Cell. Mol. Life Sci. (2015) 72:1651–1662
DOI 10.1007/s00018-014-1818-6

Cellular and Molecular Life Sciences

REVIEW

A nuclear odyssey: fibroblast growth factor-2 (FGF-2) as a regulator of nuclear homeostasis in the nervous system

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Received: 28 October 2014 / Revised: 10 December 2014 / Accepted: 19 December 2014 / Published online: 1 January 2015

Abstract Nuclear localization of classical growth factors is a well-known phenomenon but still remains a molecular and cellular conundrum. Fibroblast growth factor-2 (FGF-2) is an excellent example of a protein which functions as an extracellular molecule involved in canonical receptor tyrosine kinase signaling as well as displaying intracellular functions. Paracrine and nuclear functions are two important sides of the same protein. FGF-2 is expressed in isoforms with different molecular weights from one mRNA species. In rodents, all of these isoforms become imported to the nucleus. In this review, we discuss structural and functional aspects of FGF-2 isoforms in the nervous system. The nuclear odyssey of FGF-2 is reflected by nuclear dynamics, localization to nuclear bodies such as nucleoli, binding to chromatin and engagement in various protein interactions. Recently discovered molecular partnerships of the isoforms shed light on their nuclear functions, thereby greatly extending our knowledge of the multifaceted functions of FGF-2.

Keywords FGF signaling · Isoforms · Neurotrophic factor · Nuclear protein · SMN · Chromatin · Splicing · Spinal muscular atrophy (SMA) · Parkinsońs disease

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Abbreviations

CB	Cajal body
SMN	Survival of motoneuron protein
SMA	Spinal muscular atrophy
FGF	Fibroblast growth factor
snRNP	Small nuclear ribonucleoprotein particle

The dawn of nuclear FGF-2

The fibroblast growth factor (FGF) family represents a large group of signaling molecules and comprises 22 members [1] encoded by the same number of genes [2]. One group of FGFs was primarily identified and purified from bovine pituitary glands [3, 4] and later subdivided into acidic FGF (FGF-1) and basic FGF (FGF-2) using isoelectric focusing [5]. Based on the tissue of origin or target cell specificity, FGF-2 was given different names like basic FGF (bFGF), pituitary cationic FGF, pituitary brain FGF, cartilage-derived growth factor (CDGF), heparin-binding growth factor β (HGFβ), eye-derived growth factor 1 (EDGF-1), or astroglial growth factor 2 (AGF-2) and classified as class 2 heparin-binding growth factors (class 2 HBGFs) [6]. Later, basic FGF was renamed to fibroblast growth factor-2 [7].

FGF-2 is expressed in different isoforms from one mRNA species with distinct molecular weights. In addition to 18 kDa FGF-2 [FGF-2^{18}, denoted as low-molecular weight (LMW-FGF-2)] another two high molecular weight isoforms have been described as 21 and 23 kDa FGF-2 (FGF-2^{21} and ^{23}, together denoted as HMW-FGF-2) in rodents [8–10]. In humans, besides 18 kDa FGF-2, even four high molecular weight isoforms are expressed. Translation either starts at a conventional AUG start codon for 18 kDa FGF-2 or alternative upstream CUG start

codons for HMW-FGF-2 isoforms from a single mRNA species [11–15]. The HMW-FGF-2 isoforms show N-terminal sequence extensions compared with the 18 kDa isoform. However, all isoforms comprise the 18 kDa core sequence (Fig. 1). The FGF-2 mRNA has interesting features permitting highly regulated translation: although this mRNA exhibits a classical cap for translational initiation, an internal ribosomal entry site (IRES) in the 5′ untranslated region allows additional means of translational control [16]. The IRES is used preferentially for regulation of protein expression, especially in brain tissue [17] and at least partly determines expression of specific isoforms. However, also the 3′ untranslated region takes part in alternative initiation of translation as well as overall destabilization of the mRNA [18, 19].

Release of FGF-2 from the cell into the extracellular space has been an enigma for a long time. Although lacking a conventional signal peptide sequence, 18 kDa FGF-2 is released in an energy-dependent exocytotic mechanism bypassing the Golgi/ER pathway [20, 21]. This process is induced by the binding of cytoplasmic FGF-2 to phosphoinositides at the plasma membrane [22] followed by the oligomerization of FGF-2 and formation of a lipidic membrane pore [23] and finally the release on the cell surface [24]. It should be clearly noted that export of 18 kDa FGF-2 may not be an exclusive characteristic of this low molecular weight isoform. Release of HMW-FGF-2 isoforms from the cell has not been widely considered as a mechanism providing additional functional complexity of the isoforms as paracrine factors. However, HMW-FGF-2 becomes released in a highly regulated angiotensin and caspase-1 dependent manner from cardiac non-myocytes [25, 26]. This paracrine action induces hypertrophy of cardiac myocytes and other deleterious, e.g., pro-inflammatory downstream events [27].

