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RBM45: Molecular, Cellular, and Evolutionary Biology

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Review Article

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Introduction

RNA-binding motif protein 45 (RBM45)* is an RNA recognition motif-type (RRM)-binding domain (RBD) containing protein (RBDP) conserved throughout clade Metazoa (reviewed in [1], [2]). Therefore, I will restrict my attention to mammalian Rbm45, principally *Homo sapiens* (human) RBM45. The RBDP family of proteins are often multifunctional having roles in RNA metabolism modulating mRNA splicing, trafficking, translatability, and longevity. RBDPs have also been found in RNA-dependent and RNA-independent protein: protein interactions influencing carcinogenesis and neuropathogenesis (reviewed in [3]). RBM45 has been strongly associated with toxic cytoplasmic and nuclear inclusion bodies in the neurodegenerative disorders frontotemporal lobar degeneration (FTLD), amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD) [1,4]. This review will examine the molecular genetics, biochemistry, and cellular and tissue distribution of RBM45 as well as its role in nucleic acid metabolism and pathophysiology. I will conclude with a brief overview of the evolutionary history of RBM45 and an examination into future research on this versatile protein.

Molecular Genetics of RBM45

Molecular cloning of *Rbm45* from rat, mouse, and human was accomplished by Tamada *et al.* in 2002 [5]. These researchers cloned the rat *Rbm45* cDNA from an embryonic day 17 fetal brain tissue expression phage library using a polyclonal antibody against an RRM oligopeptide. Subsequently, in the same study, they cloned the mouse and human orthologues using expressed sequence tags and information gleaned from a human genome database. Price and coworkers [6] demonstrated that the human *RBM45* locus spans approximately 17 kb containing 10 exons on chromosome 2q31.2. Bioinformatic analysis of 243 nt of the 5′-UTR identified a TATA-less promoter with an initiator element (INR) at position -2 to +5 that conformed to the canonical PyPyAN(T/A)PyPy (where Py is any pyrimidine, e.g., cytosine or thymine, and N is any nucleotide) sequence overlapping a putative transcriptional start site. Upstream of the INR at position -156 is a CpG island, a proximal promoter element, suggesting a possibility for epigenetic control of *RBM45* expression by means of cytosine methylation. Furthermore, 889 nt of genomic DNA downstream of exon 10 was analyzed and found to contain an embryonic cytoplasmic polyadenylation signal, consistent with *RBM45*'s developmental regulation, upstream of a canonical

polyadenylation consensus signal. Downstream of the polyadenylation consensus signal is a polyadenylation cleavage site and a U-rich motif. All the exon-intron junctions conformed to consensus splice donor/acceptor sites. Control modules for transcription could be elucidated by fusing the *RBM45* 5′-UTR to a reporter gene such as luciferase or green fluorescent protein and assaying function of deletion and point mutations in yeast and/or cell culture. Concurrently, candidate transcription factor binding sites in the 5′-UTR could be identified by bioinformatics and protein binding evaluated by electrophoretic mobility shift assay.

The *RBM45* locus encodes a 476 amino acid protein with a molecular mass of ~53 kDa [5,6] with three RNA-recognition motifs (RRM) (see the figure). RRM1 spans residues 26-106 and is contained in exon 1, RRM2 consists of residues 121-195 bridging exons 2 and 3, and RRM3 contains residues 392-464 and is the product of exons 8 and 9 [2,5,6]. Additionally, RBM45 has a homo-oligomer assembly (HOA) domain, an intrinsically disordered region, between RRM2 and RRM3 (amino acid residues 258-318) coded by exons 5 and 6 [2,6,7]. Exon 6 also codes for a nuclear export signal (NES) consisting of a clique of two leucine residues (amino acids 329 and 330) [2,8]. At the extreme C-terminus of the protein, after RRM3, there is a monopartite nuclear localization signal (NLS) at residues 469-472 encoded by exon 9 [2,7,8] (see discussion below on the protein structure of RBM45). Exon 10 is a non-coding exon [6].

