RNA BIOLOGY
2024, VOL. 21, NO. 1, 1–17
https://doi.org/10.1080/15476286.2024.2332855

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REVIEW

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Role of RNA binding proteins of the *Drosophila* behavior and human splicing (DBHS) family in health and cancer

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ABSTRACT

RNA-binding proteins (RBPs) play crucial roles in the functions and homoeostasis of various tissues by regulating multiple events of RNA processing including RNA splicing, intracellular RNA transport, and mRNA translation. The *Drosophila* behavior and human splicing (DBHS) family proteins including PSF/SFPQ, NONO, and PSPC1 are ubiquitously expressed RBPs that contribute to the physiology of several tissues. In mammals, DBHS proteins have been reported to contribute to neurological diseases and play crucial roles in cancers, such as prostate, breast, and liver cancers, by regulating cancer-specific gene expression. Notably, in recent years, multiple small molecules targeting DBHS family proteins have been developed for application as cancer therapeutics. This review provides a recent overview of the functions of DBHS family in physiology and pathophysiology, and discusses the application of DBHS family proteins as promising diagnostic and therapeutic targets for cancers.

ARTICLE HISTORY
Revised 19 February 2024
Accepted 15 March 2024

KEYWORDS
DBHS family; PSF/SFPQ; NONO; PSPC1; neurodegenerative diseases; cancers

1. Introduction

RNA-binding proteins (RBPs) play important roles in post-transcriptional gene regulation at multiple steps, such as splicing of mRNA precursors (pre-mRNAs), alternative splicing, subcellular RNA transport, and mRNA translation [1–7]. In addition, RBPs are key players in biosynthesis of several membrane-less organelles (MLOs) that condensate specific molecules to facilitate the efficient biochemical reactions or suppress the functions of some molecules by sequestering them [8].

In humans, the *Drosophila* behavior and human splicing (DBHS) family consists of three RBPs: polypyrimidine tract-binding protein-associated splicing factor (PSF)/splicing factor proline/glutamine rich (SFPQ), non-POU domain-containing octamer-binding protein (NONO)/nuclear RNA-binding protein, 54 kDa ($p54^{nrb}$) and paraspeckle component 1 (PSPC1)/paraspeckle protein 1 (PSP1).

DBHS family proteins have been indicated to modulate gene regulation at posttranscriptional, transcriptional, and epigenetic levels. In addition, DBHS family proteins have been shown to modulate DNA damage response and homologous recombination [9]. Regarding MLO formation, DBHS proteins are core components of MLOs called paraspeckles, which are involved in transcriptional and posttranscriptional gene regulation [9–14]. DBHS family proteins are ubiquitously expressed and play important roles in normal physiology of some tissues, including neural tissues. Notably, recent studies have demonstrated that DBHS family proteins regulate cancer-specific gene expression to promote the progression in some cancers, suggesting that DBHS family proteins may be promising for diagnostic and therapeutic targets for cancers. Importantly, some recent studies have developed small molecules inhibiting DBHS family proteins and have examined their potential application in cancer therapy [15–19].

In this review, we briefly introduce the molecular mechanisms of DBHS family proteins, and then introduce the recent knowledges on the roles of DBHS family proteins in physiology and pathophysiology in diseases including cancers, particularly through gene regulation, and discuss the application of DBHS family proteins in clinical cancer management.

2. Molecular mechanisms of DBHS family proteins

Regarding the structures of DBHS family proteins, these proteins contain the conserved DBHS region, which is composed by tandem RNA recognition motifs (RRM1 and RRM2), a NonA/paraspeckle (NOPS) domain, and a C-terminal coiled-coil domain. The *N*- and C-terminal regions adjacent to the DBHS region are low-complexity regions (LCRs) in which a few types of amino acids are highly enriched and are predicted to be intrinsically disordered (Figure 1) [9,20]. Structural and biochemical analyses demonstrated that the formation of DBHS family protein homodimer and heterodimer is mediated by reciprocal interactions between the RRM2, NOPS and coiled-coil domains. The interaction surface is distributed extensively throughout the DBHS proteins, suggesting that these proteins act as obligatory heterodimer as well as homodimer. On the other hand, dimerization of DBHS family proteins is known as a dynamic phenomenon, and DBHS family

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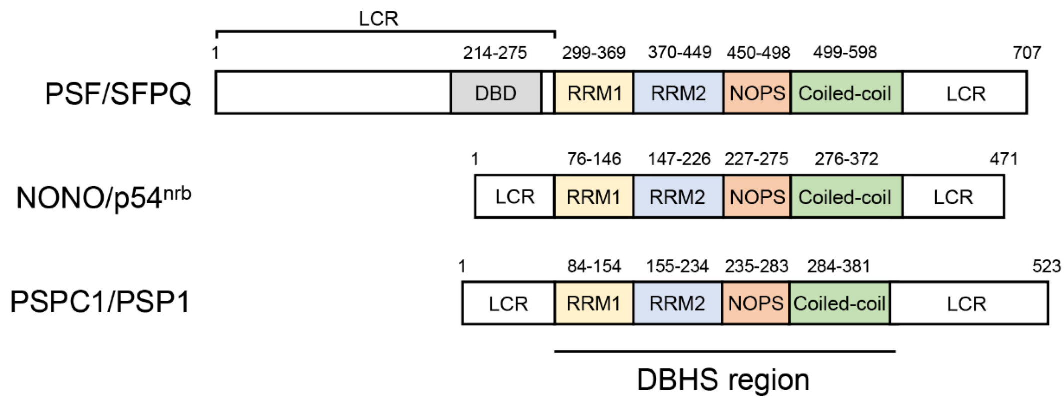

Figure 1. Representation of domain structures of human *Drosophila* behavior/human splicing (DBHS) family proteins. Human DBHS family consists of polypyrimidine tract-binding protein-associated splicing factor (PSF)/splicing factor proline/glutamine rich (SFPQ), non-POU domain-containing octamer-binding protein (NONO)/nuclear RNA-binding protein, 54 kDa ($p54^{nrb}$) and paraspeckle component 1 (PSPC1)/paraspeckle protein 1 (PSP1). DBHS family proteins contain the conserved DBHS region that is involved in RNA recognition and the formation of DBHS family protein homodimer and heterodimer. The *N*- and C-terminal regions adjacent to the DBHS region are low-complexity regions (LCRs) that are involved in the regulation of phase separation. DBD, DNA-binding domain; LCR, low-complexity region; NOPS, NonA/paraspeckle domain; RRM1, RNA recognition motif 1; RRM2, RNA recognition motif 2.

proteins can easily change the partner to form the different types of dimers. In addition, it is proposed that DBHS family proteins form oligomer through the interactions between coiled-coil domains, and drive phase separation, a key mechanism for biomolecule assembly, through LCRs [9,20–27].

DBHS family proteins play crucial roles in multiple steps of gene expression, including transcription, RNA splicing and editing, RNA transport, and translation. In addition, DBHS family proteins are involved in DNA damage response and homologous recombination (reviewed in Ref. 9). In this regard, a recent study proposed a novel mechanism of PSPC1 in transcriptional regulation. This study showed that PSPC1 drives phase separation through its C-terminal LCR, forming molecular condensates with the basal transcription factor TATA-binding protein (TBP) and RNA polymerase II. These condensates promote RNA polymerase II binding to transcription sites and its C-terminal domain (CTD) phosphorylation by cyclin-dependent kinases (CDKs). Subsequently, RNA polymerase II with a hyper-phosphorylated CTD dissociates from these condensates and participates in efficient transcription elongation [27]. Although this model is primarily based on *in vitro* biochemical experiments and needs to be validated by *in vivo* analyses, it highlights the significance of DBHS family proteins in gene expression.

DBHS proteins are also known as core components of MLOs called paraspeckles. Paraspeckles are nuclear MLOs observed in a small subpopulation of particular types cells in mammals [28,29]. Paraspeckle formation requires an architectural long noncoding RNA *nuclear enriched abundant transcript 1* (*NEAT1*) [30–32]. Alternative transcriptional termination generates a short isoform (*NEAT1_1*, 3.7 kb in humans) and a long isoform (*NEAT1_2*, 22.7 kb in humans) of *NEAT1*. *NEAT1_2*, not *NEAT1_1*, is indispensable for paraspeckle formation [33]. Functional analysis of *NEAT1_2* demonstrated that the middle domain of *NEAT1_2*, which contains multiple binding sites for NONO and SFPQ, is necessary and sufficient for the paraspeckle formation, and that the recruitment of NONO dimer to this domain of *NEAT1_2* is a key event for paraspeckle formation [34].

Phase separation is a driving force to form molecular condensates and MLOs [35,36]. For example, the liquid-liquid phase separation (LLPS), a type of phase separation, plays important roles in the formation of molecular condensates with non-ordered structures. On the other hand, paraspeckles have core-shell structures [37,38], and these structures are assembled through another type of phase separation called micellization [10]. More than 60 paraspeckle proteins are identified and include some RNA-binding proteins that contain the LCR [39,40]. Among them, RNA-binding motif 14 (RBM14) and fused in sarcoma (FUS) are demonstrated to drive phase separation through their LCRs, promoting paraspeckle formation [39]. Of note, a recent study revealed the roles of the LCRs of SFPQ in paraspeckle formation. This study showed that the C-terminal LCR of SFPQ promotes phase separation and is essential for paraspeckle formation, whereas the N-terminal LCR of SFPQ has the opposite effects. In addition, the C-terminal LCR, not N-terminal LCR, is shown to be required for the condensation of SFPQ at sites of DNA damage [20]. These results indicate that further characterization of LCRs will be important to elucidate phase separation-related functions of DBHS family proteins.

