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Biological Roles of Fibroblast Growth Factor-2*

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I. Introduction

FIBROBLAST growth factor-2 [basic FGF (bFGF); FGF-2] is a member of the FGF family that comprises nine members (reviewed in Refs. 1 and 2). This FGF prototype has pleiotropic effects in different cell and organ systems. FGF-2 is a potent angiogenic molecule *in vivo* and *in vitro* stimulates smooth muscle cell growth, wound healing, and tissue repair (1, 3). In addition, FGF-2 may stimulate hematopoiesis (reviewed in Refs. 4 and 5) and may play an important role in the differentiation and/or function of the nervous system (reviewed in Refs. 6–8), the eye (reviewed in Ref. 9), and the skeleton (10, 11). In this review, we have focused on recent observations that relate to the mechanism of action and function of FGF-2. First, we discuss the structure of FGF-2. We then discuss the mechanism of action and the release of FGF-2. Finally, we summarize recent findings on the role of FGF-2 in embryonic and organ development.

II. Structure of FGF-2

FGF-2 was first identified as a 146-amino acid protein isolated from the pituitary (12). When FGF-2 cDNAs were cloned (13, 14), an AUG codon was found in the proper context to initiate translation of a protein of 155 amino acids, and no in-frame AUG codons were found upstream. Therefore, translation was predicted to initiate at this AUG codon. However, FGF-2 molecules both longer and shorter than that predicted from the cDNA sequence were found in guinea pig brain, rat brain, liver, human placenta, prostate, and several types of cultured cells (15–22). The shorter forms are derived from the 155-amino acid FGF-2 by proteolytic degradation (16). The origin of higher molecular weight forms (196, 201, and 210 amino acids) was elucidated by *in vitro* transcription/translation analysis that revealed that CUG codons, 5′ to the AUG codon used for the translation initiation of the 155-amino acid form, were used as initiation codons for the larger species (23, 24). Alternative translation occurs by internal ribosomal entry sites in the FGF-2 mRNA (25). When the FGF-2 cDNA is expressed in cells, the AUG- and three CUG-initiated forms migrate on SDS-PAGE gels with molecular masses of 18, 22, 22.5, and 24 kDa, respectively. The forms initiated using the CUG codons (22, 22.5, and 24 kDa) are predominantly localized in the nucleus, whereas the AUG-initiated form (18 kDa) is localized primarily in the cytoplasm (26–29). This may depend, however, upon the specific cells examined and the levels of FGF-2 expressed.

Using recombinant protein, several groups have determined the three-dimensional structure of crystalline 18-kDa FGF-2 (30, 31). FGF-2 contains 12 anti-parallel β-sheets organized into a trigonal pyramidal structure. Several domains may be important for FGF-2 function. Residues 13–30 and 106–129 are believed to represent the receptor-binding sites (32, 33). The inverse RGD sequences PDGR and EDGR are possibly involved in the modulation of mitogenicity (34). Two potential phosphorylation sites occur; one at serine 64 and the other at threonine 112. Serine 64 and threonine 112 can be phosphorylated by protein kinase A and protein kinase C, respectively (35). The cellular kinases responsible for FGF-2 phosphorylation may be localized both in the nucleus and at the cell surface (36, 37). FGF-2 contains four cysteines; however, there are no intramolecular disulfide bonds (38).

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The unique feature of the high molecular weight (HMW) FGF-2 forms, which distinguishes them from 18-kDa FGF-2, is the amino-terminal extension. In the largest form, this sequence contains nine Gly-Arg repeats. At least six of the arginines in these Gly-Arg motifs are methylated (Refs. 39 and 40 and our unpublished data). Neither the exact number nor the functional significance of the methylated arginines is known, but these modified residues may be involved in nuclear transport or retention. The nuclear targeting of HMW FGF-2 resides in the amino-terminal extension as this sequence, which when joined to other proteins, targets them to the nucleus (26, 28). However, the nuclear targeting sequence of SV40 T-antigen joined to 18-kDa FGF-2 does not allow nuclear accumulation of the fusion protein (41). This indicates that 18-kDa FGF-2 *per se* contains inhibitory sequences for nuclear transport, which can be overcome by the presence of the N-terminal extension.

III. Mechanism of Action of FGF-2: Extra- and Intracellular Signaling

A. Exogenous 18-kDa FGF-2

FGF-2 interacts with specific cell surface receptors. Four major receptor families have been identified; FGFR1 (flg), FGFR2 (bek), FGFR3, and FGFR4 (1, 42). These receptors share common features including a cytoplasmic conserved tyrosine kinase domain, a transmembrane domain, and an extracellular ligand-binding domain. Spliced variants exist that differ in the composition of the extracellular ligand-binding domain, which can contain two or three immunoglobulin (Ig)-like loops (43, 44). Additional splice-variants, containing sequence modifications in the intracellular portions of the protein, have been described (43, 44). The nine FGFs differ in their abilities to signal through the different FGF receptor variants, which is an essential mechanism for regulating FGF activity (45). The FGF receptor families are well conserved as FGFR1 to FGFR4 have been identified in species as primitive as *Drosophila*, *C. elegans*, and Medaka fish (46).

The mechanism of FGFR interaction has been investigated at three levels: 1) ligand specificity, 2) extracellular conditions that modify ligand/receptor interaction, and 3) interactions between the ligand-activated receptor and substrates generating the intracellular signals.

The ligand specificity of FGFR1-3 resides in the C-terminal half of Ig domain III. Whereas for FGFR4, one exon encodes the C-terminal half of Ig domain III, for FGFR1-3, three alternatively spliced exons, IIIa, IIIb, and IIIc, encode this region. The expression of IIIa leads to a secreted form, whereas IIIb and IIIc encode membrane-anchored forms. For FGFR1, there is a secreted receptor isoform containing IIIa and membrane-anchored isoforms containing IIIb and IIIc (47). A possible role of the secreted FGFR1 is as a natural competitor for FGF binding, which may regulate FGF-induced responses. For FGFR2 and R3, receptor isoforms containing IIIb and IIIc exons have been reported (48–51). Expression of the alternative exons in this position seems to regulate ligand specificity. The preferential usage of one of the exons over the other may depend upon the presence of a titratable repressor. This mechanism has been suggested for the FGFR2 gene that is generated by splicing of the IIIb or IIIc exons (52). The second Ig loop domain, for which there are no alternative spliced forms, may also contribute to binding specificity (53). In contrast, the first Ig loop domain, which is only present in some receptor forms, may alter the affinity of the receptor without changing its specificity (54).

A cell adhesion molecule (CAM) domain has been identified in FGFR1 (55). CAMs such as neural adhesion molecule L1 or neural CAM (NCAM) may signal via the FGFR by interacting with the CAM domain (56–61). Antibodies to the acidic box domain inhibit L1 and NCAM-induced neurite outgrowth. Antiserum raised against the CAM homology domain of the receptor also blocks outgrowth on L1- or NCAM-expressing substrate cells. Moreover, peptides of the CAM domain in the FGFR corresponding to a specific CAM block neurite outgrowth on the appropriate adhesion molecule. Finally, neurite outgrowth is induced by a soluble L1-Fc chimera. This effect can be inhibited by anti-FGF receptor antibodies and by pharmacological reagents that interfere with the FGFR-dependent signal transduction pathway.

A fifth FGFR has been described that is structurally distinct from the other FGFRs (62). It is an integral membrane protein containing an extracellular domain with 16 cysteine-rich repeats. The ligand specificity of this receptor subtype is not known although it can bind FGF-1, -2, -3, and -4. The cysteine-rich receptor does not have intrinsic signaling properties and its function is not presently known. Another FGFR isoform is the E-selectin ligand ESL-1 (63), which is 94% identical to the cysteine-rich receptor except for 70 amino acids at the N terminus. Fucosylation of this receptor appears to be necessary for binding to E-selectin. Binding studies with FGF-2 and ESL-1 have not been performed and it is, therefore, not known whether fucosylation is also important for FGF-2 binding.

