Scientific Research Publishing

World Journal of Neuroscience, 2018, 8, 303-313
http://www.scirp.org/journal/wjns
ISSN Online: 2162-2019
ISSN Print: 2162-2000

Identification and Physiochemical Analysis of ERK Interacting Proteins Using Bio-Computational Tools

Khuleshwari Kurrey, Vijay Paramanik*

Cellular and Molecular Neurobiology & Drug Targeting Laboratory, Department of Zoology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh, India
Email: *vijayparamanik@gmail.com

How to cite this paper: Kurrey, K. and Paramanik, V. (2018) Identification and Physiochemical Analysis of ERK Interacting Proteins Using Bio-Computational Tools. *World Journal of Neuroscience*, **8**, 303-313.
https://doi.org/10.4236/wjns.2018.82024

Received: March 28, 2017
Accepted: May 19, 2018
Published: May 22, 2018

Abstract

ERK (Extracellular Signal Regulated Kinase) or MAP kinase is an intracellular signaling molecule. ERK is involved in regulation of various functions *i.e.* cell proliferation, cell migrations, cell survival and many more. It gets activated in response of various stimuli like growth factors, cytokines, virus, second messengers, transforming agents and carcinogens. While transferring signals from cell surface receptors to cell nucleus, ERK interacts with a numbers of proteins. Physiochemical and functional characterization of these proteins is little known. Thus, we attempted to study physiochemical and functional properties of ERK interacting proteins using bio-computational tools. ExPASy and SOSUI server suggested 22 ERK interacting proteins. Physical and chemical parameters of these ERK interacting partners indicated higher percentage of hydrophobic amino acid and leucine as major constituent. Moreover, the instability index indicated that four proteins are stable in over wide range temperature *in vitro,* and remaining eighteen proteins were found unstable. In addition, SOSUI server showed that fifteen proteins were soluble and six are trans-membrane in nature.

Keywords

Extracellular Signal Regulated Kinase (ERK), GRAVY, SOSUI, STRING, STITCH, ExPASy

1. Background

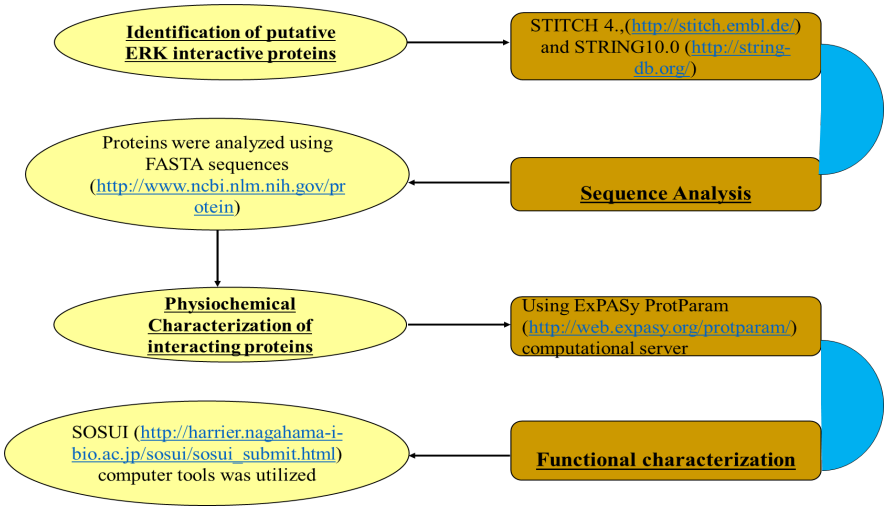
ERK (Extracellular Signal Regulated Kinase) or MAP kinase is an intracellular signaling molecule. It is associated to regulate various cell functions, namely cell proliferation, cell migrations, cell survival and many other biological functions.

ERK gets activated in response to the response of various stimuli such as different growth factors, cytokines, virus, second messengers, transforming agents and carcinogens. ERK helps to transfer signals from cell surface receptors to nucleus of the cells. However, it's still undefined how single ERK pathway regulates verity of specific and different cellular functions [1]. ERK interacts with wide range of proteins to perform specific function. It is important to line up the action of ERK with its interactive proteins at molecular level. Thus the physiochemical and functional properties are needed.

