Original Article

The molecular responses of human embryonic kidney cells to abnormal expression of RNA binding motif protein 10: cues to molecular mechanism and function

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Abstract: This study was aimed to illustrate the role of RNA binding motif protein 10 (RBM10) in the human embryonic kidney (HEK293) cells. The RNA sequencing data following RBM10 knockdown (KD) or over-expression (OE) in HEK293 cells were downloaded and reanalyzed. Differentially expressed genes (DEGs) were screened and divided into four groups, including RBM10 KD induced up- and down-regulated, RBM10 OE induced up- and down-regulated groups. In addition, direct (differentially expressed in RBM10 OE and KD groups) and indirect DEGs (differentially expressed in one groups) were identified, and protein-protein interaction (PPI) network for them was constructed. In the end, pathway enrichment analysis of genes was performed. Total 268 DEGs (169 up- and 99 down-regulated) in RBM10 KD group and 113 DEGs (38 up and 75 down-regulated) in RBM10 OE group were obtained, of which, 34 DEGs were direct genes. A direct-indirect PPI network with 339 nodes including 18 direct DEGs, 156 indirect DEGs and 165 intermediate genes was gained. SAT1 (spermidine/spermine N1-acetyltransferase 1), HMBOX1 (homeobox containing 1), CLOCK (clock circadian regulator) and CCND2 (cyclin D2) with high degree were direct genes. Genes in PPI network were closely associated with signaling pathways related to cell cycle and cancers. In conclusion, RBM10 may be involved in the regulation of gene expression of CLOCK, SAT1 and CCND2. RBM10 may interact with these genes to involve in gene expression and congenital disease occurrence through multiple signaling pathways including dopaminergic synapse and Wnt and Hippo signaling pathway.

Keywords: RNA binding motif protein 10, human embryonic kidney cells, differentially expressed genes, pathways

Introduction

The RNA-binding motif protein 10 encoded by the RBM10 gene, contains two RNA recognition motifs, two zinc fingers and one G patch motif [1]. The motifs are found in RNA-binding proteins which involved in pre-mRNA splicing [2, 3]. RBM10 granules located at the sites of transcription and splicing and is related to transcriptional activity [4]. RBM10 is considered involved in gene expression process via alternative splicing [5].

RBM10 is considered a disease-causing gene and the mutation of it is closely related with multiple human congenital disorders [6]. In addition, the loss of function of RBM10 could result in various diseases, such as cleft palate, malformation of the heart (TARP syndrome) and lung adenocarcinoma [6-8]. Recent evidence suggests that RBM10 plays a regulatory role in alternative splicing and shows the ability to mediate apoptosis [5, 9].

Human embryonic kidney (HEK) 293 cells have been widely used as a mammalian expression system. In the present study, the RNA sequencing data following RBM10 knockdown (KD) or overexpression (OE) in HEK293 cells were downloaded. The differentially expressed genes (DEGs) induced by the aberrant expression of RBM10 were analyzed. The functions of DEGs were investigated and the protein-protein interaction (PPI) network was constructed. Our findings may help illustrate the role of RBM10 in the HEK cells.
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Figure 1. Venn diagrams of differentially expressed genes induced by abnormal expression of RNA Binding Motif Protein 10 (RBM10). KD.UP: RBM10 knockdown induced up-regulated genes. KD.DOWN (KD): RBM10 knockdown induced down-regulated genes. OE.UP: RBM10 over expression induced up-regulated genes. OE.DOWN: RBM10 over expression induced down-regulated genes.

Materials and methods

Data source and DEGs

The data analyzed in our paper was derived from the previously published reports (GSE4-4976) [10]. In the original paper, the effect of RBM10 knockdown (KD) and over expression (OE) on splicing regulation in HEK293 cells were analyzed. We collected and analyzed the original information of differential gene expression that induced by RBM10 KD and OE in HEK293 cells following compared to the controls, and the fold-change of each gene was computed. These DEGs were screened with the thresholds of fold-change > 1.5 and FDR (false discovery rate) < 0.05.

Classification of DEGs

DEGs were divided into four groups, including RBM10 KD induced up-regulated genes (KD.UP) and down-regulated genes (KD.DOWN), RBM10 OE induced up-regulated genes (OE.UP) and down-regulated genes (OE.DOWN).

