Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Review Article **DOI: 10.26479/2019.0502.38**

RBM3: A COLD-INDUCIBLE GENE AND A POTENTIAL THERAPEUTIC TARGET FOR CANCER AND PARKINSON DISEASE

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ABSTRACT: The performance of any gene expression occurs best at its normothermic that is 37°C and any deviation i.e. heat and cold stress can lead to downregulation of many proteins which in turn affect in the cell survival which might result in cell death. To overcome such disastrous effect there are a certain set of proteins which will respond in rescuing the cells from apoptosis and one such set is cold-inducible genes. Cold-Inducible genes are those genes whose expression gets elevated at hypoxia condition. The differential expression of the gene differs from cell to tissue type and the authenticity of it being a biomarker can be a major set forward in acting as a potential therapeutic target for diseases, associated with the RBM3.

KEYWORDS: RBM3, Neurodegeneration, stress response, cell cycle, cancer.

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1.INTRODUCTION

The organism had developed sophisticated cellular mechanisms to combat the changing environmental temperature. Change in lipid composition, a decrease in the rate of protein synthesis and cell proliferation are the cellular responses which are adopted by the mammals and other organisms under cold stress condition[1],[2],[3],[4]. Though there is plenty of research done on the cold-inducible gene, yet the molecular mechanism has not been understood completely. The regulation in the expression of the gene is done post-transcriptionally and during transcription which is mediated by many proteins and one such protein is RNA Binding protein [5],[6],[7],[8] whose characterization had led to the identification of different RNA binding motifs in the family. The RBM3 is involved in positive regulation of mRNA splicing via spliceosome, positive regulation of

translation, RNA processing and responses to stress leading to differential expression of the gene. Moreover, the earliest discovery was in the Cyanobacterium and plants, from which the isolation of family of protein also known as Glycine-rich RNA binding protein family with one carboxyl terminal Glycine-rich domain and one amino-terminal domain CS-RBD some of which is induced by cold and oxygen stress i.e. RBM3 and CIRBP in the animal[9][10],[11],[12],[13],[14].

Cold-inducible gene

RBM3 and CIRBP are two cold-inducible genes in mammals known till date. AT 32°C RBM3 is expressed in the Sertoli cell while CIRBP expressed in the germ cells in the scrotum of the mouse indicating that though both are induced in cold stress but there are differences in the degree of distribution both on the basis of the type of tissue and level of cold stress-induced leading to different functions. In hypoxia condition, the expression of the RBM3 and CIRP gene expression is upregulated and does not require HIF1 for the same [9],[15]. RBM3 is a cold-inducible gene whose expression gets elevated at hypothermia and hypoxia condition. It is located at Xp11.23 with the gene size of approximately 6813bp, 7 exons, 157aa and localized specifically on the nucleus, cytoplasm, and dendrites. RBM3 amino acid sequence given below

MSSEEGKLFVGGLNFNTDEQALEDHFSSFGPISEVVVVKDRETQRSRGFGFITFTNPEHA
SVAMRAMNGESLDGRQIRVDHAGKSARGTRGGGFGAHGRGRSYSRGGGDQGYGSGRYY
DSRPGGYGYGYGRSRDYNGRNQGGYDRYSGGNYRDNYDN

Cell stress response

As and when there is a deviation from the physiological condition such as exposure to the cold stress the enzymatic reaction, diffusion, membrane transport rate will automatically be reduced. The net physiological effect on the cell is quite similar for both cold and heat stress which includes denaturation and misfolding of the proteins, rate of cell cycle reduced, reduction of protein synthesis due to inhibition of transcription and translation, change in membrane permeability leading to decrease in intracellular potassium ions and increase in cytosolic Na^+ and H^+ etc. are result of cellular physiological response to cold stress. In the mammalian cell the level of RBM3 expression reaches highest at moderate to mild hypothermia (28°C -34°C), and drops significantly upon deep hypothermia (15°C -25°C) and decreases significantly at hyperthermia (39°C -42°C) in the cultured cell in vitro. The RBM3 expression is highly sensitive towards the change in temperature specifically in a neuronal cell, even a drop of 1 °C from 37°C to 36°C is sufficient to show differential expression of RBM3 [16]. During physiological or mild hypothermia condition there is tight physical interaction of the small proportion of RBM3 with the 60S in the brain which may lead to the decrease in the microRNA levels thereby preventing more dramatic reduction of the protein synthesis. Further, the mechanism by which the RBM3 expression increases protein synthesis requires further investigation. RBM3 also plays a role in stress response in the presence of IRES within the 5'UTR of its mRNA which is utilized during hypothermia thereby elevating the rbm3

production [17],[18].