With increasing knowledge in bioinformatics, the computational server is used to determine physical, chemical and functional properties of proteins. These services are useful to facilitate researchers to frame numerous experiments at molecular level. Although physiochemical and functional studies of various proteins and drugs have been done by many workers using bioinformatics tools, [2] computational study on ERK interactive proteins is little studied. Thus, we focused to study physiochemical and functional characterization of ERK interactive proteins using computational tools. Such studies are helpful to understand ERK dependent signal transduction.

2. Methodology

The methodology was followed in this manuscript as under:



2.1. Identification of Putative ERK Interactive Proteins

ERK interactive proteins were retrieved by using STITCH 4., which is a hugearchives of protein-chemical interaction (http://stitch.embl.de/) [3] and STRING 10.0 (http://string-db.org/), is a huge archives of protein-protein interactions [4] [5]. These tools predict 42ERK interactive proteins. Physiochemical analysis of 22 commonly predicted proteins by both servers were done (**Table 1**).

2.2. Sequence Analysis

The proteins were analyzed using FASTA sequences and proteins accession

Table 1. Interacting proteins of ERK retrieved from STITCH and STRING.

S.N.	NCBI Accession no.	Gene Name	Protein Name
1	NP_004433.2	EPHB2	ephrin type-B receptor 2 isoform 2 precursor
2	NP_620407.1	MAPK1	mitogen-activated protein kinase 1
3	NP_060033.3	IL17RD	interleukin-17 receptor D precursor
4	NP_001093207.1	WDR83	WD repeat domain-containing protein 83
5	AAH15221.1	TESC	tescalcin
6	AAI36277.1	MAP3K4	mitogen-activated protein kinase kinasekinase 4
7	EAX07758.1	MAP3K6	mitogen-activated protein kinase kinasekinase 6
8	AAQ89028.1	ULBP2	UL16 binding protein 2
9	AAK13081.1	ULBP1	UL16 binding protein 1
10	CAG38739.1	DUSP14	dual specificity phosphatase 14
11	BAA34369.1	DUSP6	dual specificity phosphatase 6
12	AAI08905.1	RHAMM	hyaluronan-mediated motility receptor
13	NP_001136254.1	MAGI3	membrane-associated guanylate kinase
14	CAG46533.1	PEA15	phosphoprotein enriched in astrocytes 15
15	AAH95453.1	WARS	tryptophanyl-tRNA synthetase, cytoplasmic
16	AAH60837.1	DUSP9	dual specificity phosphatase 9
17	NP_005912.1	MAP3K1	mitogen-activated protein kinase kinasekinase 1
18	AAK13083.1	ULBP3	UL16 binding protein 3
19	NP_067004.3	SLAMF7	SLAM family member 7 isoform a precursor
20	NP_002410.1	MAP3K11	mitogen-activated protein kinase kinasekinase 11
21	NP_065789.1	KIDINS220	kinase D-interacting substrate, 220 kDa
22	Q6VAB6.2	KSR2	kinase suppressor of ras 2

number (http://www.ncbi.nlm.nih.gov/protein) [6] [7] [8] [9] from NCBI.

2.3. Physiochemical Characterization of Interacting Proteins

Using ExPASy ProtParam (http://web.expasy.org/protparam/) computational server, primary structure and physiochemical characters of proteins, such as amino acids number (AA), Molecular weight of protein, Theoretical pI, Amino acids compositions of protein, total number of negatively charged residues (Asp + Glu), total number of positively charged residues (Arg + Lys), extinction coefficient, instability index, aliphatic index and Grand Average Hydropathicity (GRAVY) were observed.

2.4. Functional Characterization

The nature of proteins whether it is soluble or trans membrane in character, SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html) computer

tools was utilized to analyze them [10].

3. Results

3.1. Physiochemical Characterization

ERK interactive protein's amino acids (AA) composition was presented in Table 2 and Table 3. Most of the ERK interacting proteins are hydrophilic in nature. AA composition indicates that higher percentage of hydrophobic amino acid leucine. This is due to presence of all the hydrophobic proteins inside the cell membrane and hydrophilic amino acids on the outside. Sequence length of protein represents basic nature of protein [6] [7] [8].

Theoretical pI (isoelectric point) (Table 4) value of 17 proteins *i.e.* EPHB2, MAPK1, IL17RD, WDR83, TESC, MAP3K4, MAP3K6, ULBP2, ULBP1, DUSP6, RHAMM, KIDINS220, PEA15, WARS, DUSP9, MAP3K1, SLAMF7 is less than 7. Hence, these all are acidic in nature and other 5 proteins *i.e.* KSR2, DUSP14, MAGI3, ULBP3 and MAP3K11 pI value is greater than 7. Hence, these all are basic in nature. pI value may be utilized for making buffer system when these proteins are to be purified in solution by isoelectric focusing method [6] [7] [8].

