

State-of-the-Art Review

Fibroblast Growth Factors and Their Receptors in Hematopoiesis and Hematological Tumors

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ABSTRACT

Fibroblast growth factors (FGFs) belong to a family of pleiotropic heparin-binding growth factors. They exert multiple functions on various cell types of mesodermal and neuroectodermal origin, affecting cell proliferation, motility, survival, and differentiation. FGF's exert their activity by interacting with tyrosine kinase receptors (FGFRs) and cell-surface heparan sulfate proteoglycans. This article reviews recent studies on the role of the FGF/FGFR system in embryonic hematopoietic development, hematopoiesis, and hematological tumors. FGFs exert both autocrine and paracrine functions in these biological processes by acting on blood cells and their precursors and accessory cells in the bone marrow, including stromal and endothelial cells.

THE FIBROBLAST GROWTH FACTOR/FIBROBLAST GROWTH FACTOR RECEPTOR SYSTEM

FIBROBLAST GROWTH FACTORS (FGFs) represent a family of heparin-binding polypeptides. The two prototypic members of the FGF family (acidic and basic FGF, now named FGF1 and FGF2, respectively) were isolated approximately 30 years ago by heparin-affinity chromatography. Since then, more than 20 members have been identified (1; see also the Cytokine Family Database at: <http://cytokine.medic.kumamoto-u.ac.jp>). FGFs induce different biological responses in a variety of cell types of mesodermal and neuroectodermal origin, affecting cell proliferation, differentiation, motility, and survival (2). Also, some members of the FGF family, including FGF1 and FGF2, exert a potent angiogenic activity in vitro and in vivo (2). FGFs share a significant amino acid sequence homology in their central core that folds in 12 antiparallel β -strands, leading to the formation of a cylindrical barrel (3,4). Their conformation al-

lows the interaction with a dual receptor system represented by high-affinity tyrosine kinase receptors (FGFR) and low-affinity heparan sulfate proteoglycan (HSPG) binding sites (5).

FGF involvement ranges from embryonic development to neovascularization in physiological and pathological conditions. During embryogenesis, FGFs induce cell division starting at as early as the fifth cell division in preimplantation embryos (6), regulate gastrulation (7), are required for mesoderm formation (8), and regulate the migration of cells that have undergone an epithelial-mesenchymal transition (9). Accordingly, FGF4^{-/-} and FGF8^{-/-} mutations are embryonically lethal in mice (9,10).

FGFs are also relevant in organogenesis of the nervous system, lungs, and limbs. At least 10 members of the FGF family are expressed in the developing central nervous system (CNS): FGF2 and FGF15 are generally expressed throughout the developing CNS, where FGF8 and FGF17 are tightly localized to specific regions of the developing brain and are expressed only in the embryo during the

early phases of proliferation and neurogenesis (11). Mice carrying the *FGF10*^{-/-} mutation die at birth due to the lack of lung development. The trachea is formed, but subsequent pulmonary branching morphogenesis is disrupted. In addition, mutant mice have complete truncation of the fore- and hind-limbs (12). Various FGF family members can induce the formation of an ectopic limb in chick embryo, including FGF1, FGF2, FGF4, FGF8, and FGF10 (13–17), although differences have been observed with respect to the efficiency and the stage specificity of their effects. Nevertheless, all of the FGFs act by initiating the normal cascade of gene expression that leads to limb formation (18).

Angiogenesis is a multistep process that plays a key role in different physiological and pathological conditions, including embryonic development, wound repair, inflammation, and tumor growth (19). It begins with the degradation of the basement membrane by activated endothelial cells that will migrate and proliferate, leading to the formation of solid endothelial cell sprouts into the stromal space. Then, vascular loops are formed and capillary tubes develop with deposition of new basement membrane and accessory cell recruitment (20). FGF1, FGF2, and FGF4 are positive regulators of angiogenesis in vitro and in vivo (2,21). Thus, FGFs may represent a target for antiangiogenic therapy (22).