Exome sequencing was employed to ascertain *RBM45* as a candidate gene for the neurodegenerative diseases FTLD and ALS [9]. A p.Arg183* nonsense mutation, segregating in an autosomal dominant manner, was identified in a family with familial FTLD but was absent from unaffected individuals. The persistence of the mutant mRNA, suggesting at least partial escape from nonsense mediated decay, indicates the possible formation of a truncated protein lacking an NLS which would fail to be localized to the nucleus; the degradation of the mutant mRNA and/or translation of a truncated protein would lead to a loss-of-function phenotype. Additionally, these workers mapped an NLS variant, p.Lys456Arg, in a patient with ALS; computer modeling predicted this conservative amino acid

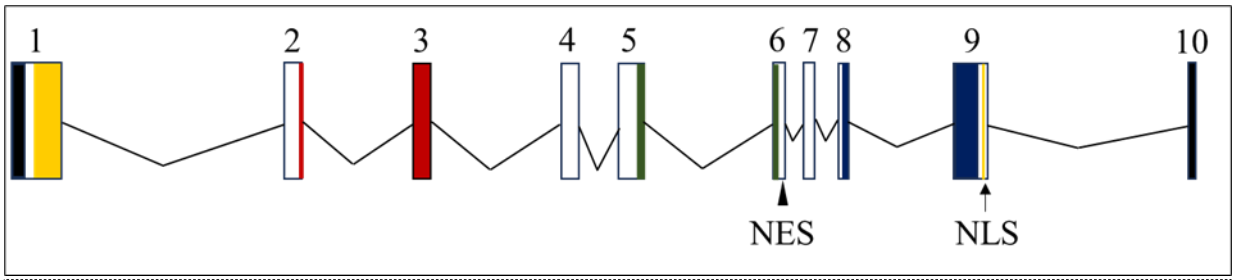

Figure 1. Genomic architecture of human *RBM45*. Schematic representation of exons 1-10 of *RBM45*. Vertical boxes represent exons, and the solid V-shaped lines represent introns. The diagram shows the relative sizes of the exons and introns. The introns and exons are scaled by type; the intron width scale is half that of the exon width scale. The DNA sequences encoding RNA-recognition motifs (RRM) I, II, and III, and the homo-oligomer assembly (HOA) domain, are indicated by yellow, red, green, and blue fill, respectively. The DNA sequences encoding the monopartite nuclear localization signal (NLS) are indicated by a vertical gold line and an upward pointing arrow; the nuclear export signal (NES) is indicated by an arrowhead. Non-coding exon sequences are shaded black.

substitution to be deleterious, though empirical evidence of a significant change in nuclear-cytoplasmic shuttling was inconclusive, indicating that further investigation is required.

Protein Structure of RBM45

The RBM45 RRMs contain ~ 90 amino acids characterized by the proto-typical βαββαβ topology and the archetypical two conserved aromatic residues; these RRMs also have the conserved consensus 8 residue ribonucleoprotein domain 1 (RNP1) and the 6 residue RNP2 necessary for RNA binding [5,10]. RBM45 RNA-binding sequence specificity is not well-characterized despite considerable effort. RBM45 was initially reported to have high affinity to poly(C) and poly(G) RNA [5]. Further work demonstrated that full-length RBM45 bound GACGAC [11] and ACGC [12] RNA sequences, presumably through interactions with the RRM domains, though not explicitly determined. Direct evidence for RRM binding to RNA came from the observation of RBM45 RRM2 mediating high affinity (K_D = 33nM) association with the RNA octanucleotide sequence GGGACGGU from intronic sequence of human parvovirus B19 [13]. Chen and coworkers [10] provided the first direct evidence RRM1 and RRM2 recognize RNA/ssDNA through identification of the high affinity binding site: GGACGG. Through co-crystallization of the N-terminal tandem RRM1-RRM2 and an 11-nt CGA CGGGACGC ssDNA, these workers demonstrated that RRM1 and RRM2 physically interact with a GAC motif of ssDNA and RNA. Molecular modeling of the RBM45/ssDNA complex [14] unveils that this interaction is most likely driven by hydrophobic interactions. More recently, Chen *et al.* have demonstrated that RRM3 interacts specifically with a GACG motif [15] similar to but not identical to RRM1 and RRM2. The identification of the GAC(G) motif should help future investigations into physiologically relevant RNA targets for RBM45.