Regarding the functions of paraspeckles, paraspeckles act as molecular sponges that sequester multiple proteins and RNAs to regulate gene expression. For example, paraspeckles suppress the SFPQ functions as a transcriptional regulator by sequestering it [11,12]. Moreover, *Neat1* knockout mice exhibit the defects of mammary gland development [41], and nearly half of these mice show the impairment of corpus luteum formation [42], which may suggest the significance of paraspeckles in maternal reproduction system. However, some studies indicate that *Neat1* can exert paraspeckle-independent functions [43,44], pointing out that integrated analyses of multiple paraspeckle components are essential to elucidate the roles of paraspeckles.

3. Roles of DBHS family proteins in physiology and pathophysiology

3.1. Regulation of pre-mRNA splicing/RNA processing in cells and tissues

DBHS family proteins have been shown to play important roles in gene regulation in a number of normal cells and tissues (Figure 2, Table 1). In neural cells, SFPQ has been demonstrated as a key molecule in alternative splicing of pre-mRNAs. For example, SFPQ regulates alternative splicing of *microtubule associated protein tau* (*Mapt*) that encodes tau proteins. The exons 9, 10, 11, and 12 of human *MAPT* encode microtubule-binding repeat sequences, and the exclusion and inclusion of exon 10 produce 3-repeat tau (3 R-T) and 4-repeat tau (4 R-T), respectively. The 4 R-T/3 R-T ratio is tightly regulated and its imbalance is associated with tauopathy, a class of neurodegenerative diseases characterized by neuronal and/or glial tau-positive inclusions [45,64]. SFPQ has been shown to bind to a stem-loop structure at the 5' splice site downstream of *MAPT* exon 10 and promote the exclusion of this exon [45]. In

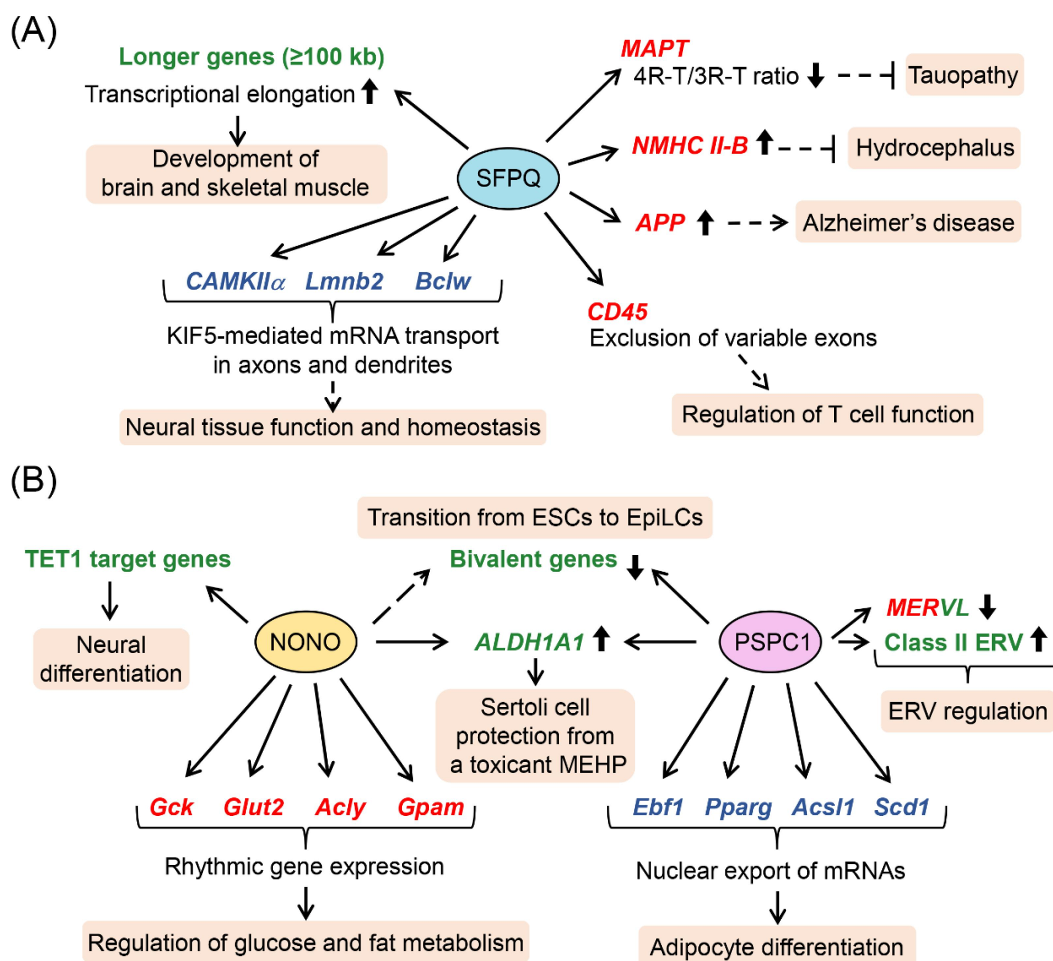

Figure 2. Schematic representation of roles of DBHS family proteins in cells and tissues. (a) polypyrimidine tract-binding protein-associated splicing factor (PSF)/ splicing factor proline/glutamine rich (SFPQ) regulates neural gene expression posttranscriptionally and transcriptionally. SFPQ-mediated gene regulation plays important roles in the development and homeostasis of neural tissues and cells and in the pathophysiology of neural diseases. SFPQ is also involved in the development of skeletal muscle and modulation of T cell function. (b) Non-POU domain-containing octamer-binding protein (NONO) posttranscriptionally regulates the rhythmic expression of glucose metabolism-related genes, such as *glucokinase* (*Gck*) and *glucose transporter type 2, liver* (*Glut2*), and fat metabolism genes, such as *ATP-citrate-lyase* (*Acly*) and *mitochondrial glycerol-3-phosphate acyltransferase* (*Gpam*), in liver, contributing to the homeostasis of glucose and fat metabolism. In addition, NONO promotes the ten-eleven translocation 1 (TET1)-mediated 5-hydroxymethylcytosine modification of neural genes, facilitating neural differentiation of embryonic stem cells (ESCs). Paraspeckle component 1 (PSPC1) enhances nuclear export of mRNAs associated with adipogenesis to promote adipocyte differentiation. In addition, PSPC1 forms a complex with a 5-methylcytosine (5mC) dioxygenase ten-eleven translocation 2 (TET2) and is involved in transcriptional and posttranscriptional regulation of endogenous retrovirus (ERV), such as *murine endogenous retrovirus-L* (*MERVL*) and class II ERVs. NONO and PSPC1 transcriptionally upregulate the expression level of *aldehyde dehydrogenase 1 family member a1* (*ALDH1A1*) to protect Sertoli cells from a toxicant mono-(2-ethylhexyl) phthalate (MEHP). NONO/PSPC1 complex is also suggested to be involved in epigenetic regulation of bivalent genes during the transition from embryonic stem cells (ESCs) to epiblast-like stem cells (EpiLCs). Posttranscriptional and transcriptional target genes of DBHS family proteins are shown in red and green, respectively. Genes regulated by DBHS family proteins during subcellular mRNA transport are shown in blue. *Acly*, *ATP-citrate-lyase*; *Acsl1*, *acyl-coenzyme a synthetase long chain family member 1*; *ALDH1A1*, *aldehyde dehydrogenase 1 family member a1*; *APP*, *amyloid-β precursor protein*; *Bclw*, *B-cell lymphoma-2* (*bcl-2*)*-like* 2; *CaMKIIa*, *calmodulin-dependent protein kinase II α*; *CD45*, *cluster of differentiation 45*; DBHS, *Drosophila* behavior and human splicing; *Ebf1*, *early B-cell factor 1*; EpiLCs, epiblast-like stem cells; ERV, endogenous retrovirus; ESCs, embryonic stem cells; *Gck*, *glucokinase*; *Glut2*, *glucose transporter type 2, liver*; *Gpam*, *mitochondrial glycerol-3-phosphate acyltransferase*; KIF5, kinesin family member 5; *Lmnb2*, *lamin B2*; *MAPT*, *microtubule associated protein tau*; MEHP, mono-(2-ethylhexyl) phthalate; *MERVL*, *murine endogenous retrovirus-L*; *NMHC II-B*, *nonmuscle myosin heavy chain II-B*; NONO, non-POU domain-containing octamer-binding protein; *Pparg*, *peroxisome proliferator activated receptor γ*; PSPC1, paraspeckle component 1; *Scd1*, *stearoyl-coenzyme A desaturase-1*; SFPQ, splicing factor proline/glutamine rich; 3R-T, 3-repeat tau; 4R-T, 4-repeat tau; TET1, ten-eleven translocation 1; TET2, ten-eleven translocation 2.

Table 1. Roles of DBHS family proteins in gene regulation in cells and tissues.

