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Deciphering brain-specific transcriptomic expression of *Ezr*, *Rad* and *Msn* genes in the development of *Mus musculus*

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ABSTRACT

Ezrin, Radixin and Moesin (ERM) are critical membranous component involved in cross-linking of actin filaments. Moesin (*Msn*) is recognized as a pivotal protein involved in regulation of cell signalling events associated with the maintenance of epithelial integrity, actin organization and polarity. Radixin (*Rad*) is known to cell-to-cell adherens junction as a barbed end-capping protein whereas ezrin (*Ezr*) is recognized at cell adhesion, motility, apoptosis and phagocytosis. The current study for the first time reports the transcriptional and RNA secondary structural variations among brain-specific ERM genes. Firstly, we analyzed brain-specific transcriptomic expression in selected embryonic and postnatal developmental stages (E10.5, E14.5, E18.5, P0.5, P3.5, P5.5, P10.5 and P20.5) of *Mus musculus*. Among designated developmental stages, *Ezr* has highest fold difference in early embryonic and postnatal stages (E10.5, P0.5 and P5.5). *Rad* showed a similar pattern of high expression especially at embryonic stages (E10.5 and E18.5) and postnatal (P0.5 and P5.5), however, *Msn* exhibited non-significant fold differences in comparison to controls leading to its curial role in development. Furthermore, computational prediction of ERM coding mRNA transcripts, reveals compact and less dynamic *Msn* secondary structure and pseudoknots configurations, in contrast to *Ezr* and *Rad*. Conclusively, transcriptomic levels are greatly associated with compact base pairing organization of its secondary structures. These findings open a new domain to understand the occurrence of ERM-specific cytoskeleton proteins during developmental stages.

1. Introduction

The cytoskeleton is the most diverse cellular network of the cell. It is an intricate communication of microfilaments and microtubules in the cytoplasm that controls cell shape, maintains intracellular organization, and in cellular mobility (Goldman et al., 2008). Among cytoskeleton molecules, least is known about ERM members. the role of ERM proteins is recently highlighted in cellular environmental microfilaments (Thomas, 2011) and found to be crucial in cellular migration and proliferation, this finding provided information in the involvement of ERM members in cancer and in serval neurological disorders (Yaming et al., 2015). Previously, ezrin (*Ezr*) 81-KDa was identified as a constituent of chicken microvilli (Bretscher, 1983) later on also found in lungs, kidneys, epithelial and mesothelial cells (Louvet-Vallée, 2000). *Ezr* found cytosolic proteins which are present in the juxtamembrane region. The localization of *the Ezr* protein is evident for its dynamic activity and cellular actions (Sarrió et al., 2006). The second member of ERM family radixin (*Rad*) 82-KDa was first isolated from mammalian hepatocytes and has found end-capping actin-modulating protein (Amieva et al., 1994). Interestingly, *Rad* molecule was noticed on synaptic membrane interaction with GABAA receptors. Thus, indirectly involved in neuronal regulation (Loebrich et al., 2006). Moesin (*Msn*) 78-KDa is a heparin-binding protein and was identified as third ERM members as membranous protrusions and involve in cell growth regulation and mobility. The cellular movement is also greatly influenced by the localization of mRNA within the cell which creates a cellular polarity for different transportational and cytoskeleton proteins to be synthesis at various ends of the cellular structure. Along with abundant evidence of proteins sorting and selective transportation to different polarized areas of cell, an increasing volume of evidence have suggested a promising role of mRNA concentration at respective positions with their secondary and tertiary structures influence have been reported. (Liao et al., 2015). Therefore, we explored ERM member's co-transcriptional levels in *Mus musculus* brain tissues and their mRNA sequence's secondary structures by using computational biology servers, which are directly and indirectly involved in most of cellular signalling and survival processes such as cell division, adhesion, polarity, migration and cellular movement facilitated by cytoskeletal proteins