Between the N-terminal RRM1 and RRM2 and the C-terminal RRM3 lies a pseudo-RRM domain [5] identified as a HOA domain (residues 258-318) that promotes oligomerization of RBM45 [7]. The HOA domain mediates protein:protein complex formation, not only self-aggregation but also direct interaction with TDP-43 and FUS, two proteins tightly linked to causation of FTLD and ALS. The RBM45:TDP-43 interaction is RNA-dependent, while the RBM45:FUS binding is through direct protein:protein binding. Furthermore, the HOA domain directs the co-localization of RBM45:TDP-43 to stress granules induced by oxidative stress. Interestingly, the RBM45:TDP-43 interaction also requires both the HOA domain and RRM3 for its activity [7]. A large-scale immunoprecipitation/mass spectrometry study identified 132 binding partners for RBM45 in HEK293 cells. These binding partners interacted with RBM45 primarily through the HOA domain in an RNA-independent and RNA -dependent manner [16]. In contrast, RRM1 and RRM2 were necessary and sufficient to mediate the RNA-independent protein:protein interaction between RBM45 and KEAP1, a modulator of antioxidant response in ALS [17], demonstrating the multifunctionality of these domains.

RBM45 undergoes nuclear-cytoplasmic trafficking via an NLS and NES. The monopartite NLS maps just downstream of RRM3 at amino acids 469-KRQR-472 matching the consensus NLS basic amino acid sequence K(K/R)X(K/R) [7,8]. The NES is composed of a clique of two tandem leucine residues (329-LL-330) [8] in the context of a 15 amino acid domain rich in hydrophobic residues [2].

Cellular Compartmentalization of RBM45

Endogenous RBM45 localizes predominantly to the nucleus [7] in the human neuroblastoma cell line SH-SY5Y [18]. Expression of FLAG-tagged RBM45 in PC12 cells, a rat cell line composed of embryonic neuroblastic and eosinophilic cells derived from a pheochromocytoma [19], localizes RBM45 predominantly in the cytoplasm with a minority of cells showing subcellular localization to

both the cytoplasm and nucleus [5]. Similarly, overexpression of EGFP-tagged RBM45 in Neuro2A cells, a mouse neuroblastoma cell line [20], localized the protein to the nucleus with a minority of cells (~10%) accumulating EGFP-RBM45 into cytoplasmic granules [7], suggesting that overexpression of RBM45 leads to cytoplasmic stress granules like those found in the cells from ALS patients. Work performed in the same laboratory [21], analyzing physiological levels of RBM45, did not detect these cytoplasmic granules, suggesting they are an artifact of the protein construct lacking an NLS and being overexpressed to non-physiological levels from a plasmid-based system. Proteomic analysis of stress granules from ALS and FTLD model systems did not identify RBM45 as a constituent [22], supporting the artifactual nature of these cytoplasmic granules in RBM45 overexpression systems.

In normal motor neurons from lumbar spinal cord tissue, endogenous RBM45 exhibits punctate staining in the cytoplasm and nucleus. In contrast, RBM45 from motor neurons derived from ALS and FTLD patients exhibit an orbicular, coiled pachytene morphology throughout the cytoplasm, reminiscent of the staining pattern of TDP-43 and FUS, two proteins strongly associated with FTLD and ALS pathology. In fact, these RBM45 positive cytoplasmic inclusions contain TDP-43. Confocal microscopy of normal hippocampus reveals a speckled pattern of RBM45 expression in the nucleus of dentate granule cells [4]. RBM45 is induced to exit the nucleus and form cytoplasmic granules in the presence of acute oxidative stress inducers (e.g., paraquat or H_2O_2) in cultured primary rat neurons and in human SH-SY5Y cells, but not HEK-293 cells or undifferentiated NSC-34 cells [17], suggesting that RBM45 localization in response to a variety of cell stressors is cell-type specific. Conversely, the nuclear itinerary of RBM45 is reinforced under conditions of chronic cellular stress. Collins and coworkers [21] analyzed RBM45 cellular distribution under conditions that induce cellular stress (e.g., sodium arsenite, heat shock at 42 ^{o}C) in human embryonic kidney 293 cells (HEK293) [23]. Under basal conditions, RBM45 exhibited diffuse nuclear staining with slight cytoplasmic staining in HEK293 cells. RBM45 did not co-localize with subnuclear membraneless organelles, regions of liquid-like or gel-like phase separation [24], such as nuclear speckles, nuclear gems, nuclear stress bodies, Cajal bodies, or cytoplasmic stress granules, as assessed using the appropriate marker protein (e.g., SMN for nuclear gems) [21]. In contrast, when HEK293 cells were subjected to a variety of stressors (e.g., serum deprivation, genotoxic stress, heavy metal stress etc.), nuclear RBM45 mobilized into nuclear stress bodies (NSBs). RBM45 association with NSBs is RNA-dependent and required an intact NLS and direct interaction of NSB pericentromic heterochromatic satellite III repeat transcripts with the RRM2 and RRM3 domains. siRNA-mediated knockdown of RBM45 demonstrated that RBM45 is not essential for NSB formation but confirmed that NSBs do indeed contain RBM45 as a constituent as measured by its colocalization with the NSB marker protein SAFB. Significantly, these authors [21] demonstrated a statistically significant increase in NSBs in dentate gyrus tissue cells and lumbar spinal cord motor neurons from patients affected by FTLD, ALS, and AD versus normal unaffected neurological controls. In contrast to neuropathogenic spinal cord, RBM45 demonstrates nucleolar localization in CNS motor neurons. The function of nucleolar RBM45 is not known; however, these workers hypothesize that this subnuclear fraction of RBM45 is not available for mobilization into NSBs. The role of these cytoplasmic and nuclear RBM45-containing inclusions is murky but these data suggest that RBM45 recruitment into NSBs disrupts the normal subcellular localization-dependent activity(ies) of RBM45 and associate RBM45-containing NSBs in neuropathologies.