Depending on the isoform or more precisely on the length of the N-terminal extension, FGF-2 plays diverse roles in different tissues and cell types. In the 1970s, FGF-2 was initially purified from pituitary glands [3, 4] due to high concentrations of the growth factor in the brain indicating that FGF-2 has a specific role in the nervous system [3, 4]. FGF-2 has been then considered to be a neurotrophic factor due to in vitro and in vivo promoted survival, proliferation, and regulation of differentiation of central or peripheral neurons [28, 29]. For example, FGF-2 is retrogradely transported in the hypoglossal nerve [30]. Retrograde axonal transport was before shown for nerve growth factor (NGF) as the prototype of a neurotrophic factor [31–33]. In this review, we focus on the roles of FGF-2 isoforms in the nervous system and provide an overview about recently described molecular mechanisms of FGF-2 isoforms in the nucleus. These nuclear FGFs display a remarkable dynamics in the interchromosomal space—compared to a nuclear space odyssey—where they interact with a number of proteins which were recently identified. Identification of molecular partner proteins defines new functional roles of FGF-2 isoforms in signaling.

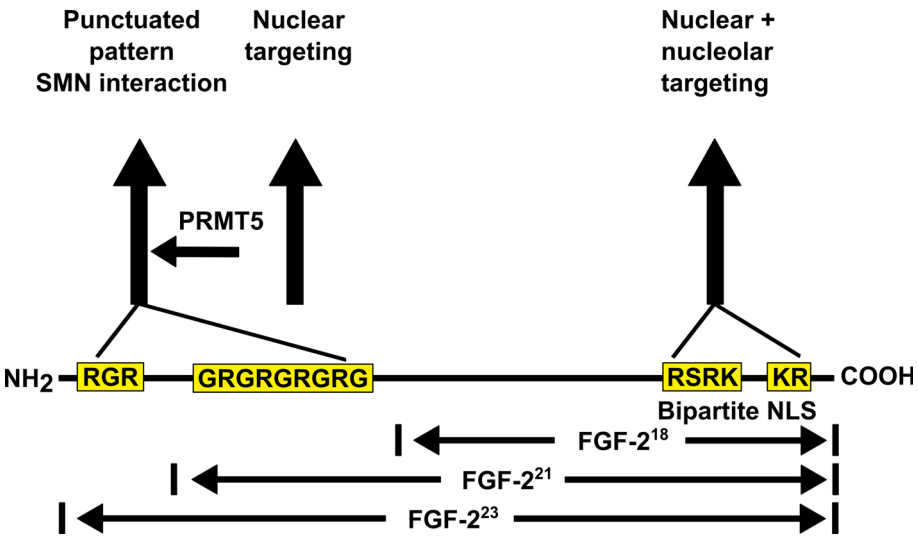

Fig. 1 Primary structures of FGF-2 isoforms and distribution of RG motifs in rodent FGF-2 isoforms. A bipartite nuclear NLS, comprised by splitted arginine–lysine (RK) motifs, is important for nuclear targeting of all isoforms and nucleolar targeting of FGF-2^{18} [81, 83]. Nuclear localization of HMW-FGF-2 isoforms is mediated by a large conserved arginine–glycine (RG) sequence [84, 85]. In this model, a critical threshold of positive charges in the N-terminal FGF-2 extension is required for nuclear localization and chromatin association [89] which could be eventually responsible for transcriptional modification. Furthermore, the N-terminal extension of HMW-FGF-2 promotes SMN binding [81, 118]. The FGF-2^{23}-SMN interaction is severely affected by symmetrical dimethylation by PRMT5 [89]

FGF-2 isoforms in the nervous system

The FGF-2 isoforms are not equally distributed in different brain regions. Diverse structures of the central (CNS) and peripheral nervous system (PNS) display distinct FGF-2 profiles with specific expression of isoforms in development and after tissue damage. In the adult rat cerebellum, cortex and spinal cord, specific expression patterns of FGF-2 isoforms have been found and these profiles become particularly regulated during development. This indicates diverse functions of the corresponding protein isoforms in formation, maturation and organization of the central nervous system [34].