FGFs interact on the cell surface with membrane-spanning tyrosine kinase (TK) receptors (FGFRs) classified as subclass IV (23,24). The first FGFR to be characterized (*FGFR1/flg*) (25) was a single membrane-spanning molecule with three extracellular immunoglobulin (Ig)-like domains, an acidic box located between the first and the second Ig-like loop, a transmembrane domain, a juxtamembrane region, and an intracellular catalytic TK domain split by a 14-amino-acid insertion (Fig. 1). Since then, three other genes encoding TK-FGFRs have been discovered: *FGFR2/bek* (26), *FGFR3* (27), and *FGFR4* (28). Several RNA alternative spliced FGFR variants that structurally differ in the number of Ig-like loops and/or in the absence of the intracellular domain (soluble forms) were also described (21). The major growth factor bind-

ing site of FGFR appears to be located in the second half of the third Ig-like loop; three variants of this region, encoded by different exons, have been described as IIIa, IIIb, and IIIc. The IIIa sequence seems to be unique for the FGFR1 soluble receptor form (21,29), whereas IIIb and IIIc are found in FGFR1, FGFR2, and FGFR3 membrane-spanning molecules (21,30–31). Binding studies indicate that IIIc variants show a broad spectrum of ligands (21,30,32,33); other isoforms seem to be more specific, as in the case for FGFR2 (IIIb) isoform interaction with FGF7 and FGF10 (33,34).

As a general mechanism, binding of growth factors to cognate TK receptors and subsequent conformation alteration of the extracellular domain leads to receptor oligomerization (23). The interaction between adjacent cytoplasmic domains leads to receptor autophosphorylation and activation of kinase function by allosteric mechanisms (23). Phosphorylated tyrosine residues serve as docking sites for downstream signal transduction molecules containing either Src-homology 2 (SH2) or phosphotyrosine-binding (PTB) domains, leading to the switch of various intracellular signals (35–39). The multiple signal transduction pathways activated by FGFRs include, among others, the ras pathway (40), Src family protein tyrosine kinases (41), phosphoinositide 3-kinase (PI-3K) (40,42), and the phospholipase C- γ (PLC- γ) pathway (40,42). Seven autophosphorylation sites in the intracellular domain of FGFR1 have been identified (43,44). Among them, phosphorylated tyrosine 463, located in the juxtamembrane region, binds to the small adaptor molecule Crk (45), whereas phosphorylated tyrosine 766 binds PLC- γ (43); however, PLC- γ activation appears to be dispensable for both cell proliferation and differentiation induced by FGFs (46,47). Tyrosines 653 and 654 are instead crucial for receptor TK activity and their neutralization hampers receptor autophosphorylation (44). The biological function(s) of the other autophosphorylation sites and their relationship with FGFR-activated signal transduction pathways remain uncertain, although tyrosine 730 appears to be critical for FGF-induced urokinase up-regulation (48) and its counterpart in

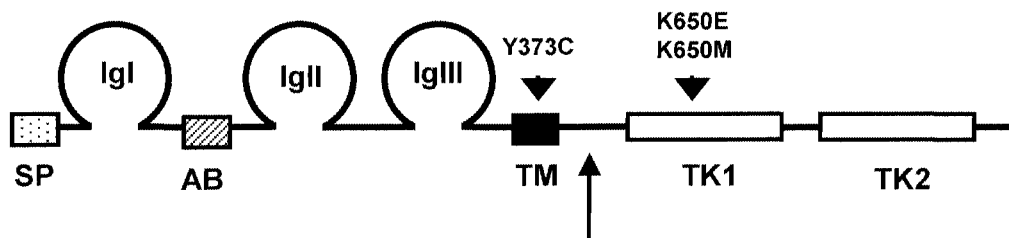


FIG. 1. Schematic representation of FGFRs. The extracellular domain contains a signal peptide (SP), two or three Ig-like (Ig) domains with an acid box (AB) followed by the transmembrane (TM) domain and intracellular domain comprising two TK subdomains interrupted by a kinase insert. The vertical arrow indicates the localization of the FGFR1 breakpoint in the 8p12 myeloproliferative disorder; arrowheads indicate FGFR3 missense mutations in MM. See text for further details.

FGFR3, tyrosine 724, is of importance for mitogenesis and for Stat and PI-3K activation (49).

FGF signaling through high-affinity FGFRs is modulated by HSPG molecules. Indeed, FGFs bind HSPGs that can act as activators or suppressors of growth factor activities. A large body of biochemical and cellular evidence points to a direct role for heparin/heparan sulfate in the formation of an active FGF-FGFR signaling complex. Accordingly, heparan sulfate is essential for FGF function during *Drosophila* development (50).