Cell cycle

The overexpression of RBM3 has observed in both the nucleus and cytoplasm of human cancer tissues occurred in pancreas, breast, colon, lung, ovary, prostate cancer. Overexpression of RBM3 by generating stable RBM3 expressing cell results in the higher growth rate of the cells that induces anchorage-independent growth when overexpressed and thereby inducing oncogenic transformed cell. In contrast, the down-regulation of the RBM3 by RBM3 specific siRNA in HCT116 cells led to the 50% reduction of the RBM3 expression. The expression of the RBM3 regulates the expression of the COX2, VEGF, IL8 and resulting in a reduction of capillary formation leading to loss of tumor growth. Depletion of RBM3 leads to inhibition of cell growth by increasing the number of cells arrest at the G2/M phase. The suppression of RBM3 leads to an increase in the level of protein expression of the cdc25c which catalysis the activation of CDK1/cyclin B kinase, a rate-limiting step in mitosis [18],[19]. The kinase i.e. Chk1 and Chk2 are associated with the DNA damage checkpoint which is activated by phosphorylation of Ser345/ser317 and Thr68 respectively. [19],[20],[21]. Moreover, the phosphorylation at Ser 15 of p53 results in activation of apoptosis and G2/M checkpoints[22]. Immunoblot of RBM3 depleted cells in culture and tumor shows increased phosphorylation of Chk (ser345), chk (Thr68) and p53 (ser15) resulting in caspase-mediated apoptosis in the G2/M phase. Therefore, RBM3 is considered a novel proto-oncogene and is essential for cell cycle progression [23].

Effect (Why RBM3 is important)

In breast cancer, the expression of RBM genes in the X chromosome is associated with the expression of the apoptotic genes and some of these genes significantly correlates with the expression of VEGF and CD105 [24]. The overexpression of RBM3 is associated with cell survival in breast cancer and its progression, especially in ER-positive tumors. Therefore, RBM3 is a good prognosis for patients who do not need adjuvant systemic chemotherapy [25]. In HPV negative oropharyngeal carcinomas when compared to the normal oral epithelium there is down-regulation of the RBM3 which is involved in apoptosis. Knocking down or Reduction of the expression of the RBM3 in LNCaP and PC-3 cells is failed to induce apoptosis in prostate cancer cell line instead it induced cell cycle arrest prior to S and G2 M phase. The RBM3 and CIRBP enhanced the DNA damage induced by the chemotherapy in the prostate cancer cell line [26]. In hypoxia condition, the overexpression of RBM3 leads to the colon cancer survival by COX2 signaling mechanism. Under serum deprivation condition the RBM3 overexpression rescued the cells from death. Therefore, on adverse condition, the RBM3 plays a crucial role in restoring the translational efficacy [27]. The expression of RBM3 is a good prognostic marker for EOC epithelial ovarian cancer both at protein and mRNA Level and decrease in the RBM3 effects the platinum sensitivity [28] RBM3 expression upregulates during the muscle atrophy to preserve the remaining

muscle mass which can act as a therapeutic target for rescuing the muscle loss [29]. Moreover, RBM3 stimulates differentiation of osteoblast via ERK signaling pathway when exposed to the hypothermic condition. Therefore, RBM3 is potent prognosis marker in ovarian cancer, colon cancer, prostate cancer, breast cancer, and malignant melanoma.

Clinical importance

As and when we learned the importance of the overexpression of the RBM3 in the prostate, breast and colorectal cancer had led to the improved cell survival, therefore, the molecular mechanism study had become more important and to be elucidated in detail. When there are cancerous tumors which are not suitably resecting surgically, and moreover the conventional chemotherapy drug doesn't help against cancer. Therefore, there is compulsive need of radiotherapy sensitive cancer cells for treating cancer but due to frequent exposure to IR which induces an alteration in the protein and gene expression level leading to reduced sensitivity to IR resulting in radiation resistance, or radioresistance ultimately leads to recurrence of the tumor. The upregulation of RBM3 inhibits the apoptotic response through AKT/Bcl-2/PI3K signaling pathway thereby enhancing the radioresistance. RBM3 can be a novel protein for predicting radioresistance for the treatment of NPC Nasopharyngeal carcinoma [30]. In a neuron, RBM3 plays a highly specific role in enhancing translation thereby increasing global translation [31]. The excessive accumulation neurotoxicity like NO induces neuronal cell death by causing disease like Parkinson disease PD, Alzheimer's, multiple sclerosis and stroke. Mild hypothermia leads to RBM3 mediated neuroprotection from NO-induced apoptosis which is independent of COX2 signaling [32]. In neurodegenerative diseases like Alzheimer's, PD the expression of RBM3 by mild hypothermia had mediated in neuronal protection [33]. Mild hypothermia is used as a therapeutic measure to induce neuroprotection against brain injuries by rescuing the cells from undergoing apoptosis [34],[35],[36]. There is differential expression of RBM3 at the spatial-temporal region of the brain and the expression decreases at a high extent when we reach adulthood [36]. Therefore, clinical research is going on to boost the expression of the RBM3 in the brain after an injury [37]. Therefore, RBM3 can be used as a potential biomarker and a therapeutic target for many diseases.

