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PROSPECTS

Basic Fibroblast Growth Factor (FGF-2): The High Molecular Weight Forms Come of Age

Pey-Jen Yu,[1] Giovanni Ferrari,[1] Aubrey C. Galloway,[1] Paolo Mignatti,[1,2] and Giuseppe Pintucci[1]*

[1]The Seymour Cohn Cardiovascular Research Laboratory, Department of Cardiothoracic Surgery, New York University School of Medicine, New York, New York
[2]Department of Cell Biology, New York University School of Medicine, New York, New York

Abstract After over thirty years from its discovery, research on basic fibroblast growth factor (FGF-2) keeps revealing new aspects of the complexity of its gene expression as it evolved in the eukaryotic organisms. The discovery of multiple forms of FGF-2 generated by alternative translation from AUG and non-canonical CUG codons on the same mRNA transcript has led to the characterization of a low molecular weight (LMW) FGF-2 form and various high molecular weight (HMW) forms (four in humans). In this review, we discuss the biochemical features and biological activities of the different FGF-2 forms. In particular, we focus on the properties that are unique to the HMW forms and its biological functions. J. Cell. Biochem. 100: 1100–1108, 2007. © 2006 Wiley-Liss, Inc.

Key words: fibroblast growth factor; HMW FGF-2; alternative translation; arginine methylation; nuclear growth factors

Basic fibroblast growth factor, the prototypic member of a family of 22 proteins, was first purified as a heparin-binding polypeptide from bovine pituitary and subsequently characterized as a basic protein of 18 kDa [Abraham et al., 1986]. Later identified as fibroblast growth factor-2 (FGF-2), it is a ubiquitous growth factor conserved throughout the eukaryotic world. As implied by its name, FGF-2 is a strong promoter of fibroblast proliferation; however, most of the interest in FGF-2 stems from the pleiotropic effects of its gene products. FGF-2 is one of the most potent angiogenic factors, possesses neuro-protective properties, and is implicated in vascular remodeling and tumor metastasis [Bikfalvi et al., 1997]. The analysis of the human FGF-2 cDNA sequence upstream of the 5′ AUG revealed the existence of at least three (two in rodents) additional CUG initiation codons located on the same mRNA. These alternative initiation codons originate 22, 22.5, and 24 kDa (20.5 and 21 kDa in rodents) additional forms of FGF-2, collectively referred to as high molecular weight (HMW) FGF-2, as opposed to low molecular weight (LMW, 18 kDa) FGF-2. An additional 34 kDa (~30 kDa in rodents) form induced under conditions of cellular stress is generally poorly translated [Delrieu, 2000]. HMW FGF-2 forms, therefore, are colinear extensions of 18 kDa FGF-2 that contain the full LMW FGF-2 sequence. Similar to other proteins generated by alternative initiation codons on the same mRNA [Touriol et al., 2003], the various FGF-2 forms are differentially distributed in the cell and their subcellular localization controls their biological activity [Sorensen et al., 2006] (Fig. 1).

In this review, we will discuss the differences in the generation, processing and localization of the FGF-2 forms. We will also focus on the unique biological functions of HMW FGF-2 and its role in human physiology and pathology.

MECHANISMS OF TRANSLATION

Alternative splicing of FGF-2 mRNA generates FGF-2 diversity in chickens. However, despite the presence of two introns within the gene [Sorensen et al., 2006], no splice variants of

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*Correspondence to: Giuseppe Pintucci, PhD, The Seymour Cohn Cardiovascular Research Laboratory, New York University School of Medicine, 530 First Avenue, NB-15W16, New York, NY 10016.
E-mail: pintug01@med.nyu.edu

