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The Bioinformatics Analysis of Disc Large Homolog-4 (DLG-4) as Parameters in Neuroplasticity

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Abstract

Disc Large Homolog-4 (DLG-4), also known as Post Synaptic Density 95 (PSD95) is believed to have an important role in the function and organization of the synapse. DLG-4 is the main protein structure at the synapse that is able to bind to various molecules on the surface of the postsynaptic membrane. This review aims to determine the biomolecular characteristics of DLG-4 and the role of DLG-4 as an important parameter in neuroplasticity. Biomolecular characteristic analysis was obtained through various bioinformatics websites namely NCBI, PROTPARAM, TMHMM, PEPTIDE CUTTER, NETNGLYC, TARGETP and KEGG PATHWAY. The DLG-4 gene is located on chromosome 17p13.1 and has 25 exon. The DLG-4 protein consists of 764 amino acids with a molecular weight of 80.47 kDa. DLG-4 has a structure of 3 N-terminal PDZ domains, a Src Homology 3 (SH3) domain and a guanilate kinase-like C-terminal domain. This protein has a stability score of 48.61. The aliphatic index is 85.42. DLG-4 is found inside, outside, and on transmembranes, as determined by THMM. There are 37 enzymes that are predicted to be able to split the DLG-4 protein. The DLG-4 protein shows 3 potential sites of glycosylation of amino acids. The target location of the DLG-4 protein is mostly in other locations (other = 0.6556), secretory pathway (0.3328), and only slightly in mitochondria (0.0116). The DLG-4 protein is strongly associated with the glutamatergic system in postsynaptic neurons. The glutamatergic system is concerned with the molecular mechanism of memory development and cognitive function, long-term potentiation (LTP). These findings support the fact that DLG-4 protein plays a role in neuroplasticity mechanisms in the brain.

Keywords: DLG-4, PSD-95, Bioinformatics, Neuroplasticity

1. Introduction

In recent decades, the understanding of the adult human brain has significantly evolved. The adult brain is no longer considered a 'fixed' organ. Various factors such as stress, hormones, neurotransmitters, growth factors, specific medications, environmental stimulation, learning, and aging can alter the structure and function of nerves.[1] The term neuroplasticity was first introduced by Santiago Ramon y Cajal in 1894, defining it as non-pathological changes in the structure of adult brain tissue. For a broader definition, neuroplasticity is the ability of neurons to adapt in response to changes in both external and internal environments.[2]

Adaptation to the environment triggers neuronal activation, leading to modifications in neuronal circuits through two mechanisms: strengthening or efficacy of synaptic transmission and triggering the growth of new synaptic tissue. Effective synaptic activity reflects the amount of information entering the brain, thus permanent changes in synapses play a crucial role in the processes of learning and memory formation.[2,3]

Most synapses that contact with neurons are located on dendritic spines. Dendritic spines are believed to be the smallest neuronal structures capable of transmitting nerve signals at synapses.[4] Dendritic spines have three important function in the nervous system: maintaining Long Term Potentiation (LTP), regulating Ca2+, and amplifying synaptic signals. [5] Thus, dendritic spines play a significant role in the constant changes that

form the plasticity of neurons, which is the basis for the processes of adaptation, learning, and memory.[4,5,6]

In general, dendritic spines consist of a head, about 1 micron in length, attach to a dendrite through a stalk or neck.[7] The head is the most important part of the dendritic spine, containing numerous neurotransmitter receptors and signal transduction molecules essential for synaptic transmission. Therefore, many researchers believe that a larger head size in dendritic spines corresponds to a more robust synaptic connection. The outer part of the dendritic spine head comprises a complex protein structure known as synapse-associated protein (SAP) or postsynaptic density protein (PSD).[8]

PSD is a megaorganelle on the cell membrane located in the head of the dendritic spine, approximately occupying 10% of its outer part. PSD has a disc-like structure with a length of 200-800 nm and a thickness of 30-50 nm. Generally, the postsynaptic density (PSD) functions in mediating the connection between pre- and postsynapses, activating postsynaptic receptors, and a series of biochemical reactions that occur postsynaptically. The PSD group is classified into four based on molecular weight: PSD 95, PSD 93 (Chapsyn-110), SAP 97, and SAP 102. Among these four types of PSD, DLG-4/PSD-95 is the protein most abundantly found in the head of dendritic spines . [8,9,10]

This review aims to determine the biomolecular characteristics of DLG-4 and the role of DLG-4 as an important parameter in neuroplasticity.

