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Review Article

High molecular weight FGF2: the biology of a nuclear growth factor

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Abstract. Fibroblast growth factor 2 (FGF2) is one of the most studied growth factors to date. Most attention has been dedicated to the smallest, 18kDa FGF2 variant that is released by cells and acts through activation of cell-surface FGF-receptor tyrosine kinases. There are, however, several higher molecular weight (HMW) variants of FGF2 that rarely leave their producing cells, are retained in the nucleus and act independently of FGF-receptors (FGFR). Despite significant evidence documenting the expression and intracellular trafficking of HMW FGF2, many important questions remain about the physiological roles and mechanisms of action of HMW FGF2. In this review, we summarize the current knowledge about the biology of HMW FGF2, its role in disease and areas for future investigation.

Keywords. High molecular weight, FGF2, nuclear, signalling, Api5, SMN.

The biogenesis of HMW FGF2

FGF2 is a growth factor that exists in several isoforms differing in their N-terminal extensions, subcellular distribution and function. The smallest, an 18kDa FGF2 low molecular weight (LMW) variant is released by cells and acts through activation of cell-surface FGF-receptors, whereas the HMW (22, 22.5, 24 and 34 kDa) FGF2s localize to the nucleus and signal independently of FGFR [1, 2]. This review will focus specifically on nuclear HMW FGF2 signalling, its molecular mechanisms and its physiological and pathological function.

Regulation of FGF2 transcription and translation

The biogenesis of HMW FGF2 is complex and regulated at both transcriptional and translational levels. *FGF2* transcription yields several mRNAs that differ in the length of their large 3'-untranslated region (UTR). Analysis of five different FGF2 mRNA 3'-UTRs revealed profound differences in their stability, leading to discovery of a 122-nt long destabilizing element located upstream of the second poly(A) site as well as a translational enhancer between the fourth and eighth poly(A) sites, which not only

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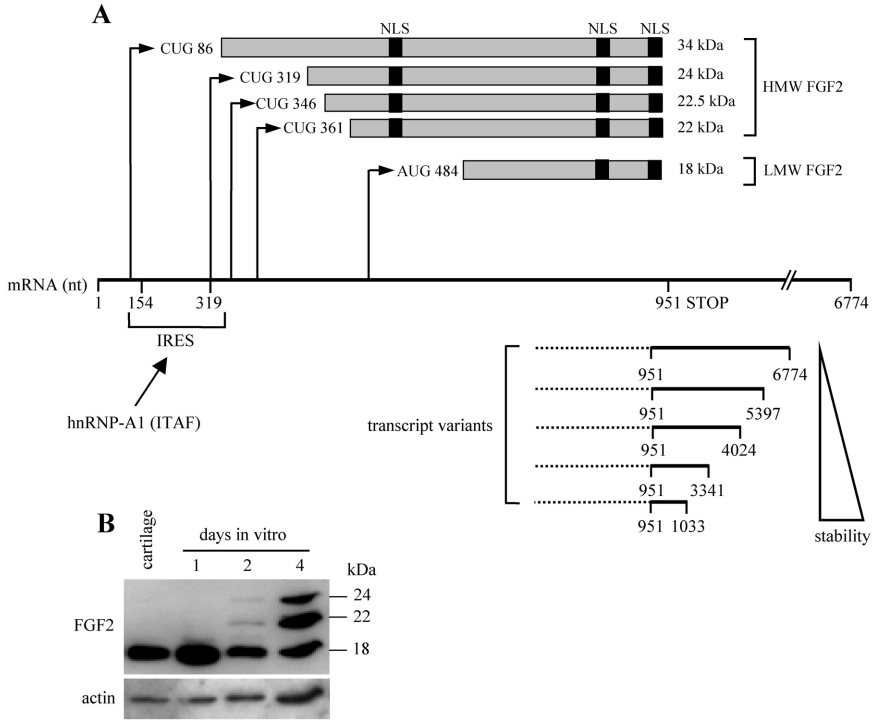