Table 2. Amino Acids (AA) Composition (in %) of ERK Interacting Proteins Followed ExPASy server.

AA	EPHB2	MAPK1	IL17RD	WDR83	TESC	MAP3K4	KSR2	MAP3K6	ULBP2	ULBP1
Ala	7.2%	6.9%	6.4%	6.0%	5.1%	6.8%	4.3%	8.7%	7.7%	7.0%
Arg	5.5%	5.3%	4.7%	6.7%	7.0%	6.2%	6.0%	7.9%	4.1%	3.7%
Asn	4.7%	5.3%	3.4%	2.2%	4.7%	3.1%	4.1%	2.0%	2.0%	2.9%
Asp	5.2%	6.7%	5.3%	7.6%	7.0%	5.7%	3.8%	4.0%	4.9%	4.5%
Cys	2.6%	1.9%	3.2%	4.4%	0.9%	1.9%	2.5%	2.3%	3.7%	3.3%
Gln	4.5%	3.3%	5.0%	3.2%	3.7%	4.5%	4.8%	6.0%	4.1%	4.5%
Glu	6.0%	5.8%	5.7%	6.0%	11.7%	8.6%	7.1%	6.7%	4.9%	6.1%
Gly	6.9%	4.7%	6.9%	10.2%	5.6%	5.5%	4.6%	7.1%	6.5%	5.3%
His	1.5%	3.6%	3.2%	1.9%	2.8%	2.5%	3.9%	2.4%	2.0%	3.3%
Ile	6.4%	7.8%	3.5%	1.6%	6.5%	5.1%	5.1%	3.3%	4.9%	3.7%
Leu	6.9%	11.9%	9.6%	10.5%	7.9%	9.5%	9.5%	12.9%	12.6%	13.1%
Lys	3.9%	6.4%	5.8%	4.4%	4.7%	6.4%	6.8%	2.4%	5.7%	7.0%
Met	3.5%	2.5%	1.8%	1.0%	5.1%	2.7%	1.9%	1.6%	3.7%	3.3%
Phe	3.9%	3.6%	5.3%	2.9%	6.1%	3.6%	3.3%	3.2%	4.1%	5.7%
Pro	4.8%	5.6%	6.0%	4.1%	3.3%	6.2%	9.7%	7.9%	6.1%	6.1%
Ser	7.7%	4.4%	9.5%	8.9%	7.0%	8.2%	8.2%	7.1%	6.5%	4.9%
Thr	6.4%	3.9%	4.3%	5.1%	4.2%	4.5%	6.0%	4.8%	7.3%	6.1%
Trp	1.4%	0.8%	1.1%	1.6%	0.5%	1.3%	1.5%	1.0%	2.8%	4.1%
Tyr	4.0%	5.3%	3.2%	2.9%	1.9%	2.5%	1.8%	2.8%	1.6%	1.2%
Val	7.3%	4.2%	6.1%	8.9%	4.2%	5.2%	5.2%	5.9%	4.9%	4.1%
Pyl	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sec	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 3. Amino Acids (AA) Composition (in %) of ERK Interactive Proteins Followed ExPASy server.

