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Mini Review

Emerging roles of RNA and RNA-binding protein network in cancer cells

*Mee Young Kim, Jung Hur & Sunjoo Jeong**

National Research Lab for RNA Cell Biology, BK21 Graduate Program for RNA Biology and Department of Molecular Biology, Dankook University, Gyeonggi-do 448-701, Republic of Korea

Recent advances in RNA biology reveal unexpected diversity and complexity of cellular RNA metabolism. RNA-binding proteins (RBPs) are essential players in RNA metabolism, regulating RNA splicing, transport, surveillance, decay and translation. Aberrant expression of RBPs affects many steps of RNA metabolism, significantly altering expression of RNA. Thus, altered expression and dysfuncting of RBPs are implicated in the development of various diseases including cancer. In this minireview, we briefly describe emerging roles of RBPs as a global coordinator of post-transcriptional steps and altered RBP as a global generator of cancer related RNA alternative splicing. Identification and characterization of the RNA-RBP network would expand the scope of cellular RNA metabolism and provide novel anti-cancer therapeutic targets based on cancer specific RNA-RBP interaction. [BMB reports 2009; 42(3): 125-130]

INTRODUCTION

Following the discovery of the DNA structure half a century ago, RNA was long considered as a supporting player during cellular information transfer from DNA to proteins. However, recent discoveries on new cellular RNAs and their novel functions have shaken the ground of traditional molecular cell biology and sparked intense interest on RNA cell biology (1). Interested readers should consult the inspiring essays and comprehensive reviews on RNA in the February 20, 2009 issue of the journal *Cell*, which is timely devoted to breakthrough research on RNA. It is beyond the scope of this minireview to extensively survey all the exciting new findings on RNA biology. Rather, we focus on a discussion of the novel functions of RNA-binding proteins (RBPs) and the altered RNA-RBP network in cancer.

*Corresponding author. Tel: 82-31-8005-3196; Fax: 82-31-8005-3164; E-mail: sjsj@dankook.ac.kr

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RBPs

RNA cannot exist alone in cells, but are stably assembled with many RBPs and other proteins, which exist as ribonucleoprotein complexes (RNPs). RBPs are essential players in RNA metabolism and regulate of RNA splicing, localization, surveillance, decay and translation. As many RNAs and a large number of RBPs are generated in the cells, biogenesis of RNPs and their functional roles must be orchestrated with great fidelity.

There may be many thousands of RBPs in vertebrates, each of which has one or, more often, multiple RNA-Binding Domains (RBDs) usually associated with RNA in a sequence- or structure-dependent manner (2). More than 40 RBDs have been identified so far, such as well-characterized RNA Recognition Motifs (RRM), K-Homology (KH) domain, double-stranded RNA Binding Motif (dsRBM), RGG (Arg-Gly-Gly) box, DEAD/DEAH box and Piwi/Argonaute/Zwille (PAZ) domains (3). Numbers of RBPs are expected to increase if other types of RBDs are added to the list. In fact, bioinformatic analysis using previously identified RBD motifs predicts that 2-8% of genomes encode RBPs in lower eukaryotes (4).

Roles of RBPs in RNA metabolism

Gene expression is initiated from transcription and continues to post-transcriptional RNA metabolism, which includes RNA splicing, end-formation, export, surveillance, decay and translation. Traditionally, gene expression has been regarded as being composed of separate and independent post-transcriptional steps. Current views on gene expression, however, stress the interconnectivity and coordination of these steps (5, 6). Many RBPs such as heterogeneous nuclear RNPs (hnRNPs) and Ser/Arg rich proteins (SR proteins) are associated with mRNA as mRNPs in RNA metabolism, and functionally regulate multiple steps between transcription and translation by dynamic remodeling of RNPs (7). For example, the SR protein that is a genuine alternative splicing factor SF2/ASF acts from alternative splicing to translation (8, 9), which suggests a long RNA metabolic journey of a single RBP and its fine tuning by phosphorylation (10). Multiple roles of RBPs would provide a means of interconnecting the post-transcriptional steps, as pro-

posed by an extremely simplified model shown in Fig. 1.

As proposed as part of the RNA regulon hypothesis (11), multiple cellular RNAs might be coordinately regulated during post-transcriptional regulation. RBPs can be regarded as a key regulator of the RNA regulon, expanding the scope of RBPs to multiple target RNAs (Fig. 2). The significance of RBPs on such a RNA network is exampled by Pumillio-Fem3 binding Factor (PUF) RBPs in yeast, which shows that functionally related mRNAs are extensively and distinctly associated with different PUF proteins (12). Therefore, RBPs are likely to serve as a coordinator of functionally related RNA molecules by binding to consensus RNA elements in target RNA molecules (Fig. 2). Identification of RBP binding RNA elements can be experimentally studied by various methods such as Systemic Evolution of Ligands by EXponential enrichment (SELEX) technique and cross-linking immunoprecipitation (CLIP) method. Global analyses of RNA using these technologies should increase our understanding of the complex life cycle of the cellular RNA-RBP network. For example, SF2/ASF binding RNA elements of nuclear and cytoplasmic target RNAs were recently identified by CLIP (13).