Figure 1. The biogenesis of FGF2 and its regulation. *(A)* FGF2 transcription yields up to five transcripts differing in the length of their 3‘ UTR. This UTR contains several elements regulating transcript stability, with the longest transcript being the least stable. FGF2 mRNA generates five protein variants via alternative translational initiation. Four HMW FGF2 (34, 24, 22.5 and 22 kDa) variants are initiated by the CUG codons located upstream of the LMW FGF2 (18 kDa) that is initiated by the AUG. Only the 34 kDa HMW FGF2 is translated by the conventional cap-dependent mechanism, whereas all the downstream variants require internal ribosomal entry, with the IRES located between nucleotides 154 and 319. This site is also a binding site for the heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) that represents the only IRES *trans*-acting factor (ITAF) identified so far (see section 1.1. for details). As all the FGF2 variants belong to the same ORF, the HMW FGF2 s differ from LMW FGF2 by their N-terminal extension that contains an additional nuclear localization sequence (NLS). *(B)* Although the mechanism of IRES-controlled translational initiation of FGF2 is well described, the regulation of specific initiation of 24, 22.5 and 22 kDa HMW FGF2, such as shown in human fetal chondrocytes (isolated from femoral cartilage and cultivated *in vitro* for the indicated times) [14], is not known yet. FGF2 was determined by western immunoblotting. Actin serves as the loading control. The molecular weights of FGF2 isoforms are indicated [14].

enhances the global translation of FGF2 but also selectively upregulates the translation of HMW FGF2 variants [3, 4] (Fig. 1A). The longest UTR is used by primary cells, in contrast to short UTRs preferred by transformed cells *in vitro*, allowing for precise post-transcriptional regulation of FGF2 production in primary cells in contrast to transformed cells that express FGF2 in a constitutive manner, perhaps contributing to their transformed phenotype [3, 4].

In addition to the long 3’-UTR, the 5’-UTR leader of FGF2 mRNA is also lengthy, measuring 485 nt in humans. Analysis of this sequence revealed three additional potential CUG initiation codons (positions 320, 347 and 362 nt from the 5’-end) in frame with the AUG-initiated open reading frame (ORF) (positioned 485 nt from the 5’-end) coding for 18 kDa LMW FGF2, which originally seemed to be the only bioactive variant of FGF2 [5]. The CUG initiation codons give rise to three additional high molecular weight FGF2 variants (22, 22.5 and 24 HMW FGF2) [6, 7]. Later, a fifth FGF2 variant (34 kDa HMW FGF2) was described being initiated from a CUG at position 86 from the 5’-end [8], although this variant appears poorly translated in normal conditions. Thus, a single FGF2 mRNA can give rise to a total of five protein variants through a process of alternative translation, with the HMW FGF2 s being linear N-terminal extensions of the LMW 18 kDa variant (Fig. 1A).

How is the translation of FGF2 variants regulated? According to the current model of translation, the 40S ribosomal subunit is first recruited to the 5’-end cap structure of the mRNA, followed by linear scanning of the mRNA sequence in the 3’ direction until an initiation codon in a favorable sequence context is found. This so-called cap-dependent translation has severe limitations when mRNA forms stable secondary structures between two or more initiation codons that cannot be easily linearized by eIF4A RNA helicase [9]. In the case of FGF2, its 485

nt 5'-UTR is not only unusually long but also more than 80 % GC-rich in some regions permitting the formation of a stable secondary structure that prevents ribosomal scanning [9]. Therefore, an alternative mechanism exists to allow for translation of the four FGF2 variants located downstream of the 34 kDa HMW FGF2, which is the only variant of FGF2 translated in a cap-dependent manner due to its proximity to the 5'-end of the mRNA [8]. Vagner et al. showed that 18 kDa LMW FGF2 as well as the 22, 22.5 and 24 kDa HMW variants of FGF2 are translated independently of the cap mechanism and that this process requires a sequence element located between nt 154 and 319 of the FGF2 leader, which has features of an internal ribosomal entry site (IRES) [10]. According to the IRES translational initiation model, the ribosomal 40S subunit binds FGF2 RNA internally at the IRES site located between 154 and 319 nt of the FGF2 5'-UTR to allow translation of both HMW and LMW FGF2. Further analyses revealed two additional factors contributing to the FGF2 IRES activity. First, an intramolecular G-quartet motif, located between nt 57–108, functions as a *cis*-acting positive translational element. Second, ribonucleoprotein hnRNP-A1 binds the IRES and is required for IRES-dependent FGF2 translation, functioning as an IRES *trans*-acting factor [9, 11].