DBHS family protein	Target genes	Cell type	Function
Regulation of pre-mRNA splicing/RNA processing			
SFPQ	*MAPT*	Neural cells	Promotes exon 10 exclusion to decrease 4R-T/3R-T [45,46]
	APP	Neural cells	Posttranscriptionally upregulates APP levels [47,48]
	NMHC II-B	Neural cells	Promotes exon N30 inclusion of *NMHC II-B*, contributing to normal migration of facial neurons [49]
	CD45	T cells	Promotes exclusion of *CD45* variable exons, modulating TCR signalling [50,51]
NONO	glucose metabolism-related genes such as *Gck and Glut2*, and fat metabolism-related genes such as *Acly* and *Gpam*	Hepatic cells	Posttranscriptionally regulates the rhythmic expression of its target RNAs to modulate glucose and fat metabolism [52]
PSPC1	*MERVL*	ESCs	Posttranscriptionally downregulates the expression of *MERVL* through TET2-mediated 5-hydroxymethylcystosine modification of *MERVL* transcripts [53]
Transcriptional regulation			
PSPC1	*MERVL* and class II ERVs	ESCs	Epigenetically regulates *MERVL* and class II ERVs [53]
SFPQ	Longer genes (≥100 kb)	Neural cells Skeletal muscles	Promotes the transcriptional elongation on longer genes [54–56]
NONO	Genes associated with neural differentiation	ESCs	Promotes the recruitment of TET1 onto neural genes, increasing TET1-mediated 5-hydroxymethylcystosine modification of these genes [57]
NONO and PSPC1	Some bivalent genes, including *Eomes* and *Fgf5*	ESCs EpiLCs	Repress the transcription of some bivalent genes by promoting the recruitment of PRC2 to these gene promoters [58]
	ALDH1A1	Sertoli cells	Promote the transcription of *ALDH1A1* in Sertoli cells, contributing to survival of the cells treated with a toxicant MEHP [59]
Subcellular RNA transport			
SFPQ	*CaMKIIa*, *Lmnb2*, and *Bclw*	Neural cells	Modulates axonal transport of these gene transcripts [60–62]
PSPC1	Adipogenesis-related genes such as *Ebf1*, *Pparg*, *Acsl1*, and *Scd1*	Differentiating adipocytes	Facilitates nuclear export of target mRNAs to promote adipocyte differentiation [63]

Acly, ATP-citrate-lyase; Acsl1, acyl-coenzyme A synthetase long chain family member 1; ALDH1A1, aldehyde dehydrogenase 1 family member a1; APP, amyloid-β precursor protein; Bclw, B-cell lymphoma-2 (Bcl-2)-like 2; CaMKIIa, calmodulin-dependent protein kinase II α; CD45, cluster of differentiation 45; DBHS, *Drosophila behavior and human splicing; Ebf1, early B-cell factor 1; Eomes, eomesodermin*; EpiLCs, epiblast-like stem cells; ERV, endogenous retrovirus; ESCs, embryonic stem cells; *Fgf5, fibroblast growth factor 5; Gck, glucokinase; Glut2, glucose transporter type 2, liver; Gpam, mitochondrial glycerol-3-phosphate acyltransferase*; KIF5, kinesin family member 5; *Lmnb2, lamin B2; MAPT, microtubule associated protein tau*; MEHP, mono-(2-ethylhexyl) phthalate; *MERVL, murine endogenous retrovirus-L; NMHC II-B, nonmuscle myosin heavy chain II-B*; NONO, non-POU domain-containing octamer-binding protein; *Pparg, peroxisome proliferator activated receptor γ*; PRC2, polycomb repressive complex 2; PSPC1, paraspeckle component 1; *Scd1, stearoyl-coenzyme A desaturase-1*; SFPQ, splicing factor proline/glutamine rich; 3 R-T, 3-repeat tau; 4 R-T, 4-repeat tau; TCR, T cell receptor; TET1, ten-eleven translocation 1; TET2, ten-eleven translocation 2.

addition, it has been shown that SFPQ forms a complex with another splicing factor FUS to regulate the *MAPT* exon 10 exclusion. Hippocampus-specific SFPQ- or FUS-knockdown mice exhibited frontotemporal lobar degeneration (FTLD)-like behaviours, accumulation of phosphorylated tau in neural cells, reduced adult neurogenesis, and hippocampal atrophy accompanied with neuronal cell loss [46].

SFPQ has also been shown to posttranscriptionally upregulate the expression level of *amyloid-β precursor protein* (*APP*), a representative gene related to Alzheimer's disease (AD), in neural cells [47,48].

In addition, SFPQ exists in a complex with another splicing factor, RNA-binding feminizing locus on X protein 1 homolog 3 (Fox-3), or neuronal nuclei antigen (NeuN), which promotes the inclusion of exon N30 into *nonmuscle myosin heavy chain II-B* (*NMHC II-B*) mRNA. The exon N30-inserted isoform of *NMHC II-B* contains an insert of 10 amino acids near the ATP-binding region of the myosin heavy chain and regulates normal migration of facial neurons. Homozygous deletion of the exon N30 has been shown to cause abnormal facial neuron migration and accumulation of facial neurons under the ventricular surface near their origin, leading to hydrocephalus [49,65].

In T lymphocytes, SFPQ modulates alternative splicing of *cluster of differentiation 45* (*CD45*) that encodes a transmembrane tyrosine phosphatase involved in T cell receptor (TCR) signalling. In addition, the phosphorylation of threonine 687 (T687) in SFPQ has been implicated to affect the alternative splicing activity. In resting T cells, a kinase glycogen synthase kinase 3 (GSK3) phosphorylates SFPQ T687 to induce the SFPQ binding to thyroid hormone receptor-associated protein 3 (THRAP3), or thyroid hormone receptor-associated protein, 150 kDa subunit (TRAP150), competitively inhibiting RNA binding of SFPQ. In contrast, SFPQ is released from TRAP150 by reduced GSK3 activity in activated T cells, and promotes skipping of *CD45* variable exon, which decreases the phosphatase activity of CD45 and maintains T cell homoeostasis [50,51].

Meanwhile, it has been suggested that NONO posttranscriptionally regulates the rhythmic expression of its target mRNAs with the feeding/fasting cycle in mouse liver. In these organs, NONO binds primarily to intronic sequences proximal to promoters, and loss of NONO impairs rhythmic expression of mRNAs, not pre-mRNAs, of NONO target genes. Considering that NONO is involved in splicing of some pre-mRNAs, these results suggest that NONO-mediated pre-mRNA splicing may promote the rhythmic expression of these genes, and further studies are important to elucidate the mechanisms of NONO in rhythmic gene expression. NONO target genes in liver include glucose metabolism-related genes such as *glucokinase* (*Gck*) and *glucose transporter type 2, liver* (*Glut2*), and fat metabolism-related

genes such as *ATP-citrate-lyase* (*Acly*) and *mitochondrial glycerol-3-phosphate acyltransferase* (*Gpam*). Consistent with the roles of NONO target genes, NONO-deficient mice exhibit metabolic defects with impaired glucose tolerance, impaired triglycerides synthesis, and increased dependency on fat as an energy source during fasting. Impaired glucose tolerance is rescued by restored NONO expression in liver, suggesting the importance of NONO-mediated oscillations of gene expression in normal glucose homoeostasis [52].

In mouse embryonic stem cells (ESCs), PSPC1 is involved in the posttranscriptional regulation of endogenous retroviruses (ERVs). PSPC1 forms a complex with a 5-methylcytosine (5mC) dioxygenase ten-eleven translocation 2 (TET2) and mediates 5-hydroxymethylcytosine (5hmC) modification of *murine endogenous retrovirus-L* (*MERVL*) transcripts, destabilizing them. PSPC1/TET2 complex also modulates *MERVL* transcription through the recruitment of histone deacetylases onto *MERVL* loci, and activates the transcription of class II ERVs through 5hmC-mediated DNA demethylation. Consistent to these findings, silencing of *PSPC1* and *TET2* increases *MERVL* expression levels in early-stage mouse embryos. However, PSPC1 and TET2 are dispensable for early development and knockdown of these genes does not significantly delay embryonic development, at least until the blastocyst stage [53]. Further studies are required to clarify the physiological significance of PSPC1/TET2-mediated ERV regulation.

Regarding the roles of DBHS family proteins in posttranscriptional gene regulation as mentioned above, the mechanisms of DBHS family proteins have been analysed by the individual protein. Considering that the DBHS family proteins have been suggested to act as obligatory homodimer or heterodimer, it is important to analyse these mechanisms from the viewpoints of their homodimer and heterodimer.

3.2. Transcriptional and epigenetic gene regulation in cells and tissues

In the developing mammalian brain, extra-longer genes containing long introns (≥100 kb) are transcribed. SFPQ is essential for transcription of such longer genes and cotranscriptionally binds to long introns. SFPQ binds to cyclin-dependent kinase 9 (CDK9) that phosphorylates serine 2 of heptad repeats in CTD of RNA polymerase II, which promotes transcriptional elongation [54,55]. Homozygous knockout of SFPQ by the Nestin-Cre system resulted in the loss of most of the dorsal portion of the brain, including the cerebral cortex, in E18.5 mouse embryos [54]. In addition, SFPQ is required for transcriptional elongation of extra-long genes such as *Dystrophin* in skeletal muscles. In this tissue, SFPQ also affects the expression of energy metabolism pathway genes, and skeletal muscle-specific SFPQ deficiency causes decreased mitochondrial respiratory complexes and growth defects in skeletal muscles [56]. As SFPQ/NONO heterodimer is suggested to interact with CTD of RNA polymerase II [66], SFPQ and NONO may cooperatively promote transcriptional elongation of extra-long genes.

On the other hand, NONO has been demonstrated to be involved in epigenetic gene regulation for neural differentiation. By analyses using mouse ESCs, NONO has been shown to bind to 5mC dioxygenase TET1 and promote TET1-mediated 5hmC modification of neural genes, increasing the expression levels of these genes [57]. PSPC1 also forms a complex with TET1 and is involved in epigenetic gene regulation. During the transition from ESCs to epiblast-like stem cells (EpiLCs), PSPC1/TET1 complexes bind to some bivalent promoters marked by tri-methylation of histone H3 lysine 4 and lysine 27 (H3K4me3 and H3K27me3, respectively), including those of *eomesodermin* (*Eomes*) and *fibroblast growth factor 5* (*Fgf5*), and repress the expression of these bivalent genes. PSPC1/TET1 complexes mediate transcriptional repression by promoting the recruitment of a histone methyltransferase polycomb repressive complex 2 (PRC2) to these bivalent promoters, independent of the histone modification activity of TET1. The expression of these bivalent genes increases by NONO silencing, suggesting that NONO may be involved in this mechanism. On the other hand, *Neat1* silencing decreases the expression of these genes. *Neat1* silencing does not substantially affect PRC2 levels on these gene promoters, while it suppresses PRC2 binding to these gene transcripts, suggesting that *Neat1* may enhance these gene expression by facilitating transcripts-mediated dissociation of PRC2 from the promoters [58]. However, the precise mechanisms and the functional relationships of PSPC1, TET1, NONO, and *Neat1* remain unclear and require further analysis.