In the CNS, FGF-2 is widely expressed in different structures [35, 36]. However, most studies address expression on mRNA level and not the expression of FGF-2 protein isoforms. Therefore, our knowledge of isoform distribution and expression is still limited in the nervous system. All three FGF-2 isoforms are expressed in the striatum and substantia nigra of adult rats [37]. This could indicate a role of FGF-2 in supporting differentiation or survival of neurons in these structures. Since degeneration of substantia nigra neurons occurs in Parkinson's disease (PD), expression of FGF-2 or its specific isoforms could be a means of attenuating degeneration. In a model of PD, chemically lesioned mice display increased recovery of striatal dopaminergic (DA) neurons after intrastriatal application of 18 kDa FGF-2 [38, 39]. Accordingly, mice deficient of all FGF-2 isoforms show reduced numbers of DA neurons in contrast to FGF-2 transgenic mice which display an increase [40]. Moreover, HMW-FGF-2 expressing Schwann cells increase the survival of rat dopaminergic mesencephalic neurons in co-culture experiments in vitro as well as exogenously applied FGF-2^{21} and FGF-2^{23} [41]. Moreover, co-transplantation of HMW-FGF-2 expressing Schwann cells improve in vivo the restoration, reinnervation and survival of dopaminergic micrografts in a rat model of Parkinson's disease, compared with FGF-2^{18} expressing Schwann cells [42]. In the rat cortex, FGF-2^{21} has been mainly found at embryonic day 18 (E18) and day of birth (postnatal day 0, P0). In contrast, no FGF expression is detected in the adult cortex. However, in the rat cerebellum, FGF-2^{18} and FGF-2^{21} have been found highly expressed between E18 and P7. After this point of time, only FGF-2^{18} remains strongly expressed and no high molecular weight isoforms have been found in the adult cerebellum. In the spinal cord, FGF-2^{18} and FGF-2^{21} are expressed at E18 and P0. This pattern changes afterwards—also in contrast to the cerebellum: here, in the adult spinal cord exclusively both HMW-FGFs are highly expressed and LMW-FGF-2 displays only moderate expression levels [34]. Rat oligodendrocytes are affected by the application of FGF-2, terminal differentiation in vitro [43] as well as myelin production in vivo [44] is severely reduced. Consistently, FGF-2 deletion in mice promotes oligodendrocyte regeneration in vivo [45]. Expression of FGF-2^{23} in pyramidal neurons of the rat hippocampus results in increased detection of two nuclei in these cells. The same has been found for postganglionic neurons and non-neuronal cells, followed by degeneration [46]. Interestingly, transfection of this isoform into postmitotic sympathetic neurons resulted in bi- or multinucleated phenotypes supporting a function of FGF-2^{23} as a positive regulator of karyokinesis [46]. Interestingly, this phenotype has also been observed in a non-neuronal system: cardiac myocytes also exhibit binuclear morphology upon transfection with HMW-FGF-2 [47]. Furthermore, the expression profiles of FGF-2 isoforms can be pharmacologically modulated. The antidepressants desipramine and fluoxetine increase FGF-2 protein levels in vivo in rat frontal cortical neurons and shift particularly the intracellular HMW-FGF-2 localization from the nucleus into the cytoplasm [48]. The antidepressant amitriptyline increases FGF-2 mRNA transcription and equally the expression of low and high molecular weight isoforms in vitro only in rat cortical astrocytes but not in cortical neuron-enriched cultures [49].

In the PNS, FGF-2 mRNA has been found upregulated after peripheral nerve lesion in rat sensory ganglia [50, 51]. After hypoglossal nerve transection, all FGF isoforms are downregulated 3 days after lesion and then upregulated 14 days after lesion above control levels [52]. Axotomy of the rat superior cervical ganglion (SCG) results in a fivefold increased FGF-2^{18} and FGF-2^{23} and a threefold increased FGF-2^{21} protein level [53]. In the lesioned rat sciatic or saphenous nerves, FGF-2 displays neurite-promoting functions [54–56]. The same improvement can be observed in the regenerating rat sciatic nerve by transplantation of HMW-FGF-2 over-expressing Schwann cells [57]. Indeed, after lesion of spinal ganglia or sciatic nerve FGF-2 protein isoforms are differentially upregulated, indicating isoform-specific physiological functions in the regeneration of injured peripheral nerves [58–60]. FGF-2 knockout in mice leads to increased regeneration and myelination close to the injury site after sciatic nerve lesion with indications of augmented Wallerian degeneration, suggesting crucial functions of FGF-2 in early peripheral nerve regeneration [61]. After sciatic nerve transection leaving a certain gap length, HMW-FGF-2 over-expressing Schwann cells promote sensory nerve recovery and long-distance myelination in vivo in contrast to inhibitory effects of FGF-2^{18} over-expressing Schwann cells on myelination [62]. In contrast, neither LMW- nor HMW-FGF-2 overexpressing Schwann cells affect the recovery after facial nerve transection in adult rats [63]. Taken

together, the results demonstrate specific roles of FGF-2 isoforms in regeneration both in the CNS and the PNS.