As the FGF2 IRES is positioned just upstream of the four FGF2 ORFs, it is unlikely to regulate the relative translation of the different FGF2 variants [10]. The IRES alone can not fully explain profound differences in expression of FGF2 variants observed in human aortic epithelial cells, skin fibroblasts or chondrocytes that express either 18 kDa LMW FGF2 alone or all four (LMW and HMW) FGF2 variants depending on the cultivation conditions [12–14] (Fig. 1B). This could be a result of the existence of a second IRES located between HMW and LMW FGF2 initiation codons; however this option was ruled out [9]. At this time, the factors that regulate selective translation of HMW FGF2 variants are yet to be identified, although the cap-binding protein eIF-4E may play a role in this process [13, 15].

Subcellular distribution of the FGF2 variants

Growth factors are typically protein molecules that are released from a producer cell and signal via activation of their cognate receptors located at the surface of the recipient cell. Among the FGF2 variants, LMW 18 kDa FGF2 acts according to this paradigm, being released from cells through an unclear mechanism that is independent of the endoplasmic reticulum (ER)-Golgi system, involves exocytosis and requires ATP [16–18]. Although HMW FGF2 s can also be released by cells [18], these variants are mostly intracellular and accumulate in the nucleus after translation [19, 20]. The profound differences in the intracellular sorting of the FGF2 variants appear to be the major determinant of their functional diversity, with LMW FGF2 being an extracellular signalling molecule that acts via activation of transmembrane FGFR, whereas the HMW FGF2 variants serve a nuclear, FGFR-independent intracrine function [2].

In addition to HMW FGF2 s, a fraction of both exogenous and endogenous LMW FGF2 can also reach the nucleus, together with internalized cell-surface FGFR and/or complexed with the microtubule-associated translokin [21–27]. To distinguish between the nuclear signalling of HMW FGF2 and LMW FGF2, it is at first important to note the differences in their distribution within the nucleus. In Schwann cells or NIH3T3 cells, the LMW FGF2 showed highly ordered localization into the Cajal bodies and nucleoli, whereas HMW FGF2 was distributed in a punctuate pattern in the nucleoplasm and periphery of nucleoli, and co-localized with DNA and mitotic chromosomes [28, 29].

Such differences are likely to originate from different routes of translocation, i.e. translokin- and/or FGFR-dependent in case of extracellular LMW FGF2, FGFR-independent in case of cytoplasmic HMW FGF2; and/or from different nuclear localization signals (NLS) in the HMW and LMW FGF2 sequences. Analyses of FGF2 deletion mutants revealed a total of three NLSs. Two are located at the C-terminus of FGF2, between amino acids 116–129 and 147–155 of LMW FGF2 [27, 30] (Fig. 1A). In addition to the C-terminal NLSs that are present in all FGF2 variants, the N-terminal extension of HMW FGF2 contains a potent NLS as well. When the N-terminal extension was fused to beta-galactosidase, chloramphenicol acetyl-transferase or pyruvate kinase, the resulting chimeric protein also accumulated in the nucleus demonstrating that the N-terminal extension contains a NLS sufficient for nuclear translocation of HMW FGF2 [20, 31, 32]. This NLS lies within the evolutionary conserved glycine-arginine repeat motif within an N-terminal extension of HMW FGF2 [32, 33], that contains several methylated arginine residues. This methylation appears required for efficient HMW FGF2 nuclear localization [34–36].

Cell and tissue phenotypes modulated by HMW FGF2

The overlap of HMW and LMW FGF2 actions

Differential intracellular sorting determines the functional differences between LMW and HMW FGF2

rather than intrinsic differences in protein capabilities [2]. For example, in endothelial cells or cardiomyocytes, exogenously added HMW FGF2 behaved similarly to LMW FGF2 in FGFR or heparin binding, activation of ERK kinase, stimulation of rRNA synthesis, and induction of both cell proliferation and chemotactic movement [37, 38]. LMW FGF2, on the other hand, can mimic the HMW FGF2 phenotypes, such as growth in low serum conditions, when artificially targeted to the nucleus [29].