In Sertoli cells, testicular somatic cells essential for testis formation and spermatogenesis, NONO and PSPC1 are involved in a stress response through transcriptional regulation. It has been suggested that the abundance of NONO and PSPC1 in Sertoli cells is increased by treatment with the Sertoli cell toxicant mono-(2-ethylhexyl) phthalate (MEHP), and that these DBHS family proteins promote the transcription of *aldehyde dehydrogenase 1 family member a1* (*ALDH1A1*) to protect Sertoli cells from apoptotic cell death [59].

3.3. Subcellular RNA transport

DBHS family proteins have been demonstrated to modulate RNA transport involved in gene regulation. SFPQ plays crucial roles in subcellular RNA transport in neural cells. In neural cells, some mRNAs form complexes, or granules, with multiple proteins and are transported to axons and dendrites. These processes enable local translation of these mRNAs and establishment of local proteomes, contributing to functions and homoeostasis of highly polarized neural cells [5]. Microtubule-dependent motors, kinesins and dyneins, drive mRNA transport in axons and dendrites. A previous study showed that SFPQ is a component of RNA-containing granules associated with kinesin family member 5 (KIF5) proteins and modulates KIF5-mediated transport of *calmodulin-dependent protein kinase II α* (*CaMKIIα*) mRNA in dendrites [60]. Later, SFPQ was shown to bind to 3ʻ-untranslated regions (3ʻ-UTRs) of *lamin B2* (*Lmnb2)* and *B-cell lymphoma-2* (*Bcl-2*)*-like* 2 (*Bclw*) mRNAs and promote their axonal trafficking and local translation in distal axons, protecting against axonal degeneration [61]. Recently, it has been

proposed that the R208H mutation in KIF5A associated with Charcot–Marie–Tooth disease type 2 (CMT2), a hereditary neuropathy, induces axonal degeneration by disrupting the interaction of KIF5A with SFPQ and suppressing axonal *Bclw* mRNA transport [62]. NONO was also found as an interacting protein of KIF5 proteins [60], suggesting that SFPQ/NONO heterodimer may play roles in mRNA transport in neural cells.

Meanwhile, PSPC1 has been shown to promote nucleocytoplasmic export of mRNAs during adipogenesis. During adipocyte differentiation, PSPC1 expression level is increased and PSPC1 facilitates nuclear export of mRNAs transcribed from adipogenesis-related genes such as *early B-cell factor 1* (*Ebf1*), *peroxisome proliferator activated receptor γ* (*Pparg*), *acyl-coenzyme A synthetase long chain family member 1* (*Acsl1*), and *stearoyl-coenzyme A desaturase-1* (*Scd1*) [63]. Nuclear export of mRNA is generally a motor-independent process and requires nuclear export factors that allow mRNAs to diffuse through the nuclear pore complex to cytoplasm [4]. An RNA helicase DEAD-box helicase 3 X-linked (DDX3X) has suggested to act as a nuclear export factor for PSPC1 target mRNAs upon adipocyte differentiation. Interestingly, PSPC1 overexpression promotes adipocyte differentiation, while SFPQ and NONO overexpression hardly affects it [63], suggesting that PSPC1 homodimer may play important roles in nuclear export of adipogenesis-related mRNAs.

3.4. Cytoplasmic SFPQ mislocalization and stress granules

SFPQ is predominantly localized in the nucleus of normal neural cells while cytoplasmic SFPQ increases in neural cells in the brains of AD and FTLD patients. A familial FTLD-associated tau P301L mutant is partly involved in the alteration of subcellular SFPQ localization [67]. In addition, cytoplasmic SFPQ mislocalization has also been observed in motor neurons of amyotrophic lateral sclerosis (ALS) patients [68]. Multiple mutations of an RNA-binding protein TAR DNA-binding protein 43 (TDP-43) are linked to ALS, and a study using transgenic pigs expressing the human TDP-43 M337V mutant has shown that the TDP-43 M337V mutant partially induces cytoplasmic mislocalization of SFPQ [69]. In addition, a recent study has reported that SFPQ mutations (N553H and L534I) found in familial ALS patients partially increase cytoplasmic aggregates of SFPQ [70,71].

Interestingly, cytoplasmic SFPQ is reported to be located with membrane-less organelles called stress granules in the brains of AD patients [72]. Stress granules are composed of multiple RNAs and proteins, including translationally arrested mRNAs, translation-associated factors, and RNA-binding proteins, and are considered as organelles involved in mRNA metabolism and translational control. A recent study has indicated that long transcripts of some AD-associated genes are enriched in stress granules and the possibility that stress granules modulate the expression of these AD-associated genes [73]. In general, stress granules are formed in response to some stresses, such as oxidative stress and heat-shock stress, and are quickly disassembled when stress is removed. However, in neurons in diseased brain tissues with chronic stress, stress granules become persistent structures. Interestingly, stress granules have been shown to enhance the formation of insoluble tau aggregates, suggesting the roles of stress granules in progression of tauopathy, including AD [74,75]. In the brains of AD patients, SFPQ is colocalized with tau aggregates in stress granules, suggesting that SFPQ may be involved in stress granule-mediated tau aggregation [72]. However, whether cytoplasmic SFPQ is simply a cause of neurodegenerative diseases or is involved in the development and progression of the diseases remains to be elucidated. Further studies are required to elucidate the roles of SFPQ in the cytoplasm and stress granules of neural cells. Unlike SFPQ, cytoplasmic mislocalization of NONO and PSPC1 has not been observed in the brains of neurodegenerative disease patients [76], while a proteomic analysis has identified all DBHS family proteins as components of mammalian stress granules [77]. Thus, the roles of NONO and PSPC1 in neural stress granules would also be interesting issues to understand neural physiology and pathophysiology.

4. Multiple functions of DBHS family proteins in cancer pathophysiology

4.1. SFPQ- and NONO-fusion genes

DBHS family genes have been demonstrated to play important roles in cancer pathophysiology by multiple mechanisms (Table 2). Regarding *SFPQ* and *NONO*, these genes are reported to form fusion genes that may exert oncogenic functions. For example, in Xp11.2 translocation renal cell carcinoma (XP11.2 tRCC), a type of sporadic RCCs, *SFPQ* and *NONO* are found to form fusion genes with a MiT (microphthalmia transcription factor) family member gene, *transcription factor binding to IGHM enhancer 3* (*TFE3*) [100]. In these fusion genes, the N-terminal parts of *SFPQ* and *NONO* genes, including the region encoding the RRMs, are fused with the C-terminal regions of *TFE3*, including the sequences encoding the basic helix-loop-helix (bHLH) and leucine zipper (LZ) domains.

A recent study suggested a model in which *SFPQ-TFE3* fusion gene modulates the transcription of *insulin receptor substrate 1* (*IRS1*), activating the phosphatidylinositol-3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway associated with cell proliferation and survival [101].

Regarding the roles of *NONO-TFE3*, silencing of this fusion gene was indicated to suppress the colony formation of UOK109 XP11.2 tRCC cells on the soft agar [102]. In addition, recent studies suggested the roles of NONO-TFE3 in mitochondrial metabolism. For example, NONO-TFE3 forms a complex with another MiT family protein transcription factor EB (TFEB) and upregulates the transcription of *nicotinamide riboside kinase 2* (*NMRK2*), enhancing mitochondrial respiration in Xp11.2 tRCC cells [103]. Surprisingly, NONO-TFE3 increases *NMRK2* mRNA expression, but rather suppresses NMRK2 protein expression through a NONO-TFE3 target, long noncoding RNA *metastasis associated lung adenocarcinoma transcript 1* (*MALAT1*). In NONO-TFE3 tRCC, *NMRK2* mRNA act as a functional RNA, which interacts with miRNAs *miR-26b*

Table 2. Roles of DBHS family proteins in gene regulation in cancers.

DBHS family protein	Target genes	Cancer type	Function
Regulation of pre-mRNA splicing/RNA processing			
SFPQ, NONO	*AR*	Prostate cancer	Promote the splicing of *AR* to upregulate the expression levels of full-length AR and AR-V7 [78]
	Multiple spliceosome genes, including *SF1* and *SF3B2*	Prostate cancer	Posttranscriptionally upregulate the expression levels of some spliceosome genes [78]
	BIN1	Hepatocellular carcinoma	Promote exon 12a inclusion to produce BIN-L isoform [79]
	glycolysis-associated genes, including *PGK1* and *LDHA*	Hepatocellular carcinoma	Promote splicing of some glycolysis-related genes to enhance aerobic glycolysis [80]
	SETMAR	Bladder cancer	Promote exon 2 inclusion of *SETMAR* to produce SETMAR-L isoform [81]
	CASP9	Ovarian cancer	Modulate alternative splicing of *CASP9* to upregulate the abundance of CASP9b isoform [82]
SFPQ, PSPC1	*ESR1 and SCFD2*	ER-positive breast cancer	Posttranscriptionally upregulate the expression levels of *ESR1* and *SCFD2* [83,84]
SFPQ	*ASPM* and *TRA2B*	ER-positive breast cancer	Posttranscriptionally upregulates the expression levels of *ASPM* and *TRA2B* [83]
	SLC7A11	Glioblastoma	Promotes the splicing of *SLC7A11* to suppress ferroptosis [85]
NONO, PSPC1	*GPX1*	Glioblastoma	Promote the splicing of *GPX1* to suppress apoptosis [18]
NONO	*SKP2* and *E2F8*	Breast cancer	Posttranscriptionally upregulates the expression level of *SKP2* and *E2F8* [86]
	STAT3	Breast cancer	Binds to and Increases *STAT3* mRNA [17]
	MycN	Neuroblastoma	Posttranscriptionally upregulates the expression level of *MycN* [87]
	GATA2 and *HAND2*	Neuroblastoma	Promotes the splicing of *GATA2* and *HAND2* [88]
	HIF-1/2 target genes	Hepatocellular carcinoma	Posttranscriptionally modulates the expression level of HIF-1/2 target genes [89]
Transcriptional and epigenetic gene regulation			
SFPQ, NONO	Some genes including *CTBP1* and *p53*	Prostate cancer	Repress the target gene transcription by promoting the histone H3 deacetylation [90]
SFPQ	*CXCL8*	Glioblastoma	Suppresses the transcription of *CXCL8* [91]
	FOSL	Nasopharyngeal cancer	Activates the transcription of *FOSL1* [92]
NONO	TAZ target genes	Glioblastoma	Promotes TAZ-mediated transcriptional gene regulation [93]
	HIF-1/2 target genes	Hepatocellular carcinoma	Promotes HIF-1/2-mediated transcriptional gene regulation [89]
	Ets-1	Gastric cancer	Upregulates the transcription of *Ets-1* [94]
PSPC1	EMT- and cell stemness-related genes, including *Snail* and *Nanog*	Breast cancer Hepatocellular carcinoma Lung cancer	Forms a complex with Smad2/3 and modulates the recruitment of Smad2/3 to target genes [95]
	β-catenin target genes	Hepatocellular carcinoma	Promotes β-catenin-mediated transcriptional gene regulation [96]
Regulation of IRES-mediated translation			
SFPQ	*CK1α*	HCT-116 colorectal cancer cells	Promotes the IRES activity of *CK1α* [97]
SFPQ	*RUNX2* and *VEGFA*	Colorectal cancer	Suppresses the IRES activity of *RUNX2* by sequestering PTBP2 [98]
SFPQ	*c-Myc*	Cervical cancer	Suppresses the IRES activity of *c-Myc* by sequestering PTB [99]