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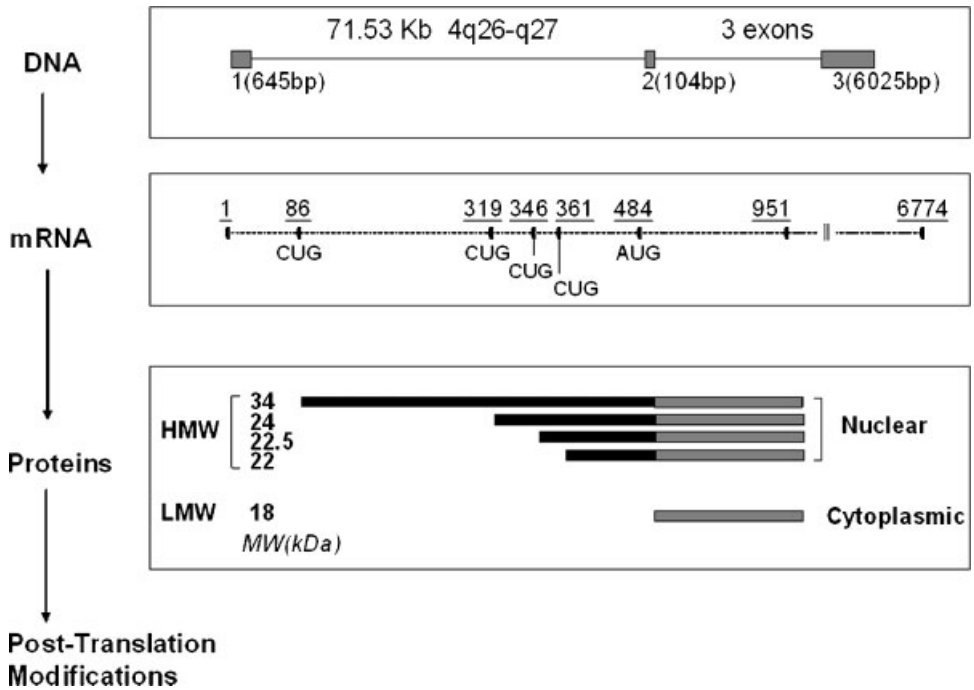

Fig. 1. Schematic representation of the multiple levels of regulation of FGF-2 expression. The human *FGF-2* gene, a 71.53 kb sequence located on chromosome 4, contains three exons (gray boxes) that lead to transcription of a unique mRNA. Alternative translation initiation from a canonical AUG codon (484) or from four CUG codons (86, 319, 346, and 361) on the same mRNA generates five different forms. LMW FGF-2 (18 kDa, gray rectangle) is mostly cytoplasmic and is released from cells despite lacking a signal peptide sequence. The HMW forms of FGF-2 (34, 24, 22.5, and 22 kDa) all contain LMW FGF-2 and can also be released from cells although they are predominantly nuclear due to their N-terminal extensions. Thirty-four kiloDaltons FGF-2 is generally poorly translated. Post-translational modifications, including arginine methylation specific for the HMW forms and proteolytic processing of both HMW and LMW FGF-2, add further complexity to the many levels of regulation of *FGF-2* gene expression.

FGF-2 have been described in mammals, in which various forms of FGF-2 are generated by alternative translation initiation via two very different mechanisms. The synthesis of 18 kDa FGF-2 is the result of ribosome binding to the 5′-end capped structure of the mRNA and subsequent scanning of the mRNA to initiate protein synthesis from the most favorable AUG (methionine) codon [Sorensen et al., 2006]. Conversely, the HMW forms of human FGF-2 are generated by an internal ribosome entry site (IRES) mechanism that allows their synthesis starting from alternative CUG (leucine) initiation codons whose recognition is facilitated by the formation of hairpin-loop structures within the mRNA [Vagner et al., 1995]. Interestingly, unlike the other HMW FGF-2 forms, the 34 kDa form of FGF-2 is synthesized by a cap-dependent and IRES-independent mechanism [Delrieu, 2000].

Although the translational mechanism for the generation of the different FGF-2 forms has been elucidated, the regulatory mechanisms for their expression remain elusive. The relative ratios and cellular distribution of the different FGF-2 forms vary according to the cell type and developmental stage during embryogenesis [Riese et al., 1995]. Increased translation of HMW FGF-2 is found in differentiated cells [Cowan et al., 2003] and varies with different cellular conditions and stimuli (Table I). Expression of HMW FGF-2 decreases in a cell-density-dependent manner in normal human skin fibroblasts (HSFs), but is retained constitutively in SK-HEP-1 cells and HSFs transformed by SV40 large T antigen [Galy et al., 1999]. In addition, conditions like stress, or exposure to cytokines, catecholamines or estrogens alter the balance of FGF-2 expression in favor of the HMW forms [Stachowiak et al., 1994; Delrieu, 2000; Garmy-Susini et al., 2004]. Using a rat osteoblastic cell line, Sabbieti et al. [2005] showed that prostaglandin F2α upregulates HMW FGF-2 expression through a mechanism mediated by extracellular signal-regulated kinase (ERK)-2 expression. Similarly, we have recently found that PDGF-BB induces rat vascular smooth muscle cell