As many common *in vitro* cell models expressing HMW FGF2 also seem to export it [18, 39–41] (P. Krejci, unpublished), the exogenous signalling of HMW FGF2 (i.e. LMW FGF2-mimicking) is very likely contributing to the observed phenotypes of putative nuclear HMW FGF2 signalling. It is unclear to what extent extracellular HMW FGF2 exists naturally *in vivo*, although its release from over-expressing and/or dying cells was documented in at least one pathological condition, hairy cell leukemia [42]. In this section, we will discuss the cell- and tissue-phenotypes that appear to be regulated by HMW FGF2 nuclear signalling.

HMW FGF2 expression and cellular actions *in vitro*

HMW FGF2 expression is relatively common *in vitro*, being detected in cultured cells of bone, cartilage, endothelial, blood, neuronal, glial and liver origin [13, 14, 39, 43–47]. This ubiquitous expression may represent an adaptation to *in vitro* culture, as primary cells seem to express only LMW FGF2 in contrast to transformed cells that express both LMW and HMW FGF2 [12]. In our experiments, freshly isolated murine heart, muscle, skin, lung, spleen and kidney cells all upregulated HMW FGF2 within the first week of *in vitro* cultivation (P. Krejci and W. R. Wilcox, unpublished). Similarly, human fetal chondrocytes upregulate HMW FGF2 expression within 48 hours after transition to tissue culture [14] (Fig. 1B).

To date, the effects of HMW FGF2 have been examined in only a limited number of cell types *in vitro* (Table 1). While these cells were scored for HMW FGF2 influence on the basic cell culture characteristics such as proliferation, migration, and apoptosis, the effect of HMW FGF2 on other, more cell-type specific functions remains largely unknown. Similarly, our knowledge of the mechanism of HMW FGF2 signalling in cells is poor, with the molecular basis of the cell phenotypes mediated by HMW FGF2 being mostly undefined.

The proliferative activity of HMW FGF2 has been well documented in NIH3T3 and A31 fibroblasts, adult bovine aortic endothelial (ABAE) cells, cardiac myocytes and ROS17/2.8 osteosarcoma cells [39, 48–53]. In NIH3T3 cells, this proliferation also takes place in low-serum conditions [29, 49, 50]. In addition to proliferation, HMW FGF2 induces a phenotype of radioresistance in NIH3T3 cells as well as HeLa cervical cancer cells, which is accompanied by G2 delay and hypophosphorylation of p34cdc2 kinase in the latter cells [50, 54].

Little is known about HMW FGF2 effect on cell differentiation. HMW FGF2 appears to influence cell fate decisions in human embryonal stem cells (hESC), where its expression is downregulated upon differentiation, and its knock-down in undifferentiated cells induces differentiation [55] (P. Dvorak, unpublished). In cultured avian Schwann cell precursors isolated from the neural crest, HMW FGF2 caused trans-differentiation into melanocytes [56]. In rat calvarial osteoblasts, the expression of HMW FGF2 increased as the cell-state became more differentiated [57]. In contrast, over-expression of HMW FGF2 failed to induce differentiation in a PC12 *in vitro* cell model of neuronal differentiation [58].

HMW FGF2 expression and phenotypes *in vivo*

The *in vivo* expression pattern of HMW FGF2 is poorly defined to date, although it is more relevant than *in vitro* expression. FGF2 transcripts are found in most developing and adult human tissues including brain, heart, lung, skeletal muscle, pancreas and others [59], but limited data is available about HMW FGF2 expression in these tissues. In transgenic mice expressing bicistronic luciferase vector capable of monitoring the activity of the FGF2 IRES, IRES activity was limited to the adult brain and testis, suggesting a restricted expression of FGF2 protein *in vivo* [60]. Furthermore, the HMW FGF2 expression may be absent or restricted even in tissues that produce FGF2 protein. In human epiphyseal growth plate cartilage, HMW FGF2 is expressed only by proliferating chondrocytes in contrast to LMW FGF2 that is produced throughout the growth plate [14]. In addition to cartilage, HMW FGF2 was found in heart, aorta, testis, lung, brain, peripheral nerves, adrenal gland and eye tissue [61–67].