It can be reasonably hypothesized that genes both differentially expressed and showed opposite expression trend in RBM10 KD and OE groups were likely to be directly perturbed by the abnormal expression of RBM10. These genes were defined as the direct genes. While the remaining DEGs, which only differentially expressed in RBM10 KD group or in RBM10 OE group were considered indirect genes. This perturbation may be indirectly relevant to the aberrant expression of RBM10 and would be induced by the aberrant expression of direct DEGs.

PPI network construction

Biological General Repository for Interaction Datasets (BioGRID) is a collection of gene-gene interaction information [11]. It was used to construct the PPI network of direct and indirect genes in this study. Briefly, we hypothesized that the differential expression of indirect genes were induced by the differential expression of direct genes. The propinquity of a node to another within the network was expressed as path lengths [12, 13]. A pair of nodes with small path length in network tends to be densely connected. Based the large scale data on protein interaction across human provided by BioGRID 3.4, the direct and indirect gene pairs with shortest-path length (shortest path distance) no more than two were collected for PPI network construction. The direct-indirect PPI network was visualized by Cytoscape software [14].

Pathway enrichment analysis

ClueGO as a Cytoscape (version 3.2.1) plug-in is widely used for functional (e.g. gene ontology) and pathway enrichment analysis for a large scale of genes [15]. It uses kappa statistics to determine the link between functional terms based on a predefined kappa score level and creates functionally grouped network with terms. In addition, CluePedia [16] is another Cytoscape plug-in. It extends ClueGO functionality by rendering those networks with other biological data including known, in silico and experimental data.

In order to further analyze the direct-indirect PPI network, DEGs in the PPI network were subjected to KEGG pathway enrichment analysis.
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Results

Classification of DEGs

Based on the raw expression values, total 268 DEGs (169 up-regulated genes and 99 down-regulated genes) in RBM10 KD group and 113 DEGs (38 up-regulated genes and 75 down-regulated genes) in RBM10 OE group was screened compared to the control group (Supplementary Table 1). The classification of DEGs was displayed in Figure 1.

Among the 335 DEGs, 34 of them were direct DEGs, including 21 KD.UP and OE.DOWN genes, such as ACTR3C (ARP3 actin-related protein 3 homolog C (yeast)) and BDKRB2 (bradykinin receptor B2), and 13 KD. DOWN and OE.UP genes, such as DOK3 (docking protein 3) and DRD4 (dopamine receptor D4).

Direct-indirect PPI network

Based on the human gene interaction network, the direct-indirect gene pairs with the shortest and a functionally organized pathway term network was created. FDR (false discovery rate) < 0.05 and kappa score = 0.4 were set as the cutoff value.

Figure 2. The direct-indirect protein-protein interaction (PPI) network of differentially expressed genes (DEGs). Red nodes represent direct DEGs. Green nodes represent indirect DEGs. Blue nodes represent intermediate genes. The size of a node indicates the degree of a gene. Edges refer to the interaction between two nodes.
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path length ≤ 2 were collected for PPI network construction. The direct-indirect PPI network with 339 nodes and 747 edges were exhibited in Figure 2. There were 18 direct DEGs, 156 indirect DEGs and 165 intermediate genes. All the 18 direct genes in the PPI network were listed in Table 1, among which five genes had relatively high degree, such as RBM10 (RNA binding motif protein 10), SAT1 (spermidine/spermine N1-acetyltransferase 1), HMBOX1 (homeobox containing 1), CLOCK (clock circa-dian regulator) and CCND2 (cyclin D2).

Pathway enriched by DEGs and the pathway term network

The functional term network (Figure 3) showed pathways enriched by DEGs in the PPI network. Especially, the genes in the PPI network were closely functionally associated with multiple signaling pathways (e.g. PI3K-Akt signaling pathway, FoxO signaling pathway or p53 signaling pathway), cell cycle and cell division, and various cancers (e.g. viral carcinogenesis, proteoglycans in cancer, prostate cancer or lung cancer).

Discussion

RBM10 has been proposed to be a disease-related gene and plays a crucial role in disease progression [6-8]. However, the molecular effects of RBM10 in disease process remain to be clarified clearly via further investigations. In this paper, our data showed that there were 268 DEGs in RBM10 KD cells and 113 DEGs in RBM10 OE cells. The differential expression of 34 genes was found to be induced by abnormal expression of RBM10, directly. PPI network highlighted five direct DEGs with high degree of connectivity, such as RBM10, SAT1, HMBOX1, CLOCK and CCND2. Moreover, multiple disease-related pathways were enriched by DEGs.

