor, in the treatment of patients with moderate rheumatoid arthritis: results of a phase Ib/IIa randomised, double-blind, placebo-controlled, dose-escalation trial, Ann. Rheum. Dis. 74 (6) (2015) 1058–1064.
[92] J.F. Liu, et al., Randomized Phase II trial of Seribantumab in combination with paclitaxel in patients with advanced platinum-resistant or -refractory ovarian cancer, J. Clin. Oncol. 34 (36) (2016) 4345–4353.
[93] H. Kourlas, P. Abrams, Ranibizumab for the treatment of neovascular age-related macular degeneration: a review, Clin. Ther. 29 (9) (2007) 1850–1861.
[94] N.C. Buyukkaramikli, et al., Ramucirumab for treating advanced gastric cancer or gastro-oesophageal junction adenocarcinoma previously treated with chemotherapy: an evidence review group perspective of a NICE single technology appraisal, Pharmacoeconomics 35 (12) (2017) 1211–1221.
[95] A. Sommer, et al., Preclinical efficacy of the auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors, Cancer Res. 76 (21) (2016) 6331–6339.
[96] R. Ronca, et al., Antiangiogenic activity of a neutralizing human single-chain antibody fragment against fibroblast growth factor receptor 1, Mol. Cancer Ther. 9 (12) (2010) 3244–3253.
[97] G. Veggiani, et al., Single-domain antibodies that compete with the natural ligand fibroblast growth factor block the internalization of the fibroblast growth factor receptor 1, Biochem. Biophys. Res. Commun. 408 (4) (2011) 692–696.
[98] H. Fan, et al., Selection of peptide ligands binding to fibroblast growth factor receptor 1, IUBMB Life 54 (2) (2002) 67–72.
[99] X. Wu, et al., Identification of a novel peptide that blocks basic fibroblast growth factor-mediated cell proliferation, Oncotarget 4 (10) (2013) 1819–1828.
[100] M.D. Ballinger, et al., Semirational design of a potent, artificial agonist of fibroblast growth factor receptors, Nat. Biotechnol. 17 (12) (1999) 1199–1204.
[101] F. Maruta, et al., Identification of FGF receptor-binding peptides for cancer gene therapy, Cancer Gene Ther. 9 (6) (2002) 543–552.
[102] G.A. Rao, et al., Evaluation of the transfection property of a peptide ligand for the fibroblast growth factor receptor as part of PEGylated polyethylenimine polyplex, J. Drug Target. 16 (1) (2008) 79–89.
[103] A. Sokolowska-Wedzina, et al., High-affinity internalizing human scFv-Fc antibody for targeting FGFR1-overexpressing lung cancer, Mol. Cancer Res. 15 (8) (2017) 1040–1050.
[104] S. Moshitch-Moshkovitz, et al., Sorting polyclonal antibodies into functionally distinct fractions using peptide phage display: 'a library on top of a library', J. Immunol. Methods 242 (1–2) (2000) 183–191.
[105] L.L. Abler, M.D. Sheets, Expression of scFv antibodies in Xenopus embryos to disrupt protein function: implications for large-scale evaluation of the embryonic proteome, Genesis 35 (2) (2003) 107–113.
[106] J. Zhou, et al., Identification and validation of FGFR2 peptide for detection of early Barrett's neoplasia, Oncotarget 8 (50) (2017) 87095–87106.
[107] Y. Yin, et al., Redesigning a monospecific Anti-FGFR3 antibody to add selectivity for FGFR2 and expand antitumor activity, Mol. Cancer Ther. 14 (10) (2015) 2270–2278.
[108] A.W. Tolcher, et al., A phase I, first in human study of FP-1039 (GSK3052230), a novel FGF ligand trap, in patients with advanced solid tumors, Ann. Oncol. 27 (3) (2016) 526–532.
[109] M. Korc, R.E. Friesel, The role of fibroblast growth factors in tumor growth, Curr. Cancer Drug Targets 9 (5) (2009) 639–651.
[110] H. Fan, et al., Selection of peptide ligands that bind to acid fibroblast growth factor, IUBMB Life 49 (6) (2000) 545–548.
[111] X. Dai, et al., Identification of a novel aFGF-binding peptide with anti-tumor effect on breast cancer from phage display library, Biochem. Biophys. Res. Commun. 445 (4) (2014) 795–801.
[112] J. Tao, et al., Selection and characterization of a human neutralizing antibody to human fibroblast growth factor-2, Biochem. Biophys. Res. Commun. 394 (3) (2010) 767–773.
[113] X. Wu, et al., Isolation of a novel basic FGF-binding peptide with potent anti-angiogenetic activity, J. Cell. Mol. Med. 14 (1-2) (2010) 351–356.
[114] W. Luo, et al., P7 peptides targeting bFGF sensitize colorectal cancer cells to CPT-11, Int. J. Mol. Med. 33 (1) (2014) 194–200.
[115] L. Fan, et al., A novel FGF2 antagonist peptide P8 with potent antiproliferation activity, Tumour Biol. 35 (10) (2014) 10571–10579.
[116] W. Wang, et al., Screening a novel FGF3 antagonist peptide with anti-tumor effects on breast cancer from a phage display library, Mol. Med. Rep. 12 (5) (2015) 7051–7058.
[117] T.T. Yen, D.T. Thao, T.L. Thuoc, An overview on keratinocyte growth factor: from the molecular properties to clinical applications, Protein Pept. Lett. 21 (3) (2014) 306–317.
[118] J. Movassat, B. Portha, Early administration of keratinocyte growth factor improves {beta}-cell regeneration in rat with streptozotocin-induced diabetes, J. Endocrinol. 195 (2) (2007) 333–340.
[119] B. Jafari, et al., Identification of novel single-domain antibodies against FGF7 using phage display technology, SLAS Discov. (2017) p. 2472555217728520.
[120] G.J. Li, et al., Keratinocyte growth factor phage model peptides can promote human oral mucosal epithelial cell proliferation, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 116 (2) (2013) e92–7.
[121] N.A. Sunmonu, K. Li, J.Y. Li, Numerous isoforms of Fgf8 reflect its multiple roles in the developing brain, J. Cell. Physiol. 226 (7) (2011) 1722–1726.
[122] W. Wang, et al., Screening a phage display library for a novel FGF8b-binding peptide with anti-tumor effect on prostate cancer, Exp. Cell Res. 319 (8) (2013) 1156–1164.
[123] A. Benet-Pages, et al., An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia, Hum. Mol. Genet. 14 (3) (2005) 385–390.
[124] S. Fukumoto, Actions and mode of actions of FGF19 subfamily members, Endocr. J. 55 (1) (2008) 23–31.
[125] M.S. Razzaque, The FGF23–Klotho axis: endocrine regulation of phosphate homeostasis, Nat. Rev. Endocrinol. 5 (11) (2009) 611–619.
[126] T. Huang, et al., Selection of a novel FGF23-binding peptide antagonizing the inhibitory effect of FGF23 on phosphate uptake, Appl. Microbiol. Biotechnol. 99 (7) (2015) 3169–3177.

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