The major extracellular nonsignaling molecules involved in FGF activity are heparan sulfate proteoglycans (HSPGs). Several groups have reported that heparan sulfates are required for FGF signaling (64–70). This claim is based on a number of experiments. First, mutant Chinese hamster ovary (CHO) cells, which lack heparan sulfates, fail to bind FGF-2 when transfected with FGFR1 but bind FGF-2 when heparin is added to the medium (64, 65). Second, the lymphoid cell line BaF3, which does not synthesize heparan sulfates, neither binds nor responds to FGF-2 when transfected with the cDNA encoding FGFR1 (66, 67). Cells expressing receptor respond to FGF-2 when incubated in the presence of heparin. Third, NIH 3T3 cells treated with chlorate, which blocks heparan sulfate sulfation, no longer respond to exogenous FGF-2 (68). Fourth, FGF-2 effects on myogenic differentiation are dependent on heparan sulfate (68, 69). However, Roghani *et al*. (71) reported that the myeloid cell line 32 D, which lacks heparan sulfates, binds FGF-2 in the absence of exogenous heparin or heparan sulfate when transfected with FGFR1. FGF-2 addition to these cells also induces c-*fos* activation. Other studies with the purified FGFR1 extracellular domain have demonstrated that heparin is not required for FGF-2 binding to high-affinity receptors (72–74). Cell surface HSPGs may modulate the action of FGF-2 by increasing its affinity for its receptor (71, 74), thereby enhancing the FGF/FGFR interaction. Further studies indicate that heparin and

heparan sulfates may both increase the surface concentration of FGF-2 as well as assist in ligand dimerization (67, 75–78).

FGF-2 is internalized via high- and low-affinity sites (79–81). FGF-2 internalized by either high- or low-affinity binding sites may have different intracellular fates (82). FGF-2-saporin chimeras are internalized in NMuMG cells by low-affinity binding molecules without killing the cells. However, when these cells, which normally do not express high-affinity receptors, are transfected with FGFR1, FGF-2-saporin kills the cells. Thus, the high-affinity FGFR1 targets the chimera to a cytosolic compartment where the toxin kills the cells, but the low-affinity sites do not permit toxin internalization to the same compartment.

A heparin-binding site was identified proximal to the third Ig-like loop of the FGFR1 (83). A point mutation in this sequence abrogated both heparin- and ligand-binding activities of the receptor. This suggests that a ternary complex of heparan-sulfate proteoglycan, FGF, and FGF receptor exists. N-Syndecan from neonatal rat brain binds FGF-2 but not FGF-1 (84). N-Syndecan and FGF-2 are both abundant in the neonatal rat brain, suggesting that N-syndecan may function as a coreceptor during nervous tissue development. Aviezer *et al*. (85) reported that the basal lamina proteoglycan perlecan promotes FGF-2 receptor binding, mitogenesis, and angiogenesis suggesting that perlecan may be a major FGF-2 low-affinity receptor. Nugent and Edelman (86) measured the on and off rates for these two classes of binding sites. Whereas the on-rate constants are similar for high- and low-affinity binding sites, the off-rate constant is 23-fold greater for HSPG than for the high-affinity receptor. Therefore, differences in dissociation constant (K_d) result from the faster off-rate for FGF-2 release from the HSPG sites *vs.* the high-affinity receptor. Gao and Goldfarb (87) recently reported that heparin and the heparin analogs fucoidan and dextran sulfate directly activate FGFR4, but not FGFR1 (87). Heparin binds with strong affinity to FGFR4 at loop 2 of the extracellular domain.

The association of FGF-2 with FGF-binding proteins in the blood or the matrix may influence FGF-2 activity and/or bioavailability. In the blood, FGF-2 associates with α_2-macroglobulin or truncated FGFR1 (88, 89). In addition, two truncated forms of FGFR1 of 85 and 55 kDa are associated with the matrix (90). Taken together, the above data indicate that FGF action is regulated extracellularly at 1) the plasma membrane by cooperative interaction of heparan sulfates and FGFR, 2) the matrix by either direct interaction or indirect action via truncated FGFR1, and 3) the blood by interaction with α_2-macroglobulin or truncated FGFR1.

FGF-2 stimulates receptor tyrosine-kinase autophosphorylation and receptor association with putative substrates such as phospholipase C-γ (PLC-γ). Mutation of a single amino acid abolishes the association with PLC-γ but not mitogenesis (91, 92). Specifically, a point mutation at Tyr-766 in the FGFR1 expressed in PC12 cells eliminates FGF-induced stimulation of phosphatidyl inositol hydrolysis (93). However, neither the ras-dependent activation pathway nor neurite outgrowth are affected. Thus, phosphatidyl inositol hydrolysis does not seem necessary for cell differentiation in PC12 cells. Similarly, the role of PLC-γ activation after FGFR1 occupancy was examined in mesoderm induction in *Xenopus* (94). Chimeras of the extracellular domain of platelet-derived growth factor (PDGF) receptor and the intracellular and transmembrane domains of either the wild type FGFR1 or the FGFR1 mutated in the PLC-γ-binding domain so as to inhibit receptor association with PLC-γ were constructed (94). Both receptors mediated mesoderm induction in *Xenopus* animal caps demonstrating that PLC-γ activation by the FGF receptor is not required for FGF-stimulated mesoderm induction. In addition to Tyr-766, other autophosphorylation sites in FGFR1 have been described (95). These include six sites at Tyr-463, Tyr-583, Tyr-585, Tyr-653, Tyr-654, and Tyr-730. Among these, only Tyr-653 and Tyr-654 are important for biological activity. Both wild type and FGFR1 mutated at the remaining four nonessential tyrosines phosphorylate Shc and an unidentified Grb2-associated phosphoprotein of 90 kDa (pp90). Binding of Grb2/Sos complex to phosphorylated Shc and pp90 may link FGFRs to the Ras-signaling pathway. Another substrate of the activated FGFR1 is the phosphoprotein cortactin, which binds to filamentous actin, and is phosphorylated by the src oncogene (96). Activated FGFR1 may associate with c-*src* that, in turn, phosphorylates cortactin. Cortactin may act as a link between the FGFR1 signal transduction pathway and the cytoskeleton.

A long-term mechanism of FGF-2 and FGF-1 action involving growth factor translocation into the nucleus has been proposed (97, 98). To achieve maximal DNA synthesis, cells require exposure to FGF-1 for at least 12 h (98). In cells stimulated by FGF-1, FGFR1 is phosphorylated and translocated perinuclearly (99). This correlates with an accumulation of FGF-1 in the nucleus. Thus, signaling through the FGF receptor may occur at the plasma membrane and after receptor translocation to an intracellular site. Mutation of the putative nuclear targeting sequence of FGF-1 abolishes cell growth but retains the ability to bind and activate the FGFR (100). However, further studies suggested that this effect may result from the structural instability of the deletion mutant (101). FGF-1 fused to diphtheria toxin and translocated into toxin-resistent cells, which lack functional FGFRs, stimulates DNA synthesis (102). This result supports a receptor-independent mechanism for FGF-1 stimulation of proliferation. This may also be true for FGF-2 as both growth factors bind to similar receptors. Therefore, signals important for cell proliferation may initiate at an intracellular (nuclear) level either through the FGF receptor or independent of it. Quarto and Amalric (103) reported that FGF-2 stimulated plasminogen activator expression in L6 rat myoblasts devoid of functional FGFR. They suggested that the signal is transduced through heparan sulfate proteoglycans. How this might be achieved is not understood.

Changes occur in FGFR expression levels during proliferation, differentiation, or malignant transformation. Exponentially growing cells generally express higher receptor levels than do subconfluent or confluent cells. During differentiation, FGFR expression may increase or decrease according to the cell type. For example, Moscatelli (104) has shown that cell surface FGFR increase in F9 teratocarcinoma cells when differentiation is induced with retinoic acid or cAMP. This increase may be due to a loss of FGF expression in differentiated cells, thus inhibiting down-regulation of FGF receptors. In contrast, Pertovaara *et al*. (105) reported that retinoic acid-induced differentiation of human embryonic carcinoma cells led to a loss of FGFR4 mRNA expression and

a down-regulation of FGFR2 and FGFR3 mRNAs. However, the level of FGFR1 remained unchanged. Mummery *et al*. (106) observed down-regulation of FGFR2 but not of FGFR1, R3, or R4 in differentiated human embryonic carcinoma cells. In embryonic stem cells, however, FGFR1, R2, and R3 are up-regulated during differentiation. FGFR4 was only expressed after differentiation in cells resembling parietal endoderm. In skeletal muscle, FGFR expression is greatly reduced after differentiation (107). These examples illustrate that during differentiation, changes in FGFR expression are variable and, therefore, no general conclusion can be derived.

Another new isoform of the FGFR family that has been cloned (108, 109) is the kinase named FREK (FGF receptor-like embryonic kinase) that can be alternatively spliced in two or three IgG forms (108). FREK is expressed during the elongating primitive streak in the rostral and lateral epiblast and in Hensen's node. From day 2.5, FREK is expressed in various ectoderm- and mesoderm-derived structures. It is expressed at high levels in skeletal muscle, beginning in the early myotome and later in all skeletal muscles of the embryo. From day 9, FREK levels decrease dramatically. However, expression is maintained in satellite cells of the adult muscle. The natural ligand for the FREK receptor is not known, although it can bind FGF-2. Skeletal muscle satellite cells express higher levels of FREK mRNA than do epiphyseal growth plate chondrocytes (109). Differentiation reduces the levels of FREK.