Pheochromocytoma cells (PC12) are an excellent model of neuronal differentiation and neurotransmitter vesicle release due to their origin from modified sympathetic ganglia cells in the adrenal gland. During postnatal development of rat adrenal medulla, a peak expression of FGF-2^{21} at postnatal day 28 has been observed [64]. Glucocorticoids like dexamethasone are suggested to trigger the differentiation of adrenergic chromaffin cells out of noradrenergic sympathoadrenal precursor cells [65]. In the rat adrenal medulla similar to PC12 cells, but not in non-neuronal tissues, dexamethasone enhances in vivo specifically the expression of the FGF-2^{21} isoform indicating a neuron specific physiological function [66, 67]. Overexpression of FGF-2^{18} alone in PC12 cells is sufficient to start differentiation and outgrowth of long processes in contrast to cells expressing HMW-FGF-2 which develop a round-shaped morphology [68]. Moreover, in immortalized Schwann cells, morphology and growth are altered to shorter processes after overexpression of HMW-FGF-2, compared with Schwann cells expressing FGF-2^{18} or control cells [68]. However, stimulation with 18 kDa FGF-2 enhances proliferation of cultured mouse and rat Schwann cells [69–72].

Besides the neurotrophic functions of FGF-2 and its isoforms, diverse functions in other organs have been observed. For example, after myocardial infarction in a rat model, FGF-2^{18} promotes angiogenesis and cardioprotection in contrast to HMW-FGF-2 which promoted deleterious effects [27, 73]. Furthermore, FGF-2^{18} enhances bone formation, whereas HMW-FGF-2 has an inhibitory effect [74–76]. Interestingly, HMW-FGF-2 expression in fibroblasts leads to growth reduction and lower levels of proliferating cell nuclear antigen (PCNA) [77, 78] indicating widespread isoform-specific FGF-2 functions in different tissues.

The mission of FGF-2: nuclear import and engagement

FGF-2^{18} has been found in the nucleus as well as in the cytoplasm, whereas HMW-FGF-2 isoforms localize predominantly in the nucleus of different cell types in isoform-specific nuclear localization patterns [79, 80]. However, the distribution of nuclear FGF-2^{18} is clearly different from the distribution of HMW-FGF-2 indicating different structural elements of the isoforms responsible for nuclear localization [79, 81]. HMW-FGF-2 isoforms show a punctuated pattern in the nucleus and a chromatin association, whereas 18 kDa FGF-2 localizes to nucleoli [79, 82].

All isoforms share a nuclear localization signal (NLS) in the C-terminal core sequence (Fig. 1). For the purpose of comparison between the isoforms, we define here the sequence of 18 kDa FGF-2 as a "core" since this primary structure is common to all isoforms. The first part of the bipartite NLS comprises Arg^{116}, Arg^{118} [81] and Lys^{119} [83]. Together with the second part (Lys^{128} and Arg^{129}), these amino acid clusters are designated as a non-classical bipartite NLS, not only mediating nuclear import but also nucleolar accumulation of FGF-2^{18} [81, 83]. HMW-FGF-2 isoforms comprise a second nuclear localization signal in their N-terminal extensions. Fusion of the HMW extension to chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP) or β-galactosidase (β-gal) results in nuclear accumulation of the fusion proteins in contrast to control constructs [81, 84, 85]. Conserved glycine-arginine-glycine (RGR) sequence motifs in HMW-FGF-2 cause nuclear localization [81, 84, 85]. Basic peptide sequences, containing mainly arginine and lysine residues serve as nuclear localization sequences due to the potential to build an import complex with importin-α and importin-β, followed by a transfer through a nuclear core complex into the nucleus [86, 87]. Insertion of the RGR motif into FGF-2^{18} leads to increased nuclear localization of the protein, supporting the supposed role of the amino acid sequence [88]. FGF-2^{21} and FGF-2^{23} differ in only one RGR motif. However, this single element is not sufficient for nuclear localization of the protein. It has been suggested that the positive charge of this sequence regulates the punctuated patterns of the HMW-FGF-2 isoform. Deletion of a hydrophobic stretch between RGR clusters and mutation of two arginine to histidine residues, retaining a positive charge, does not change the nuclear distribution of FGF-2^{23} [89]. It has been suggested that a threshold of positive charge regulates chromatin association primarily as a result of certain number of arginine residues in the N-terminal FGF-2 extension [89] (Fig. 1). Interestingly, the chromatin-binding high-mobility group proteins A1/A2 (HMG-A1/A2) also comprise RGR motifs as extended motifs with a proline residue forming RGRP motifs. These motifs are reminiscent of the RGR-rich primary structure of HMW-FGF-2 [90]. It has been shown that these structures designated as AT-hooks in HMGA proteins bind to AT-rich DNA [91].