2. CONCLUSION

The striking finding after careful references in all the papers revealed:

The maintenance of stable temperature is important for the proper molecular function in the brain. As far as we know there were only 2 studies related to brain temperature in PD vs Normal individual. The temperature measurement of Visual cortex and centrum semiovale was done using Proton magnetic resonance spectroscopy (1H MRS). The visual cortex showed that the PD patient has 1.161℃ increased temperature than control participants and the temperature of centrum semiovale of PD patient have increased the temperature of 0.301°C compared to control participants. These might be due to impaired brain temperature control which led to an increase in brain temperature

[38]. The second research on brain temperature using ventricular CSF which was done using MR-DWI thermometry, and found out that the male PD have 0.9°C temperature variation compared with control while in female PD have no significant temperature variation compare to normal participants. This indicates that there is cerebral thermic homeostasis impairment occurred in PD, which is due to mitochondrial dysfunction that is reported in PD by Rango et.al 2014. There are reports that due to reduced activity of electron transport complex leading to reduced consumption of oxygen and glucose had resulted in defective mitochondrial oxidative phosphorylation in mitochodriopathy. Moreover, differential expression of RBM3 i.e. 5 fold reduction in the PD brain sample of substantia nigra proved to be PD associated gene and can be a potential therapeutic target [39]. The above reports stating about an increased brain temperature in PD patient which supports the fact that RBM3 gene being cold-inducible gene is highly sensitive to the temperature. The SNPs variation had led to the change in the expression of the gene. Therefore, research had shown that a decrease in the temperature of the brain that is in the hypothermic condition in brain cell line had helped in neuroprotection. The pathway analysis showed that when RBM3 was introduced with the Parkinson causative gene in the string, RBM3 did not show any interaction with the PD causative gene while when RBM3 was introduced with the CIRBP gene (which is also a cold-inducible gene and relative differential expression was seen in brain with that of RBM3) and PD causative gene which results in interaction of RBM3 with that of PD causative gene was observed. The involvement of RBM3 with that of PD causative gene in stress regulation, neurodegeneration, Parkinson disease interaction is shown in figure 1 A, B and C. Therefore, we can hypothesize that RBM3 might be acting as a translational coactivator or coexpression by sharing the same transcriptional factor and helping the PD causative genes to express normally. The cold shock proteins are induced by cold stress or chemicals i.e. erythromycin, fusidic acid, chloramphenicol, tetracycline, and spiramycin which are involved in various cellular processes such as transcription, translation, etc. The RBM3 being one of cold-inducible gene and a translational regulatory protein which is highly sensitive to different temperatures therefore, deviation in its expression will affect the overall expression of the PD causative gene. Therefore, it is very crucial to understand the role of RBM3, its relation with CIRBP expression and its pathways involved which can act as a blood biomarker and a major therapeutic target in treating Parkinson disease.

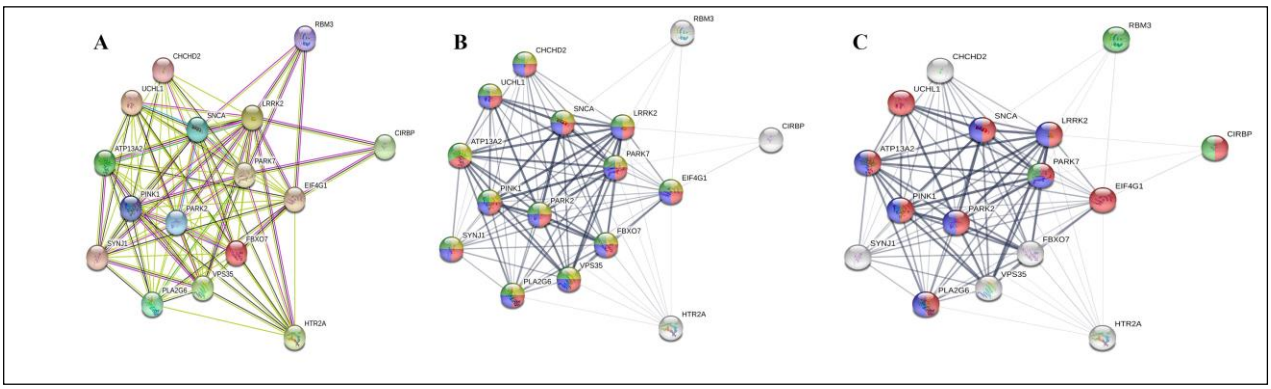

Figure 1. Interaction of RBM3 with PD causative proteins, Colored node represents different proteins, Line color indicates the type of interaction evidence i.e. Pink=experimetally determined, Green=gene neighbourhood, Sky blue=curated database, Yellow =textmining, Black=coexpression, Interaction score- low confidence 0.150, PPI enrichment p-value: < 1.0e-16 (**A).** Line thickness indicates the strength of data support, Yellow=node Mitochondrial dysfunction in Parkinson's disease, Blue=node Parkinson disease, Red=node Parkinsonism, and PPI enrichment p-value: < 1.0e-16 **(B)**. Green=stress response, Blue=cellular response to stress, Red =response to stress and PPI enrichment p-value: < 1.0e-16**(C)**.

ACKNOWLEDGEMENT

The author would like to thank the Tibetan Children Village and Tibet Fund for providing the scholarship.

CONFLICT OF INTEREST

None.

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