B. Endogenous 18-kDa and HMW FGF-2

The different subcellular localization of HMW and 18-kDa FGF-2 supports the potential for different roles for these FGF-2 isoforms. Cell fractionation and immunofluorescence of either 3T3 or COS cells transfected with FGF-2 cDNAs, BAE cells, or hepatoma cells expressing different FGF-2 isoforms indicated that HMW FGF-2 is predominantly localized in the nucleus, whereas 18-kDa FGF-2 is primarily localized in the cytoplasm (26–29).

Endogenous FGF-2 may play a role in cell growth, migration, and differentiation. Sato and Rifkin (110) and Biro *et al*. (111) demonstrated that migration of bovine capillary endothelial cells is inhibited by FGF-2 antibodies, implying that endothelial cell migration is affected by extracellular FGF-2. Mignatti *et al*. (112, 113) showed that movement of NIH 3T3 cells transfected with wild type FGF-2 cDNA is increased compared to that of control cells and that movement is inhibited by FGF-2 antibodies. BHK cells (114) and BALB/c 3T3 cells (115) transformed by overexpression of 18-kDa FGF-2 also exhibit increased growth. NIH 3T3 cells expressing HMW FGF-2 isoforms have altered growth properties (116); cells with high copy numbers of HMW FGF-2 cDNA grow like transformed cells, whereas cells with low copy numbers are growth inhibited.

We have attempted to answer the question of whether specific forms of FGF-2 induce specific phenotypes by examining cells expressing unique forms of FGF-2 (117). Cells expressing only 18 kDa were more migratory than control cells, whereas cells expressing only HMW FGF-2 migrated at a level similar to control cells. Cells expressing only HMW FGF-2 exhibited increased saturation densities, growth in soft agar, and growth in low serum. Cells expressing only 18-kDa FGF-2 also grew to high saturation densities and grew in soft agar but did not grow in low serum. An increase in migration and receptor down-regulation correlated with the presence of extracellular 18-kDa FGF-2. Supertransfection of cells expressing HMW FGF-2 with a cDNA encoding 18-kDa FGF-2 caused an increase in migration and cell surface-associated 18-kDa FGF-2. Overexpression of a dominant negative FGF receptor inhibited migration and decreased saturation density and soft agar growth of cells expressing 18 kDa FGF-2 but did not inhibit growth of cells expressing HMW FGF-2. These data demonstrate that endogenous 18 kDa and HMW FGF-2 can have different biological roles; the former affects cell growth and cell migration; the latter affects cell growth but not migration. Estival *et al*. (118) also showed that adrenocortical cells transfected with the cDNA encoding HMW FGF-2 display enhanced proliferation. Furthermore, Biro *et al*. (111) demonstrated that FGF-2 accumulates in the nucleus before the onset of DNA synthesis. In addition, the presence of the 24-kDa FGF-2 form is present in human pituitary adenomas, whereas in the normal pituitary gland only the 18-kDa form is present (119). These data are consistent with a role of HMW FGF-2 in cell growth and tumorigenesis. Another potential intracellular role of endogenous FGF-2 is trans-differentiation of neural crest-derived Schwann cell precursors into melanocytes (120) as antisense oligonucleotides for FGF-2, but not FGF-2 antibodies, inhibited trans-differentiation. However, it has not been established which form of FGF-2 is responsible for this effect.

The intracellular molecules that interact with endogenous FGF-2 are not known. Nakanishi *et al*. (121) showed *in vitro* that pgk gene transcription is regulated by high concentrations of 18-kDa FGF-2. FGF-2 stimulates the phosphorylation of nucleolin by CKII in an *in vitro* system (122). In addition, Prats *et al*. (123) using the radiation inactivation method showed that FGF-2 is present in the nucleus in two complexes: HMW FGF-2 in a complex of 320 kDa and 18-kDa FGF-2 in a complex of 130 kDa. These results indicate that intracellular FGF-2 may associate with other molecules.

Several factors regulate the expression of endogenous FGF-2. Phorbol-myristate acetate (PMA) enhances FGF-2 mRNA and protein expression in human umbilical vein and bovine adrenal cells (124, 125), and forskolin induces the expression of all FGF-2 isoforms in these cells. FGF-2 accumulates preferentially in the cytosol after PMA stimulation, whereas forskolin induces nuclear accumulation. Interleukin-1 and TGF-β regulate FGF-2 expression (126, 127). p53 May regulate FGF-2 expression as well because cotransfection of p53 and FGF-2 cDNAs into human glioblastoma or hepatocellular carcinomas revealed that the FGF-2 promoter is responsive to p53 (128). Whereas wild type p53 represses FGF-2 expression, mutant p53 enhances FGF-2 expression.

IV. Release of FGF-2

The mechanism of FGF-2 release remains one of the more intriguing questions in FGF biology. Neither FGF-1, -2, or -9 possesses a signal sequence and neither FGF-1 nor -2 is released by the classic signal sequence pathway. One

view of how FGF-2 is released from cells is through passive processes such as cell death, wounding, or chemical injury (129). Haimovitz-Friedman *et al*. (130) reported that FGF-2 can be released into the medium after irradiation. Factors such as fibrin-split products also induce FGF-2 release (131). In addition, endothelial cells exposed to polymorphonuclear leukocytes release FGF-2 by an undefined mechanism (132).

A second view is that a nonclassic release pathway exists for the release of proteins, such as FGF-2, that lack secretion signals. Mignatti *et al*. (112) demonstrated that migration of an isolated single cell expressing FGF-2 can be inhibited by FGF-2-neutralizing antibodies. Thus, a mechanism other than cell death can lead to FGF-2 release. Because FGF-2-dependent cell migration was not inhibited by drugs affecting the classic secretion pathway, FGF-2 release may be via a novel mechanism (113). Kandel *et al*. (133) demonstrated that an angiogenic "switch" occurs in fibrosarcoma progression at a point when FGF-2 begins to be released. Florkiewicz *et al*. (134) have shown that FGF-2 is exported from COS-1 cells by an energy-dependent, non-ER/Golgi pathway. COS-1 cells transfected with all four FGF-2 isoforms only released 18-kDa FGF-2. Export was not inhibited by brefeldin A. Data on FGF-1 support the view that an alternative transport mechanism is involved in release as heat shock induces release in NIH 3T3 cells transfected with a cDNA encoding FGF-1 (135). Maciag *et al*. (136) reported that mutation of cysteines abrogated the extracellular appearance of FGF-1. These data suggest that FGF-1 may be released by a mechanism involving heat shock proteins and disulfide bonds. These requirements may also be associated with FGF-2 release but need to be tested.

V. Roles of FGF-2 in Development and Differentiation in Various Organ Systems

FGF-2 has been proposed to have an important function in the development and function of numerous organ systems (Table 1). The following is a summary of recent findings emphasizing the pleiotropic role of this molecule.

A. Mesoderm induction

FGF plays a crucial role in mesoderm induction in *Xenopus* (137–147). FGF signals are required for activin-mediated mesoderm induction (141, 142), as injection of a dominant negative FGFR1 mRNA into the *Xenopus* oocyte greatly diminishes the ability of activin to induce certain markers of mesoderm formation such as Xbra or Mix1. However, other markers are less sensitive to the expression of the dominant negative FGFR, suggesting that the activin signal is partly independent of FGF activity. However, mesoderm formation in response to Xbra may require FGF signaling (143) because mesoderm induction by overexpression of Xbra is blocked by introduction of a dominant negative FGFR (143). A different approach using transgenic mice lacking expression of FGFR1 shows the involvement of the FGF signaling pathway in embryogenesis. The early growth defects and aberrant mesodermal patterning in these mutant mice suggest that FGFR1 is required during mouse embryogenesis for both proper embryonic cell proliferation and pattern formation (148, 149).

The intracellular signals required for mesoderm induction are beginning to be identified. Protein kinase C (PKC) induction alone is not sufficient for mesoderm induction (94, 144). Rather, PKC activation may be part of a negative feedback mechanism because PKC activation inhibits mesoderm induction by FGF-2 (144). Mesoderm induction by FGF probably involves the mitogen-activated protein kinase (MAPK) cascade (145). MAPK phosphatase-1 mRNA injection into oocytes leads to severe defects in gastrulation and posterior development and blocks FGF-dependent mesoderm induction. This can be overcome by simultaneous injection of wild type MAPK. These data indicate that FGF induces two types of signals: one that is dependent upon MAPK and stimulates mesoderm formation, and one that involves PKC and may shut-off FGF signaling, thus arresting the FGF effects on mesoderm induction.