The heterogeneity of HSPGs makes these macromolecules some of the most complex substances of structured tissues (51). They consist basically of a protein core linked to qualitative and/or quantitative different oligosaccharide chains. Cell surfaces and the immediate neighboring structures, such as basement membranes, contain several classes of HSPGs that can interact with the binding counterpart via their saccharide chains, protein core, or both (51). Cellular HSPG comprise syndecans, transmembrane proteoglycans that carry both heparan sulfate and chondroitin sulfate chains near the cell surface, and glypicans that are attached to the plasma membrane via a glycosyl-phosphatidyl-inositol (GPI) linkage (52). Pericellular HSPGs comprise agrin, which is very abundant in most basement membranes, perlecan, with a widespread tissue distribution and a very complex structure, and a third class that encompasses collagen type XVIII, a hybrid collagen-HSPG molecule that is a constituent of most basement membranes and is particularly abundant in kidneys and the peripheral nervous system (52). Physiologically, cells can induce or suppress HSPG expression, which in turn modulates the nature and strength of signaling from the various FGFs stored in the extracellular matrix (ECM). For instance, perlecan induces high-affinity binding of FGF2 to heparan sulfate-deficient cells and augments FGF2 mitogenic activity (53), whereas glypican-1 inhibits the binding of FGF7 to its receptor (54). It has also been proposed that cell-surface HSPGs mediate the binding of FGFs to FGFRs, whereas free HSPGs inhibit the formation of the FGF/FGFR complex (55,56). HSPGs also serve as important reservoirs for biologically active FGFs (57) that can be released from the substratum by the concerted action of proteases (including stromelysin, collagenase, plasmin, and elastase) and heparanases (58).

FGF/FGFR SYSTEM IN EMBRYONIC HEMATOPOIETIC DEVELOPMENT

Hematopoiesis is a complex two-stage process by which mature blood cell of distinct lineages are produced from pluripotent stem cell (HSCs) during the entire adult life span (59–61). The first stage is represented by the differentiation of HSCs from early mesodermal cells; the

second stage is represented by the further differentiation of the multipotent stem cells into progenitors (HPC) that become committed to develop into cells of the different lineages, defined as burst-forming units (BFUs) and colony-forming units (CFUs).

During the embryonic ontogeny, HSCs first arise in the yolk sac blood islands (ventral blood islands in *Xenopus*) (primitive hematopoiesis) and then in the fetal liver, spleen, and bone marrow (definitive hematopoiesis) (62). Recently, an intraembryonic source of HSCs has been identified in the para-aortic splanchnopleura (63–65) and aorta-gonad-mesonephros (dorsal lateral plate in *Xenopus*) (66–68), which contains progenitors capable of long-term multilineage reconstitution. These pre-liver sites derive from mesodermal germ layer cells forming in the gastrulating embryo (69,70) and represent the main site of blood development that involves the induction, proliferation, and differentiation of the ventral mesoderm.

The survival, growth, migration, and differentiation of HSCs is regulated by the differential expression of transcription factors (i.e., SCL/tal-1, GATA-1,2, Lmo-2, Cbfa2, rbt2) (71–75) and by a network of hematopoietic growth factors, colony-stimulating factors (CSF), and interleukins (ILs) released by lymphocytes, monocytes/macrophages, fibroblasts, and stromal cells (76–78). Among these growth factors that can play either negative or positive functions, the FGF family members play a crucial role.

Extensive work on *Xenopus* embryos shows that the FGF signaling pathway is important in the formation of some derivatives of ventro-posterior mesoderm (79,80) and in the proliferation of hematopoietic progenitors. Xu et al. (81) had observed that ventral injection of embryonic FGF (eFGF) mRNA suppresses ventral blood island formation, whereas expression of the dominant negative form of FGFR in the lateral mesoderm results in a dramatic expansion of ventral blood islands. Bone morphogenetic protein-4 (BMP-4), a member of the transforming growth factor- β (TFG- β) family, induces erythropoiesis and acts in a concentration-dependent manner. At low levels, BMP-4 induces CD34⁺ stem cells to proliferate and differentiate, whereas at high levels the long-term repopulating capacity and stem cell phenotype is prolonged (82). eFGF has been shown to inhibit erythropoiesis induced by BMP-4 (81), thus indicating that FGF and BMP-4 signaling pathways may interact in the decision of ventral mesoderm to form blood. BMP-4 signaling is controlled in the extracellular space by action of dorsalizing molecules (like noggin, chordin, and follistatin) and intracellularly by the antagonizing action of activin and FGF. FGF signaling might regulate hematopoietic development downstream of BMP-4 by modulating the expression of BMP-4-activated genes. Indeed, BMP-4 induces the expression of Mix.1, a gene that induces hematopoiesis in the animal cap assay (83,84), and a