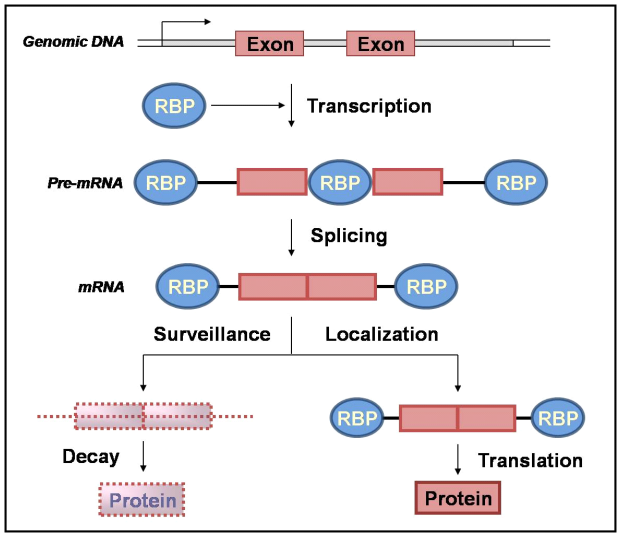

Fig. 1. Multifunctionality of RBP in RNA metabolism. RNA undergoes several steps of regulation from transcription to translation such as splicing, localization, surveillance, decay and translation. RBP binds to RNA and regulates multiple steps of RNA metabolism.

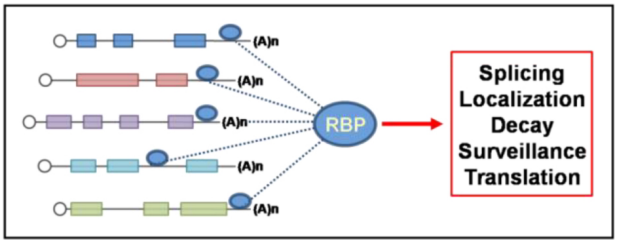

Fig. 2. Post-transcriptional RNA regulon. Multiple cellular RNAs are coordinately regulated by specific RBPs during post-transcriptional steps as a RNA regulon. RBPs regulate splicing, localization, surveillance, decay or translation of mRNAs by interaction with sequence- or structure-dependent manner in the RNA.

Altered RNA-RBP network in disease

Based on the proposed functional significance of cellular RNA-RBP network, mutations that disrupt either the RNA or RBPs are supposed to be deleterious to gene expression and may cause disease (14). Compared to normal cells, altered expression of RBPs in diseased cells might cause dramatic changes of cellular RNP composition. For example, if RBPs are mutated or overexpressed in cancer cells, they might associate with many incorrect target RNA molecules due to altered target affinity, which might lead to formation of aberrant RNPs in cancer cells (Fig. 3). Functional alterations of such events would have a global impact on the gene expression profile of cancer cells, even showing more significant effects than altered DNA binding proteins for improper transcription.

If RBPs or RBP binding elements are mutated in cells, processes of RNA metabolism would be changed as aberrant alternative splicing, unregulated decay, malfunctioning surveillance, mislocalization and unregulated translation of target RNAs. Such aberrant RNA molecules could generate aberrant and pathogenic proteins. Effects of altered RBPs are most clearly seen in neuronal cells than in any other cell type. Identification of large numbers of target RNAs for neuron specific RBPs (i.e., Nova) have greatly enhanced our understanding of the RNA-RBP network in differentiated as well as

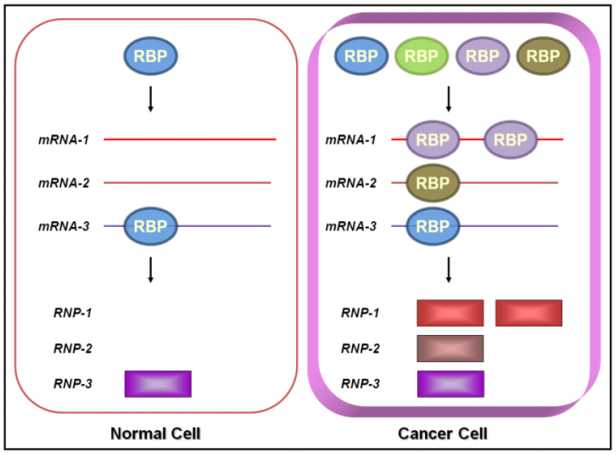

Fig. 3. Aberrant formation of RNPs in cancer cells. RBPs bind their target RNAs and form specific RNPs in normal cells. However, altered expression or overexpression of a RBP changes the affinity of that RBP to the target RNA molecules, so as to associate with wrong target RNA molecules and form aberrant RNPs in cancer cells.

in diseased cells (15).