TABLE I. Factors that Control the Expression of HMW FGF-2

Factor	Effect on HMW	Cells	Reference
Cell density	↓	Human fibroblasts	Galy et al., 1999
Transformation, stress (heat shock, oxidative)	↑	Human fibroblasts	Delrieu, 2000
Cytokines (IL-1β, TNF-α, EGF)	↑	Rat astrocytes	Delrieu, 2000
Aging	↑	Rat osteoblasts	Cowan et al., 2003
Activation of cholinergic receptors	↑	Bovine adrenal medullary cells	Stachowiak et al., 1994
17-β-Estradiol	↑	Rat microvascular endothelial cells	Garmy-Susini et al., 2004
Prostaglandin F2α	↑	Rat osteoblastic cell line	Sabbieti et al., 2005
PDGF-BB	↑	Rat aortic smooth muscle cell	Pintucci et al., 2005

expression and nuclear accumulation of HMW FGF-2 through sustained activation of ERK-1/2 [Pintucci et al., 2005].

LOCALIZATION OF FGF-2 FORMS

All *FGF-2* gene products lack a classical signal peptide that directs secretion through the endoplasmic reticulum-Golgi pathway, a surprising feature for a growth factor whose biological effects mostly depend on interaction with cell membrane receptor tyrosine kinase(s) (RTK). This unexpected feature is also found in interleukin-1 beta (IL-1β), which is released from cells by unconventional mechanisms [Rubartelli et al., 1990]. LMW FGF-2 is predominantly found in the cytosol, but is also released from cells and found associated with heparan-sulfate proteoglycans in the extracellular matrix [Moscatelli, 1987]. This interaction has profound biological consequences as it mediates the formation of a ternary complex with FGF receptor(s) [Spivak-Kroizman et al., 1994]. In contrast, HMW FGF-2 forms predominantly localize to the nucleus by virtue of nuclear localization sequences (NLS) in their N-terminal extension [Bikfalvi et al., 1997; Arese et al., 1999; Delrieu, 2000]. These features have indicated a link between the subcellular compartmentalization and biological functions of HMW and LMW FGF-2. However, LMW FGF-2 also becomes associated with the nucleus, after it is internalized following binding to the cell membrane receptors [Delrieu, 2000]. Recently, a nuclear 16 kDa FGF-2 form derived from LMW FGF-2 has been described [Sorensen et al., 2006]. Conversely, the HMW forms are present not only in the nucleus but also in the cytosol and in the extracellular environment [Mignatti et al., 1991; Piotrowicz et al., 1997; Taverna et al., 2003]. We observed that an antibody raised against a 17-mer N-terminal peptide of HMW FGF-2 (a generous gift from Dr. Daniel Rifkin, NYU School of Medicine) precipitated HMW but not LMW FGF-2 from cells expressing either FGF-2 form. However, the same antibody co-precipitated HMW and LMW FGF2 from cells that expressed both FGF-2 forms (our unpublished results). This finding suggested that HMW and LMW FGF-2 can associate, either directly or indirectly, and that a reciprocal piggy-back mechanism may translocate each form either to the nucleus or the cytoplasm depending on factors such as their relative expression. It has therefore been tempting to speculate that HMW and LMW FGF-2 may serve different functions by controlling each other's biological activity depending on their relative concentration and/or localization [Pintucci et al., 2005; Quarto et al., 2005]. Furthermore, the observation that a number of growth factors can be targeted to the nucleus with or without their receptors has strengthened the hypothesis that products of the *FGF-2* gene may act through alternative mechanisms of action in addition to interacting with their cell membrane receptors [Stachowiak et al., 2003]. However, the role of specific receptor(s) for FGF-2 in the nucleus or in the perinuclear space remains elusive.