AR, androgen receptor; ASPM, assembly factor for spindle microtubules; BIN1, bridging integrator 1; CASP9, caspase 9; CK1α, casein kinase 1 α; CRPC, castration-resistant prostate cancer; *CTBP1, C-terminal binding protein 1; CXCL8, C-X-C motif chemokine ligand 8*; DBHS, *Drosophila behavior and human splicing; E2F8, E2F transcription factor 8*; ER, oestrogen receptor; *ESR1, estrogen receptor 1; Ets-1, v-ets erythroblastosis virus E26 oncogene homolog 1; FOSL1, FOS like antigen 1; GPX1, glutathione peroxidase 1*; HIF-1/2, hypoxia-inducible factor-1/2; IRES, internal ribosome entry site; *LDHA, lactate dehydrogenase A*; NONO, non-POU domain-containing octamer-binding protein; *Oct4, octamer-binding protein 4; PGK1, phosphoglycerate kinase 1*; PSPC1, paraspeckle component 1; PTB, polypyrimidine tract-binding protein; PTBP2, polypyrimidine tract-binding protein 2; *RUNX2, runt-related transcription factor 2; SCFD2, sec1 family domain-containing 2; SETMAR, SET domain and mariner transposase fusion protein; SF1, splicing factor 1; SF3B2, splicing factor 3b subunit 2*; SFPQ, splicing factor proline/glutamine rich; *SKP2, S-phase-associated kinase 2; SLC7A11, solute carrier family 7 member 11*; Smad2/3, Sma- and Mad-related protein 2/3; *Sox2, SRY-box transcription factor 2; STAT3, signal transducer and activator of transcription 3*; TAZ, transcription factor PDZ-binding motif; *TGFB1, transforming growth factor-β1*; TNBC, triple-negative breast cancer; *VEGFA, vascular endothelial growth factor A*.

and *miR-181a*, long noncoding RNA *growth arrest-specific 5* (*GAS*), and Hsp10 proteins to regulate their functions, promoting mitochondrial respiration and energy production [104].

NONO-TFE3 was also suggested to increase mitochondrial biosynthesis and metabolism by forming the positive feedback loop with nuclear respiratory factor 1 (NRF1), a key transcription factor for metabolic genes [105]. Meanwhile, another study suggested that NONO-TFE3 promotes aerobic glycolysis by modulating *hypoxia-inducible factor 1A* (*HIF1A*) [106]. Further studies are thus required to better understand the roles of *NONO-TFE3* in metabolic reprogramming.

Besides RCC, *SFPQ-TFE3* fusion genes are also found in the Xp11 translocation perivascular epithelioid cell neoplasms (PEComas) [100,107,108], whereas *NONO-TFE3* fusion genes are found in the Xp11 translocation PEComas and cutaneous microcystic/reticular schwannomas [108,109].

In addition, *SFPQ* forms fusion genes with *Abelson tyrosine-protein kinase 1* (*ABL1*) in tumours of B-cell acute lymphoblastic leukaemia (B-cell ALL) [110,111]. *SFPQ-ABL1* overexpression enhances the sensitivity to some anticancer reagents, including tyrosine kinase inhibitors (TKIs) and apoptosis-inducing Bcl-2 homology 3 (BH3) mimetics [112], suggesting the potential roles of *SFPQ-ABL1* in diagnosis and therapy of B-cell ALL.

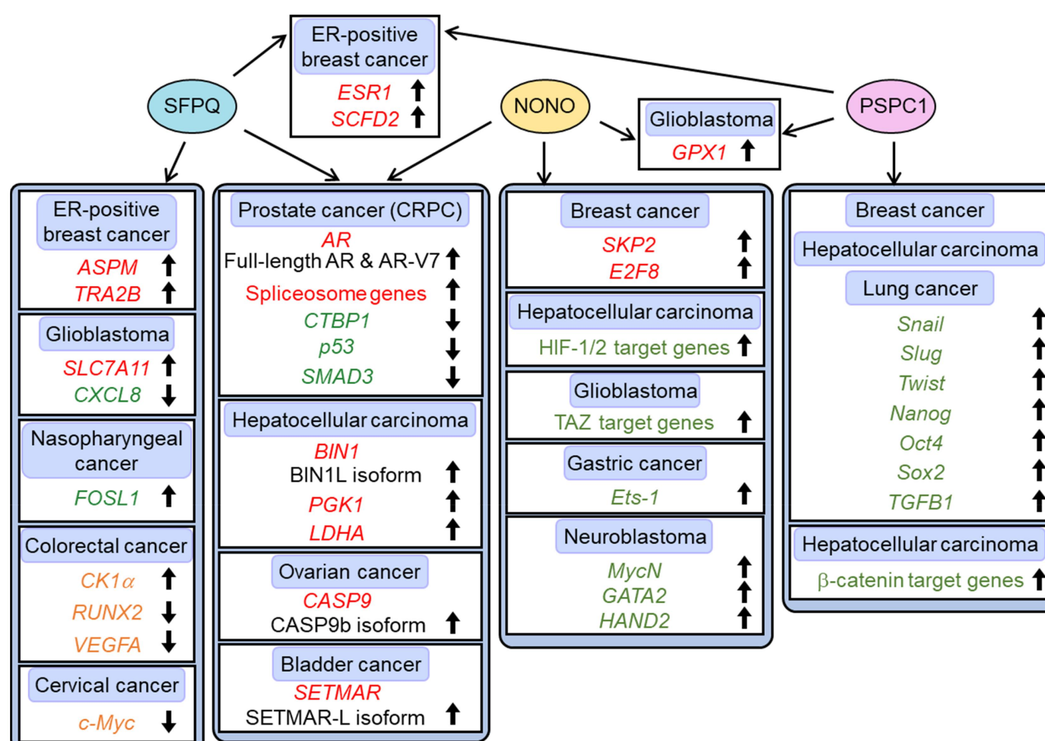

Figure 3. Diagram of gene regulation by DBHS family proteins in cancers. Posttranscriptional and transcriptional target genes of DBHS family proteins are shown in red and green, respectively. Genes regulated by DBHS family proteins during the internal ribosome entry site (IRES)-mediated translation are shown in orange. *AR, androgen receptor; ASPM, assembly factor for spindle microtubules; BIN1, bridging integrator 1; CASP9, caspase 9; CK1a, casein kinase 1 a;* CRPC, castration-resistant prostate cancer; *CTBP1, C-terminal binding protein 1; CXCL8, C-X-C motif chemokine ligand 8; E2F8, E2F transcription factor 8;* ER, oestrogen receptor; *ESR1, estrogen receptor 1; Ets-1, v-ets erythroblastosis virus E26 oncogene homolog 1; FOSL1, FOS like antigen 1; GATA2, GATA-binding protein 2; GPX1, glutathione peroxidase 1; HAND2, heart and neural crest derivatives expressed 2;* HIF-1/2, hypoxia-inducible factor-1/2; *LDHA, lactate dehydrogenase A; MycN, neuroblastoma MYC oncogene*; NONO, non-POU domain-containing octamer-binding protein; *Oct4, octamer-binding protein 4; PGK1, phosphoglycerate kinase 1;* PSPC1, paraspeckle component 1; *RUNX2, runt-related transcription factor 2; SCFD2, sec1 family domain-containing 2; SETMAR, SET domain and mariner transposase fusion protein*; SFPQ, splicing factor proline/glutamine rich; *SKP2, S-phase-associated kinase 2; SLC7A11, solute carrier family 7 member 11; SMAD3, Sma- and Mad-related protein 3; Sox2, SRY-box transcription factor 2; STAT3, signal transducer and activator of transcription 3*; TAZ, transcription factor PDZ-binding motif; *TGFB1, transforming growth factor-β1*; TNBC, triple-negative breast cancer; *VEGFA, vascular endothelial growth factor A*.

4.2. Regulation of cancer-specific pre-mRNA splicing/RNA processing

DBHS proteins have been shown to regulate cancer-specific splicing and RNA processing of target RNAs (Figure 3, Table 2). For example, SFPQ and NONO promote the progression of castration-resistant prostate cancer (CRPC), a type of hormone-refractory prostate cancer, by regulating pre-mRNA splicing of androgen receptor (AR). AR is a member of nuclear hormone receptor superfamily and functions as a ligand-dependent transcription factor. The full-length AR protein consists of four core domains: the N-terminal domain (NTD), the DNA-binding domain (DBD), the hinge region, and the C-terminal ligand-binding domain (LBD). Alternative splicing of *AR* pre-mRNA produces a splicing variant called AR-V7 that lacks the hinge region and LBD. AR-V7 is a constitutively active AR isoform which contributes to the progression of CRPC [113,114]. In CRPC cells, SFPQ and NONO regulate the splicing of *AR* to increase the full-length AR and AR-V7 proteins. Moreover, SFPQ and NONO posttranscriptionally upregulate the expression levels of multiple spliceosome genes such as *splicing factor 1* (*SF1*) and *splicing factor 3b subunit 2* (*SF3B2*). These results suggest that SFPQ/NONO complex contributes to the establishment of an aberrant splicing profile to promote CRPC progression [78].