number of GATA-binding and homeobox transcription factors (85–88). Probably, FGF suppresses BMP-4-triggered GATA-2 expression, which induces erythroid differentiation in the ventral mesoderm and enhances the expression of PV.1, which inhibits erythropoiesis. The expression of the dominant interfering form of PV.1 enhances blood development, thus confirming these results. In addition, a negative interaction between PV.1 and GATA-2 presumably delineates the region of blood development (81). Thus, relative levels of FGF and BMP-4 signaling regulate the decision of ventral mesoderm to form blood by controlling the expression levels of downstream transcription factors. The expression patterns of eFGF and FGFR (89) agree with the role of FGF signaling in the ventral blood formation in *Xenopus*. eFGF is expressed before and at the initiation of mesoderm formation (during the gastrula stage). Likewise FGFR is ubiquitously expressed in the early developing embryo (89).

The importance of the cooperation between FGF and BMP-4 signaling pathways is also demonstrated by experiments of ectopic expression of BMP-4 in totipotent ectoderm explants (animal caps) (90). This induces the expression of the transcription factors GATA-1, GATA-2, SCL, and globin. Overexpression of BMP-4 induces a low number of erythroid cells. In contrast, a large number of erythroid cells are observed when ectoderm is treated with BMP-4 and activin or FGF. Ectopic expression of GATA-1 also induces abundant erythroid cells in ectoderm treated with FGF. Coinjection of GATA-1 and a dominant negative BMP receptor does not induce erythroid cells in animal explants treated with FGF, showing that GATA-1 requires the BMP-4 signaling pathway to function (90). GATA-1 does not ventralize whole embryos and is not a patterning molecule, but it is capable of cooperating with FGF to induce erythroid cells in animal caps.

At present it is not known how FGF differentially regulates mesoderm induction and transcription factors downstream of BMP-4. Mesoderm induction probably involves the mitogen-activated protein kinase (MAPK) cascade (91). Indeed, MAPK phosphatase-1 mRNA injection into oocytes leads to severe defects in gastrulation and posterior development and blocks FGF-dependent mesoderm induction. Smad 1 might be the target of MAPK that phosphorylates multiple serine residues of the protein and hampers its nuclear translocation.

The role of FGF as mesoderm induction molecule is also supported by studies in murine embryonic stem (ES) cells. Cultured ES cells can give rise to blast colony-forming units (BL-CFCs), which are thought to represent the hemangioblast (a bipotent cell that expresses Flk-1 and SCL). ES cells in which the FGFR1 gene has been knocked out differentiate poorly and produce fewer hematopoietic colonies compared with wild-type cells

(93,93). Embryoid bodies (EBs) generated from FGFR1^{-/-} cells express normal levels of BMP-4 but have reduced SCL, Flk-1, c-Kit, and globin expression. Addition of FGF2 during EB differentiation increases the numbers of BL-CFCs/hemangioblasts and Flk-1⁺ cells produced. This effect is enhanced by the addition of activin A. These data suggest that FGF2 and activin A, singularly or in concert, positively regulate hematopoietic development and that BL-CFC generation requires FGF-mediated signals.

FGF/FGFR SYSTEM IN HEMATOPOIESIS

Several members of the FGF family are implicated in the hematopoiesis process. Indeed, FGFRs are expressed on nearly every cell type of hematopoietic origin tested so far (94). FGFR4 is expressed by erythroid precursors (95), whereas FGFR2 is expressed mainly by histiocytes (96) and megakaryocytes (MKs) (97). This latter cell type also expresses FGFR1 (97). FGFR1, FGFR3, and FGFR4 can be detected on immature hematopoietic precursors from biopsies of normal and fibrotic bone marrow (BM) (96). The bulk of experimental data suggest that FGFRs are poorly expressed on early hematopoietic cells and that their expression increases on more mature cells (98,99). However, some groups have reported that the expression of FGFR1 decreases rapidly during the process of differentiation of the various hematopoietic lineages (100), this discrepancy possibly reflecting differences in the experimental conditions adopted by the various authors (e.g., composition of the cytokine cocktails).