2. Methods

Information about the genetic features of DLG-4 was sourced from the National Center for Biotechnology Information's website, www.ncbi.org, utilizing the gene ID NC_000017.11 and the protein ID NR_135527.1. The exploration of DLG-4 and its involvement in neuroplasticity pathway involves the use of bioinformatics tools. The 'NCBI' website provides details on the structure, location, and expression of DLG-4.

Chemical-physical analysis involves the application of 'PROTPARAM'; the 'PROTSCALE' website emphasizes hydrophobicity; 'TMHMM' provides information on transmembrane protein topology; protease prediction is conducted using 'PEPTIDE CUTTER'; NETNGLYC is utilized to predict the glycosylation index, and the 'TARGETP' homepage facilitates the examination of the location of the target protein.

3. Result

3.1. Structure, location and gene expression

DLG-4 is encoded by the DLG-4 gene located on chromosome 17p13.1 (Chromosome number 17 short arm region 13 subunit 1), consisting of 25 exons.

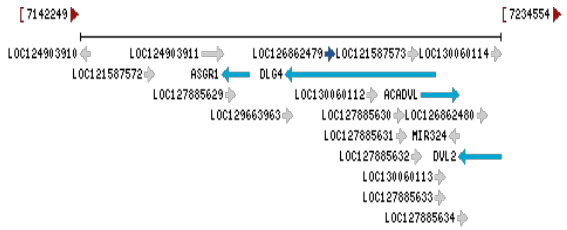


Figure 1. Location of DLG-4 Gene

Based on RNA sequencing conducted on tissue samples from 95 human individuals, DLG-4 is expressed in various tissues, particularly in the brain, with a total TPM (Transcripts Per Million) of 34.6. This implies that brain tissue is enriched with the DLG-4 protein.

3.2. DLG-4 Protein

The DLG-4 protein consists of 764 amino acids with the amino acid sequence seen in figure 3.

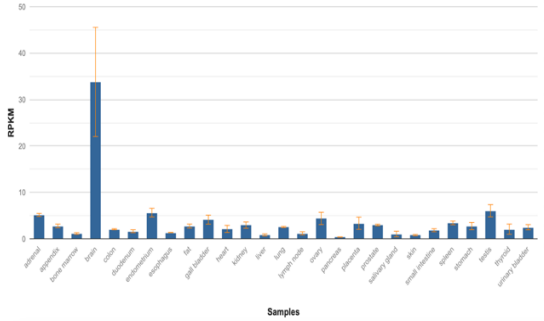


Figure 2. Gene Expression of DLG-4

msqrpraprs alwllappll rwapplltvl hsdlfqalld ildyyeasls esqkyrygde
dtpplehspa hlpnqanspp vivntdtlea pgyvngtege meyeeitler gnsglgfsia
ggtdnphigd dpsifitkii pggaaaqdgr lrvndsilfv nevdvrevth saavealkea
gsivrlyvmr rkppaekvme iklikgpkgl qfsiaggvqn qhipgdnsiy vtkiieggaa
hkdgrlqigd kilavnsvgl edvmhedava alkntydvvy lkvakpsnay lsdsyappdi
ttsysqhldn eishssylgt dyptamtpts prryspvakd llgeedipre prrivihrgs
tglgfnivgg edgegifisf ilaggpadls qelrkgdqil svngvdlrna sheqaaialk
naggtvtiia qykpeeysrf eakihdlreq lmnsslgsgt aslrsnpkrg fyiralfdyd
ktkdcgflsq alsfrfgdvl hvidasdeew wqarrvhsds etddigfips krrverrews
rlkakdwgss sgsqgredsv lsyetvtqme vhyarpiiil gptkdrandd llsefpdkfq
scvphttrpk reyeidgrdy hfvssrekme kdiqahkfie aggynshlyg tsvqsvreva
eqgkhcildv sanavrrlqa ahlhpiaifi rprslenvle inkriteeqa rkafdratkl
eqeftecfsa ivegdsfeei yhkvkrvied lsgpyiwvpa rerl

Figure 3. Sequence of DLG-4 (NR_135527.1)