AA	DUSP9	MAP3K1	ULBP3	SLAMF7	MAP3K11	RHAMM	DUSP14	DUSP6	KIDINS220	MAGI3	PEA15	WARS
Ala	8.3%	8.4%	7.8%	3.9%	8.4%	5.9%	7.6%	4.5%	6.1%	4.9%	3.8%	7.2%
Arg	8.1%	6.4%	6.6%	3.9%	9.3%	3.6%	7.1%	4.7%	5.5%	5.1%	4.6%	4.2%
Asn	3.4%	3.8%	1.6%	5.1%	1.8%	5.2%	3.5%	6.3%	4.9%	4.5%	3.1%	3.6%
Asp	3.9%	3.8%	5.7%	3.9%	4.5%	4.0%	3.0%	6.6%	5.7%	5.8%	7.7%	8.3%
Cys	2.1%	2.4%	2.5%	2.7%	1.3%	1.1%	2.0%	2.4%	1.6%	1.1%	0.8%	1.3%
Gln	3.6%	4.5%	4.5%	3.0%	4.1%	8.8%	3.0%	3.9%	3.6%	4.9%	2.3%	4.2%
Glu	7.3%	6.9%	5.3%	6.9%	7.4%	15.2%	3.5%	6.3%	6.5%	7.6%	12.3%	5.1%
Gly	8.6%	6.5%	6.1%	6.3%	9.4%	2.2%	7.1%	6.3%	6.5%	8.6%	1.5%	5.5%
His	1.6%	2.5%	2.5%	2.1%	1.4%	1.5%	4.0%	1.0%	2.3%	2.5%	1.5%	2.3%
Ile	2.3%	4.0%	4.1%	6.0%	3.0%	3.4%	7.6%	4.7%	5.7%	5.2%	9.2%	6.8%
Leu	14.8%	9.0%	12.3%	10.7%	9.6%	13.8%	8.1%	12.6%	11.0%	7.2%	11.5%	7.2%
Lys	2.1%	4.5%	5.3%	5.7%	3.1%	13.7%	4.0%	3.9%	5.8%	8.8%	10.0%	7.4%
Met	1.3%	2.4%	3.7%	2.1%	1.5%	1.8%	3.5%	2.1%	2.0%	1.7%	1.5%	2.8%
Phe	3.9%	2.6%	5.3%	2.7%	2.8%	2.2%	3.5%	5.0%	2.9%	2.4%	2.3%	7.0%
Pro	8.1%	7.0%	5.3%	6.3%	12.0%	1.5%	6.1%	5.5%	5.4%	7.6%	5.4%	5.3%
Ser	10.2%	12.0%	7.0%	8.4%	8.0%	7.0%	7.1%	11.3%	9.7%	8.2%	8.5%	7.2%
Thr	2.6%	5.4%	5.3%	7.8%	3.9%	3.9%	5.1%	4.5%	5.7%	5.0%	6.9%	5.5%
Trp	0.8%	0.9%	3.7%	1.8%	2.5%	0.3%	2.0%	0.8%	1.2%	0.7%	0.8%	0.6%
Tyr	2.6%	1.6%	2.0%	3.6%	1.2%	1.4%	4.0%	2.6%	2.5%	1.8%	3.1%	3.2%
Val	4.4%	5.6%	3.3%	7.5%	4.7%	3.4%	8.1%	5.0%	5.5%	6.4%	3.1%	5.1%
Pyl	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sec	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

3.2. Extinction Coefficients (EC)

The extinction coefficient of ERK interacting proteins at 280nm ranged from 11460 $M^{-1}\cdot cm^{-1}$ to 188310 $M^{-1}\cdot cm^{-1}$ in comparison to the concentration of Cys, Trp and Tyr (Table 4). Extinction coefficients of KIDINS220, MAP3K4, EPHB2, MAP3K11, MAP3K6, MAP3K, KSR2 were very higher indicating presence of high concentration of aromatic amino acid. Extinction coefficient of PEA15 was as lower 11460 $M^{-1}\cdot cm^{-1}$ indicating that the presence of aromatic amino acids is low. The EC values are useful in the quantitative study of protein-protein and protein-ligand interactions in solution [6] [7] [8].

3.3. The Instability Index (II)

It gives estimation of protein stability *in vitro*. Protein having II value less than 40 the protein is measured as stable in laboratory condition whereas proteins II value higher than 40 is defined as unstable. ExPASy ProtParam result for II of ERK interacting proteins represent that only 4 proteins out of 22 is stable and remaining 17 are unstable [6] [7] [8].

Table 4. Physiochemical properties of Interacting Proteins of ERK.