Immunohistochemical studies have shown the presence of FGF2 associated with stromal cells in normal marrow (101). In vitro studies confirmed that BM stromal cells, as well as MKs, express FGF2, whereas immature and mature cells of the granulocyte series are weaker producers (102). In partial contrast with these latter findings, the capacity of myeloblasts, erythroblasts, and megakaryoblasts to produce FGF2 has been reported (103). Once secreted, FGF2 binds to GPI-anchored HSPGs present on the surface of BM stromal cells (104) or to other HSPGs species present in the ECM of the same cells (102). Immobilized FGF2 can be released in the extracellular environment by endogenous and/or exogenous GPI-phospholipases and by proteases (104). At this regard, FGF2 stimulates BM stromal fibroblasts to produce plasminogen activators with consequent generation of plasmin that, in turn, digests the core protein of HSPGs releasing FGF2 itself and other hematopoietic cytokines (105). Interestingly, heparin, which is structurally related to heparan sulfates, modulates the biological activity exerted by FGF2 in hematopoietic cells (106). Finally, plasmin converts inactive TGF- β and IL-1 β to their

active forms that regulate hematopoiesis in tight cooperation with FGFs (see below).

The capacity of hematopoietic cells to express both FGFs and their receptors suggests a functional role of these growth factors. Indeed, *in vivo* experiments demonstrated that FGF1 (107) and FGF2 (108) increase survival and hematopoietic recovery in irradiated mice. Accordingly, FGF7 and FGF10 protect bone marrow during therapeutical irradiation (109). Moreover, administration of recombinant FGF9 (110) or adenovirus-mediated transfer of FGF4 (111) stimulate murine thrombopoiesis.

As already mentioned, FGF and FGFR expression is widely distributed on stromal cells and different hematopoietic precursors, thus suggesting that FGFs can regulate hematopoiesis directly by acting on hematopoietic cells, and/or indirectly by stimulating stromal cells to produce and release hematopoietic cytokines. This latter possibility is supported by the observation that FGF2 enhancement of megakaryocytopoiesis can be abrogated by anti-IL-6 antibodies (96). FGF2 induces proliferation of BM stromal cells (112,113). In these cells, FGF2 also causes prominent morphological changes, it modifies the expression of several proteins related to cytoskeleton or ECM organization (114) and induces plasminogen activator production (105). Finally, FGF4 increases the growth rate of BM stromal cells and delays their senescence (115). The extracellular signal-regulated kinases 1 and 2 are phosphorylated in response to FGF2 in these cells (116), thus suggesting that the activation of this second messengers is, at least in

part, responsible for FGF-induced biological responses in BM stromal cells.

FGFs may also act directly onto hematopoietic cells at different stages of differentiation. Indeed, FGF1 (117) and FGF2 (118,119) sustain stem cell proliferation and promote their self-renewal. On the other hand, FGF2 increases the generation of colonies of noncommitted hematopoietic progenitors from stem cells (92,120). These cells show defective hematopoietic development when carrying mutated, nonfunctional FGFR1 (92).

The capacity of FGF1 and FGF2 to induce stem cells proliferation and differentiation indicates a very early involvement of FGFs in the hematopoietic process. However, some reports suggest that FGFs regulate only the late stages of hematopoietic cells differentiation (98,99). Again, this discrepancy may be due to different, possibly nonphysiological experimental conditions adopted by the various Authors.

Among committed hematopoietic cells, MKs express FGFRs and respond to FGF1 (97) and FGF2 (121) in terms of proliferation. The mitogenic activity exerted by FGF2 in MKs is not abrogated by antibodies directed against granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-6 (121), thus confirming that FGFs exert a direct effect in these cells. On myeloid progenitor cells, FGF2 induces the production of IL-6 (99) and counteracts the suppressive effect of TGF- β (122).

FGFs have been demonstrated to drive the differentiation of the hematopoietic cells (Fig. 2). FGF1 induces granulopoiesis (123) and megakaryocytopoiesis (97,106).