Cell death or injury, including sub-lethal damage, have been described as major mechanisms for FGF-2 release into the extracellular environment, and have been invoked to explain exercise-induced muscle hypertrophy as an FGF-2-dependent process. Such mechanisms of release occur in numerous instances in which tissue injury is accompanied by a raise of FGF-2 concentrations [D'Amore, 1990; Lindner and Reidy, 1991; Pintucci et al., 1999]. However, cell death or injury is not the sole mechanism controlling FGF-2 release. Mignatti et al. [1991] showed that viable, single NIH 3T3 cells overexpressing all FGF-2 forms actively release FGF-2, which promotes cell migration through an autocrine mechanism. Several reports have confirmed the presence of HMW FGF-2 forms in the extracellular matrix [Piotrowicz et al., 1997;

Taverna et al., 2003] and in our laboratory we have detected HMW FGF-2 in the conditioned medium of transfected NIH 3T3 cells or bovine capillary endothelial cells exposed to oxidative stress (our unpublished results).

HMW FGF-2 METHYLATION

In 1991, Burgess et al. [1991] provided direct evidence that the N-terminal extension of HMW FGF-2 contains characteristic RG (or RGG) motifs that are methylated on arginine residues. Such post-translational modification, mediated by specific protein arginine methyltransferases (PRMTs), was later found to direct the nuclear localization of HMW FGF-2 [Pintucci et al., 1996]. We also found that upon methionine starvation followed by a pulse-chase with radiolabeled methionine the apparent molecular weight of the HMW forms of FGF-2 increased during their nuclear accumulation. In contrast, the electrophoretic mobility of LMW FGF-2 remained unchanged. The observed mass shift of HMW FGF-2 was abolished by general inhibitors of methyltransferases [Pintucci et al., 1996], indicating that methylation affects the electrophoretic properties of HMW FGF-2. It was later shown that 24 kDa FGF-2 contains eight methylated arginines whereas 22.5 and 22 kDa FGF-2 contain only five [Klein et al., 2000] (Fig. 2).

RG or RGG domains are found in a variety of nuclear and nucleolar proteins and represent a consensus sequence for arginine methylation [Bedford and Richard, 2005]. Although the functional significance of arginine methylation of HMW FGF-2 has not been fully elucidated, the discovery of its role in the nuclear accumulation of HMW FGF-2 opened a new way to look at both *FGF-2* gene expression and the biological role of this specific post-translational modification. Several reports have subsequently confirmed that the intracellular localization of a number of eukaryotic proteins is controlled by similar mechanisms involving arginine methylation [Bedford and Richard, 2005].

BIOLOGICAL FUNCTIONS OF THE *FGF-2* GENE PRODUCTS

In Vitro Studies

In addition to exhibiting different subcellular localizations, the FGF-2 forms also exhibit different biological functions. The use of cDNA microarray analysis of transfected NIH 3T3 cells has shown that endogenous overexpression of either human HMW or LMW FGF-2 induces expression of different genes, a finding that provides insight into the generation of unique phenotypes by the different FGF-2 forms [Quarto et al., 2005]. Selective expression of LMW FGF-2 promotes cell transformation, proliferation, and migration by autocrine and paracrine mechanisms that depend on specific cell membrane receptor(s). In contrast, HMW FGF-2 induces NIH 3T3 cell transformation through a receptor-independent mechanism, promotes the formation of multinucleate giant cells, and does not induce cell migration [Quarto et al., 1991; Bikfalvi et al., 1995]. The transforming activity of HMW FGF-2 has been confirmed in different experimental models [Bikfalvi et al., 1997; Delrieu, 2000; Sorensen et al., 2006]. While the expression of both LMW and HMW FGF-2 forms results in cell transformation as measured by enhanced saturation

Fig. 2. Amino acid sequence of the human FGF-2 N-terminal extension. The leucine (L) residues initiating 34, 24, 22.5, and 22 kDa FGF-2 and the methionine (M) residues initiating 18 kDa FGF-2 are bold typed. The eight arginine residues undergoing methylation are both bold typed and underlined.