Tissue Localization of RBM45

Tamada *et al.* [5], who discovered RBM45, showed by northern blot analysis that rat *Rbm45* has robust expression in adult brain followed by small amounts in kidney and spleen, barely detectable amounts in heart and lung, and no detectable expression in skeletal muscle or liver. Analysis of steady-state levels of mouse Rbm45 mRNA in adult tissues by qRT-PCR, a more sensitive assay than northern blot, confirms highest expression in brain with heart, spleen, lung, kidney, small intestine enterocytes, and liver having three-fold to six-fold reduced expression compared to brain tissue and skeletal muscle and colon having 10-fold lower expression compared to brain [6]. Tamada and coworkers [5] further parsed the expression of Rbm45 mRNA in adult rat brain showing highest expression in cerebellum followed by olfactory bulb, then hippocampus, with no detectable expression in brain stem or cerebral cortex. These workers also assessed expression of rat Rbm45 mRNA in embryonic day 12 (E12), E16, E21, post-partum day 1 (P1), and P7 whole brain. Rbm45 mRNA expression was highest in the embryonic samples, peaking at E16 with P7 expression being approximately equal to adult brain. These data reveal a spatio-temporal developmental regulation of Rbm45 in rat brain. The developmental regulation of rodent Rbm45 was further demonstrated in cultured neural precursor cells, rat PC12 and mouse P19, where Rbm45 mRNA expression was highest in the undifferentiated state and then decreased when the cells were induced to differentiate with nerve growth factor (PC12 cells) or retinoic acid (P19 cells). The spatial localization of rat Rbm45 mRNA in the CNS was determined by *in situ* hybridization. In E16 rat embryo, robust expression was detected in the subventricular zone and neural tube, while in P3 rat, strong signals were detected in pyramidal neurons from the hippocampus, cortical neurons, and neurons of the dentate gyrus. Rat Rbm45 mRNA expression overlapped with Nestin, a marker for neural stem cells, and NeuN, a marker for neural cells. Together with the observation of highest Rbm45 mRNA expression at stage E16 of development, these authors suggest that rat Rbm45 expression would be limited to neurons and neural stem cells but not glial cells of the nervous system.