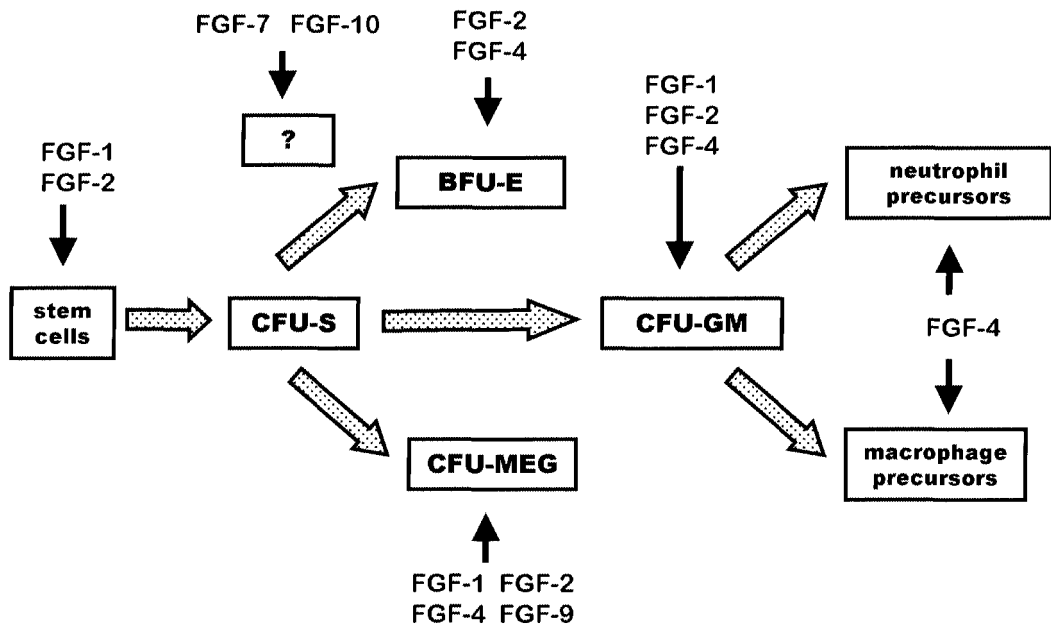


FIG. 2. FGFs in hematopoiesis. Various FGFs may exert their biological function acting at different steps of the adult hematopoiesis hierarchy. See text for further details.

FGF2 and FGF4 are implicated in erythropoiesis (95) and myelopoiesis (124) and in the consequent generation of mature neutrophils and macrophages (112,115,125). FGF2 also enhances MK colony formation and maturation (106,126).

FGFs usually act in cooperation with others cytokines (127). Indeed, when combined with TGF- β , interferons, or epidermal growth factor (112,128), FGF2 inhibits hematopoiesis. On the contrary, FGF2 synergizes with GM-CSF and erythropoietin to increase the formation of non-committed, early colony-forming units (120). These cells can be then directed to myelopoiesis by a cocktail of FGF2, stem cell factor (SCF), and GM-CSF (124). A further step toward MK generation can be obtained by IL-6 (94) or by the combination of FGF2 with HGF (126). The generated MKs can be then induced to proliferate by FGF2 or FGF1 together with IL-3 (97) and GM-CSF (129). Alternatively, myeloid progenitors can be driven to the generation of cells of the neutrophil-granulocyte series by stimulation with FGF2, GM-CSF, and G-CSF (125). Finally, erythroid progenitors can be obtained by stimulation of myeloid precursor with FGF2, SCF, and insulin (95).

FGFs have been implicated mainly in myeloid differentiation. However, their capacity to affect BM stromal cells, stem cells, and tumor cells in leukemias and lymphomas (see below) suggests a possible role of FGFs also in lymphopoiesis. Indeed, FGF2 induces a dramatic increase in clonogenicity of SR-4987 cells derived from murine BM stroma that may represent a very early differentiation stage of B cells (130).

It should be pointed out that the effect exerted by FGFs on hematopoietic cells might be exploited therapeutically. Indeed, FGFs may ameliorate *ex vivo* expansion of human BM cells prior to transplantation to obtain a long-lasting restoration of normal hematopoiesis in leukemic patients. FGFs may also protect BM and guarantee hematopoiesis after immunosuppressive treatment or during radio/chemotherapy in patients with tumors (107,108). Relevant to this latter possibility is the observation that FGF7 and FGF10 exert their radioprotective effect on BM *in vivo* in the absence of adverse effects on tumor growth (109).

FGF/FGFR SYSTEM IN HEMATOLOGICAL TUMORS

Several experimental and clinical evidences implicate FGFs and their receptors in the genesis and/or progression of blood tumors. FGFR3 and FGFR4 were originally cloned from the human K562 leukemia cell line (28). Since then, several experimental results indicate that human leukemia cells express at least one receptor among FGFR1, FGFR3, or FGFR4 (131–134), thus indicating

that FGFs may exert paracrine and autocrine functions in hematological tumors (see below). Also, alterations of FGFR expression and activity may play a role in the genesis and progression of these malignancies.