Although FGF signaling participates in mesoderm induction and in the maintenance of mesoderm during gastrulation, the form(s) of FGF responsible is not known. Isaacs *et al*. (146, 147) suggested that *Xenopus*-derived embryonic FGF (eFGF) is the mesodermal inducer. Injection of eFGF or FGF-2 mRNA into oocytes revealed that eFGF is more potent than FGF-2 in mesoderm induction, perhaps because eFGF contains a signal sequence and is, therefore, more efficiently released than FGF-2. FGF-2 is as potent as eFGF in mesoderm induction when applied to embryonic caps. FGF-2 may play a role in mesoderm induction because, first, FGF-2, like eFGF,

TABLE 1. Putative functions of FGF-2 in different organ systems

Organ	Putative functions
Brain	Neuronal differentiation and survival
Blood vessel	Angiogenesis, smooth muscle cell proliferation
	Atherogenesis, blood pressure control
Lung	Branching morphogenesis, fibrosis
Limb	Limb development
Muscle	Myogenesis
Bone	Osseous healing, chondrogenesis
Hematopoiesis	Stimulation of granulopoiesis, megakaryocytopoiesis, stem cell survival
	Antiapoptotic effect
Reproductive system	Spermatogenesis
Eye	Photoreceptor survival and transduction
Skin	Melanogenesis
	Morphogenesis of the suprabasal keratinocytes
	Tissue repair

In several organ systems other members of the FGF family may play the indicated roles.

is expressed before and at the initiation of mesoderm formation; second, FGF-2 may function intracellularly; third, FGF-2 is exported via an alternative pathway that is activated at selected times during embryogenesis.

FGFR1 and R2 homologs have been cloned in *Drosophila* (150). *Drosophila* FGFR1 and R2 (DFR1 and DFR2) have three and five Ig-like domains in the extracellular region, respectively. DFR1 expression is specific to mesodermal primordium and invaginated mesodermal cells. At later stages, putative muscle precursor cells and cells in the central nervous system express DFR1. DFR2 is expressed in endodermal precursor cells, central nervous system (CNS) midline cells, and certain ectodermal cells such as those of the salivary duct and the trachea. The specific roles of DFR1 and R2 in mesoderm formation are not known.

B. Angiogenesis

FGF-2 induces endothelial cell proliferation, migration, and angiogenesis *in vitro* (for review see Ref. 1). FGF-2 regulates the expression of several molecules thought to mediate critical steps during angiogenesis. These include interstitial collagenase, urokinase type plasminogen activator (uPA), plasminogen activator inhibitor (PAI-1), uPA receptor, and β1 integrins (for reviews see Refs. 151–153). These molecules may be involved in the invasive phenotype displayed by endothelial cells during angiogenesis. Angiogenesis induced by FGF-2 also involves $\alpha v\beta 3$ integrin because antibodies directed against this integrin subtype block angiogenesis *in vitro* and *in vivo* (154). In addition, the extracellular matrix provides tensional signals to the FGF-2-activated endothelial cells to allow capillary cord formation (155).

Flamme and Risau (156) performed an interesting study to determine how endothelial and hematopoietic cell lineages emerge. They showed that FGF-2 induces differentiation of both endothelial cells and hematopoietic cells from dissociated quail epiblasts *in vitro*. In long-term cultures, the induced endothelial cells give rise to vascular structures. Based on this study, FGF-2 may be important for embryonic vascular development.

FGF-2 was initially regarded as the tumor angiogenesis factor (for review see Ref. 1). This view was challenged by the discovery of vascular endothelial cell growth factor (VEGF; for review see Ref. 157). FGF-2 is present in many tissues, tumor cells, and cell lines including rhabdomyosarcoma, fibrosarcoma, and glioma cells (1, 133, 158–160). However, FGF-2 is released by most cell types with low efficiency. Therefore, it has been proposed that other factors such as VEGF regulate tumor angiogenesis (161–163). VEGF may induce the endothelium to produce FGF-2, which may control angiogenesis as a secondary autocrine or intracrine cytokine.

Li *et al.* (164) reported that the cerebrospinal fluid of children and adults with brain tumors contains an angiogenic activity identical to FGF-2. The presence of FGF-2 in the cerebrospinal fluid correlates with the amount of tumor microvessel formation. Thus, FGF-2 may mediate angiogenesis in brain tumors and, together with microvessel quantification in biopsied tumors, provide prognostic information for the outcome of the disease. Nguyen *et al.* (165) analyzed FGF-2 levels in urine samples from 950 patients with a wide variety of solid tumors, lymphomas, or leukemias. Patients with active local cancers had intermediate FGF-2 levels, whereas patients with active metastatic cancer had high FGF-2 levels. This indicates that urine levels of FGF-2 may be of significance in monitoring cancer patients.

Tumor angiogenesis usually occurs in a hypoxic environment (166). This is in apparent contradiction with an inhibition of endothelial cell proliferation, migration, and FGF-2 expression observed during hypoxia. However, during hypoxia, macrophages release FGF-2, and this activity stimulates the growth of hypoxic endothelial cells (167). This may explain neovascularization in tumor angiogenesis mediated by paracrine FGF-2.

How can the action of FGF-2 in angiogenesis be modulated? We have investigated the opposing effects of TGF-β on FGF-2 activity in BAE cells (for review see Ref. 168). TGF-β inhibits FGF-2-induced cell migration and protease production. FGF-2 stimulates uPA expression, which, in turn, activates latent TGF-β. Activated TGF-β stimulates PAI-1 synthesis, which inhibits uPA, shutting down subsequent TGF-β formation. This creates a loop regulating both TGF-β activation and FGF-2 activity. In addition, TGF-β is a biphasic regulator of FGF-2-induced angiogenesis (169, 170). At low concentrations, TGF-β stimulates FGF-2 action *in vitro*, whereas, at high concentrations, it inhibits FGF-2 action *in vitro*.

Several inhibitors of angiogenesis have been described. These include heparin (171), heparinase (171, 172), platelet factor-4 (173, 174), suramin (175, 176), angiostatic steroids (177), thalidomide (178), and angiostatin (179). Among the heparinases, only heparinase I and III inhibit FGF-2-induced angiogenesis *in vitro* and *in vivo* (172). Tissue inhibitor of metalloproteinase-2 inhibits FGF-2-induced human microvascular endothelial cell proliferation (180). Interferon-α and -β down-regulate FGF-2 expression in human renal, bladder, prostate, colon, and breast carcinomas (181). This might account for the benefit observed after interferon treatment in these vascularized neoplasms. However, interferon-α and interleukin-2, in combination, stimulate endothelial cell growth and *in vivo* angiogenesis (182).

Like FGF-2, FGF-1 stimulates angiogenesis *in vitro* and *in vivo* (183). However, FGF-1 is not significantly expressed in endothelial cells, which suggests a paracrine mechanism of action. Smooth muscle cells express FGF-1, which may play a role in the induction of neovascularization within the atherosclerotic lesion (183, 184). It is also possible that other members of the FGF family, if expressed at appropriate levels in specific sites, will be angiogenic as they can bind to the same receptors as FGF-1 and -2.

C. Vessel wall

FGF-2 stimulates smooth muscle cell proliferation (for review see Ref. 3). An elegant series of experiments on the role of FGF-2 in neointimal cell proliferation and atherogenesis were performed by Reidy and co-workers (185, 186), who demonstrated that the infusion of neutralizing antibodies to FGF-2 after balloon injury of the rat aorta inhibits neointimal cell proliferation. By *in situ* hybridization, FGF-2 mRNA was

detectable at the wound edge of the endothelial cell layer and in migrating or proliferating smooth muscle cells. Expression of FGF-2 mRNA and FGFR1 mRNA was observed in replicating endothelial and smooth muscle cells. In agreement with these results, Casscells *et al.* (187) observed that FGFRs were up-regulated in smooth muscle cells after vessel injury. Up-regulation of FGFR expression renders smooth muscle cells susceptible to the lethal effects of FGF-2 coupled to saporin. Furthermore, FGF-2 and FGFR1 mRNAs are up-regulated in human atherosclerotic arteries, and increased mRNA expression is specifically associated with neovascularization of the atheromatous lesion (188). Thus, in injured arteries, the FGF-2 ligand/receptor system may be involved in neointimal formation. In support of this hypothesis, FGF-2 was found to be released after vessel injury (189).