Several studies addressing the function of HMW FGF2 *in vivo* have been performed to date (Table 2). LMW and HMW FGF2 both stimulate endothelial cell proliferation but differ in their effect on cell migration, which is increased by LMW FGF2 in contrast to HMW FGF2 that has an inhibitory effect [40]. This inhibition requires an estrogen receptor and is mediated by the amino terminal extension of 24 kDa HMW FGF2 [68, 69]. Moreover, a recent study reports a thrombin-mediated cleavage of the amino terminal extension of HMW FGF2. This abolishes its inhibitory effect on endothelial cell migration [70], thus adding another level of regulation of the exog-

Table 1. HMW FGF2 cellular actions *in vitro*.

cell line (type/origin)	phenotype	reference
ABAE (endothelia)	downregulation of FGFR, immortalization	[39]
	growth in higher cell density, no soft-agar colony formation	
NIH3T3 (embryonal fibroblast)	growth impairment and formation of multinucleated cells at low levels, transformation at high levels	[98]
	no induction of migration, low-serum growth, no downregulation of FGFR	[49]
	low-serum growth, high density culture, radioresistance	[50]
	low-serum growth, decreased migration, cytotoxic drug resistance	[52]
	proliferation, soft-agar growth	[38, 51]
	low-serum growth	
HeLa (cervical cancer)	**radioresistance**	[54]
Schwann cells (brain)	**flat and large cell morphology**	[58]
PC12 (adrenal gland)	**no induction of neuronal differentiation**	
dopaminergic neurons (brain)	*increased survival, neurotoxin protection	[72]
sympathetic neurons (cervical ganglion)	**induction of multinuclear phenotype**	[95]
Schwann cells (neural crest)	transdifferentiation into melanocytes	[56]
BAEC (endothelial) MCF-7 (breast cancer)	*inhibition of migration	[97]
A31 (fibroblast)	proliferation, high-density growth	[52]
ROS 17/2.8 (osteosarcoma)	proliferation	[53]
AR4–2J (pancreatic cancer)	low-serum growth	[92]
HEK293 (embryonal kidney)	**chromatin compaction, apoptosis**	[94]
cardiac myocytes	stimulation of proliferation at low doses; inhibition of proliferation, chromatin compaction and cell death at high doses	[99]
	proliferation, decreased myosin accumulation, **chromatin condensation**	[48]
	***increase in size**	[37, 69]
GM7373 endothelial cells	*FGF-receptor binding, cell proliferation and chemotactic movement	[36]
MDA-MB-231 (breast cancer)	reduction of colony formation in soft agar	[93]

Highlighted in bold are the phenotypes regulated by HMW FGF2 that are different from those regulated by LMW FGF2, as compared directly in the same study. * – phenotypes generated by extracellular HMW FGF2 addition.

Table 2. HMW FGF2 cell/tissue actions *in vivo*.

animal/tissue/cell	phenotype	reference
mice (ABAE cells)	**no tumor formation in nude mice**	[39]
mice (NIH3T3)	tumor formation in nude mice at a high level of expression	[103]
NBT-II (bladder carcinoma)	**survival and lung metastasis formation in nude mice**	[101, 79]
heart	***induction of cardiac hypertrophy**	[38]
endothelial cell	**necessary for estradiol-mediated angiogenesis**	[67]
sciatic nerve	***enhanced regeneration after injury**	[77, 78]
mice	inhibition of angiogenesis by the amino terminal fragment of HMW FGF2	[71]

Highlighted in bold are the phenotypes regulated by HMW FGF2 that are different from those regulated by LMW FGF2, as compared directly in the same study. * – phenotypes generated by extracellular HMW FGF2.

enous HMW FGF2. The HMW FGF2-mediated inhibition of endothelial cell migration was confirmed *in vivo* [69, 71] and one physiological role of HMW FGF2 may lie in the negative regulation of angiogenesis.

In cardiac muscle, HMW FGF2 accumulates upon the exposure to stressful stimuli such as isopreterenol or hypothyroidism [72, 61]. When injected into the infarcted rat heart, HMW FGF2 but not LMW FGF2 induced significant cardiac hypertrophy by increasing the overall size of cardiomyocytes, possibly via induction of cardiotrophin-1 [38, 73].