In hepatocellular carcinoma cells, SFPQ and NONO have been indicated to regulate alternative splicing of *bridging integrator 1* (*BIN1*). Two BIN isoforms, BIN1-S and BIN1-L, are produced by exclusion and inclusion of exon 12a, respectively, and BIN1-S is predominantly produced and suppresses the transcriptional activity of c-Myc in normal liver tissues. On the other hand, in hepatocellular carcinoma cells, SFPQ, NONO, and DExH-box helicase (DHX9) promote the inclusion of exon 12a and increase the expression of BIN1-L isoform. BIN1-L interacts with polo-like kinase 1 (PLK1) to increase its stability, promoting the progression of hepatocellular carcinoma [79]. In addition, SFPQ/NONO complex is shown to regulate pre-mRNA splicing of glycolysis-associated genes such as *phosphoglycerate kinase 1* (*PGK1*) and *lactate dehydrogenase A* (*LDHA*), and enhance aerobic glycolysis in hepatocellular carcinoma [80].

In some cancers, SFPQ and NONO have been shown to exert tumour-suppressive roles. For instance, SFPQ and NONO regulate alternative splicing of *SET domain and mariner transposase fusion protein* (*SETMAR*) in bladder cancer cells. SETMAR is a histone methylase and its splice isoforms are produced by the alternative splicing of exon 2 [115]. SFPQ

and NONO promote the inclusion of exon 2 to produce SETMAR-L isoform to induce the SETMAR-L-mediated repression of metastasis-associated genes, including *peroxiredoxin 4* (*PRDX4*), *SET domain-containing 7* (*SETD7*), and *glucosidase II α subunit* (*GANAB*), suppressing bladder cancer metastasis [81].

Furthermore, SFPQ and NONO are also indicated to modulate alternative splicing through protein–protein interaction rather than RNA-binding. Caspase 9 (CASP9) is an initiator caspase that proteolytically cleaves and activates effector caspases that cleave multiple target proteins to execute apoptosis [116]. In epithelial ovarian cancer cells, such as MDAH and OVCAR8, SFPQ complexed with NONO binds to a splicing factor, serine and arginine rich splicing factor 2 (SRSF2) and prevents SRSF2 recruitment to *CASP9* transcripts. It enhances the skipping of exons 3–6 to produce anti-apoptotic isoform CASP9b, increasing resistance to platinum treatment [82].

Meanwhile, the posttranscriptional gene regulation by SFPQ/PSPC1 complex plays crucial roles in breast cancer pathophysiology. Most of breast cancers express a nuclear hormone receptor, oestrogen receptor (ER), which is an oestrogen-dependent transcription factor to promote the progression of ER-positive breast cancer [117]. It is notably demonstrated that SFPQ posttranscriptionally upregulates the expression of *estrogen receptor 1* (*ESR1*), which encodes an ER family protein ERα. In addition, *sec1 family domain-containing 2* (*SCFD2*), *assembly factor for spindle microtubules* (*ASPM*), and *transformer 2 β homolog* (*TRA2B*) genes are identified as SFPQ target and their expression levels are also posttranscriptionally increased by SFPQ [83]. A recent study showed that PSPC1 forms a complex with SFPQ and contributes to the posttranscriptional regulation of *ESR1* and *SCFD2* in breast cancer cells [84]. Immunohistochemical analyses show that combinations of SFPQ/PSPC1/SCFD2 immunoreactivities are potently associated with poor prognosis in ER-positive breast cancer patients [84]. In addition, SCFD2 enhances the resistance to antiestrogenic drug, 4-hydroxy tamoxifen (4-OHT) in ER-positive breast cancer cells and promotes 4-OHT-resistant breast cancer tumour growth in mouse xenograft models [83,84]. These findings suggest that SFPQ/PSPC1/SCFD2 can be a promising diagnostic and therapeutic target in antiestrogen-resistant ER-positive breast cancer.

In addition, SFPQ is involved in splicing of a ferroptosis-related gene, *solute carrier family 7 member 11* (*SLC7A11*). Ferroptosis is a programmed cell death triggered by phospholipid peroxidation and regulated by cellular redox systems [118]. Analysis using U87MG and U251 glioblastoma cells demonstrated that an RNA-binding protein nuclear factor κB-activating protein (NKAP) binds to the N^6-methyladenosine (m^6A) -containing site of *SLC7A11* transcripts and recruits SFPQ to promote the splicing of *SLC7A11* [85]. SLC7A11 acts as a cystine/glutamate antiporter to promote the synthesis of reduced glutathione (GSH). GSH is converted to oxidized glutathione (GSSG) under the catalytic action of glutathione peroxidase 4 (GPX4) to neutralize the oxidative substances in the cell membrane, which suppresses ferroptosis [119]. The roles of SFPQ homodimer and heterodimer in this splicing event remain unclear and require further studies.

As for NONO/PSPC1 complex, it has been shown to suppress apoptotic cell death by modulating the splicing of an antioxidant gene, *glutathione peroxidase 1* (*GPX1*). Consistently, NONO silencing increases reactive oxygen species (ROS) level and induces apoptotic cell death in U251 and P3 glioblastoma cells [18].

NONO is also implicated in posttranscriptional gene regulation in breast cancer and neuroblastoma cells. For example, NONO posttranscriptionally regulates *S-phase-associated kinase 2* (*SKP2*) and *E2F transcription factor 8* (*E2F8*) in ER-positive breast cancer MCF-7 cells and triple-negative breast cancer MDA-MB-231 cells that are negative for progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)/ErbB2 along with ER [86]. SKP2 is an E3 ubiquitin ligase involved in degradation of cyclin-dependent kinase (CDK) inhibitor p27. p27 is a cell cycle regulator to prevent the activation of cyclin E/CDK2, cyclin D/CDK4, and cyclin D/CDK6 complexes, which promotes cell cycle arrest in the G1 phase [120,121]. E2F8 belongs to the E2F family and regulates the expression of cyclin E1 and cyclin E2, both of which form complexes with CDK2 to promote G1/S transition of cell cycle [122]. Consistent with functions of these genes, NONO silencing suppresses G1/S transition of cell cycle and cell proliferation. Based on immunohistochemical analyses, NONO immunoreactivity is significantly associated with poor prognosis in patients with breast cancer [86]. In addition, *signal transducer and activator of transcription 3* (*STAT3*) is identified as NONO target in triple-negative breast cancer cells. NONO binds to *STAT3* mRNA to increase *STAT3* mRNA abundance, and also interacts with STAT3 proteins to increase the stability of these proteins. NONO-mediated STAT3 upregulation is suggested to promote cell stemness and the resistance to an anticancer drug doxorubicin [17]. In neuroblastoma cells, NONO forms a complex with *long non-coding upstream neuroblastoma MYC oncogene* (*lncUSMycN*), a long non-coding RNA whose gene locus is located upstream of the *neuroblastoma MYC oncogene* (*MycN*) locus, and posttranscriptionally upregulates *MycN* expression [87]. In addition, a recent study showed that NONO is involved in pre-mRNA splicing of *GATA-binding protein 2* (*GATA2*) and *heart and neural crest derivatives expressed 2* (*HAND2*) that encode super-enhancer-related transcription factors controlling the gene expression programme of neuroblastoma [88,123,124]. NONO LCRs as well as RRM1 are required for these splicing events, suggesting the significance of NONO-mediated phase separation in splicing of some pre-mRNAs. To clarify the detailed functions and mechanisms of NONO in neuroblastoma cells, it will be important to elucidate the role of other DBHS family proteins in these cells.

4.3. Transcriptional and epigenetic gene regulation in cancers

DBHS family proteins regulate transcriptional events by multiple mechanisms. For example, SFPQ and NONO have been shown to epigenetically regulate transcription through long

noncoding RNA-protein complex formation. In prostate cancer cells, an AR-induced long noncoding RNA *C-terminal binding protein 1-antisense* (*CTBP1-AS*) forms a complex with SFPQ, NONO, and histone deacetylases. By promoting the histone H3 deacetylation, *CTBP1-AS*/SFPQ/NONO complex is involved in transcriptional repression of multiple genes, including AR corepressor *C-terminal binding protein 1* (*CTBP1*), cell cycle regulator *p53*, and transcription factor *Sma- and Mad-related protein 3* (*SMAD3*), contributing to prostate cancer progression [90].