Brogi *et al.* (190) showed that all cells of arteries contain FGF-1 and FGF-2. However, FGF-1 mRNA was detected in only one of five control arteries tested, whereas all five atheromatous arteries contained FGF-1 mRNA. FGF-2 mRNA was expressed in both control and atheromatous arteries. Immunolocalization revealed abundant FGF-2 in control arteries but little in plaque. FGF-1 immunoreactivity was absent in control arteries but was high in atheroma-containing arteries. All arterial cells and arteries contained FGFR1. Only smooth muscle cells and control vessels had FGFR2 mRNA, although endothelial cells and some arteries contained FGFR4 mRNA. These data suggest that FGF-1, but not FGF-2, may be important in atherogenesis. However, FGF-2 may play a role in the early stages of formation of the atherosclerotic lesion, whereas FGF-1 is active at a later stage.

The fate of FGF-2 applied to the vessel wall was examined by Edelman *et al.* (191) who characterized intravenously injected ^{125}I-labeled FGF-2 *vs.* controlled perivascular released growth factor. Whereas intravenously administered FGF-2 was rapidly cleared from the circulation, FGF-2 from slow release polymers was delivered to the extravascular space without transendothelial transport for longer periods of time. The deposition of FGF-2 delivered by the slow release system was 40 times greater than by intravenous administration. Thus, an intact endothelium is not required for FGF-2 to reach the subendothelium, and this passage is not mediated by transendothelial transport. Systemic administration of FGF-2 in rats lowers the blood pressure (192). This hypotensive action is due to the induction of nitric oxide synthesis and/or ATP-sensitive potassium ion channels. Therefore, FGF-2 may play a role in the regulation of blood pressure and may be of therapeutic use in the treatment of hypertension. FGF-2 improves myocardial function in chronically ischemic porcine hearts (193). Periadventitial administration of FGF-2 in a gradual coronary occlusion model resulted in an improvement of coronary blood flow and a reduction in the infarction size. Furthermore, intracoronary injection of FGF-2 improved cardiac systolic function and reduced infarction size in a canine experimental myocardial infarct model (194).

D. Lung

Lung development is another example of branching morphogenesis. Expression of FGF-2 and FGFRs was studied in the developing rat fetal lung (195). During development, FGF-2 immunoreactivity is localized to cells of the airway epithelium, basement membranes, and extracellular matrix. FGFRs are also detectable in the airway epithelial cells, mainly in the branching areas starting from day 13. During the embryonic and pseudoglandular stages of lung development, the expression of FGFRs increases. At the saccular stage, no FGFRs are detectable. During postnatal development, FGF-2 immunoreactivity is found in the developing airway epithelium basement membrane (196). Peters *et al.* (197) showed that lung development, specifically branching, was impaired in transgenic mice when a dominant negative FGFR mRNA was targeted to the lung by use of a surfactant promoter. This receptor shows a high specificity for FGF-7; therefore, a molecule identical or related to FGF-7 must be responsible for branching morphogenesis during lung development. However, targeted disruption of the FGF-7 gene yields mice with normal lung structure (198). *Drosophila* FGFR1 is required for the migration of tracheal cells during embryogenesis (199).

The expression of a number of genes may be regulated by FGF-2 during lung development. There is a burst of elastin synthesis by interstitial fibroblasts that coincides with the period of alveolar septal elongation during lung differentiation. FGF-2 negatively regulates elastin synthesis, and the inhibition of FGF-2 activity by neutralizing anti-FGF-2 antibodies increases elastin synthesis (200). Thus, a decrease in FGF-2 expression during lung development may stimulate elastin synthesis and alveolar septal elongation. In the adult lung, FGFRs seem to be expressed at low levels because binding studies on lung tissue membrane preparations failed to detect significant levels of high affinity FGFR (201).

Fuks *et al.* (202) demonstrated that the intravenous administration of FGF-2 in C3H/HeJ mice before and after irradiation inhibited apoptosis in endothelial cells and protected the mice against lethal radiation pneumonitis. Thus, FGF-2 is an efficient radioprotector in nonhematopoietic tissue and may prevent radiation-induced pneumonitis and fibrosis.

In a model of acute intraalveolar granulation tissue formation after lung injury, FGF-2 mRNA and protein were detected in macrophages obtained after bronchioalveolar lavage (203). Tissue sections from a patient who died after lung injury showed FGF-2 immunoreactivity in numerous macrophages. In addition, FGF-2 expression can be increased in lung fibroblasts by PDGF and TGF-β (204). Similar results were reported by Henke *et al.* (205), who isolated FGF-2 from bronchoalveolar lavage fluid from patients with acute lung injury.

E. Hematopoiesis

Several groups reported that FGF-2 as well as FGF-1 stimulates hematopoiesis in *in vitro* systems and, therefore, may play a role in both normal and pathological hematopoiesis (for review see Refs. 4 and 5). Wilson *et al.* (206) showed that myelopoiesis is enhanced by FGF-2 in long-term bone marrow cultures. Low concentrations of FGF-2 (0.2–2 ng/ml) increased the number of cells from the neutrophil-granulocyte series, stromal cells, adherent hematopoietic foci, gran-

ulocyte, colony-stimulating factor-, and granulocyte macrophage-colony-stimulating factor-responsive progenitor cells. The mechanism of the FGF-2 effect on granulopoiesis is not understood. Several possibilities may be considered. First, FGF-2 may stimulate granulopoiesis by inducing the production of a secondary cytokine such as interleukin-1 (IL-1), IL-6, granulocyte macrophage colony-stimulating factor, or IL-3. Second, FGF-2 may suppress the action of growth inhibitors such as interferons or TGF-β. Third, FGF-2 may inhibit the expression of growth factor receptors as has been described for IL-1 (207). Fourth, FGF-2 may directly stimulate the growth and differentiation of stem cells or granulocytic progenitors. This is supported by the observation that the proliferation of peripheral blood stem cells is stimulated by FGF-2 (208). More recently, Gabrilove *et al.* (209) demonstrated that FGF-2 is synergistic with stem cell factor in augmenting committed myeloid progenitor cell growth.

FGF-2 is a stimulator of megakaryocytopoiesis (4, 210–213) and acts additively, if not synergistically, with IL-3. Han *et al.* (210), Bikfalvi *et al.* (211), and Avraham *et al.* (213) found this effect is mediated via IL-6, whereas Bruno *et al.* (212) found that the regulation is mediated through IL-3. The reasons for these differences are probably the different experimental conditions used and the different species studied.

FGFRs are detectable in bone marrow and on megakaryocytic cells. [^{125}I]FGF-2 binds to murine megakaryocytes as visualized by a single cell autoradiographic assay and is cross-linked to human erythroleukemic cells (HEL) (211). In addition, FGFR1 and FGFR2 mRNA is detectable by Northern blotting in murine bone marrow and HEL cells and/or by PCR in purified megakaryocytes, platelets, megakaryocytic-like cells, T cells, B cells, and granulocytes. These findings are supported by the work of Katoh *et al.* (214) who identified FGFR2 transcripts in K562 cells and platelets. In contrast, Armstrong *et al.* (215) demonstrated the presence of only FGFR4 in megakaryocytic cell lines. These authors also cross-linked FGF-2, but not other FGFs, to K562 cells.

The effect of FGF-2 on erythroid progenitors has not yet been investigated, but results using K562 cells, which acquire some erythroid characteristics upon differentiation, indicate that FGF-2 may block differentiation. Burger *et al.* (216) showed that FGF-2 antagonizes the induction of hemoglobin synthesis by TGF-β and inhibits the expression of glycophorin A. Allouche *et al.* (217) have shown that FGF-2 exhibits an antiapoptotic effect in K562 cells differentiated with hemin or PMA.

FGF-2 is a potent mitogen for human stromal cells and delays their senescence (218). Also, macrophage-colony-stimulating factor production and release in murine bone marrow-derived stromal cells (TC 1) are stimulated by FGF-2 (219). Brunner *et al.* (220) showed that bone marrow stromal cells are a storage site for FGF-2 and that treatment of these cells with plasmin or phospholipase C (PLC) liberates FGF-2 in an active form. However, it is likely that *in vitro* endogenous phospholipase D and not PLC is responsible for FGF-2 release (221). Thus, the stromal cell layer and the matrix may act as growth factor reservoirs.