Arginine residues in the N-terminus of HMW-FGF-2 can be methylated, affecting the intracellular distribution of the protein [89, 92, 93]. FGF-2^{23} becomes methylated by protein arginine methyltransferase 5 (PRMT5) [89] (Fig. 2). PRMT5 protein complexes in the cytoplasm are designated as methylosomes [94] and responsible for symmetrical methylation of Sm proteins resulting in the assembly of small nuclear ribonucleoproteins (snRNPs) by the survival of motoneuron (SMN) protein [95–97]. SnRNPs are composed of Sm proteins in a ring-like structure as well as a catalytic small uridine-rich RNA

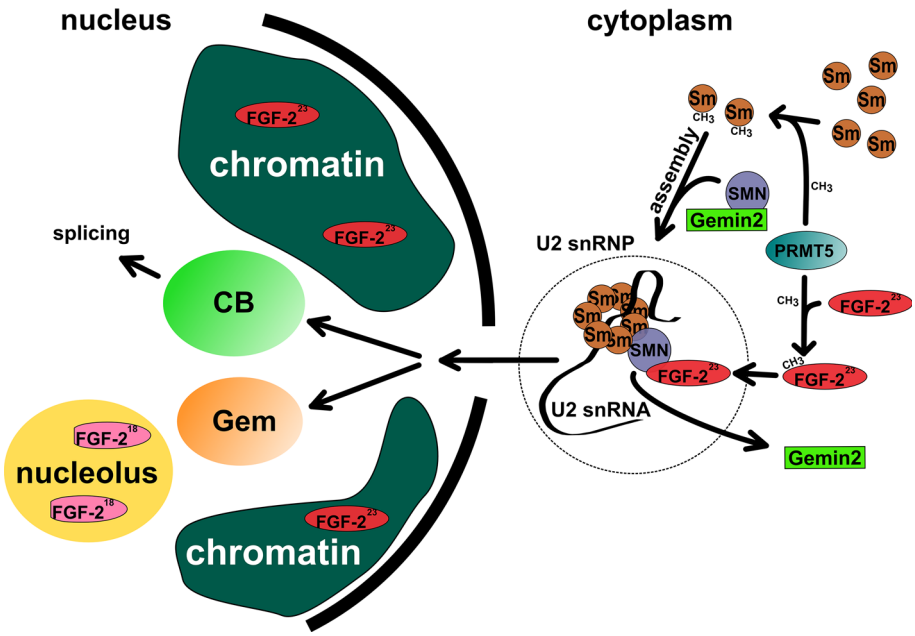

Fig. 2 FGF-2^{23} interaction with the Survival of Motoneuron (SMN) protein. SMN is an assembly factor for small nuclear ribonucleoprotein particles (snRNPs) composes of catalytic RNA molecules (U RNAs, shown here for the U2 snRNA) and Smith antigen (Sm) proteins. FGF-2^{23} becomes arginine-methylated at its N-terminus by Protein Arginine Methyltransferase 5 (PRMT5) [89] as well as the Sm proteins. FGF-2^{23} binds to SMN thereby competing with Gemin2 for binding to the N-terminus of SMN [81, 118]. The snRNP translocates to Cajal bodies (CBs) in the nucleus where different snRNPs undergo a subsequent master assembly into tri-snRNP complexes which later engage in pre-mRNA splicing. SMN dissociates from the complex forming small nuclear bodies called nuclear gems [110]. CBs and gems are highly mobile structures moving in the nuclear space between the chromatin domains [127, 136–141]. The low molecular weight FGF-2^{18} isoform does not bind to SMN but translocates to the nucleolus, whereas FGF-2^{23} also localizes to chromatin [81]