3.3. Analysis of Transmembrane Level of DLG-4 Protein

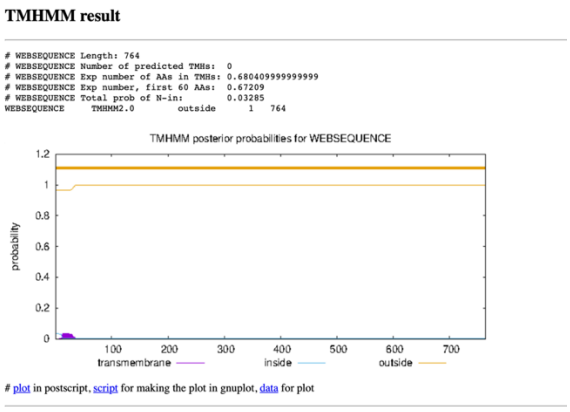


Figure 4. The results of TMHMM for DLG-4 protein

3.4. Analysis of Hydrophobicity Level of DLG-4 Protein

The hydrophobicity level of the protein was determined using the Hphob.App method (PROTSCALE).

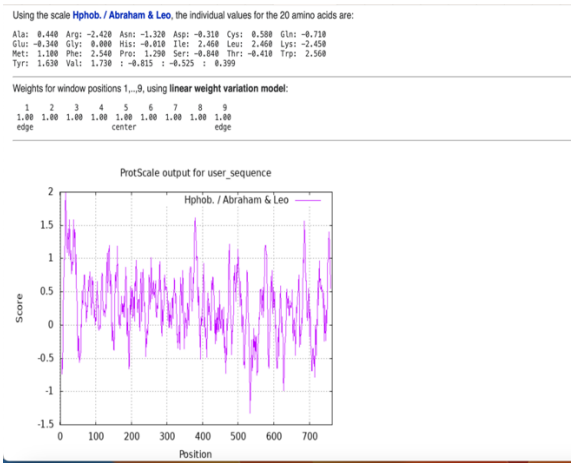


Figure 5. The hydrophobicity level of DLG-4 protein

3.5. Analysis of the Target Location of DLG-4 Protein

Target P 2.0 is used to predict the subcellular location of a protein. The target location is determined based on the N-terminal groups of chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP), and secretory pathway signal peptide (SP).

The above findings indicate that DLG-4 protein is primarily localized in other areas (other = 0.6556) and the secretory pathway (0.3328), with only a small fraction in the mitochondria (0.0116).

3.6. Physical-Chemical Analysis of DLG-4 Protein

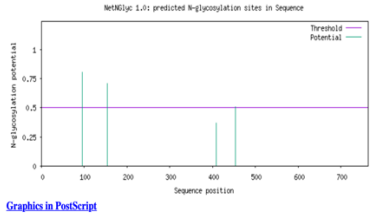
The Physical-Chemical Analysis of DLG-4 Protein is shown in the table 1.

3.7. The Analysis of Predictions by Protease (Peptide Cutter)

There are 24 enzymes out of a total of 31 enzymes listed on the website https://web.expasy.org/cgibin/peptide_cutter/peptidecutter.pl were predicted to be able to

cleave DLG-4 protein : Arg-C proteinase, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, BNPS-Skatole, Caspase1, Chymotrypsin-high specificity, Chymotrypsin-low specificity, Clostripain, CNBr, Factor Xa, Formic acid, Glutamyl endopeptidase, Hydroxylamine, Iodosobenzoic acid, LysC, LysN, NTCB (2-nitro-5-thiocyanobenzoic acid), Pepsin (pH1.3), Pepsin (pH>2), Proline-endopeptidase, Proteinase K, Staphylococcal peptidase I, Thermolysin and Trypsin.

3.8. Analysis of Glycosylation Index Prediction (NETNGLYC)



SeqName	Position	Potential	Jury agreement	N-Glyc result
Sequence	95 NGTE	0.8032	(9/9)	+++
Sequence	154 NDSI	0.7080	(9/9)	++
Sequence	409 NASH	0.3690	(8/9)	-
Sequence	453 NSSL	0.5076	(5/9)	+

Figure 6. Glycosylation Index Prediction Result of DLG

The graph above illustrates the prediction of N-glycosylation sites on the protein chain. The X-axis represents the protein length from the N-terminal to the C-terminal. Positions where the potential line (vertical line) crosses the threshold line (horizontal line = 0.5) indicate glycosylated proteins. DLG-4 protein shows three potential glycosylation sites at amino acid positions 95 (potential glycosylated 0.8032), 154 (potential glycosylated 0.7080), and 453 (potential glycosylated 0.5076).