As mentioned above, human *RBM45* contains a predicted embryonic cytoplasmic polyadenylation signal [6]. Currently, no RNA-seq data is available for human fetal brain tissue from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/gene/129831#gene-expression; accessed 2024 August 18); however, it is reasonable to conjecture that qRT-PCR analysis of RBM45 mRNA levels from brain tissue of spontaneously aborted human fetuses would show developmentally regulated expression like that observed in the rat. RNA-seq data from NCBI (https://www.ncbi.nlm.nih.gov/gene/129831; accessed 2024 August 18) reveals very little difference in RBM45 mRNA expression between adult tissues. Similarly, data from The Human Protein Atlas (https://www.proteinatlas.org/ENSG00000155636-RBM45/tissue; accessed 2024 August 18 [25]) shows expression of RBM45 protein in all major organ systems except for lung tissue. Interestingly, 55% of lung cancer patients were positive for RBM45 protein. Similarly, RBM45 protein was not detected in normal cervix, but 90% of patients show RBM45 protein expression in cancer of the cervix [25]. These observations suggest an activation or unmasking of RBM45 mRNA for translation in these carcinomas.

Nucleic Acid Metabolism Mediated by RBM45

The speckled pattern of RBM45 nuclear localization in FTLD, ALS, AD, and normal hippocampus [4] suggests a role in mRNA splicing further reinforced by the discovery of a protein: protein interaction network with various hnRNPs, SNRPs, DDX39B (spliceosome RNA helicase), and RBMX [16]. Subsequently, RBM45 was revealed to bind to intron splicing enhancers and acting as a scaffold protein essential for the splicing and maturation of human parvovirus B19 [13]. More recently, Choi *et al.* [26] showed that RBM45 regulates gene expression through control of mRNA splicing via

N^6-methyladenosine-dependent and N^6-methyladenosine-independent pathways. They identified 1,868 RNA targets by cross-linking and immunoprecipitation sequencing. Of these targets, roughly one-third bound to N^6-methyladenosine-enriched sites. Furthermore, CRISPR-Cas9 *RBM45* knockout in differentiating SH-SY5Y cells altered the abundance of differentially regulated RNAs. RBM45 bound these RNA targets in intronic regions and did not alter alternative splicing but instead directed positive and negative regulation of constitutive splicing of pre-mRNAs through an unknown mechanism. These results demonstrate that RBM45 regulates constitutive splicing during *in vitro* neurodifferentiation. Intriguingly, RBM45-containing spliceosome complexes can be regulated by miRNA-4454 in response to insulin within prostate tissue [27], adding an additional layer of metabolic control of RBM45 splicing activity. Further investigation into the precise mechanism by which RBM45 and splicing factors interact during the splicing process will be a fruitful area of study in the future.

Nucleolar localization of RBM45 [21] and protein:protein interactions with rRNA processing proteins (e.g., NCL, RPS19) [16] are consistent with a role in rRNA metabolism. Algorithmic analysis [28] of RBM45 amino acid sequence does not predict the presence of a nucleolar localization sequence (Henderson, J.O. unpublished data), intimating a role for a nucleolar-specific protein binding partner mediating RBM45 distribution to the nucleolus. Other RNA metabolic processes, implicated by Li and colleague's [16] RBM45 protein:protein interaction network study, meriting future experimental validation are: regulation of translation (e.g., eIF2, eIF4), regulation of mRNA stability (e.g., ELAVL1, DHX9), mRNA deadenylation-dependent decay (e.g., PABPC1, EIF4A1), and nuclear mRNA export (e.g., hnRNP-A1, hnRNP-K). These authors have laid the groundwork for in-depth exploration of the dynamic interaction of RBM45 with these different factors in control of gene expression, neurogenesis pathways, and cellular metabolism.

RNA editing is a post-transcriptional molecular process whereby the nucleotide sequence of an mRNA is enzymatically modified resulting in an amino acid change or change in sequence for binding partners. An *in silico* transcriptomics approach has identified RBM45 as the most common RBDP bound to A-to-I RNA editing sites in granulosa cells associated with polycystic ovary syndrome (PCOS) [29]. Empirical validation of the targets of A-to-I editing has not yet been done, though it is interesting to note that RBM45 interacts with GRY-RBP (SYNCRIP) and hnRNPC1 [16], proteins known to modulate C-to-U editing ([30], reviewed in [31]). Significantly, many of the proposed targets of A-to-I editing in PCOS are affiliated with the apoptotic pathway [29]; RBM45 has been previously identified to be upregulated 5-fold in cardiomyocytes resistant to hypoxia-induced apoptosis [32]. Based on these protein:protein interactions, bioinformatic predictions, and known association of RBM45 with apoptotic pathways, the proposed functional role for RBM45 in A-to-I editing will be a fertile area of research in the future.