molecule (U snRNAs) [98]. Symmetrically dimethylated RG motifs are preferred binding sites for the SMN protein [97] due to an aromatic cage in the SMN Tudor domain mediating dimethyl-arginine recognition [99]. Therefore, it was supposed that methylated FGF-2^{23} could also be a target and interaction partner of SMN like the Sm proteins. This is indeed the case: symmetrically dimethylated FGF-2^{23} does not only show an interaction with SMN but also is additionally targeted to the nucleus. As expected, FGF-2^{18} does not interact with SMN. This clearly confirms that the N-terminal extension of FGF-2^{23} is the molecular interface responsible for direct molecular partnering with SMN. Inhibition of methyltransferases by 5′-deoxy-5′-methylthioadenosine (MTA) or adenosine dialdehyde (AdOx) causes a dysregulation of the nucleus/cytoplasmic distribution of FGF-2 towards a cytoplasmic localization [89, 93] showing a clear effect of FGF-2 methylation on intracellular localization [59, 62]. Due to the strong influence of FGF-2 methylation on SNM binding, it has been suggested that the FGF-2^{23}-SMN interaction influences FGF-2 nuclear import. Since FGF-2^{18} does not interact with SMN, the nuclear transport of this isoform may be based on a different mechanism. How could the nuclear transport of FGF-2^{18} be mediated? The protein Translokin (Tlk) has been shown to interact with FGF-2^{18} but not with the high molecular weight isoforms. Reduced Tlk expression leads to reduced nuclear FGF-2 accumulation emphasizing the existence of FGF-2 isoform-specific nuclear import mechanisms [100]. However, treatment of Swiss 3T3 cells with FGF-2^{18} results in importin-β-dependent internalization of the FGF receptor 1 (FGFR1) [101, 102]. Internalization and nuclear translocation of ligands with the respective growth factor receptors are not an exclusive mechanism of the FGF-2/FGFR1 interaction but can be shown for different ligand/receptor interactions like epidermal growth factor (EGF)/EGF receptor (EGFR) or interferon γ (IFNγ)/INFNγ receptor (INFGR) [103]. FGFR1 interacts directly with importin-β, but uses an adapter protein that could be HMW-FGF-2 [104]. Immortalized pancreatic stellate cell line 1 (PS1) cells in a model for pancreatic cancer display a correlation of FGF-2/FGFR1 nuclear accumulation and the ability to invade into the extracellular matrix. Inhibition or RNAi knock-down of FGFR1 leads to cytoplasmic FGF-2/FGFR1 localization and a reduction in proliferation and cell invasion, suggesting a potential novel therapeutic usability of FGFR1 inhibition in pancreatic cancer [105]. Prostaglandin $F_{2\alpha}$ incubated immortalized rat osteoblastic Py1a cells display an increased binding of FGF-2 and FGFR2, a co-localization with importin-β and a nuclear translocation suggesting

a role of FGF-2/FGFR2 nuclear trafficking in bone metabolism [106]. Following the translocation, different nuclear localization sequences and isoform-specific nuclear localization patterns of FGF-2 point to diverse functions of this nuclear growth factor.

Another protein that is symmetrical methylated by PRMT5 and thereby affected in its affinity to SMN in a similar manner as FGF-2^{23} is the Cajal body protein coilin [107]. Cajal bodies (CBs) are nuclear structures involved in the maturation of snRNPs, a relevant step in spliceosome assembly [98, 108, 109]. Furthermore, SMN is concentrated in a second nuclear compartment called Gemini of CBs (gem) that could have a role in RNA metabolism [110]. Both of these SMN containing nuclear bodies are sensitive for expression of FGF-2^{23} [111, 112]. However, there are even more binding partners and proposed specific roles of different FGF-2 isoforms, in particular inside the cell nucleus.

Nuclear FGF-2 and beyond—the interaction partners

Besides the canonical signaling via cell surface receptors and signaling cascades, there is an increasing number of intracellular and intranuclear FGF-2 functions that have been recently discovered. In the nucleus, FGF-2^{18} localizes to nucleoli of NIH 3T3 cells [79] and additionally to Cajal bodies in rat immortalized Schwann cells [81]. In contrast, HMW-FGF-2 shows a chromatin-associated localization pattern accompanied by chromatin condensation in cell lines as well as in primary cells indicating different nuclear functions of individual FGF-2 isoforms [79, 81, 82, 113].

Intracellular FGF-2 specifically interacts with different nuclear proteins providing potential involvements in multifaceted nuclear regulations. Depending on the respective FGF-2 binding domain involved, the interactions can be isoform-specific or include all isoforms. The interaction of FGF-2 with the splicing factor SF3a66 depends on the 18 kDa core sequence comprised by all FGF-2 isoforms, since a direct interaction can be shown for both FGF-2^{23} and FGF-2^{18} [114]. Isoform-specific binding was found for the binding to the ribosomal protein L6/TAXREB107, bearing at least one binding site for all FGF-2 isoforms but an additional binding site for HMW-FGF-2 only, strongly increasing the affinity of the larger isoforms to the ribosomal protein [115]. This is similar to high affinity interaction of HMW-FGF-2 with fibroblast growth factor-2 (FGF-2)-interacting factor (FIF). For FIF interactions, FGF-2^{18} was not detectable after co-precipitation with FIF antibody [116] indicating a dependence of the N-terminal extension of HMW-FGF-2 for binding to FIF. FIF is also called apoptosis inhibitor-5 or antiapoptotic protein 5 (Api5) and is found to be upregulated together with HMW-FGF-2 in peripheral blood mononuclear cells (PBMCs) of patients with B cell chronic lymphoid leukemia (B-CLL) supposing a possible role of the binding partners in the pathology of the disease [117].