S.N.	Protein	Number of amino acids	Molecular weight	Theoretical pI	Total number of negatively charged residues (Asp + Glu)	Total number of positively charged residues (Arg + Lys)	Extinction coefficients $M^{-1}\cdot cm^{-1}$, at 280	The instability index (II)	Aliphatic index	Grand average of hydropathicity (GRAVY)
1	EPHB2	987	110030.2	5.49	110	92	136735	37.88 stable	80.11	−0.202
2	MAPK1	360	41389.7	6.50	45	42	45185	39.71 stable	95.94	−0.287
3	IL17RD	739	82410.5	6.78	81	78	81260	56.54 Unstable	75.21	−0.346
4	WDR83	315	34342.6	5.36	43	35	41785	42.56 Unstable	78.86	−0.305
5	TESC	214	24749.8	4.84	40	25	11585	49.58 Unstable	73.83	−0.568
6	MAP3K4	1608	181691.7	5.92	230	203	176975	51.45 Unstable	78.81	−0.509
7	KSR2	950	107632.3	8.95	103	122	103830	61.10 Unstable	75.93	−0.600
8	MAP3K6	1288	142596.0	6.70	133	138	127015	58.54 Unstable	88.79	−0.254
9	ULBP2	246	27367.9	6.93	24	24	44960	49.34 Unstable	90.04	0.019
10	ULBP1	244	27996.5	7.07	26	26	59970	40.06 Unstable	84.39	−0.162
11	DUSP14	198	42333.7	9.62	13	22	34170	37.79 Stable	92.07	−0.012
12	DUSP6	381	22254.9	4.75	49	33	31900	52.25 Unstable	86.48	0.265
13	RHAMM	725	84175.5	5.68	139	125	26400	53.72 Unstable	83.17	−0.988
14	KIDINS220	1771	196542.1	6.19	217	199	188310	44.54 Unstable	87.17	−0.369
15	MAGI3	1481	162948.8	8.26	199	205	96230	48.35 Unstable	71.65	−0.746
16	PEA15	130	15068.1	4.93	26	19	11460	64.55 Unstable	93.77	−0.625
17	WARS	471	53178.5	5.83	63	55	39225	40.34 Unstable	76.65	−0.345
18	DUSP9	384	41934.6	5.96	43	39	31900	67.23 Unstable	88.20	−0.245
19	MAP3K1	1512	164469.9	7.93	161	165	115010	63.95 Unstable	75.07	−0.425
20	ULBP3	244	27949.3	8.20	27	29	57325	46.78 Unstable	81.23	−0.236
21	SLAMF7	335	37420.9	6.02	36	32	51380	37.55 Stable	90.72	−0.188
22	MAP3K11	847	92687.9	8.40	101	105	131025	70.83 Unstable	70.89	−0.582

3.4. Aliphatic Index

Aliphatic index of a protein is considered as the relative volume captured by aliphatic side chains (alanine, valine, isoleucine, and leucine). It is considered as a positive factor for the increase of thermal stability of annular proteins. Aliphatic index of ERK interactive proteins limit from 62.34 to 107.02. Both MAP3K11 and MAGI3 have lower thermal stability representing their more flexible structure as compared to other proteins (**Table 4**). The high aliphatic index of other proteins indicates their stability in high range of temperature [6] [7] [8].

3.5. A GRAVY (Grand Average of Hydropathy)

GRAVY is understood as the total hydropathy values of all the amino acids of a protein, divided by the number of amino acids residues in the sequence. ExPASy Protparam server for GRAVY indicated that ERK interactive proteins are hydrophilic in nature except ULBP2. Lowest GRAVY value of DUSP14 suggests its better communication with water [6] [7] [8].

3.6. Functional Characterization

The SOSUI server data expressed ERK interacting proteins namely EPHB2 (2 trans membrane), IL17RD (2 trans membrane), ULBP2 (2 trans membrane), ULBP1 (1 trans membrane), KIDINS220 (4 trans membrane) and SLAMF7 (2 trans membrane) are membrane protein. However, MAPK1, WDR83, TESC, MAP3K4, KSR2, MAP3K6, DUSP6, DUSP14, MAGI3, PEA15, WARS, DUSP9, MAP3K1, ULBP3, MAP3K11 and DUSP4 are classified as a soluble protein (**Table 5**).

4. Discussion

There are several genes responsible for the long term synaptic plasticity. ERK is one of these gene involved in long term learning and memory. During this process, a cascade of regulatory immediate early genes is recruited to particular gene including ERK [11] [12]. These genes are required for long-term synaptic plasticity and long-term memory formation. For gene expression, chromatin remodeling is crucial event. Studies from several researchers have established that chromatin remodeling is involved in learning and memory, long-term neuronal responses, drug addiction, stress, epilepsy and depression [13] [14]. For performing any functions ERK interacts with a host of proteins. Hence the ERK interacting proteins are crucial and involved in almost all brain related disorders and diseases.

The chromatin remodeling is done by modification at the level of histones like acetylation and methylation. histone acetylation is a reversible modification of lysine residues within the amino-terminal domains histone. It is done by histone acetyltransferase (HAT), which transfers acetyl groups from acetyl-coenzyme A to the lysine residue. In the other hand, histone deacetylase (HDAC) acts in the

Table 5. Functional characterization of ERK interactive proteins.