Perhaps unexpectedly, RBM45 also functions in DNA transactions. RBM45 is part of a DNA-damage response (DDR) pathway dependent on its interaction with FUS and PAR in an RNA-independent interaction through its HOA-RRM3 domains. Direct interaction of RBM45 with DNA in chromatin loading was not investigated; however, RBM45/FUS/PAR stoichiometry is key to RBM45 regulation of DDR. Depletion of RBM45 decreased the efficiency of DNA double-strand break repair most likely through negative regulation of histone deacetylase 1 recruitment, an important protein for efficient response to DNA damage through its role in chromatin remodeling [33]. RBM45's role in DNA repair was further amplified by the discovery of its upregulation in response to radiotherapy across 15 cancer types [34]. RBM45 has previously been shown to interact with FUS in cytoplasmic granules in FTLD/

ALS patient cells [7] and FUS has been shown to be recruited to DSB [33]; therefore, Gong and colleagues [33] suggest that perturbations in these interactions may negatively affect DDR and exacerbate pathogenesis in ALS patients. Future work with conditional down-regulation and up-regulation of neuronal RBM45 in the context of the FUS knockout mouse model [35] may shed light on the role of RBM45-influenced DNA repair in neurodegeneration.

Pathogenesis and RBM45

Dysregulation of RBM45 was first noted in a neonatal opossum model for spinal cord injury, suggesting a role in neurite outgrowth [36], and the neuropathogenic diseases FTLD, ALS, and AD (reviewed in [1]) with subsequent linkage to polycystic ovary syndrome (discussed above). More recently, perturbations in RBM45 expression have been connected to nonalcoholic fatty liver disease (NAFLD), lung cancer, hepatocellular carcinoma (HCC), and breast cancer progression. NAFLD is a constellation of liver disorders, including HCC, cirrhosis, and hepatic inflammation, characterized by the accumulation of fat (steatosis) within the liver independent of alcohol consumption [37]. Dynamic array qPCR of liver samples from patients with steatosis revealed alterations in splicing machinery proteins including upregulation of RBM45. siRNA silencing of RBM45 in HepG2, an immortalized hepatocyte cell line isolated from an HCC [38], subjected to oleic acid overload had a decrease in lipid accumulation compared to scrambled siRNA controls [37]. These data suggest that aberrant pre-mRNA splicing has a negative effect on lipid flux *in vitro* and perhaps in the liver proper.

A transcriptomic analysis of KRAS mediated alternative splicing in isogenic A549 lung a denocarcinoma cells [39] serendipitously identified WT RBM45 and RBM45^{M126I} as inducing alternative splicing events with skipped exons predominating. In fact, KRAS was second to RBM45 ^{M126I} in the number of differential splicing events induced in a lentiviral overexpression system indicating a strong association of oncogenic RBM45^{M126I} with lung carcinoma. The RBM45 ^{M126I} allele has a conserved amino acid change in RRM2 suggesting perturbation in pre-mRNA binding, though these authors did not explore the molecular biology of how the RBM45 ^{M126I} allele was inducing splicing errors. These data further support the role of RBM45 in spliceosome regulation; however, the exact molecular mechanism of RBM45 action remains unknown.

A trio of recent papers have highlighted the role of RBM45 in HCC progression and prognostication. Wu *et al.* [40] developed an RBM protein family prognostic signature to predict clinical outcomes in HCC patients. These researchers identified a cohort of 4 RBDP genes, including RBM45, that can function as independent predictors for high-risk, poor-prognosis HCC patients. Additionally, RBM45 is upregulated in HCC tissue. As a proof-of-principle, shRNA knockdown of RBM45 in an HCC cell line (MHCC-LM3) inhibited proliferation, confirming their hypothesis that RBM45 is a risk factor for HCC. The mechanism by which RBM45 elevates HCC risk may be explained, in part, by its ability to enhance the stability of the glutamine transporter, ASCT2 [41]. Bioinformatic analysis identified RBM45 as being upregulated in HCC correlating with a poor prognosis, especially in male patients, consistent with the work of Wu and colleagues [40,41]. Importantly, overexpression of RBM45 in the liver of an HCC mouse model developed tumors more quickly and in greater numbers compared to non -specific GFP controls. Metabolomics identified dysregulation of glutamine metabolism correlating with overexpression of RBM45; furthermore, RBM45 was shown to directly bind to ASCT2, via RRM3, in an RNA-dependent manner stabilizing ASCT2 expression leading to increased cellular uptake of glutamine. Overexpression of RBM45 in SK-HEP-1 cells consistently increased intracellular glutamine uptake concomitant with activation of mTORC1 through amino acid flux pathways. As