SFPQ has been indicated to act as a transcriptional factor through DNA-binding activity. For example, SFPQ has been shown to bind to the promoter of *C-X-C motif chemokine ligand 8* (*CXCL8*)/*interleukin 8* (*IL8*) genes, repressing *CXCL8* transcription. The transcriptional repressor function of SFPQ is regulated by *NEAT1* RNA [11,12]. Mechanistically, *NEAT1* derepresses *CXCL8* expression by sequestering SFPQ to paraspeckle and inhibiting SFPQ binding to the *CXCL8* promoter [11,12]. Recently, it has been indicated that *NEAT1* expression level is modulated by m^{6}A modification. In U87 and GL261 glioblastoma cells, an m^{6}A demethylase, AlkB homolog 5, RNA demethylase (ALKBH5), is induced by hypoxic stress. ALKBH5 erases m^{6}A deposition from *NEAT1* and upregulates the stability of *NEAT1*. Resultant increase of *NEAT1* abundance promotes paraspeckle formation and relocation of SFPQ from the *CXCL8* promoter to paraspeckles, which enhances CXCL8 expression. CXCL8 then facilitates tumour-associated macrophage (TAM) recruitment and the establishment of immunosuppressive microenvironment, contributing to glioblastoma progression [91]. Intriguingly, a recent study has reported that PSPC1 is a regulatory protein of ALKBH5. ALKBH5 is acetylated at lysine 235 (K235) by lysine acetyltransferase 8 (KAT8), which promotes the binding of ALKBH5 to RNA m^{6}A. PSPC1 preferentially binds to K235-acetylated ALKBH5 and further facilitates the recognition of RNA m^{6}A by ALKBH5 [125]. Thus, PSPC1 may play a role of SFPQ-mediated transcriptional regulation through modulating ALKBH5/*NEAT1* axis. It has also been reported that ALKBH5 and PSPC1 are colocalized in nuclear domains called nuclear speckles, or interchromatin granules, enriched in splicing factors, including SRSF2 [126]. This result was obtained from immunostaining experiments using *ALKBH5* knockout HeLa cells stably reexpressing FLAG-tagged ALKBH5. However, PSPC1 is a component of paraspeckles, independent domains of nuclear speckles. Moreover, given that the m^{6}A demethylation activity of ALKBH5 has been suggested to influence the proper assembly of nuclear speckles [126], it is expected that ALKBH5 may influence the properties of paraspeckles.

On the other hand, SFPQ-mediated transcriptional activation has been reported to play roles in cancer pathophysiology. For example, it has been shown that SFPQ binds to the promoter of *FOS like antigen 1* (*FOSL1*) to activate its transcription in HK1 and SUNE1 nasopharyngeal cancer cells. FOSL1 is a transcription factor of FOS family and has been indicated to promote nasopharyngeal cancer cell proliferation and invasion [127,128]. An AR-regulated long noncoding RNA *long intergenic noncoding RNA 01503* (*LINC01503*) has been indicated to promote SFPQ recruitment to *FOSL1* promoter. Silencing of *LINC01503* suppresses growth and migration of HK1 and SUNE1 cells and these defects can be rescued by *FOSL1* overexpression. In addition, silencing of *LINC01503* suppresses tumour growth and metastasis of nasopharyngeal cancer xenograft mouse models, suggesting the importance of *LINC01503*/SFPQ/FOSL1 axis in nasopharyngeal cancer progression [92].

NONO has also been shown to form multiple complexes with transcription factors to mediate transcriptional regulation. For example, in glioblastoma cells, NONO is involved in the transcriptional regulation by transcription factor PDZ-binding motif (TAZ)/WW domain containing transcription regulator 1 (WWTR1). Mechanistically, NONO interacts with TAZ to facilitate its recruitment to transcriptional enhancer regions, TAZ-TEA domain transcription factor (TEAD) complex formation, and TAZ-mediated LLPS that condensates cofactors to activate transcription. Based on Chinese Glioma Genome Atlas (CGGA) dataset, higher NONO expression is significantly correlated with shorter survival in glioblastoma patients, and higher TAZ expression is also significantly associated with poor prognosis in these patients. In addition, NONO silencing prolonged overall survival in mice with intracranially implanted LN229 glioblastoma cells stably transduced constitutively active TAZ^{4SA} mutant, suggesting that NONO-TAZ axis may be applicable to clinical management of glioblastoma [93]. In hepatocellular carcinoma cells, NONO regulates hypoxia-inducible factor-1 (HIF-1) and HIF-2 complexes [89]. NONO binds to HIF-1α, HIF-1β, and HIF-2 transcription factors, and enhances the interactions between HIF-1α and HIF-1β and between HIF-2 and p300 histone acetyltransferase, modulating transcriptional activity of HIF-1 and HIF-2 complexes. NONO also binds to transcripts of HIF-1/2 target genes and regulates these gene expression posttranscriptionally. From analysis using HepG2 and SMMC-7721 hepatocellular carcinoma cells, NONO knockout downregulates hypoxia-induced enhancement of cell proliferation and migration. Sorafenib is a multi-kinase inhibitor used to treat hepatocellular carcinoma and has been considered to induce hypoxic microenvironment by suppressing angiogenesis. Sorafenib-induced hypoxia has been suggested to be involved in sorafenib resistance through HIF pathways [129]. Importantly, the combination of NONO knockout and sorafenib treatment cooperatively inhibits tumour growth in mouse models of hepatocellular carcinoma, suggesting that NONO may be a therapeutic target for hepatocellular carcinoma with sorafenib resistance [89].

From analysis using gastric cancer cells, including MKN-45 and NCI-N87 cells, it has been demonstrated that NONO is involved in transcriptional regulation of v-ets erythroblastosis virus E26 oncogene homolog 1 (Ets-1), a transcription factor of the E26 transformation-specific (Ets) family. Ets-1 has been shown to promote growth and metastasis of multiple cancers, including gastric cancer. In gastric cancer, Ets-1 expression is upregulated by *Ets-1 promoter-associated noncoding RNA (pancEts-1)/Ets1-antisense RNA 1* (*ETS1-AS1*) that is transcribed from the proximal region of *Ets-1* locus. The experiments using MKN-45 and NCI-N87 gastric cancer cells have demonstrated that *pancEts-1* forms a transcriptional complex with NONO and another Ets family protein v-ets

erythroblastosis virus E26 oncogene homolog (ERG), which activates the Ets-1 transcription [94]. Interestingly, ERG is known to modulate growth and epithelial-mesenchymal transition (EMT) of cancer cells by regulating other genes than Ets-1. For example, in leukaemia cells, ERG transcriptionally upregulates the expression level of proviral integration site for moloney murine leukaemia virus-1 (*Pim-1*) that encodes a serine/threonine kinase, promoting cell proliferation and survival [130]. In prostate cancer cells, ERG induces the EMT by regulating the expression of a WNT receptor frizzled-4 (FZD4) [131]. Thus, comprehensive research on *pancEts-1*/NONO/ERG complex target genes may be useful to better understanding of gastric cancer pathophysiology and management.

Regarding roles of PSPC1 in transcriptional regulation in cancers, it has been reported that PSPC1 forms a complex with Smad2/3 transcription factors to control transcription of stemness and EMT-related genes in breast cancer, hepatocellular carcinoma, and lung cancer cells [95]. Smad2/3 are mediators of transforming growth factor-β (TGF-β) signalling, and PSPC1-Smad2/3 interaction is upregulated by TGF-β signalling. Inversely, PSPC1-Smad2/3 complex augments the expression of a TGF-β family ligand transforming growth factor-β1 (TGFB1), forming TGFB1 autocrine signalling. Interestingly, PSPC1 regulates the recruitment of Smad2/3 to target genes; under conditions of low PSPC1 expression levels, Smad2/3 enhances the expression of tumour suppressor genes such as *p21*, *p57*, and *death associated protein kinase 1* (*DAPK1*), while high PSPC1 abundance relocates Smad2/3 to promoters of EMT-related genes such as *Snail*, *Slug*, and those of cell stemness-related genes such as *Nanog*, *POU class 5 homeobox 1* (*POU5F1*)/*octamer-binding protein 4* (*Oct4*), and *SRY-box transcription factor 2* (*Sox2*). Consistent to these results, PSPC1 modulates growth and metastasis in breast cancer, hepatocellular carcinoma, and lung cancer xenografted in immunodeficient mice, respectively.

In addition, PSPC1 interacts with another transcription factor β-catenin to promote its transactivation function, which promotes EMT and cell stemness of hepatocellular carcinoma cells. PSPC1 also binds to and sequesters phosphorylated protein tyrosine kinase 6 (PTK6) at tyrosine 342 in the nucleus of hepatocellular carcinoma cells, which suppresses oncogenic PSPC1 functions [96]. Nuclear PTK6 has been indicated to exert tumour suppressive roles via phosphorylation of some RNA-binding proteins, including Src-associated in mitosis 68 kDa Protein (Sam68) and SFPQ. PTK6-mediated tyrosine phosphorylation induces the cytoplasmic localization of these RNA-binding proteins, leading to cell cycle arrest in BT-20 breast cancer cells [132]. Meanwhile, cytoplasmic PTK6 has been shown to facilitate migration and invasion of A431 skin squamous cell carcinoma cells and MDA-MB-231 cells [133]. Hepatic growth factor (HGF) stimulation promotes PSPC1-β-catenin interaction but suppresses PSPC1-PTK6 interaction, which facilitates nuclear location of β-catenin and cytoplasmic location of phosphorylated PTK6, enhancing cell mobility. Overexpression of PTK6-unbound PSPC1 mutant (PSPC1-Y523F) has similar effects on subcellular localization of β-catenin and phosphorylated PTK6, promoting tumour growth and metastasis of SK-hep1 hepatocellular carcinoma cells xenografted in immunodeficient mice. Importantly, overexpression of PSPC1 C-terminal 131 polypeptide (PSPC1-CT131) that binds to both PSPC1 and PTK6 inhibits tumour progression and metastasis in hepatocellular carcinoma xenograft mouse models through modulating nucleocytoplasmic shuttling of β-catenin and PTK6, suggesting PSPC1/PTK6 interaction may be promising target of hepatocellular carcinoma treatment [96].