No.	Interactive Proteins	N terminal	Trans-membrane region	C terminal	Type	Length	Characters
1	EPHB2	6	LGAALLLLPLLAAVEETLMDSTT	28	SECONDARY	23	MEMBRANE PROTEIN 2 Transmembrane helices.
2		542	PLIIGSSAAGLVFLIAVVVIAIV	564	PRIMERY	23	
	MAPK1	-	-	-	-	-	SOLUBLE PROTEIN
1	IL17RD	1	MAPWLQLCSVFFTVNACLNGSQL	23	SECONDARY	23	MEMBRANE PROTEIN 2 Transmembrane helices.
2		298	IRAVAITVPLVVISAFATLFTVM	320	PRIMARY	23	
	WDR83	-	-	-	-	-	SOLUBLE PROTEIN
	TESC	-	-	-	-	-	SOLUBLE PROTEIN
	MAP3K4	-	-	-	-	-	SOLUBLE PROTEIN
	KSR2	-	-	-	-	-	SOLUBLE PROTEIN
	MAP3K6	-	-	-	-	-	SOLUBLE PROTEIN
1	ULBP2	2	AAAAATKILLCLPLLLLLSGWSR	24	PRIMARY	23	MEMBRANE PROTEIN 2 Transmembrane helices.
2		225	TATTLILCCLLIILPCFILPGI	246	PRIMARY	22	
	ULBP1	1	MAAAASPAFLLCLPLLHLLSGWS	23	PRIMARY	23	MEMBRANE PROTEIN 1 Transmembrane helices
	DUSP14	-	-	-	-	-	SOLUBLE PROTEIN
	DUSP6	-	-	-	-	-	SOLUBLE PROTEIN
1	KIDINS220	496	QFSWLIVFLTLLLCGGLGLLFAF	518	PRIMARY	23	MEMBRANE PROTEIN 4 Transmembrane helices
2		525	GIAVSLSFLALLYIFFIVIYFGG	547	PRIMARY	23	
3		657	LPSFVIFLFIIGCIISGITLLAI	679	PRIMARY	23	
4		687	LTVNAVLISIASVVGLAFVLNCR	709	PRIMARY	23	
	MAGI3	-	-	-	-	-	SOLUBLE PROTEIN
	PEA15	-	-	-	-	-	SOLUBLE PROTEIN
	WARS	-	-	-	-	-	SOLUBLE PROTEIN
	DUSP9	-	-	-	-	-	SOLUBLE PROTEIN
	MAP3K1	-	-	-	-	-	SOLUBLE PROTEIN
	ULBP3	-	-	-	-	-	SOLUBLE PROTEIN
1	SLAMF7	1	MAGSPTCLTLIYILWQLTGSAAS	23	SECONDARY	23	MEMBRANE PROTEIN 2 Transmembrane helices
2		226	MVLLCLLLVPLLLSLFVLGLFLW	248	PRIMARY	23	
	MAP3K11	-	-	-	-	-	SOLUBLE PROTEIN
	DUSP14	-	-	-	-	-	SOLUBLE PROTEIN

reverse manner and removes the acetyl groups from lysine residues. Actually, acetylation neutralizes the basic histone tails by weakening of the histone-DNA interaction and promoting the accessibility of transcription factors. The present study revealed that all ERK interacting proteins are rich in lysine residues *i.e.* basic in nature. Further ERK interacting proteins are hydrophobic in nature which favors gene expression at transcriptional level. So these proteins may be involved in histone modification needed for chromatin dynamics and gene transcription. Furthermore, there are several kinases which are decisively involved in histone phosphorylation affecting long-term memory formation nemely ERK1/2, p38 MAP kinase, and ribosomal 6 kinase (RSK) [14]. The aforementioned findings favor our result as ERK interacted with various proteins as shown in this paper.