FGF-2^{23} but not FGF-2^{18} binds to the survival of motoneuron (SMN) protein in the nucleus with the arginine-rich N-terminal extension of this HMW isoform [81, 118]. Interestingly, the cellular SMN protein amount is linked to the pathogenesis of the monogenic, neurodegenerative disease spinal muscular atrophy (SMA) [119, 120]. A hallmark of SMA is the progressive degeneration of spinal cord motoneurons caused by disruptions of the Survival of Motor Neuron 1 (Smn1) gene. The selective death of motoneurons is currently still unexplained. The ubiquitously expressed SMN protein functions as an assembly factor for small nuclear ribonuclear protein particles (snRNPs) or small nucleolar RNPs (snoRNPs) involved in splicing [121]. In the nucleus, SMN is found in nuclear foci denoted as gemini of Cajal bodies (gems) which partly colocalize with Cajal bodies (CBs). Some SMN mutations identified in SMA patients disrupt in vitro the binding of SMN to Sm proteins, which are components of snRNPs. However, no splicing defects have been found in presymptomatic stages which are linked to motoneuron death [122].

FGF-2^{23} associates with spliceosomal subunits called small nuclear ribonucleoproteins (snRNPs) without interfering with the interaction of SMN and small nuclear RNAs (snRNAs) within the snRNPs [118] (Fig. 2). The binding of FGF-2^{23} to SMN has been mapped to the first 90 amino acid residues of N-terminus of SMN not competing with Sm protein binding to the Tudor domain [118]. However, SMN binding to nuclear proteins like gemin2 and coilin is severely affected by FGF-2^{23} causing a nuclear phenotype including alterations in nuclear body formation. Expression of FGF-2^{23} leads to a disruption of nuclear gems due to a competition of the growth factor with Gemin2 for binding to SMN [111]. Down-regulation of Gemin2 causes a similar phenotype showing that Gemin2 and the interaction with SMN are necessary for nuclear gem stabilization. FGF-2^{23} can compete with this interaction as demonstrated in vitro using a cell culture model and in vivo using FGF-2 transgenic mice [89] (Fig. 2). The loss of nuclear gems has also been observed in fibroblasts derived from patients suffering from spinal muscular atrophy [123]. Interestingly, the total number of coilin positive Cajal bodies (CBs) is not affected by FGF-2^{23} [111], but the function of CBs is as well affected by the growth factor. Coilin is a marker protein for Cajal bodies and a direct interaction partner of SMN [124–126]. The interaction of SMN and coilin is diminished by FGF-2^{23} leading to a mobilization of the SMN protein from an immobile into a fast mobile fraction and a depletion of

SMN from Cajal bodies [112]. SMN-coilin binding is an important requirement for the formation of tri-snRNPs in CBs as a crucial step of the spliceosome cycle resulting in spliceosome formation [127]. FGF-2^{23} expression leads to inhibited tri-snRNP assembly at CBs indicated by a U4 snRNP accumulation at the nuclear complex [112] (Fig. 3). These results indicate a putative role of FGF-2 as a disease modifier of SMA. In a *Drosophila* model of SMA, activation of muscle-specific FGF signaling is sufficient to rescue neuromuscular junction (NMJ) defects [128], indicating a function link between FGF-signaling and SMA pathology. Furthermore, the expressional level of FGF-2 mRNA is increased in a mouse model of SMA at disease onset [36]. In a cellular model of SMA in PC12 cells, coexpression of FGF-2^{23} antagonizes the SMN promoting effect on neurite outgrowth [129], indicating a negative regulatory effect of HMW-FGF-2 on SMA pathology. FGF-2 loss could, therefore, possibly attenuate the SMA phenotype. Similarly, FGF-2 deficiency in a mouse model for the neurodegenerative disease amyotrophic lateral sclerosis (ALS) resulted in a milder phenotype [130].

Nuclear HMW-FGF-2 induces chromatin compaction and cell death with participation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) and a mitochondrial cell death pathway [82]. Due to the high overlap of HMW-FGF-2 with DAPI stainings [81, 82], the high molecular FGF-2 isoforms can be suggested to be involved in the regulation of gene expression. Interestingly, also FGFR1 localizes to the nucleus and is part of a chromatin bound complex with CREB-binding protein (CBP) [131, 132]. Nuclear FGF-2^{23} co-localizes with FGFR1 and is suggested to be part of the chromatin bound complex with CBP and, therefore, to be directly involved in the regulation of gene transcription, in contrast to FGF-2^{18} that seems not to affect this mechanism [133]. Accordingly, FGF-2 is assumed to participate in a pathway denoted as Integrative Nuclear FGFR1 Signaling (INFS) controlling growth, proliferation and differentiation particularly in the nervous system by regulating gene transcription [104, 134].