density and growth in soft agar, only HMW FGF-2 induces cell growth in low serum [Bikfalvi et al., 1995]. Interestingly, when targeted to the nucleus by fusion to a canonical NLS, LMW induces cell growth in low serum as well as HMW FGF-2 [Arese et al., 1999], suggesting that the nuclear activity of HMW FGF-2 is mediated, at least in part, by the amino acid sequence it shares with 18 kDa FGF-2. However, while exogenous HMW FGF-2 induces plasminogen activator expression and cell growth in endothelial cells with an effect similar to LMW FGF-2, it inhibits cell migration in both endothelial and MCF-7 breast carcinoma cells [Levin et al., 2004]. In particular, a peptide encompassing the 55 amino-terminal end (ATE) of 24 kDa HMW FGF-2 plus the first 31 amino acids of 18 kDa FGF-2 (ATE + 31) was shown to inhibit cell migration without interfering with the binding of 24 kDa FGF-2 to FGF receptor(s). This finding indicates that LMW FGF-2 controls cell proliferation and migration through the cell membrane interaction with FGF receptors, whereas HMW FGF-2 exerts its antimigratory activity through a different unknown mechanism. Various reports have also cautioned that the effects of HMW FGF-2 may depend on its endogenous levels of expression, as HMW FGF-2 has also been shown to signal growth arrest [Quarto et al., 1991, 2005]. Constitutive expression of HMW FGF-2 has also been shown to induce radio-resistance in HeLa cells [Delrieu, 2000] and drug resistance in the rat-1 cell line [Dini et al., 2002].

In Vivo Studies and Patho-physiological Roles

Not surprisingly for a ubiquitous growth factor, FGF-2 has been implicated in a variety of physiological and pathological processes including angiogenesis, tumor growth, vascular remodeling, and stenosis following injury, cardiac hypertrophy, neuronal regeneration, bone development, and remodeling (Table II).

Angiogenesis. FGF-2 is one of the most potent inducers of angiogenesis [Presta et al., 2005]. This important feature has implicated FGF-2 in diverse areas of medicine including cardiovascular medicine, oncology, and wound healing. Administration of FGF-2 into the coronary arteries has been shown to be beneficial to patients with myocardial infarction, although these effects have been deemed as transient [Simons and Ware, 2003]. FGF-2 has also been implicated in controlling vascular tone [Dono et al., 1998; Zhou et al., 1998], in mediating cardiac hypertrophy [Kardami et al., 2004] and promoting the mobilization and differentiation of myocardial precursor cells [Rosenblatt-Velin et al., 2005]. Conversely,

TABLE II. Biological Roles of HMW and LMW FGF-2

Process	FGF form	Effect	Reference
Angiogenesis	LMW and HMW	• Potent inducers of angiogenesis	Presta et al., 2005
		• Control of wound healing	Ortega et al., 1998
Vascular remodeling	LMW and HMW	• Development of intimal hyperplasia	Lindner and Reidy, 1991 Nguyen et al., 1994
Cardiac hypertrophy	LMW and HMW	• Increases cardiomyocyte proliferation	Kardami et al., 2004
		• Increases cardiomyocyte hypertrophy	
	HMW	• Induces cardiomyocyte binucleation	
Neuronal regeneration	LMW and HMW	• Stimulate mitosis and differentiation of neuronal precursors and glial cells	Grothe et al., 2006
	HMW	• Mediates neurotrophic effects and neurite outgrowth following injuries	
		• Promotes axons myelination	
		• Strongly enhances reinnervation and functional recovery in a Parkinson's disease model	Timmer et al., 2004
	LMW	• Inhibitory effect on myelination of regenerating axons	Grothe et al., 2006
		• Reduced effect in a Parkinson's disease model	Timmer et al., 2004
Bone development and remodeling	LMW and HMW	• Pfeiffer syndrome (FGF-R1 mutation)	Marie et al., 2005
		• Crouzon syndrome (FGF-R2)	
		• Jackson–Weiss syndrome (FGF-R2)	
		• Achondroplasia (FGF-R3)	
		• Thanatophoric dysplasia (FGF-R3)	
Tumor growth	HMW	• Correlates with a poorer prognosis in neural tumor, pituitary adenomas, and prostatic and pancreatic cancers	Yamanaka et al., 1993; Fukui et al., 2003; Polnaszek et al., 2003

inhibition of angiogenesis through FGF-2 inhibition has been sought for limiting tumor metastases, as tumor growth beyond a 2-mm size depends on formation of a new capillary network from pre-existing blood vessels and circulating endothelial progenitor cells [Presta et al., 2005]. The role of FGF-2 in angiogenesis underscores its importance in wound healing. Besides a defective vascular tone, FGF-2 −/− mice also display a mild phenotype characterized by abnormal cytoarchitectural features of the frontal cortex [Dono et al., 1998; Ortega et al., 1998]. However, one of the most interesting features of these mice is that they display a significant delay of excisional wound healing [Ortega et al., 1998]. We showed that neutralizing antibody to FGF-2 blocks both MAPK activation and cell migration following wounding of cultured endothelial cells [Pintucci et al., 1999]. Both MAPK activation and cell migration secondary to mechanical injury are defective in endothelial cells from FGF-2 −/− mice [Pintucci et al., 2002], suggesting that FGF-2 plays a critical role in angiogenesis by controlling endothelial cell proliferation and migration at sites of wound healing.