In addition, calsyntenins (Cst) comprise a family of trans-membrane proteins with the unique potential to link extracellular proteolytic activity with intracellular calcium signaling. It is molecular class of calcium binding protein, which can bind calcium ion and being involved in cell signaling and cell-cell communication. It contains three members, calsyntenin-1, calsyntenin-2 and calsyntenin-3, which are postsynaptic membrane proteins and predominantly expressed in brain neurons. Each of the three calsyntenins exhibits a distinct neuronal mRNA expression pattern. The calsyntenin-1 is located in the postsynaptic membrane of CNS and a proteolytically processed protein of the postsynaptic membrane contains calcium-binding cytoplasmic domain. Calsyntenin-1 is a dynamic modulator of postsynaptic calcium by extracellular proteolysis. It may modulate Ca^{2+} transients locally either beneath the postsynaptic membrane or around intracellular Ca^{2+} stores, such as LTP and LTD. Recent studies showed that impairment of the coordinated metabolic regulation of APP, calsyntenins and the consequent loss of the reciprocal regulation by APP and calsyntenins in the gene transactivation in AD [15]. Almost all ERK interacting proteins are trans-membrane in nature. Thus ERK interacting proteins may be involved in metabolic regulation of APP and calsyntenins mediated pathway of AD.

Food provides energy and building blocks to the body. Its ability to prevent and protect against diseases is started to be recognized. Especially research over the past one decade has provided exciting evidence suggests the influence of dietary factors on brain functions. For example, omega-3 fatty acids rich diet is useful for cognitive processes in humans [16] and up-regulating genes that are significant for maintaining synaptic function and plasticity in rodents [17]. Further, high unsaturated fat diets are responsible for reducing molecular substrates that support cognitive processing and increases the risk of neurological dysfunctions in both humans and animals [18] [19]. The important effects of food on brain functions are cited still further research is necessary to determine underlying mechanisms of action and for therapeutic applications in humans. In this study, we found that most of the ERK interacting are rich in leucine, suggesting that their roles as transcription factor. The leucine zipper motif contains leucine residues spaced 7 amino acids apart repeating at least 3 times (L××××××L××××××L)

[20]. In the process of dimerization, parallel leucine zipper motifs interact through a coiled coil hydrophobic interface that juxtaposes 2 adjacent basic regions [21]. Further, once bound to DNA, these adjacent basic regions undergo key changes in conformation [22]. As almost ERK interacting proteins contain leucine so it may be assumed that these are involved in ERK mediated functions including learning and memory. In addition, leucine rich food could be good for cognition and healthy brain functions. Thus such findings regarding ERK interacting proteins are instrumental to understand learning and memory via ERK. Moreover, it may be helpful to target these interacting proteins during learning and memory deficit and neurodegenerative diseases.

Acknowledgements

VP acknowledges Science and Engineering Research Board (SERB), Government of India for providing financial support (Registration No.SERB/LS-485/2013). KK acknowledges SERB for Junior Research Fellowship.

References

[1] Ebisuya, M., Kondoh, K. and Nishida, E. (2005) The Duration, Magnitude and Compartmentalization of ERK MAP Kinase Activity: Mechanisms for Providing Signaling Specificity. *Journal of Cell Science*, **118**, 2997-3002. https://doi.org/10.1242/jcs.02505

[2] Vidhya, V.G., Upgade, A., Bhaskar, A. and Deb, D. (2012) In Silico Characterization of Bovine (Bostaurus) Antiapoptotic Proteins. *Journal of Proteins and Proteomics*, **3**, 187-196.

[3] Kuhn, M., Mering, V.C., Campillos, M., Jensen, J.L. and Bork, P. (2015) STITCH: Interaction Networks of Chemicals and Proteins. *Nucleic Acids Research*, **36**, D684-D688. https://doi.org/10.1093/nar/gkm795

[4] Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta. C.J., Simonovic, M., Alexander, R., Santos, A., Tsafou, P.K., Kuhn, M., Bork, P., Jensen, J.L. and Mering, V.C. (2015) STRING v10: Protein-Protein Interaction Networks, Integrated over the Tree of Life. *Nucleic Acids Research*, **43**, D447-D452. https://doi.org/10.1093/nar/gku1003

[5] Bidkar, A.P., Thakur, K.K., Bolshette, NB., Dutta, J. and Gogoi, R. (2014) In-Silico Structural and Functional Analysis of Hypothetical Proteins of *Leptospira Interrogans*. *Biochemistry & Pharmacology*, **3**, 3. https://doi.org/10.4172/2167-0501.1000136

[6] Jabalia, N., Bansal, H., Mishra, P.C. and Chaudhary, N. (2015) In-Silico Comparative Analysis of Papain Family Cysteine Protease Using Computational Tools and Servers. *International Journal of Basic and Applied Biology*, **2**, 310-314.