Candidate cells in the bone marrow that produce FGF-2 are the fibroblasts, the stromal layer, or the cells of hematopoietic lineages. Brunner *et al.* (222) reported that FGF-2 is expressed in platelets, megakaryocytes, and granulocytes using immunofluorescence and immunological techniques. Yet, FGF-2 is not detectable after metabolic labeling and immunoprecipitation. Thus, megakaryocytes may only be a storage site for FGF-2. However, FGF-2 mRNA has been detected in peripheral mononuclear cells, platelets, and leukemic cell lines with megakaryocytic features (223, 224). Furthermore, the leukemic cell line K562 expresses HMW FGF-2 and 18-kDa FGF-2. More data are needed to clarify the cell types involved in the expression of FGF-2 in the bone marrow.

Among other FGFs involved in hematopoiesis, only FGF-1 has been investigated to some extent. Megakaryocytopoiesis is stimulated by FGF-1 in a manner similar to that of FGF-2 and involves the same mechanism of action (4, 210, 211).

F. Nervous system

Several laboratories have analyzed the distribution and function of FGF-2 and FGF receptors in the central nervous system. However, a consensus on the distribution of FGF-2 or FGF receptors has not been reached. Although the neurotrophic role of FGF-2 is established, no other function in the nervous system has been assigned to FGF-2 with certitude. In the following we summarize the work on the distribution of FGF-2 and FGFRs during neural development, the putative functions of FGF-2 in the adult nervous system, and potential roles for FGF-2 in neuropathology.

FGF-2 has been localized in the nervous system in a variety of species (Table 2). The stage at which FGF-2 expression appears varies according to the species studied. In the chicken, FGF-2 immunoreactivity appears at stage E12 in the spinal chord and ganglia. Neuronal FGF-2 expression increases in intensity until the perinatal period and thereafter remains unchanged (225). FGF-2 immunoreactivity is localized at stage E2 in neuroepithelial cells (226). Specific staining is observed in young sensory neurons as well as in nonneuronal cells. *In situ* staining of the spinal cord and ganglionic neurons appears at stage E6 and increases until E10. This is followed by a subsequent decline in FGF-2 expression. In the rat, strong immunoreactivity is detectable between stages E16 and E17 in the cortex, the striatum, and in almost all neurons of the brain stem, spinal chord, and spinal ganglia (227). In the embryonic brain and hypothalamus, an abundant FGF-2 mRNA species of 1.8 kb is detectable at stages E13-E20, but little mitogenic activity is associated with the prenatal brain (228). In the newborn rat, FGF-2 immunoreactivity is found in neural subpopulations of brain stem nuclei, ventral spinal cord, and spinal ganglia. An earlier appearance of FGF-2 mRNA expression was reported by Nurcombe *et al.* (229), who showed that murine neuronal precursor cells express FGF-2 mRNA at stage E9.

The pattern of expression of different molecular mass forms of FGF-2 has been examined during the development of the nervous system (230). In the rat embryo, only the 18-kDa and the 21-kDa FGF-2 forms are detected. Expression of the 22-kDa form are first observed in the neonate and steadily increase to adult levels by 1 month of age.

In human adult brain, strong staining for FGF-2 is observed in central nervous system neurons and in cerebellar Purkinje

TABLE 2. Localization of FGF-2 in the developing and adult nervous system

During development	Rodent	Chicken	Bird
	Cortex Striatum Brain stem Hypothalamus Spinal chord Ganglia	Spinal chord Ganglia	Sensory neurons Spinal chord Ganglia
In the adult	**Rodent**		**Human**
	Septohippocampal Nucleus Cerebellum (Purkinje cells, deep nuclei) Hypothalamus Pituitary Facial nerve nuclei Trigeminal nuclei		Cortex (neurons) Cerebellum (Purkinje cells) Branching capillaries

cells (231). However, the most intense immunoreactivity is found in branching capillaries. In the adult rat brain FGF-2 immunoreactivity is observed in astrocytes, in selected neural populations, and occasionally in microglial cells (232). FGF-2 immunoreactive neuronal populations include septohippocampal nucleus, cerebellar Purkinje cells, cerebellar deep nuclei, facial nerve nucleus, and the motor and spinal subdivisions of the trigeminal nucleus and facial nerve nucleus (232).

In the adult brain all FGF-2 forms are found; the 18-, 21-, and 22-kDa forms in the rat and the 18-, 22-, and 24-kDa forms in the human (230). Tooyama and co-workers (233) localized HMW FGF-2 to a subpopulation of calbindin-negative mesenchephalic dopaminergic neurons using a specific HMW FGF-2 antibody. The immunolocalization of FGF-2 matches the distribution of the gap junction protein connexin 43 and is localized to gap junctions between astrocytes (234). FGF-2 may regulate intercellular communication at such junctions. FGF-2 is localized by immunohistochemistry and *in situ* hybridization to the hypothalamic pituitary system (235), where it is widely distributed in both the anterior and neural lobes of the pituitary. Immunoreactive FGF-2 is detected in basement membranes, pituicytes, and Herring bodies. In the hypothalamus, magnocellular neurons of paraventricular and supraoptic nuclei contain immunoreactive FGF-2. In the median eminence, immunoreactivity for FGF-2 is associated with fibers, glial, and endothelial cells. Ependymal and subependymal cells of the third ventricle show high levels of immunoreactivity and mRNA for FGF-2.

Different FGFR subtypes have been identified in the brain, and the role of low-affinity binding sites has been investigated. During mouse organogenesis, FGFR1, R2, and R3 are expressed in the germinal epithelium of the neural tube at day 9.5–16.5 post coitum (236, 237). However, at day 1 postpartum, FGFR3 is expressed diffusely and localized in cells with morphological characteristics of glia, whereas no expression of FGFR1 or R2 is found. FGFR1 exhibits a discrete neuronal expression pattern. In the adult mouse brain, FGFR1 is expressed in widespread, but specific, neuronal populations, whereas FGFR2 is primarly expressed in the fiber tracts suggesting that oligodendrocytes are the main site of FGFR2 expression (238). FGFR4 mRNA is expressed in the medial hubenular nucleus neurons, but not in other locations (239). Powell *et al.* (228) found that the expression of FGFR1 is temporally regulated. FGFR1 4.3-kb mRNA is high in embryonic rat brain between E13-E19. In the pituitary-hypothalamic system, the distribution of FGFR1 immunoreactivity matches that of FGF-2 immunoreactivity (235). Nurcombe *et al.* (229) reported the developmentally regulated interaction of heparan sulfates with FGFs. At stage E9, when FGF-2 is expressed, heparan sulfates bind preferentially to FGF-2. At stage 11, when mRNA for FGF-1 is first detectable, there is a switch to FGF-1 in the binding specificity of heparan sulfate. Although both FGFRs and low-affinity binding sites may undergo developmental regulation, Fayein *et al.* (240) found developmental modulation of high-affinity receptors but not of low-affinity binding sites. The reason for these differences is not understood.

Several functions for FGF-2 in the nervous system have been proposed. FGF-2 acts *in vitro* on both astroglial cells and neurons. Mature oligodendrocytes are induced to dedifferentiate and to proliferate by FGF-2, suggesting a mechanism for regeneration of the oligodendroglial lineage after demyelination (241). FGF-2 may have a trophic role in the noradrenaline (NA), adrenaline (A), and 5-Hydroxytryptamine cell groups of the rat brain (242). Indeed, FGF-2 immunoreactivity was shown in the perikarya of large numbers of NA nerve cells of the locus coeruleus, the NA cell groups C1, C2, and C3, 5-Hydroxytryptamine nerve cells, and all raphe nuclei. *In vitro* FGF-2 maintains the survival of single cultured neurons (8), stimulates neurite outgrowth of hippocampal neurons (243), promotes transmitter storage and synthesis in chromaffine cells (244), and promotes the survival of cholinergic neurons from fetal cerebrum (245). FGF-2 may play a role in regulating the generation of neurons and astrocytes in the developing CNS (246) because cells that can generate neurons and astrocytes contain protein and mRNA for FGFR1. Exogenous FGF-2 induces the proliferation of two progenitor cell types. The first gives rise to cells with only neuronal characteristics. The second gives rise to cells with neural and astrocytic characteristics. FGF-2 regulates the functional state of neuropeptide Y neurons in the brain (247) and induces a significant increase in neuropeptide Y production in these neurons. Neural precursors isolated from adult rat brain are induced to proliferate and to differentiate by FGF-2 (248). Neuronal production is optimal under conditions in which precursors are initially stimulated with FGF-2 and thereafter exposed to serum-free medium conditioned by the astrocytic cell line Ast-1. Finally, FGF-2 promotes long-term culture of

primary neurons (249). These studies implicate FGF-2 in the development of the nervous system. Although FGF-2 expression has been documented as indicated above, a role in brain development has not yet been found for FGF-2. Crossley *et al.* (250) have reported that the development of the midbrain is induced by FGF-8. FGF-8 is expressed in the isthmic region of chicken embryos and induces an ectopic midbrain when beads soaked in recombinant FGF-8 are implanted into the forebrain. Similar studies using FGF-2 would be necessary to ascribe a role for this FGF in brain development.