The brain is a prominent site of FGF2 expression, with HMW FGF2 being found in the spinal cord, cerebellum, cortex and substantia nigra [62, 64, 74, 75]. *In vitro*, exogenously added HMW FGF2 showed neurotrophic activity on cultures of dopaminergic neurons isolated from rat mesencephalon [76]. This was confirmed *in vivo*, where HMW FGF2, released from implanted Schwann cells,

enhanced the regeneration of injured rat sciatic nerve [77, 78]. Similar results were obtained when dopaminergic neurons were implanted with HMW FGF2-overexpressing Schwann cells into the injured rat brain, again confirming the neurotrophic activity of HMW FGF2 [75]. As the HMW FGF2 effects on both cardiomyocytes and neurons were induced by extracellular HMW FGF2, it is difficult to conclude to what extent such effects were mediated solely by nuclear HMW FGF2 signalling because of concomitant activation of FGFR-dependent signalling pathways.

There are two studies that report the *in vivo* effect of HMW FGF2 likely mediated by its nuclear signalling. Thomas-Mudge at al. [79] engineered NBTII rat bladder carcinoma cells to express 24kDa HMW FGF2 under the control of a conditional doxycycline promoter. When such cells were inoculated into nude mice, they gave rise to lung metastases in comparison with their FGF2 non-expressing counterparts, which underwent rapid apoptosis.

Garmy-Susini et al. [67] described a HMW FGF2 role in the effects of the sex hormone estradiol. Estradiol promoted angiogenesis and endothelial cell migration and proliferation in the $Fgf2^{+/+}$ mice but not $Fgf2^{-/-}$ animals. This phenotype was rescued in mice expressing intact HMW FGF2 but not LMW FGF2 ($Fgf2^{lmw-/-}$), thus demonstrating that HMW FGF2 but not LMW FGF2 signalling is necessary for positive effects of estradiol on the epithelia.

HMW FGF2 in disease

Unlike FGF23 [80], there is not yet a disease known to be associated with mutations in HMW FGF2, nor FGF2 in general. There are however two conditions where HMW FGF2 appears to play a pathophysiological role *in vivo*, B-cell chronic lymphoid leukemia (BCLL) and hairy cell leukemia (HCL).

BCLL is the most prevalent leukemia in Europe and North America and is characterized by accumulation of mature B-lymphocytes that have escaped apoptosis and undergone cell-cycle arrest in the G0/G1 phase of the cell cycle [81]. In patients suffering from BCLL, plasma FGF2 is massively upregulated when compared to controls, which correlates with the overexpression of both LMW and HMW FGF2 in the leukemic clone *in vivo* [82, 83]. While extracellular LMW FGF2 has no known autocrine function in BCLL to date [82], the intracellular HMW FGF2 content shows a strong positive correlation with the aggressiveness of disease and protects BCLL cells from experimentally-induced apoptosis [84]. HCL is a B-cell malignancy similar to BCLL [81]. The FGF2 abnormalities in HCL also resemble those of BCLL, including the importance of FGF2 for resistance to cytotoxic drugs and survival of HCL cells [42].

The molecular mechanism of HMW FGF2 action

In AR4–2J pancreatic cancer cells, expression of HMW FGF2 induces growth in low-serum conditions and is accompanied by changes in protein kinase C isoform expression and activation of extracellular signal-regulated kinase-mitogen activated protein (ERK MAP) kinase, both independent of FGFR [85]. In HMW FGF2-expressing NIH3T3 fibroblasts, the transcription of a total of 77 genes was significantly up or down-regulated when compared to LMW FGF2-expressing cells, including genes acting in the cell cycle, chromatin remodelling, transcription and cell adhesion [86]. How are these effects achieved? To date, the molecular basis underlying the intracrine signalling of HMW FGF2 remains poorly characterized.

Partners of HMW FGF2

Antiapoptotic protein 5 (Api5; FIF; AAC11)

Api5 is a nuclear protein originally identified based on its strong antiapoptotic properties [87, 88]. The Api5 transcript generates two proteins, the 55 kDa full-length Api5 and a 25 kDa, N-terminally truncated variant [89]. Api5 is highly expressed in various transformed cell lines *in vitro* and enhances matrix metalloproteinase expression, matrigel invasion and laminin adhesion in the cervical cancer model cell line CUMC6 [45, 88]. Two experimental studies have shown the potent anti-apoptotic action of Api5. Wild-type BALB/c3T3 cultures die in serum-free media in 10 days in contrast to Api5 expressing cells that survived for up to 16 weeks in the same conditions [87]. Similarly, cultured cervical cancer cells showed significantly increased survival in serum-free media when expressing Api5 [88].