FGFR1 appears to be involved in a distinct type of stem cell leukemia/lymphoma syndrome associated with chromosomal translocations involving 8p12 where the FGFR1 gene is located. Indeed, these translocations lead to the fusion of the 3' portion of the FGFR1 gene, encoding the catalytic TK domain of the receptor (Fig. 1), with various partners. To date, seven FGFR1 partners have been described and three fusion transcripts have been identified (135). They encode for chimeric proteins containing the amino-terminal leucine-rich region from the FOP gene, the amino-terminal leucine zipper motifs from CEP110 gene, or the amino-terminal zinc fingers from FIM gene fused to the TK domain of FGFR1 in carboxy terminus (136). The partner regions represent self-association domains that lead to oligomerization with consequent FGFR1 TK activation. The constitutive kinase activity triggered by dimerization mediated by the protein-protein interaction motifs of the FGFR1 protein partner and the delocalization of the fusion proteins compared to their normal counterparts may lead to tumorigenesis, presumably by inducing inappropriate recruitment in the cytoplasm of signaling substrates (137,138).

Chromosomal translocations involving the immunoglobulin heavy chain (Ig_H) locus at chromosome 14q32 represent instead a common mechanism of oncogene activation in lymphoid malignancies. FGFR3 expression is frequently upregulated in multiple myeloma (MM) as a consequence of the translocation of the most telomeric region of the short arm of chromosome 4, where the FGFR3 gene is located, to chromosome 14 in close vicinity to Ig_H promoter (139). Breakpoints occur approximately 50–120 kb centromeric to the FGFR3 gene, resulting in overexpression.

Missense point mutations in different structural domains of the FGFR3 gene are associated with genetic skeletal disorders such as achondroplasia, hypochondroplasia, and thanatophoric dysplasia types I and II (140). Interestingly, FGFR3 gene mutations have been identified also in MM cell lines and primary tumors carrying t(4;14)(p16.3;q32), suggesting that they may represent an event associated with tumor progression in MM with t(4;14). These mutations include mutations Y373C, K650E, and K650M (141,142) (Fig. 1) and lead to the constitutive autophosphorylation and ligand-independent activation of the receptor. However, they represent rare events in primary MM tumors (143).

The role of FGFR3 overexpression in MM is still largely unknown. It has been reported that expression of high levels of wild-type FGFR3 or constitutively active FGFR3 mutant triggers the murine B9 MM cell line to grow independently from IL-6 (144). However, studies

concerning the capability of FGFR3 mutants to induce a transformed phenotype in NIH 3T3 cells are controversial (145–147). Recently, to examine the oncogenic potential of FGFR3, murine BM cells were transduced with retroviral vectors harboring either the wild-type or an activated mutant form of FGFR3 (148). Mice transplanted with cells transduced with the constitutively activated FGFR3 mutant developed a marked leukocytosis and lethal hematopoietic cell infiltration within 6 weeks after transplantation. Analysis of the circulating tumor cells revealed a pre-B-cell phenotype in most mice, although immature T lymphoid or mature myeloid populations were also observed. Mice transplanted with wild-type FGFR3-expressing BM cells developed delayed pro-B cell lymphoma/leukemias approximately 1 year after transplantation, thus confirming that FGFR3 is transforming and can produce lymphoid malignancies in mice.

Interestingly, it has been shown that chronic myeloid leukemia (CML) patients also express high levels of FGFR3 mRNA in white blood cells. After stem cell transplantation and reconstitution of hematopoiesis, the expression of FGFR3 decreases and is maintained at low levels. However, FGFR3 up-regulation occurs again in

those patients that have accelerated BCR/ABL rearrangement and undergo relapse (149).

Since the observation that virus-transformed avian monocytic leukemia cells release a FGF-like activity (150), several experimental results indicate that FGFs are produced by tumor cells in hematological malignancies.

Indeed, FGF2 is expressed by several leukemia cell lines in vitro (133,151,152), including megakaryocytic tumor cells that also express hst-2/FGF6 (153). Elevated FGF2 levels have been detected in urine from patients with a variety of neoplastic diseases, including various leukemias (154), and from children with acute lymphoblastic leukemia (ALL) (155). Also, FGF2 levels are elevated in plasma from patients with B cell chronic lymphocytic leukemia (B-CLL) and CML (156). Accordingly, FGF2 is expressed by leukemic cells from CLL, CML, and hairy cell leukemia patients (156–158).