FGF-2 may play an important role in regeneration after injury of the CNS (251) as FGF-2 attenuates ischemic damage in mice (252). Transgenic mice that overexpress 18-kDa FGF-2 show a significantly higher number of surviving neurons after ischemia than do nontransgenic mice. Systemically administered FGF-2 in neonatal rats prevents neuronal damage after ischemia induced by unilateral ligation of the carotid artery (253). FGF-2 may participate in a cascade of neurotrophic events facilitating neuronal repair and survival (254). After an entorhinal cortex lesion, FGF-2 immunoreactivity increases in the outer molecular layer of the dentate gyrus ipsilateral to the lesion. In the lesion, there is an increase in FGF-2-producing astrocytes and in FGF-2 immunoreactivity. After transection of the fimbria-fornix, chronic infusion of FGF-2 preserves nerve growth factor receptors on neurons within the medial septal complex and prevents death of medial septal neurons. After partial transection of the fimbria, FGF-2 decreases cholinergic neuron disappearance by 25%. Thus, FGF-2 seems to protect cholinergic neurons from degeneration. In addition, a lesion of layer VIb of the rat cerebral cortex induces FGF-2 immunoreactivity and FGF-2 receptor expression (255). Furthermore, infusion of FGF-2 into a lesion in the motor-sensory cortex stimulates astrocyte proliferation (256). FGF-2 also reverses oxygen-induced cell death of cultured basal forebrain neurons (257). This effect seems to be specific as neither nerve growth factor nor insulin-like growth factor-II prevents cell death. The activity of choline acetyltransferase is also maintained when FGF-2 is present in the basal forebrain cultures. These data indicate that FGF-2 exhibits protective effects on different neural cell types and may play an important role in the regeneration after injury of the CNS.

Changes in FGF-2 levels and/or its receptors are associated with several pathologies of the nervous system including the neurodegenerative diseases, Alzheimer's, Huntington's, and Parkinson's. The senile plaques of Alzheimer's disease sequester FGF-2 (258, 259), and FGF-2 attenuates the neurodegenerative effects of β-amyloid (260). In addition, a functional relationship of β-amyloid precursor protein and FGF-2 in the neuronal cells has been suggested (261). In pyramidal and extrapyramidal cells of the hippocampus, in large cells of the medial septal nucleus, and in the horizontal limb of the diagonal band of Broca, β-amyloid precursor protein and FGF-2 colocalize. These findings support the concept that FGF-2 is stored in the plaques in a form that activates neuronal cells. In Huntington's disease, an increase in FGF-2 expression correlates with the severity of the disease (262). In Parkinson's disease, there is a loss of FGF-2 in the neurons of the substantia nigra (263).

Changes in FGF-2 or FGF receptors may be involved in the genesis of certain brain tumors. FGF-2 immunoreactivity has been shown in glioblastomas and astrocytomas (264). FGFR1 mRNA levels are significantly higher in human glioblastoma cells than in normal brain tissue (160, 265). In addition, intense immunoreactivity for FGFR1 is present in glioblastoma cells, but only low levels of FGFR1 are present in normal tissue (265). Endothelial cells of capillaries and large vessels within the tumor are devoid of FGFR1 immunoreactivity. Furthermore, antisense oligonucleotides for FGF-2 inhibit the autonomous growth of glioma cells in culture (158). Human astrocytomas undergo changes in their FGFR profile when they progress to a more malignant phenotype (266). Normal human brain and low-grade astrocytomas abundantly express FGFR2, whereas FGFR1 is barely detectable. Malignant astrocytomas, however, express FGFR1, including the alternatively spliced form of FGFR1 (FGFRIb) containing two Ig loops. This may indicate that malignant progression of astrocytomas is accompanied by a switch from FGFR2 to FGFRIb.

G. Reproductive system

FGF-2 modulates basal and LH/human choriogonadotrophin (LN/hCG)-stimulated Leydig cell function. This effect may be mediated directly through the interaction of FGF-2 with Leydig cells (267) as purified Leydig cells have FGFR. However, the role of these receptors in Leydig cell function must be established. Rat germ cells produce FGF-2 that may regulate Sertoli cell function (268). FGF-2 was isolated from germ cell-conditioned medium and stimulated transferrin expression in Sertoli cells. The sizes of the FGF-2 immunoreactive proteins were 24, 27, and 30 kDa. FGF-2 is thought to play a role in prostatic cell growth. Fast-growing prostatic tumors exhibit high FGF-2 expression and several spliced variants of FGFR (44). During malignant progression of epithelial cells derived from a rat prostate tumor, a switch occurs from expression of exon IIIb to exon IIIc in the FGFR2 gene. This switch results in the exclusive expression of FGFR2 (IIIc) isoform, which, unlike FGFR2 (IIIb) isoform, has high affinity for FGF-2 (269). Constitutive expression of FGF-2 and switched expression in FGFR2 isoforms may constitute an independent autocrine system driving prostatic tumor growth.

H. Skin

The proliferation and differentiation of normal human melanocytes are dependent on FGF-2 (270). Melanoma cells grow rapidly because of the overexpression of FGF-2 and the activation of FGF-2-dependent tyrosine kinases. However, melanocytes are normally not transformed by FGF-2 expression and, therefore, an additional factor must confer the malignant phenotype to melanoma cells. FGFR1 is expressed in normal human melanocytes and melanoma cells (271). Antisense oligonucleotides to FGFR1 inhibit the proliferation of normal human melanocytes and melanoma cells. This is not observed with FGF-2 antisense oligonucleotides and suggests a role for deregulation of the FGF-2 receptor and not of its ligand in melanoma progression.

When a transgene encoding a dominant negative FGFR was targeted to the suprabasal keratinocytes, the organization of epidermal keratinocytes was disrupted, the epidermis

was thickened, and keratin 6 was aberrantly expressed (272). This suggests that FGF-2 or a member of the FGF family is essential for the morphogenesis of suprabasal keratinocytes.

Kurita *et al.* (273) examined the localization of FGF-2 during wound healing in the skin and demonstrated that during mouse skin wound healing, the basal layer keratinocytes and hair bulbs at the wound edge are strongly stained with anti-FGF-2 antibodies. Several layers of keratinocytes are positively stained in the reepithelialized area. These findings suggest that germinative keratinocytes express FGF-2. A marked increase in extracellular FGF-2 immunoreactivity is seen in the postburn specimens, whereas in controls, primarily capillary endothelial cells are stained (274).

In healing-impaired diabetic mice, FGF-2 promotes wound healing (275–277). FGF-2 promotes wound healing in the pig partial-thickness skin excision model (278). Topically applied FGF-2 decreases the time of wound healing (279). In addition, fibroblasts seeded in an FGF-2-coated collagen I sponge matrix facilitate early dermal and epidermal wound healing (280). In wounds, FGF-2 induces a marked increase in endothelium and neovessels and an increase in wound collagenolytic activity (281). FGF-2 activity is detectable in wound fluids from both full- and partial-thickness wounds (282). FGF-2 encapsulated in red blood cell ghosts also accelerates incisional wound healing (283).

I. Eye

FGF-2 is localized in the eye, retina, lens, photoreceptors, aqueous and vitreous ocular media, and in the corneal epithelium (284, 285). During embryonic chicken development, expression of FGF-2 is first observed at low levels at day 5 in the retina and lens (284). At day 12, significant FGF-2 expression is seen in the neuroepithelial cells, amacrine cells, ganglion cells, photoreceptors, and the corneal epithelium. Human lacrimal tissue expresses FGF-2 and FGFR1 (286).