CLOCK encodes a basic helix-loop-helix-PAS transcription factor which plays a critical role in circadian pacemaker [17]. In this paper, the KEGG pathway enrichment analysis showed that CLOCK was closely associated with Herpes simplex infection, Dopaminergic synapse, and Circadian rhythm. It is reported that the histone acetyltransferase CLOCK is the main component of the transcriptome of herpes simplex virus 1 [18]. CLOCK histone acetyl transferase localizes at ND10 nuclear bodies and contributes to the expression of herpes simplex virus gene [19]. The herpes simplex virus is proposed to be the risk factor of congenital cataracts [20]. Besides, RBM10 has been known to cause the congenital disorders, such as cleft palate [7] and congenital cardiovascular malformations [21]. Moreover, 30% of neurons differentiated from embryonic stem cells by a stromal cell-derived inducing activity was dopaminergic and significant amounts of dopamine were produced [22]. In addition, SAT1 had a close relationship with CLOCK, it was found not only to acetylate both of spermidine and spermine which interacts with dopamine receptor, but also regulate polyamines concentration and their transport out of cells [23]. Mohammad et al. suggested that dopamine-2 receptor was associated with herpes simplex virus 1 encephalitis [24]. Therefore, RBM10 interacts with CLOCK and SAT1, and maybe through the dopamine signaling pathway to involve in gene expression and disease occurrence during embryonic development.

Furthermore, CCND2 was found enriched in pathways including HTLV-I infection, Wnt signaling pathway, Measles, Hippo signaling pathway, PI3K-Akt signaling pathway and several other signaling pathways. All of the above pathways are associated with cell growth, proliferation and apoptosis [25-27]. Besides, Heallen et al.

| Table 1. Top eighteen genes in protein-protein interaction network with high degree |
|-----------------|-----|-----|
| Node           | Degree | Type |
| RBM10          | 44   | direct |
| SAT1           | 40   | direct |
| HMBOX1         | 19   | direct |
| CLOCK          | 19   | direct |
| CCND2          | 18   | direct |
| ETV5           | 9    | direct |
| DOK3           | 7    | direct |
| LEPREL2        | 5    | direct |
| DRD4           | 5    | direct |
| TCX1D2         | 4    | direct |
| MC1R           | 4    | direct |
| TRNAU1AP       | 3    | direct |
| OLIG1          | 2    | direct |
| SLC2A3         | 2    | direct |
| UB4L1          | 2    | direct |
| BDKRB2         | 1    | direct |
| IFITM2         | 1    | direct |
| PAQR6          | 1    | direct |
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Figure 3. The functionally organized pathway term network. The size of nodes reflects pathway significance (false discovery rate, FDR): smaller FDR value, larger node size. Edge between nodes means they share common genes: wider edge, larger overlap. Pathways are classified into several function groups (different node color) because of kappa score. The most significant pathway of each group has highlighted label.
uncovered that Hippo pathway inhibited Wnt signaling pathway to restrict cardiomyocyte proliferation in the developing mouse heart [28], while, the PI3K-Akt signaling together with Wnt pathway involved in the progression regulation of cell cycle in cancer cells [29]. Moreover, the canonical and noncanonical Wnt pathways were both dysregulated by HTLV-1 bZIP factor to support proliferation and migration of adult T-cell leukemia cells [30]. In addition, CCND2 who had a dramatic periodicity in protein abundance, is one member of the cyclin family proteins and a cardiogenic GATA4 cofactor involving in cardiogenesis and human congenital heart disease [31]. Since the close interaction between RBM10 and CCND2 on abnormal expression, RBM10 may have significant effects on embryonic development by a number of cell proliferation-related signaling pathways.

In conclusion, the expression of CLOCK, SAT1 and CCND2 would be regulated by RBM10 directly. RBM10 may interact with CLOCK and SAT1, and may be involved in gene expression and congenital disease occurrence through the dopamine signaling pathway. In addition, RBM10 may also interconnect with cell cycle and cell proliferation-related proteins to impact embryonic development by multiple signaling pathways implicating CCND2. However, further research in some other types of cells as well as experimental validation studies to validate our results is still needed.

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Disclosure of conflict of interest

None.

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