The capacity of leukemic cells to express both FGFRs (see above) and their ligands raises the possibility that the FGF/FGFR system may exert autocrine function in hematological tumors (151). For instance, Bcl-2 and FGF2 expression correlates positively in CCL patients (159). This may result in an increased survival of tumor

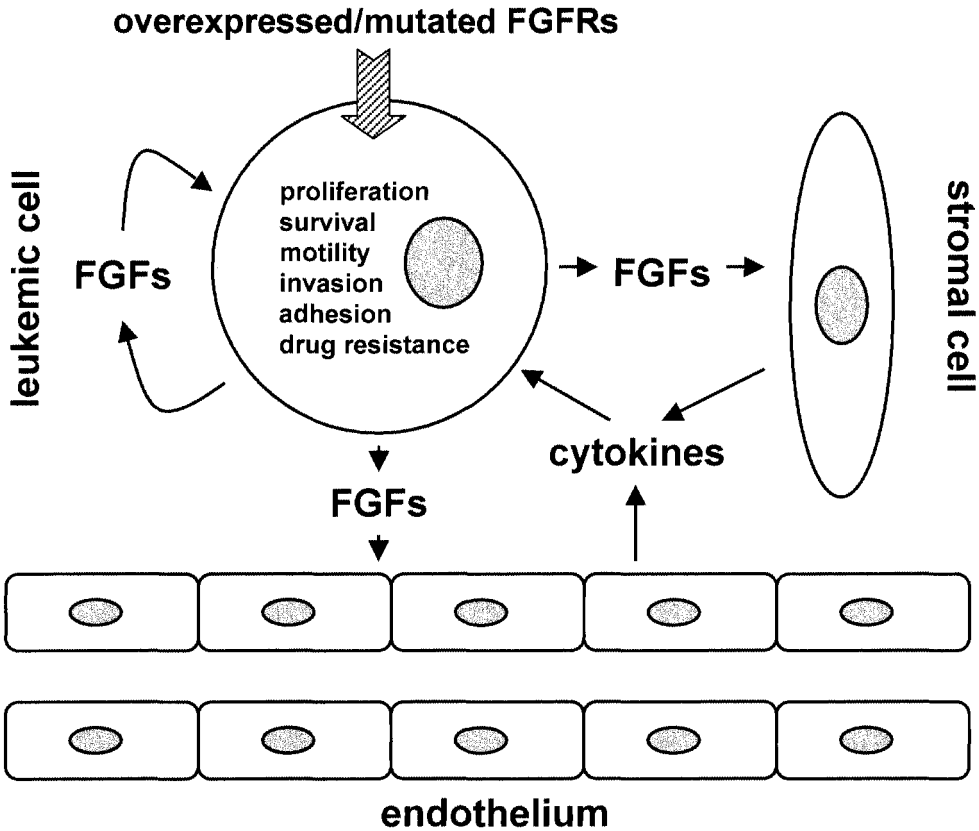


FIG. 3. Role of the FGF/FGFR system in hematological tumors. FGFs produced by leukemia cells exert autocrine and paracrine functions in bone marrow and can stimulate cytokine production by stromal and endothelial cells. Deregulation of FGFR activity may contribute to the altered behavior of tumor cells. See text for further details.

cells. Indeed, FGF2 up-regulates Bcl-2 expression, delays apoptosis, and confers an increased resistance to cytotoxic drugs in various leukemia cell lines (157,158,160). Also, FGF1 and FGF2 may stimulate the growth of leukemic cells in synergy with other hematopoietic growth factors (161,162). Thus, FGFs may affect the proliferation, survival, and antineoplastic drug susceptibility of hematological tumors in an autocrine mode of action.

An increasing body of evidence points to a role for BM angiogenesis in hematological tumors. Indeed, BM vascularization is increased in patients with acute or chronic leukemias, myelodysplastic syndromes (MDS), and MM (155,163–166). FGF2 plasma or urinary levels show a direct correlation with BM vascularity in these patients (155,164), suggesting a direct role of this growth factor in BM angiogenesis during tumor progression. Accordingly, the angiogenic activity exerted by different leukemic cell lines is inhibited, at least in part, by neutralizing anti-FGF2 antibodies (152).

Leukemic cells release endothelial growth factors other than FGFs. They include, among others, the angiogenic vascular endothelial growth factor (VEGF) (164, 167,168). Relevant to this point, is the observation that FGF2 and VEGF may exert cooperative effects on target cells (169). Also, activated endothelial cells release cytokines that stimulate leukemia cell growth (170). Thus, a reciprocal loop of stimulation exists between leukemic and endothelial cells (Fig. 3). The combined therapeutic approach targeting both tumor cells (by conventional cytotoxic agents) and endothelial cells (by angiogenesis inhibitors) or by molecules acting on both cell types may lead to synergistic antitumor effects. This mechanism of action has been hypothesized for arsenic trioxide and vinblastine in the therapy of hematological tumors (171,172).

In conclusion, deregulation of the FGF/FGFR system appears to have important implications for the genesis and progression of blood tumors.

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