[7] Mahalakshmi, K. (2015) Insilico Analysis of Proteins of *Curcuma aromatica* Salisb. *International Journal of PharmTech Research*, **8**, 51-56.

[8] Vishwanath, K.V., Pattabhiramaiah, M. and Keerthi, R. (2016) Bio Computational Analysis of Protein Sequence of Sickle Cell Anemia. *International Journal of Engineering Research and General Science*, **1**, 63-73.

[9] Verma, K.N., Verma, V., Deshwal R.K. and Yadav, N. (2014) Structural Insight into Molecular Model of Hypothetical Protein from *Trichomonas vaginalis*: A Compu-

tational Approach. *Biosciences*, **76**, 28414-28421.

[10] Hirokawa, T (1998) SOSUI: Classification and Secondary Structure Prediction System for Membrane Proteins. *Bioinformatics*, **14**, 378-379. https://doi.org/10.1093/bioinformatics/14.4.378

[11] Goelet, P., Castellucci, V.F., Schacher, S. and Kandel E.R. (1986) The Long and the Short of Long-Term Memory—A Molecular Framework. *Nature*, **322**, 419-422. https://doi.org/10.1038/322419a0

[12] Taubenfeld, S.M., Milekic, M.H., Monti, B. and Alberini, C.M. (2001) The Consolidation of New but Not Reactivated Memory Requires Hippocampal C/EBP*β*. *Nature Neuroscience*, **4**, 813-818. https://doi.org/10.1038/90520

[13] McClung, C.A. and Nestler, E.J. (2008) Neuroplasticity Mediated by Altered Gene Expression. *Neuropsychopharmacology*, **33**, 3-17. https://doi.org/10.1038/sj.npp.1301544

[14] Taniura, H., Sng, J.C. and Yoneda, Y. (2008) Histone Modifications in the Brain. *Neurochemistry International*, **51**, 85-91. https://doi.org/10.1016/j.neuint.2007.04.018

[15] Cheng, X.R., Zhou, W.X. and Zhang, Y.X. (2006) The Family of Calsyntenins: Learning and Memory Related Genes. *Progress in Physiology*, **37**, 205-210.

[16] McCann, J.C. and Ames, B.N. (2005) Is Docosahexaenoic Acid, an N-3 Long-Chain Polyunsaturated Fatty Acid, Required for Development of Normal Brain Function? An Overview of Evidence from Cognitive and Behavioral Tests in Humans and Animals. *The American Journal of Clinical Nutrition*, **82**, 281-295. https://doi.org/10.1093/ajcn/82.2.281

[17] Wu, A., Ying, Z. and Gomez-Pinilla, F. (2007) Omega-3 Fatty Acids Supplementation Restores Mechanisms That Maintain Brain Homeostasis in Traumatic Brain Injury. *Journal of Neurotrauma*, **24**, 587-1595. https://doi.org/10.1089/neu.2007.0313

[18] Greenwood, C.E. and Winocur, G. (2005) High-Fat Diets, Insulin Resistance and Declining Cognitive Function. *Neurobiology of Aging*, **26**, 42-45. https://doi.org/10.1016/j.neurobiolaging.2005.08.017

[19] Molteni, R., Barnard, J.R., Ying, Z., Roberts, C.K. and Gomez-Pinilla, F. (2002) A High-Fat, Refined Sugar Diet Reduces Hippocampal Brain-Derived Neurotrophic Factor, Neuronal Plasticity and Learning. *Neuroscience*, **112**, 803-814. https://doi.org/10.1016/S0306-4522(02)00123-9

[20] Landschulz, W.H., Johnson, P.F. and McKnight, S.L. (1988) The Leucine Zipper: A Hypothetical Structure Common to a New Class of DNA Binding Proteins. *Science*, **240**, 1759-1764. https://doi.org/10.1126/science.3289117

[21] Ellenberger, T.E., Brandl, C.J., Struhl, K. and Harrison, S.C. (1992) The GCN4 Basic Region Leucine Zipper Binds DNA as a Dimer of Uninterrupted *α* Helices: Crystal Structure of the Protein-DNA Complex. *Cell*, **71**, 1223-1237. https://doi.org/10.1016/S0092-8674(05)80070-4

[22] O'Neil, K.T. and DeGrado, W.F. (1990) A Thermodynamic Scale for the Helix-Forming Tendencies of the Commonly Occurring Amino Acids. *Science*, **250**, 646-651. https://doi.org/10.1126/science.2237415