Alternative pre-mRNA splicing is a key event that allows protein diversity from limited genomic information (16, 17). Recent analyses of the human transcriptome have revealed extreme diversity of alternative exons and extensive combinatorial powers of alternative splicing in the human genome (18-20). Considering the complexity of regulated RNA splicing, many RBPs are expected to be altered; they might be involved in disruption of splicing code and decoding machinery in diseased cells (21-23). Unregulated alternative splicing is likely to be detected in diseased cells, which is relevant to clinical diagnostics (23, 24). Indeed, at least 15% of all disease causing single base-pair mutations affect splicing and cause phenotypic differences (25, 26).

Alternative splicing in cancer

Disruption of the normal splicing code in cancer cells generates alternatively spliced mRNAs that are not produced in normal cells (27). These aberrant transcripts are part of a surveillance system such as Non-sense Mediated Decay (NMD) and are degraded before protein expression. However, some aberrant mRNAs are able to produce protein isoforms with potentially tumorigenic properties (28). There are two possible causes of such alterations in cancer-related alternative splicing (29). One is mutations in cis-splicing regulatory elements of RNA and the other concerns the alteration of RBPs involved in the regulation of splicing (24).

The number of reports on cancer-related alternative splicing continues to grow. Bioinformatic approaches also identified cancer-associated splice variants in many genes (30). Here, we will focus only on a couple of alternative splicing mechanisms known to be critical for cancer development. Most cancer-related alternative splicing seems to pertain to cell-surface expressed proteins. CD44 is a type I transmembrane glycoprotein involved in cell-cell and cell-matrix adhesions. CD44 has more than 20 known isoforms due to variable inclusion of 10 alternative exons coding for specific extracellular domains, which are associated with cancer development (31). The balance between RBPs such as hnRNP A1 and SF1/ASF, as well as signal dependent phosphorylation of Sam 68 has been proposed to be the mechanism for diverse generation of CD44 alternative splicing (32, 33).

Tenascin-C is a secreted extracellular matrix glycoprotein and specific alternative splicing is found only in some solid tumors but not in any normal tissue. Alternative splicing of exons coding for additional extracellular domains occurs only in some cancer cells. The large isoform of tenascin-C is expressed from alternatively spliced mRNA and secreted to neighboring extracellular matrix. It facilitates cell migration and metastasis of cancer cells (34).

Vascular endothelial growth factor-A (VEGF-A) is critical for angiogenesis and is highly expressed in almost every type of solid tumors. Alternative splicing of VEGF generates nine isoforms, three of which ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$) are the most predominant transcripts. These three isoforms differ in exons 6 and 7, and their affinity to heparin. Specific roles of each isoform have not been clearly understood. However, they seem to have a different but coordinated ability for tumor vascularization (35, 36).

RBPs implicated in cancer

Since RBPs are involved in the expression of diverse genes responsible for cell growth and proliferation, altered expression of RBPs might cause defects in cell physiology and lead to cancer development. Cancer is a heterogeneous disease caused by various defects in gene amplification, mutation and chromosomal rearrangement of critical oncogenes and tumor suppressors. For example, chromosomal rearrangements generate chimeric fusion RBP proteins with transcription factors. The RNA-binding RRM/RGG domain of TET family RBPs such as TLS/FUS (TransLocated in Sarcoma/FUsed in Sarcoma) and EWS (EWing Sarcoma) is frequently substituted by the DNA-binding domain of their fusion partner transcription factors (37). TET fusion proteins possess transforming potential that may be necessary and sufficient to confer oncogenicity (38-41). However, the exact tumorigenic mechanism of TET fusion proteins awaits more study.

Deregulation of splicing factors might cause alternative splicing of various transcripts in cancer cells. SF2/ASF splicing factor is an example of such an altered RBP in cancer (42). Upregulation of SF2/ASF has been revealed in various human tumors by gene amplification and overexpression of the protein. Considering the unexpected multiple functions of SR proteins starting from alternative splicing to translation (8), the unregulated expression of SF2/ASF might be influential on the expression of both RNA and protein. Furthermore, collaboration with cellular signaling could greatly increase the oncogenic potential of the SF2/ASF protein (43, 44). SF2/ASF-mediated alternative splicing inactivates tumor suppressors such as Bridging Intergrator 1 (BIN1) and activates oncogenes such as MNK2 and S6K1 kinases (42), which might be responsible for SF2/ASF-driven transformation. The study suggests that SF2/ASF is a potential proto-oncogene and that other splicing factors might also be tumorigenic in some cell types or genetic contents.