Indeed, many genes are regulated in isoform-specific processes as revealed by comparing transcriptomes of HMW- with LMW-FGF-2 expressing cells, albeit in non-neuronal NIH 3T3 fibroblasts. These mechanisms include up-regulation of genes involved in growth inhibition, tumor suppression, development as well as differentiation. In addition, down-regulation of genes involved in cell proliferation, mitogenic signaling and ribosome biogenesis has been observed [78]. HMW-FGF-2 in a complex with FGFR1 is suggested to directly up-regulate FGF23 expression in bone marrow stromal cells (BMSCs) [75]. However, isoform-dependent regulation of mRNAs in neuronal systems has also been described. Gene transcription dependent on the neuronal transcription factor Nurr-1 is amplified by FGF-2^{23} in human neuroblastoma cells [129, 135].

Outlook

The analysis of the functions of nuclear FGF-2 still remains a challenge. On the one hand, there is an increasing number of different and partly antagonistic cellular functions regulated by high and low molecular FGF-2 isoforms. The distribution of the growth factor isoforms inside the cell,

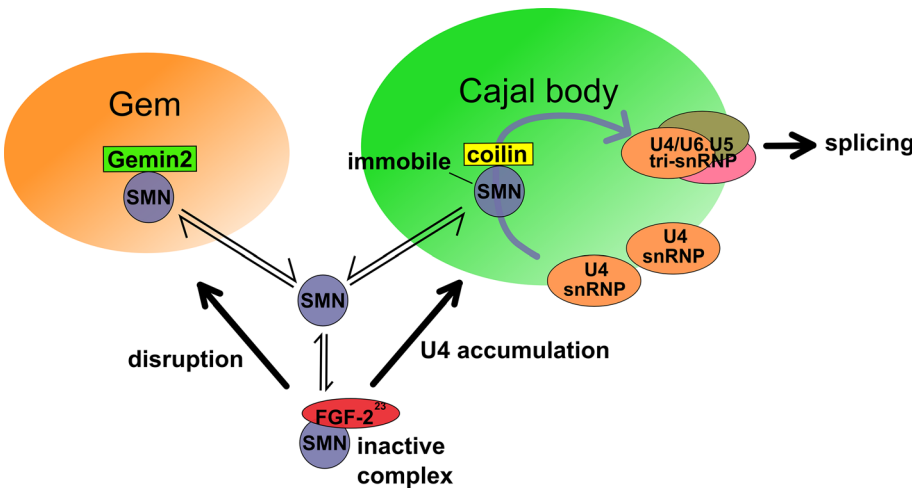

Fig. 3 FGF-2^{23} expression causes nuclear gem disruption and U4 snRNP accumulation at Cajal Bodies. Binding of FGF-2^{23} to SMN [81, 118] interferes with the binding of SMN to Gemin2 resulting in a disruption of nuclear gems [111]. Moreover, FGF-2^{23} competes with coilin for binding to SMN causing U4 snRNP accumulation in Cajal bodies [112]. The SMN/FGF-2^{23} interaction results in an inactivating protein complex disrupting the normal functions of Coilin and Gemin2, thereby putatively modifying nuclear splicing patterns [129]

the binding to proteins and the involvement in cellular processes vary depending on the length of the N-terminal extensions. On the other hand, there are specific FGF-2 functions in neuronal systems like differentiation and outgrowth of long processes that are enhanced by FGF-2[18] but inhibited by FGF-2[23]. By now, it is poorly understood how molecular complexes regulated by FGF-2 isoforms are directly engaged in these processes and—also beyond the nervous system—are involved in tissue-specific functions. A model to combine both could be that differential binding affinities and specificities of low and high molecular weight FGF-2 to other nuclear proteins involved in splicing and regulation of transcription like SMN and FGFR1 could result in altered splicing and transcription of FGF-2 dependent genes. As a consequence, differential regulation of proteins could result in tissue-specific effects. Furthermore, the binding of FGF-2[23] to the methyl transferase PRMT5 could affect the methylation of other PRMT5 targets and, therefore, directly influence protein functions with tissue dependent effects. To further analyze nuclear FGF-2 functions, it could be helpful to screen different tissues for alterations in splicing, transcription and methylation after expression of FGF-2 isoforms to better understand the connection between cellular differences and tissue-specific functions. Another interesting aspect is the positive effect of HMW-FGF-2 expressing transplanted cells on the survival, restoration and reinnervation of dopaminergic micrografts or, in the PNS, the support of nerve recovery by HMW-FGF-2 glial cells. Taken together, it is an ambitious and promising challenge to bring together isoform-specific FGF-2 functions inside the nucleus and their impact on cellular and organ function.

Acknowledgments This work was supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony.

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