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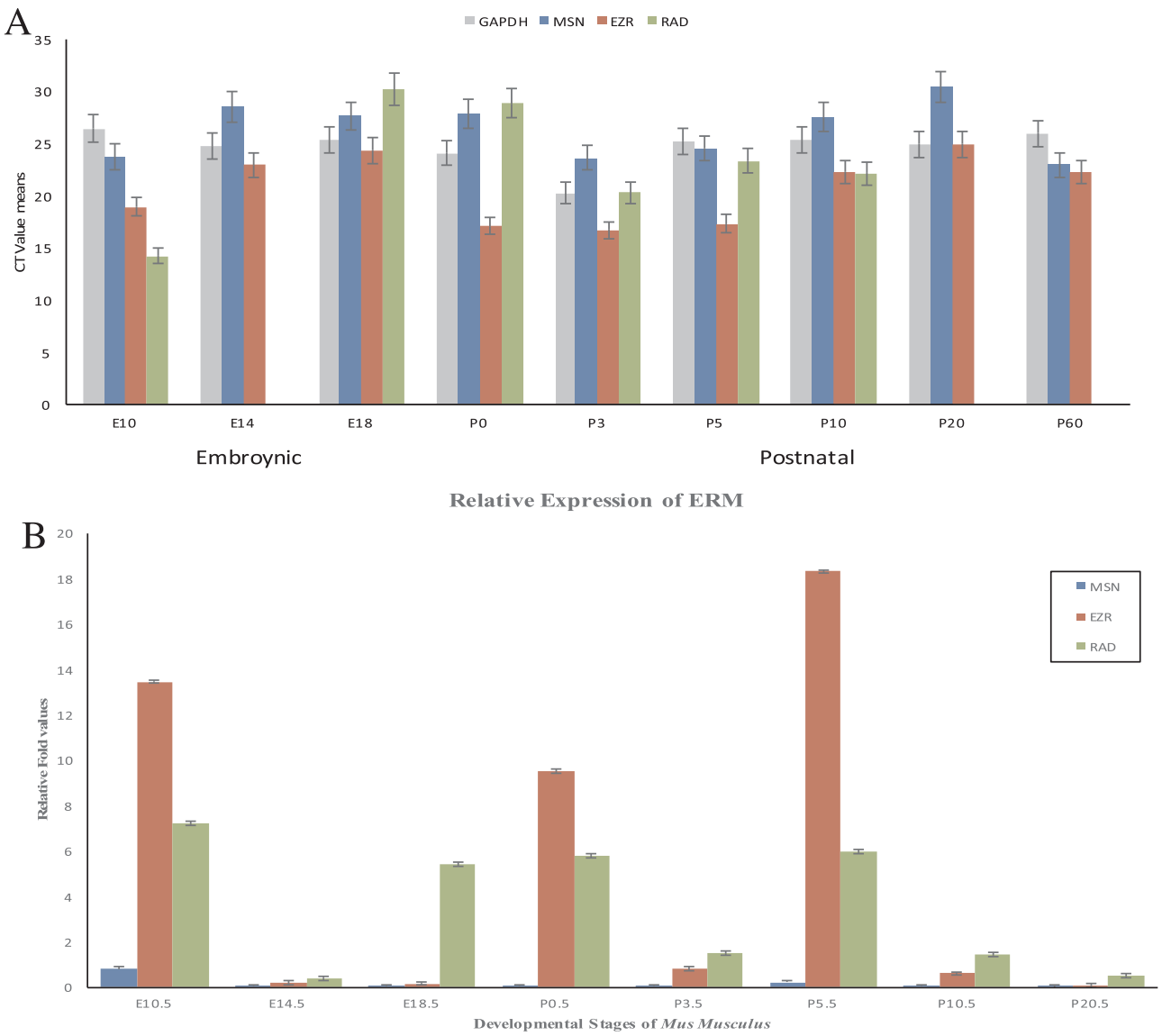

Fig. 1. ERM transcriptional expression, A.CT value means based expression profile. B. Fold chart by using ΔΔCT value analysis.

(Thomas, 2011).

2. Results

Transcriptional analysis of ERM genes in *Mus musculus* pre and postnatal brain developmental stages was done. The expression levels of each member were normalized with GAPDH. Statistical analysis by One way ANOVA, revealed variable significance level for ERM members, *Msn* and *Ezr* were significantly expressed in all selected stages. However, expression of *Rad* was significant in most of the selected stages other than P20.5 and P60.5, where it was highly non-significant (Fig. 1A).

Transcriptional fold differences were shown variably by all genes analyzed compared with calibrator (P60.5) and normalized against GAPDH. Expression of *Msn* remained significantly similar throughout all experimental stages overlying its curial importance and presented much stable expression. Whereas *Ezr* exhibited significant expression fold at E10.5 among embryonic stages and at P0.5 and P5.5 postnatal stages. *Rad* expression shows fold increase at embryonic stages (E10.5 and E18.5) and postnatal (P0.5 and P5.5). ERM expression fold analysis imprints the competitive roles of *Ezr* and *Rad* with each other however *Msn* expression has minimum variability (Fig.1B).

The RNA quantitation is depend on its stability and integrity. RNA secondary architecture structure is more conserved parameter than primary sequence, specifically for protein encoding RNA molecules and whose function depends on their structure. The approach has been utilized based on the fold and align method by using NUPACK online server (MIT, USA). Selected secondary structures were thermodynamically stable and determined on the basis of lowest free energies (minimum ΔG for *Ezr*, *Msn* and *Rad*, -492 kcal/mol, -480.80 kcal/mol and -382.20 kcal/mol respectively). ERM transcripts secondary structures were extensively variable in folding configurations which exhibits distinct mismatches, multi-branches and loops along with psudoknots. Fig. 2. also present psudo-knotted structures, which are further elucidated in the graphical presentation in Fig.3. ProbKnot algorithm predicts ERM mRNA sequence on the bases of most probable base pairing within their sequences and with themselves. Each pseudoknot based mRNA transcript configuration represents probable base pairing for each member of ERM. *Ezr* and *Rad* have a less pseudoknots in the transcripts, whereas, *Msn* shows much scattered and extensive psudoknotted structures. Hence, the distinct presence of psudoknots in transcripts effected on the integrity of molecule. Structural evidences of transcripts support the variability in expression patterns of ERM members in selected developmental stages.