would be predicted, the core component of mTORC1, mTOR [16], a serine/threonine kinase involved in cellular growth, proliferation, and apoptosis, was also elevated in these cells overexpressing RBM45 [41]. Small molecule inhibition of the RBM45:ASCT2 interaction decreased ASCT2 stability and inhibited HCC cell proliferation, further strengthening the link between RBM45 overexpression and stability of ASCT2 to HCC development and progression. RBM45 is also involved in the regulation of another mTOR pathway, mTORC2 (Rictor) [42]. Here, lipid induction in HCC activates RBM45 promoting lipid anabolism via mTORC2 and fatty acid β-oxidation by ACSL1/ACSL4. In this study, RBM45 upregulated Rictor, ACSL1, and ACSL2 by binding and stabilizing their mRNAs. siRNA mediated RBM45 (shRBM45) knockdown in an orthotopic mouse model of liver cancer significantly reduced tumor burden in these animals; combination therapy in this murine model with shRBM45 and Sorafenib synergistically reduced HCC burden. Taken together, these studies [40-42] offer the first concrete insights into how RBM45 dysregulation affects cellular pathways important for homeostasis. Furthermore, these data indicate that small molecule suppression of oncogenic RBM45, in combination with mTOR inhibitors, offers a new pathway of treatment for HCC. Questions remain as to the upstream control mechanism and signals that upregulate RBM45 in HCC.

In human breast cancer cells, RBM45's repertoire also includes epigenetic control of IRF7, one of a suite of proteins that modulate transcription of the type I interferon β (*IFNB1*) gene encoding IFN-β [43]. Type I interferons have been shown to exert anti-tumor properties through down-regulation of tumor cell proliferation, controlling the tumor microenvironment via inhibition of angiogenesis and metastasis, and stimulating antitumor immunity. These workers demonstrated that depletion of RBM45 from MCF7 human breast cancer cell mouse xenografts upregulated the expression of IFN-β leading to inhibition of breast cancer progression. Coimmunoprecipitation and proximity ligation assays identified a tripartite complex of RBM45/TRIM28/IRF7. TRIM28 is a canonical transcriptional repressor that acts as a SUMO E3 ligase to SUMOylate IRF7 and repress its activity. Lv and coworkers [43] demonstrate that RBM45 directly interacts with TRIM28 and IRF7, in an RNA-independent manner, stimulating the SUMO E3 ligase activity of TRIM28 to hyper SUMOylate IRF7 thus repressing IRF7 activation of IFN-β expression. They propose a model by which high levels of RBM45 recruit TRIM28 to the IRF7 bound *IFNB1* promoter stimulating SUMOylation of IRF7. Hyper SUMOylated IRF7 represses transcription of the *IFNB1* locus resulting in low levels of IFN-β and subsequent stimulation of tumor cell proliferation. In contrast, low levels of RBM45 would not recruit TRIM28 to IRF7 occupied *IFNB1* promoters; therefore, IRF7 would remain hypo SUMOylated, transcription of the *IFNB1* locus would be promoted, and high levels of IFN-β would inhibit tumor cell proliferation.

Resolution of the control of *RBM45* gene expression will require identification of promoter elements (described above), analysis of RBM45 mRNA half-life to test mRNA stability in diseased versus normal states, and examination of RBM45 mRNA translational efficacy in cancer and non-cancer cells and tissues. Furthermore, it is unknown whether auxiliary proteins may be influencing the downstream expression of RBM45, though there are no identified canonical binding sites for other RBDPs in the 5′- or 3′-UTR [6] of the RBM45 mRNA. As illuminated in this section, the reagents exist to dissect the transcriptional and posttranscriptional control of RBM45 in human cell culture and mouse models of disease linked to dysregulation of RBM45 expression.