Tumor growth. Overexpression of HMW FGF-2 correlates with a poor prognosis in various human cancers including neural tumors, pituitary adenomas, and prostatic and pancreatic cancers. A redistribution of the intracellular localization of FGF-2 is a feature of a number of oncogenic cells. The subcellular localization of FGF-2 is different in quiescent versus proliferating astrocytes. In normal astrocytes, FGF-2 is predominantly located in the nucleus of subconfluent proliferating cells, but is found in the cytoplasm of contact-inhibited cells. In contrast, FGF-2 is constitutively found in the nucleus of glioma cells [Delrieu, 2000]. High expression of HMW FGF-2 is associated with reduced patient survival in human astrocytic tumors [Fukui et al., 2003]. Increased FGF-2 mRNA levels have also been found in pancreatic cancer and its expression correlates with advanced tumor stage and shorter patient survival [Yamanaka et al., 1993]. FGF-2 is also expressed in prostate cancer. In particular, while poorly differentiated tumors express all forms of FGF-2, differentiated tumors appear to preferentially express HMW rather than LMW FGF-2 [Polnaszek et al., 2003].

Vascular remodeling and stenosis. FGF-2 and platelet-derived growth factors (PDGFs) play a key role in the development of intimal hyperplasia, the major cause of long-term failure of blood conduits following vascular interventions such as percutaneous transluminal coronary angioplasty or coronary artery by-pass grafting. Antibodies against FGF-2 or antisense cDNA-mediated downregulation of FGF-2 expression inhibit the development of neointimal hyperplasia in rat and rabbit models of arterial injury [Lindner and Reidy, 1991; Nguyen et al., 1994; Hanna et al., 1997]. Both strategies, however, did not clarify which form of FGF-2 promotes intimal hyperplasia, although results obtained with antibodies against 18 kDa FGF-2 implicate this domain as responsible for this process.

Cardiac hypertrophy. Work performed with animal models has implicated FGF-2 in the development of overload- and angiotensin-induced cardiac hypertrophy [Kardami et al., 2004], with increasing evidence for a specific role of HMW FGF-2. Overexpression of either HMW or LMW FGF-2 causes myocardiocyte proliferation, whereas HMW FGF-2 alone induces binucleation. Furthermore, exogenous recombinant HMW FGF-2, but not LMW FGF-2, has been shown to induce a 40% increase in the size of neonatal myocytes in culture [Kardami et al., 2004]. The role of HMW FGF-2 in cardiac hypertrophy has also been demonstrated in in vivo models. Transient increases in HMW FGF-2 have been shown in rat hearts after isoproterenol-induced cardiac injury [Kardami et al., 2004]. Intracardiac administration of HMW FGF-2 to the ischemic left ventricle during the development of myocardial infarction results in heart and cardiomyocyte hypertrophy leading to a 40% increase in left ventricle to body weight ratio 6–8 weeks after the insult [Kardami et al., 2004].

Role in the nervous system. FGF-2 plays a vital role in nerve regeneration following injury [Grothe et al., 2006]. It is expressed in glial cells and in distinct neuronal populations of the central nervous system (CNS), where it stimulates mitosis and differentiation of neuronal precursors and glial cells. HMW FGF-2 induces neurotrophic effects and neurite outgrowth following injury. In the peripheral nervous system FGF-2 is constitutively expressed in the dorsal root ganglia and peripheral nerves. Peripheral nerve injury selectively upregulates the expression of different FGF-2 forms in the dorsal root ganglia and proximal

and distal nerve stumps [Grothe et al., 2006]. In an animal model of peripheral nerve repair across long gaps, the use of grafted, genetically modified Schwann cells overexpressing either LMW or HMW FGF-2 showed distinct effects of the different FGF-2 forms. HMW FGF-2 was found to promote long-distance myelination of regenerating axons and early recovery of sensory functions. In contrast, LMW FGF-2 had an inhibitory effect on the grade of myelination of regenerating axons [Grothe et al., 2006].