Using a yeast two-hybrid system, the interaction of Api5 with FGF2 was identified [89]. Api5 showed stronger affinity for HMW FGF2 than LMW FGF2 and did not interact with FGF1, FGF3 or FGF6. Similar to Api5, HMW FGF2 has a pro-survival action in various experimental models [54, 79], implying that both HMW FGF2 and Api5 functionally cooperate. This is further suggested by our recent finding that Api5 upregulation correlates with HMW FGF2 overexpression in BCLL [83] (see section 2.4.).

As mentioned earlier (section 2.3.), HMW FGF2 plays an essential role in estradiol-mediated angiogenesis *in vivo* and endothelial cell migration and proliferation *in vitro*. RNAi-mediated downregulation of Api5

completely abolishes the effect of estradiol on endothelial cell migration in the presence of HMW FGF2, thus clearly demonstrating the functional cooperation between FGF2 and Api5 in this process [67].

Ribosomal protein L6/TAXREB107

Although both LMW and HMW FGF2 associate with the ribosomal fraction in the nucleus, this association is much more prevalent for HMW FGF2, suggesting a function of the N-terminal extension in this phenotype [19, 90]. Within the ribosome, HMW FGF2 appears to interact with the L6/TAXREB107 protein that functions as both a ribosomal component and a member of the cAMP response element binding protein / activating transcription factor (CREB/ATF) family. The HMW FGF2-L6/TAXREB107 interaction takes place between the N-terminal extension of HMW FGF2 and C-terminal region of L6/TAXREB107 and suggests a role of HMW FGF2 in ribosome biogenesis and translational control [91].

Splicing factor SF3a66 and survival of motor neurons protein (SMN)

Using a yeast two-hybrid approach, Gringel et al. [92] found an interaction of FGF2 with the splicing factor SF3a66. This interaction took place between the core sequence common to all FGF2 variants and the C-terminal region of SF3a66.

SMN is a 38 kDa protein that localizes to both the cytoplasm and the nucleus and functions as an essential factor in spliceosomal ribonucleoprotein assembly [93]. Inactivation of SMN causes spinal muscular atrophy, manifested by progressive degeneration of the motor neurons of the spinal cord and early death [94].

The interaction between HMW FGF2 and SMN was found in co-immunoprecipitation experiments, addressing the hypothesis that SMN, known to bind the GR-rich proteins, interacts with the GR-rich region of HMW FGF2 [28]. Further analyses showed that HMW FGF2, but not LMW FGF2, binds directly to the N-terminus of SMN between amino acids 1–90, which constitutes a domain involved in interaction with both RNA and spliceosomal proteins such as Gemin2/SIP2 [28, 95]. Given the essential role of both SF3a66 and SMN in the assembly of spliceosomal complexes, one of intracellular roles of HMW FGF2 may lie in the modulation of splicing.

Future prospects

Despite significant evidence documenting the biological activities of HMW FGF2, many important questions remain. These relate mostly to the physiological role of HMW FGF2 as well as to the molecular basis of its action. At the level of translation, it is not yet clear how HMW FGF2 translation is regulated following the general activation of the FGF2 IRES (Fig. 1B). Thus one specific area of future research lies in the identification of factors that govern the differential translation of the FGF2 variants.

Another important area is to understand the role of HMW FGF2 *in vivo*. Generation and detailed analysis of transgenic mice expressing LMW but not HMW FGF2 ($Fgf^{hmw-/-}$) should identify possible physiological functions of HMW FGF2 *in vivo*. As FGF2 is one of the most significant factors that maintain the pluripotency of hESCs, which also express HMW FGF2 in large quantities, self-renewal and early differentiation of hECSs may represent examples of processes potentially regulated by HMW FGF2 *in vivo* [55, 96].

To understand the physiological role of HMW FGF2 completely, we also need more insights into the molecular mechanisms of its signalling. To date, the role of HMW FGF2 in the estradiol effect on the epithelia represents the only mechanistic evidence of the HMW FGF2 action *in vivo* [67]. When adapted to *in vitro* conditions, the HMW FGF2 role in estradiol signalling could serve as an experimental model to address the molecular mechanism of HMW FGF2 signalling in detail, including the functional consequences of HMW FGF2 interaction with its partners such as Api5 or SMN.

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