Several functions have been proposed for ocular FGF-2. FGF-2 induces retinal regeneration *in vitro* (287) and protects photoreceptors from light damage (288, 289). FGF-2 induces lens epithelial cells to proliferate, migrate, and differentiate into fiber cells (285). Lens-differentiating activity identical to FGF-2 and FGF-1 is found in the vitreous, but not in the aqueous, ocular medium (285). Robinson *et al.* (290) showed that FGF-1 transgenic mice express markers consistent with lens differentiation. FGF-2 may also participate in the transduction mechanism of the photoreceptor (291). FGF-2 stimulates photoreceptor differentiation in newborn rat retinal cells, increasing the expression of opsin (292). In addition, Goreau *et al.* (293) showed that FGF-2 induces nitric oxide synthase in retinal pigmented epithelial cells. Thus, FGF-2 may participate in photoreceptor transduction, in part, by the regulation of nitric oxide production. The proliferation of corneal endothelium is synergistically stimulated by FGF-2 and corneal endothelium modulation factor (294). A synergistic inhibition of collagen IV synthesis and stimulation of FGF-2 expression with FGF-2 and corneal endothelium modulation factor has been described. Thus, endogenous FGF-2 may play a role in the growth of corneal endothelial cells.

What role may FGF-2 have in ocular pathology? The retinal pigmented epithelium is the site of the primary lesion in inherited retinal dystrophy in rats (295), a model for retinitis pigmentosa. The failure to produce trophic factors may promote photoreceptor cell death. However, when the expression of FGF-1 or FGF-2 is analyzed, no change in comparison to control is found (296). Nevertheless, retinal pigmented epithelium from rats with retinal dystrophy exhibit low FGFR numbers mainly in FGFR2 (296). The reason for the reduction in FGFR in rats with retinal dystrophy is not known.

FGF-2 accelerates healing in laser-injured retinas of New Zealand red rabbits (297). The healing of experimentally induced corneal injuries is stimulated by FGF-2 (298–303). Deepithelialized rabbit corneas heal significantly faster in the presence of FGF-2 (303). Wound healing of serum-deprived kitten corneal endothelial cells is promoted by FGF-2 but not by insulin-like growth factor (304).

FGF-7 may also have a role in skin development and differentiation as ablation of the FGF-7 gene yields mice with abnormal hair (198). This result is consistent with the original observation that FGF-7 is a growth factor for keratinocytes (305).

J. Muscle and skeleton

FGF-2 may be involved in skeletal muscle growth and differentiation. Templeton and Hauschka (306) showed that both growth and differentiation are controlled by the interaction of FGF-2 with FGFR1. FGF-2 is an inhibitor of skeletal muscle differentiation and operates by activating signaling pathways independent of PDGF-signaling pathways (307). In particular, stimulation of MAPK kinase, junB, or c-*fos* expression is not sufficient to repress skeletal muscle differentiation. In addition, heparan sulfates are required for the induction of myogenic signals by FGF-2 (68–70).

Developmental studies indicate that FGF may play an important role in muscle development. Disruption of FGF signaling by expression of a dominant negative FGFR2 results in gastrulation defects that are reflected in the lack of formation of the notocord and muscle (308). Even in embryos that show mild defects, muscle formation is impaired. The dominant negative receptor inhibits the expression of the early gene Xbra throughout the marginal zone, including the dorsal side. These data demonstrate that FGFs are involved in the earliest events of mesoderm induction. FGF-2, as well as several other FGF family members such as FGF-4 and -8, stimulates limb development (10, 11, 309–313). Cohn *et al.* (312) demonstrated that beads releasing FGF-2 induce complete limb formation. It is, however, unlikely that FGF-2 is the prime candidate for limb formation as its expression pattern does not correlate with the temporo-spatial events occurring during limb generation. FGF-4 and -8 seem to be better candidates for endogenous limb-forming molecules (312, 313).

The proliferation and differentiation of osteoblasts are stimulated by FGF-2 (314). Bovine bone cells in culture synthesize FGF-2 and store it in the extracellular matrix (315). In addition, FGF-2 induces TGF-β production in osteoblasts. This may reinforce the action of FGF-2 (316). FGF-2 enhances osseous healing in bone previously exposed to high doses of irradiation (317). *In vivo* overexpression of FGF-2 in transgenic mice results in shortening of the limbs consistent with FGF-2 inhibiting bone growth (318). Targeted disruption of

the FGFR3 gene yields mice with enhanced bone growth, suggesting that FGFR3 also negatively regulates bone growth (319, 320).

FGF-2 possibly plays a role in the genesis of muscular disorders. The absence of dystrophin in skeletal muscle reduces the plasma membrane stability and may promote FGF-2 release. Released FGF-2 may be responsible for several of the abnormalities associated with muscular dystrophy, including suppression of muscular skeletal differentiation and excessive fibrosis. Indeed, MDX mice, which serve as a model of Duchenne's myopathy, display extracellular FGF-2 surrounding myofibers compared with normal mice (321). In addition, plasma levels of FGF-2 are elevated in many muscular dystrophy patients but are undetectable in control patients (322).

K. Digestive system

FGF-2 stimulates proliferation of several intestinal epithelial cell lines (323). Thirteen human esophageal cancer cell lines were shown to contain FGF-2 mRNA and FGFR1/N-SAM (324). This suggests that FGF-2 may play an autocrine role in esophageal cancer, whereas FGF-7 may act as a paracrine mediator.

FGF-2 accelerates the healing of experimental duodenal ulcers in rats (325). FGF-2 administered orally twice daily to rats with chronic duodenal ulcers resulted in a significant acceleration of healing (83% reduction in the size of the main ulcer area and 62% complete healings). FGF-2 was more potent than cimetidine under these experimental conditions. In addition, FGF-2 is present in the human gastric or duodenal mucosa and in the bed of chronic ulcers in rats (326). Sucralfate binds to FGF-2, protects it from degradation, and elevates FGF-2 levels in the ulcer bed. This may explain the clinical utility of sucralfate. These results also indicate that FGF-2, especially the acid-stable form, is of potential use in the therapy of ulcers.

VI. Conclusions

In this article we have summarized recent information on the role of FGF-2 in a number of biological systems. What are some general conclusions we can derive from these studies on FGF-2?

At a basic functional level, there is evidence to suggest that the different molecular weight forms of FGF-2 have distinct functions. The 18-kDa form promotes cell migration and mitogenesis, whereas HMW FGF-2 controls cell growth. At the level of organs, the role of FGF-2 has been extended to the hematopoietic system, where it may have an important function. FGF-2 has not been confirmed as a regulator of physiological or pathological angiogenesis because of possible questions as to its mechanism of release. Two mechanisms may be considered. At the onset of the angiogenic switch, cells may release FGF-2, which stimulates neovascularization in a paracrine manner. Alternatively, paracrine factors such as VEGF may stimulate FGF-2 production and, subsequently, FGF-2-dependent autocrine activity. FGF-2 was identified as an important neurotrophic factor. In addition, neural activity regulates FGF-2 expression (327). In the skin, FGF-2 may contribute to melanogenesis and to the morphogenesis of suprabasal keratinocytes. In the eye, FGF-2 may be important in photoreceptor survival and may participate in photoreceptor signal transduction. The importance of heparan sulfates in FGF signaling has been emphasized, but the controversy over the extent of the involvement of heparan sulfates in FGF signaling awaits resolution. The substrates of the FGFR are beginning to be identified, and rapid progress should be made in the near future.

Second, FGFs have been identified as major mesodermal inducers and as the prime candidates for the chick limb bud apical ridge growth signal. Although FGF-2 may not play the principal role, in concert with other FGFs, it may provide signals required for mesoderm induction or maintenance and for limb bud formation.

Third, several groups have linked a deregulation of FGF-2 or FGFR to the genesis of several disease states. For example, neointimal proliferation at the initial phase of the arteriosclerotic lesion is closely linked to a deregulation of FGF-2 expression. In addition, genetic defects in FGFR1, R2, or R3 that are linked to Pfeiffer syndrome (328–331), Crouzon syndrome (332–334), Jackson-Weiss syndrome (335), Apert syndrome (336), achondroplasia (337, 338), hypoachondroplasia (339), and thanatophoric dwarfism (340) provide the first examples of FGFR abnormalities in the genesis of human diseases.

Fourth, FGF-2, or modified FGF-2s, may serve as potential therapeutic agents. For example, in coronary stenosis or myocardial infarction FGF-2 infusion improves the collateral circulation and myocardial function. In addition, FGF-2 improves the healing of duodenal ulcers and dramatically accelerates wound healing in several experimental models such as ocular or dermal wound healing. It is expected that several therapeutic applications will result from these preclinical experimental studies.

FGF-2 was initially studied by vascular biologists in an effort to understand angiogenesis, but with the realization that FGF-2 is a pleiotropic molecule, its importance in other fields, such as neurobiology, is now being appreciated.

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Although the authors have attempted to include all significant articles in this review, it is unavoidable that a certain number of articles have not been referenced. The authors apologize to those investigators whose work is not cited in this review.

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