Differential expression of FGF-2 forms may also play a role in Parkinson's disease. In a rat model of Parkinson's disease, dopaminergic neurons co-transplanted with Schwann cells overexpressing HMW FGF-2 induced enhanced reinnervation, survival and functional recovery relative to rats co-transplanted with LMW FGF-2 overexpressing cells [Timmer et al., 2004].

Bone development and remodeling. Defective FGF signaling has been implicated in a number of human dysmorphic syndromes in which bone is the primary target tissue. FGFR-1 mutations cause Pfeiffer syndrome, FGFR-2 mutations cause Crouzon and Jackson–Weiss syndromes, while mutations of FGFR-3 cause achondroplasia and thanatophoric dysplasia [Marie et al., 2005]. Recently, FGF-2 has been implicated in the control of chondrocyte differentiation and bone remodeling [Marie et al., 2005]. The observation that FGF-2 overexpression in transgenic mice causes shortening of long bones is in line with the role of FGF signaling in the dysmorphic syndromes described above [Coffin et al., 1995]. Consistent with these findings, juvenile osteoblasts from rat calvaria proliferate at a higher rate than adult osteoblasts, which show higher expression of FGF-2, particularly the 22 kDa form [Cowan et al., 2003]. This observation indicates a growth arrest-inducing role of HMW FGF-2 that could be strictly cell type-dependent.

CONCLUDING REMARKS

More than three decades after its identification, research on *FGF-2* gene expression and its control mechanisms is still providing new insights into the multiple roles of FGF-2 in physiology and pathology. The discovery of different FGF-2 forms generated by alternative translation initiation of a single mRNA has unveiled a further level of complexity to the biology of FGF-2. Here we have emphasized a potential dualism between LMW and HMW FGF-2 because the biological significance of their co-existence has been clarified only in part. Both FGF-2 forms can exert similar effects on many cell types. However, the identification of opposing role(s) of HMW and LMW FGF-2, such as their different effects on cell migration, and of the factors that affect their relative expression have generated new insights into our understanding of the biology of the *FGF-2* gene products.

Like other growth factors, FGF-2 acts predominantly through the activation of specific cell surface receptor(s). The finding that HMW and LMW FGF-2 and its receptor(s) localize within or close to the nucleus may uncover novel mechanisms of action of FGF-2. The discovery that HMW FGF-2 can exert its effects in a cell membrane receptor-independent manner is also a noteworthy addition to the many mechanisms of action of FGF-2. The N-terminal extension of HMW FGF-2 not only contains arginine motifs that are highly methylated but also serves as a nuclear localization signal for HMW FGF-2. The different biological activities of the FGF-2 forms are in part attributable to their different subcellular localization; however, the role(s) of the N-terminal extension of HMW FGF-2 remains unclear and its elucidation may provide insight into the mechanisms of action of the different FGF-2 forms.

Here we have presented a comprehensive view of the mechanisms that control the expression of the different FGF-2 forms, and their biological implications. In particular, we have focused on the unique features of HMW FGF-2 because these forms remain less understood than the canonical 18 kDa FGF-2, although there is increasing evidence for their importance in physiological and pathological states. We should also note here that, as highlighted above, post-translational processing of 18 kDa FGF-2 into a 16 kDa form occurs during its internalization into the cell and the nucleus; thus, specific intracellular proteinases must be responsible for its cleavage. Whether this 16 kDa form of FGF-2 has a role of its own and/or whether it has a privileged relationship with the FGF receptor(s) found in the nuclear and perinuclear region remains to be determined. Our group has recently found that HMW FGF-2 can be proteolytically processed into an 18 kDa-like form (Yu et al., manuscript in preparation). This novel mechanism, similar to a classical

precursor-to-mature form processing, is likely to add complexity to the mechanisms that control HMW and LMW FGF-2 expression, and will prompt further investigation of the *FGF-2* gene products and their biological roles.

After over 30 years of exciting findings on this single gene and its multiple products, the research of novel aspects and mechanisms of action of FGF-2 seems far from completed. The different forms of this ubiquitous growth factor, the regulation of their expression at the translational level, and their unique post-translational modifications are a fascinating example of the various levels of complexity of gene expression in higher eukaryotes.

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