4.4. Regulation of internal ribosome entry site (IRES)-mediated translation

Gene transcripts undergo cotranscriptional processing into mature mRNAs. For example, the 7-methylguanosine (m^7G) cap structures are cotranscriptionally added to the 5ʻ ends of mRNAs by a series of enzymes, including mRNA guanylyltransferases [134]. In general, translational initiation is cap-dependent. On the other hand, some mRNAs have been reported to have structures called internal ribosome entry sites (IRESs) that mediate cap-independent translation initiation, and IRES-mediated translation has been suggested to play roles in cancer pathophysiology [135,136]. Recently, it has been reported that the regulation of IRES activity by DBHS family proteins, especially SFPQ, is associated with cancer pathogenesis. For example, SFPQ has been indicated to modulate oncogenic rat sarcoma viral oncogene homolog (RAS) signalling by regulating an IRES activity of casein kinase 1α (CK1α). CK1α protein abundance is upregulated by oncogenic RAS signalling, and in turn CK1α phosphorylates and decreases forkhead box O3A (FOXO3A) protein, a key effector of autophagy, suppressing RAS-mediated autophagy and tumorigenesis. A CK1 inhibitor D4476 and autophagy inhibiting reagent chloroquine synergistically suppress *in vivo* tumor growth of HCT-116 colorectal cancer cells [KRAS (WT/G13D)], suggesting a potential role of CK1α in treatment of cancers with mutant RAS [137]. A recent study has demonstrated that CK1α expression is regulated by an IRES in the 5ʻ-UTR of CK1α mRNA. From analysis using HCT-116 cells, SFPQ and PSPC1 were identified as interacting proteins of this IRES, and SFPQ knockdown decreases the CK1α protein abundance, which suggests that SFPQ activates the IRES-dependent translation of CK1α [97]. SFPQ silencing suppresses *in vitro* proliferation of HCT-116 cells *via* KRAS mutation-dependent manner, and exogenous CK1α overexpression rescues this growth defect. These results suggest that SFPQ-CK1α axis may be promising therapeutic target of cancers with mutant RAS. Further experiments are required to elucidate the roles of SFPQ-CK1α axis in other RAS mutant cancer cell than HCT-116 and *in vivo* significance of SFPQ-CK1α axis. It would be of great significance to verify the roles of SFPQ/PSPC1 heterodimer in SFPQ-CK1α axis.

Meanwhile, it has been reported that SFPQ exerts a suppressive role in IRES activity by protein–protein interactions rather than binding to IRES. In colorectal cancer, a long noncoding RNA *metastasis associated lung*

adenocarcinoma transcript 1 (*MALAT1*) binds to SFPQ and releases an RNA-binding protein polypyrimidine tract-binding protein 2 (PTBP2) from SFPQ/PTBP2 complex, and SFPQ-detached PTBP2 promotes cell proliferation and migration [98]. A recent study has indicated that PTBP2 binds to IRESs in the 5‘-UTR of *runt-related transcription factor 2* (*RUNX2*) and *vascular endothelial growth factor A* (*VEGFA*) mRNAs and activates their translation, contributing colorectal cancer progression [98]. On the other hand, in cervical cancer cells, the long non-coding RNA *Arf GTPase-activating protein* (*GAP*) *with RhoGAP domain, ankyrin repeat and PH domain 1* (*ARAP1*) antisense 1 (*ARAP1-AS1*) binds to SFPQ and releases PTB from the SFPQ/PTB complex. In turn, PTB mediates IRES-dependent translational activation of *c-Myc*, promoting cervical cancer progression [99]. The roles of SFPQ homodimers and heterodimers in these IRES activation mechanisms are not fully analysed and require further research. Moreover, comprehensive identification of SFPQ-interacting proteins and RNAs by cancer type is important to elucidate mechanisms of SFPQ-mediated IRES regulation.

5. Small molecules modulating functions of DBHS family proteins

Considering the roles of DBHS family proteins in neurodegenerative diseases and cancers, small molecules modulating functions of DBHS family proteins may be applicable to the treatment of these diseases. In recent years, some studies have been reported on the development of small molecules that inhibit the function of DBHS family proteins and the application of these molecules in cancer therapy (Table 3). In recent studies, *in vitro* chemical screens based on RNA pull-down assay and alpha assay were performed to identify small molecules that affect the binding of SFPQ to *CTBP1-AS*, and compounds with structures similar to these candidate compounds were further analysed [15,16]. A chemical screen based on the pull-down assay finally identified a compound No. 10–3 (7,8-dihydroxy-4-(4-methoxyphenyl)chromen-2-one) as a small molecule that efficiently inhibits RNA binding of SFPQ. Computer-aided analysis and biochemical experiments have suggested that No. 10–3 interacts with the groove formed between coiled-coil domain and RNA-binding domain of SFPQ. No. 10–3 treatment inhibits SFPQ functions in transcriptional and posttranscriptional gene regulation and suppresses *in vitro* proliferation of 22Rv1 CRPC model cells and tamoxifen-resistant breast cancer cells. Moreover, intraperitoneal administration of No. 10–3 significantly suppresses *in vivo* tumour growth of CRPC xenograft mouse models [15]. On the other hand, an alpha assay-based screen identified C-65 (4-[2-(3 H-Imidazol[4,5-b]pyridib-2-ylsulfanyl)-acetylamino]-benzonic acid isopropyl ester) as an inhibitory molecule of SFPQ RNA binding activity, and C-65 treatment has also been shown to suppress *in vitro* proliferation of 22Rv1 cells and tamoxifen-resistant breast cancer cells, and *in vivo* tumour growth of CRPC xenograft mouse models [16]. These results highlight that SFPQ–RNA interactions are promising targets in the treatment of hormone-refractory endocrine cancers and suggest that potential therapeutic applications of No. 10–3 and C-65 in these cancers.

Recently, small molecules to inhibit NONO functions were also identified. To search small molecules suppressing NONO functions, a high-throughput screen was performed using MDA-MB-231 cells stably expressing GFP-NONO. Candidate compounds that decrease fluorescent intensity of GFP-NONO were identified, and the treatment with a candidate compound, auranofin, reduced the expression levels of endogenous NONO and its downstream target STAT3, and inhibited *in vitro* proliferation of MDA-MB-231 cells [17]. Auranofin is an oral gold-containing drug and has been clinically applied for the treatment of rheumatoid arthritis [138]. A tumour-suppressive effect of auranofin has also been shown in glioblastoma xenograft mouse models [18]. However, the importance of auranofin/NONO axis in cancer

Table 3. Small molecules targeting DBHS family proteins and their tumour-suppressive effects.

Small molecule	Target DBHS family protein	Binding site on target DBHS family protein	Effects on target DBHS family protein	Tumor-suppressive effects	Clinical application
No. 10–3 (7,8-dihydroxy-4-(4-methoxyphenyl) chromen-2-one)	SFPQ	The groove between coiled-coil domain and RNA-binding domain of SFPQ	Inhibits RNA binding of SFPQ	Suppresses *in vitro* proliferation of 22 Rv1 CRPC model cells and tamoxifen-resistant breast cancer cells [15] Impairs *in vivo* tumour growth of CRPC mouse models [15]	–
C-65 (4-[2-(3H-Imidazol[4,5-b] pyridib-2-ylsulfanyl) -acetylamino]-benzonic acid isopropyl ester)	SFPQ	Unknown	Inhibits RNA binding of SFPQ	Suppresses *in vitro* proliferation of 22 Rv1 CRPC model cells and tamoxifen-resistant breast cancer cells [16] Impairs *in vivo* tumour growth of CRPC mouse models [16]	–
Auranofin (1-Thio-*β*-D-glucopyranosatotriethy-lphosphine gold-2,3,4,6-tetraacetate)	NONO	Unknown	Decreases NONO expression level	Suppresses *in vitro* proliferation of MDA-MB -231 triple-negative breast cancer cells and glioblastoma cells [17,18] Impairs *in vivo* tumour growth of glioblastoma mouse models [18]	a treatment of rheumatoid arthritis [138]
(*R*)-SKBG-1 ((*R*)-4-(2-chloroacetyl) -N-(4-methoxybenzyl) –1-((4-methoxyphenyl) sulfonyl)piperazine-2 -carboxamide)	NONO	Cysteine 145 on NONO (covalent binding)	Enhances RNA binding of NONO	Suppresses *in vitro* proliferation of 22 Rv1 CRPC model cells and ER-positive MCF-7 breast cancer cells [19]	–

CRPC, castration-resistant prostate cancer; DBHS, *Drosophila* behavior and human splicing; ER, oestrogen receptor; NONO, non-POU domain-containing octamer-binding protein; SFPQ, splicing factor proline/glutamine rich.

progression should be carefully evaluated, as auranofin has been shown to inhibit thioredoxin reductase (TrxR) 1/2 to disrupt the reduction/oxidation (redox) system, leading to increased ROS, promotion of apoptosis, and blockage of cell proliferation [139].

Another NONO-inhibiting molecule, (*R*)-SKBG-1 ((*R*)-4-(2-chloroacetyl)-N-(4-methoxybenzyl)-1-((4-methoxyphenyl)sulfonyl)piperazine-2-carboxamide), was identified based on chemical screens for electrophilic molecules decreasing the mRNA and protein levels of full-length AR and AR-V7 in 22Rv1 cells [19]. (*R*)-SKBG-1 covalently binds to cysteine 145 on NONO and enhances NONO–RNA interactions, leading to decreased expression levels of NONO target RNAs, including transcripts of *AR*. Although (*R*)-SKBG-1 treatment has been shown to suppress the proliferation of 22Rv1 and MCF-7 cells *in vitro*, it will be of importance to verify its tumour suppressive effect *in vivo*.

6. Conclusion

In this review, we summarized the roles of the DBHS family proteins in gene regulation in several tissues and cancers. To better understand the detailed roles of DBHS family proteins, further elucidation of the functions and mechanisms of homodimers, heterodimers, and oligomers of DBHS family proteins will be important. In addition, it is required to reveal the novel roles of long non-coding RNAs in the functions of DBHS family proteins. In this regard, more extensive and detailed studies of the physiological and pathological roles of paraspeckles will be needed.

Notably, in recent years, multiple small molecules that inhibit DBHS family proteins have been identified and their application to cancer treatment has been proposed. Considering the association of aberrant gene regulation with neurodegenerative diseases, these small molecules may also be promising for the management of the diseases. Further studies on the tissue-/cell-specific and disease-specific mechanisms of DBHS family proteins will accelerate the development of new drugs targeting DBHS family proteins.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Support Project of Strategic Research Center in Private Universities from the MEXT, and grants from the Japan Society for the Promotion of Science [number 21H04829 (S.I.) and 23K08837 (T.T.)], and Takeda Science Foundation (S.I.), Japan.

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