Evolutionary History of RBM45

Orthologues of RBM45 were first identified in phylum Chordata, subphylum Craniata (human, mouse, rat, frog, Zebrafish), phylum Echinodermata (sea urchin), and phylum Arthropoda (mosquito, fruit fly)

[5,8]. The ancient origins of RBM45 were revealed when orthologues were identified in the "basal" taxa phylum Porifera, Placozoa, and Cnidaria; interestingly, no Rbm45 orthologue has yet been identified in phylum Ctenophora [2,6]. Further analysis by Vuchkovska *et al.* [2] of 36 taxa across 9 phyla (Porifera, Cnidaria, Priapulida, Mollusca, Brachiopoda, Arthropoda, Echinodermata, Hemichordata, and Chordata) revealed conservation of Rbm45 over 650 million years of evolutionary history. Rbm45 protein molecular phylogeny recapitulated most known monophylies in clade Metazoa indicating strong conservation of structure over time. In fact, the protein domains of Rbm45 are linearly conserved from sponges to humans in the order: RRM1, RRM2, HOA, RRM3, and NLS. The Rbm45 double leucine clique NES is conserved only within clade Tetrapoda of clade Craniata. Non-tetrapod craniates have a predicted NES of a hydrophobic rich (60%) 15 amino acid sequence. In contrast, Rbm45 from invertebrate species (clades Ambulacraria and Protostomia) lack a canonical NES, suggesting the NES is a novel trait in the craniate lineage; it is not known if nuclear/cytoplasmic shuttling of Rbm45 occurs in the cells of these species. *Rbm45* gene architecture analysis revealed increasing gene complexity in tandem with increasing organismal evolutionary complexity where non-bilaterians have 2 to 4 large exons with short introns, and bilaterians having 6 to 17 short exons with long introns. It is tempting to speculate that this prominent level of conservation of *Rbm45* among crown clades suggests a fundamental role in animal physiology in such processes as tissue development/organogenesis (e.g., neurogenesis), cellular homeostasis (e.g., oxidative damage response, DDR), and in chordates as a tumor suppressor.

Summary and Prospectus

RBM45 is a cosmopolitan protein with demonstrated activities in pre-mRNA splicing, mRNA stability, A-to-I RNA editing, double-strand break repair, and protein:protein interactions, whose dysregulation is associated with neuropathogenesis (ALS, FTLD, AD), PCOS, NAFLD, and cancer (lung, liver, and breast). However, fundamental areas of research remain. The determination of cis-acting elements and structural motifs within RNA targets as well as protein:protein interactions with adjuvant proteins are key areas of future research to understand the basic function of RBM45 in the metabolic processes described in this review. Furthermore, how these molecules act in tertiary and quaternary interactions and the stoichiometry of RBM45/RNA, RBM45/protein, and RBM45/RNA/protein interactions as well as their effects on subcellular localization and abundance need to be investigated. Not least of which, there is currently little understanding on how RBM45 itself is regulated at the transcriptional and post-transcriptional levels: What transcription factors regulate RBM45 expression? How is regulation of RBM45 controlled developmentally? What are the factors that modulate RBM45 mRNA and protein half-life? The field of RBM45 research would be enhanced by the creation of conditional *Rbm45* deletor mice, RBM45 transgenic lines, and *Rbm45* knock-out animals. However, many important reagents already exist to tease out the answers to these questions and offer a productive landscape for future research.

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Competing interests

The author declares no competing interests.

***Abbreviations**

RBM45 - RNA-binding motif protein 45; RRM - RNA recognition motif; RBD - RRM-binding domain; RBDP - RNA recognition motif-type binding domain protein; FTLD - frontotemporal lobar degeneration; ALS - amyotrophic lateral sclerosis; AD - Alzheimer's disease; Kb - kilobase pairs; nt - nucleotide; 5′-UTR - 5′ untranslated region; Py - pyrimidine; kDa - kilodalton; RRM - RNA recognition motif; HOA - homo-oligomer assembly; NES - nuclear export sequence; NLS - nuclear localization sequence; RNP1 - ribonucleoprotein domain; qRT-PCR - quantitative reverse transcription -polymerase chain reaction; NCBI - National Center for Biotechnology Information; PCOS - polycystic ovary syndrome; DDR - DNA-damage response; NAFLD - nonalcoholic fatty liver disease; HCC - hepatocellular carcinoma; WT - wild type; GFP - green fluorescent protein.

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