Translation of mRNA is also a regulatory point for the expression of tumor suppressors and oncogenes in cancer cells (45). Therefore, altered expression of translation factors might play critical roles in tumorigenesis. A key translation initiation factor, eIF4A cap binding protein, is a downstream target of phosphatidyl-inositol 3-kinase-Akt-mTOR pathway, and is an example of a tumor-related translation factor. The protein is highly expressed in different tumor types and seems to behave as a classical proto-oncogene.

RBPs involved in cell differentiation and proliferation might also modulate cancer cell development. An example is the

STAR (Signal Transduction Activator of RNA metabolism) family of RBPs, whose expressions are altered in cancer. The best-studied STAR is Sam68. It is a KH domain containing protein that is overexpressed in breast and prostate cancer cells (46, 47). Downregulation of Sam68 reduces the cell cycle and sensitizes cells to apoptosis, which contributes to proliferation and survival of prostate cancer cells (47).

We have recently demonstrated that the cancer-related transcription factor β-catenin can also bind RNA, thus acting as a RBP (48). In addition, overexpression and unregulated expression of β-catenin regulates RNA alternative splicing and RNA stabilization in colon cancer cells (48-50). Overexpressed β-catenin upregulates cyclinD1 and c-myc mRNA transcription as transcription factors in cancer cells. β-catenin can also regulate alternative splicing of Estrogen Receptor-β (ER-β) mRNA and stabilize Cyclooxygenase-2 (COX-2) and cyclinD1 mRNAs in colon cancer cells (Fig. 4). This is an example of how discovery of novel RBPs can lead to the demonstration of their unexpected functions in RNA metabolism. Identification and characterization of new classes of RBPs will greatly expand our understanding on cellular RNA-RBP network, and might shed light on the RNA-RBP based gene expression regulatory circuitry in the cells.

Future perspectives

Identification of RBPs responsible for specific defects in RNA metabolism is challenging. Even with the emerging roles of RBPs in the regulation of post-transcriptional steps, it is noteworthy that the list of RBPs reported to be involved in cancer is rather short so far. Considering the importance of RBPs in multiple steps of gene expression, it is still not clear why so few RBPs have been identified as oncogenes or tumor suppressors. However, after the discovery and identification of RBPs a couple of decades ago, the list of RBPs and their functions in RNA metabolism has begun to expand recently. Since the currently known RNA-RBP networks might be a snapshot of the RNA world, more extensive RNA-RBP networks await discovery (51). Advances in bioinformatic, biochemical and genetic analyses will contribute significantly to our understanding of RBPs and their associated RNAs. Since combinatorial binding of diverse sets of specific RBPs is likely to be a general feature of extensive post-transcriptional regulation in eukaryotic cells, combination of powerful technology would be required to understand complex and dynamic RNA-RBP network in cells (52, 53). Combination of high-throughput sequencing technology to CLIP method would be such an example (54). In addition, elucidation of multiple interconnected RNA metabolism and its linkage to cell signaling pathways will be exciting research areas, both in basic research field as well as in translational research for the development of therapeutic targets. Enormous opportunities exist for future identification of novel RNA-RBP networks and understanding of their pathogenic malfunction.

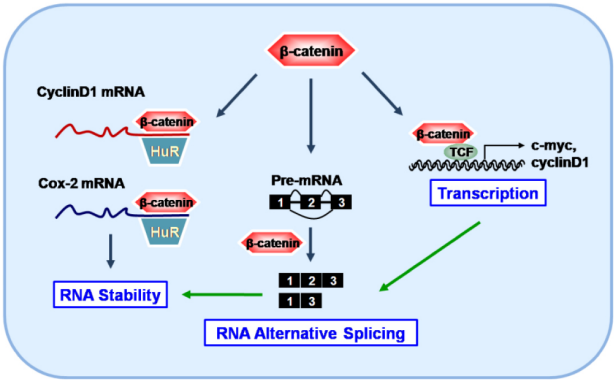

Fig. 4. Proposed roles of aberrantly overexpressed β-catenin in cancer cells. In cancer cells, aberrantly deregulated β-catenin is overexpressed and upregulates cyclinD1 and c-myc mRNA as a transcription factor. β-catenin can also regulate RNA alternative splicing of ER-β and RNA decay of COX-2 and cyclinD1 in colon cancer cells (48-50).

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