3.9. Structure analysis of DLG-4 Protein

DLG-4 is a member of the membrane-associated guanylate kinase (MAGUK), consisting of 764 amino acids with a molecular weight of 80.47 kDa. DLG-4 has a structure comprising three N-terminal PDZ domains, a Src Homology 3 (SH3) domain, and a C-terminal domain similar to guanylate kinase.

3.10. Analysis of the Role of Proteins in the Metabolic Pathway

DLG4/PSD95 is highly associated with the glutamatergic system in postsynaptic neurons. The glutamatergic system is linked to the molecular mechanisms of memory formation and cognitive function, specifically long-term potentiation (LTP).

4. Discussion

LTP is the long-term strengthening of synaptic connections in pathways activated by repeated stimulation. LTP leads to modifications in synapses, enhancing the ability of presynaptic neurons to excite postsynaptic neurons.[11] Upon adequate stimulation, presynaptic neurons release glutamate (a major excitatory neurotransmitter) that binds to two types of glutamate receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptors located in the postsynaptic neuron's plasma membrane.[12]

The binding of glutamate to AMPA receptors causes an influx of Na+ ions into the postsynaptic compartment, leading to depolarization. This depolarization leads to the release of Mg2+, which inhibits the NMDA receptor. The binding of glutamate and the removal of Mg2+ inhibition on the NMDA receptor result in the influx of Ca2+ and Na+ into the postsynaptic neuron. [13] The influx of Ca2+ is crucial for inducing the molecular mechanisms required for LTP. Calcium ions activate a series of important chemical reactions involving various protein kinases and phosphatases. [14,15]

Table 1. Physical-Chemical Characteristics of DLG-4 Protein (PROTPARAM)

Characteristics	Protein DLG-4 isoform (NR_135527.1)		
Number of amino acids	764		
Molecular weight	85059.61		
Theoretical pI	5.61		
Amino acid composition	Ala (A) 60	7.9%	
	Arg (R) 52	6.8%	
	Asn (N) 25	3.3%	
	Asp (D) 51	6.7%	
	Cys (C) 4	0.5%	
	Gln (Q) 25	3.3%	
	Glu (E) 60	7.9%	
	Gly (G) 57	7.5%	
	His (H) 24	3.1%	
	Ile (I) 54	7.1%	
	Leu (L) 63	8.2%	
	Lys (K) 37	4.8%	
	Met (M) 9	1.2%	
	Phe (F) 24	3.1%	
	Pro (P) 42	5.5%	
	Ser (S) 63	8.2%	
	Thr (T) 32	4.2%	
	Trp (W) 7	0.9%	
	Tyr (Y) 28	3.7%	
	Val (V) 47	6.2%	
	Pyl (O) 0	0.0%	
	Sec (U) 0.	0.0%	
Total number of negatively charged residues (Asp + Glu)	111		
Total number of positively charged residues (Arg + Lys)	89		
Atomic composition:	Carbon C		3767
	Hydrogen H		5916
	Nitrogen N		1062
	Oxygen O		1160
	Sulfur S		13
Formula	$C_{3767}H_{5916}N_{1062}O_{1160}S_{13}$		
Total number of atoms	11918		
Instability index:	48.61		
Aliphatic index	85.42		
Grand average of hydropathicity (GRAVY)	-0.448		

Calcium ions activate Ca2+/calmodulin-dependent protein kinase (CaMKII), causing this enzyme to undergo autophosphorylation. Consequently, its activity becomes independent of the Ca2+ concentration. This is significant because the enzyme's activity persists even after the Ca2+/CaMKII complex has dissociated. During activation, CaMKII can rapidly translocate to the postsynaptic membrane, where numerous AMPA and NMDA receptors are located. CaMKII can bind to the AMPA receptor at the GluA1 subunit, enhancing ion conductivity through the channel and is believed to be a crucial mechanism for the increased efficacy in glutamatergic synapses during the LTP process. The influx of Ca2+ also leads to the insertion of new AMPA receptors into the postsynaptic

membrane. CaMKII and PKA activated by Ca2+ stimulate the process of AMPA receptor exocytosis. The increased activity of AMPA receptors, both in terms of their quantity and increased conductivity, is a key factor in enhancing Excitatory Postsynaptic Potential (EPSP) responses during LTP, leading to stronger and more enduring signal transmission.[16]

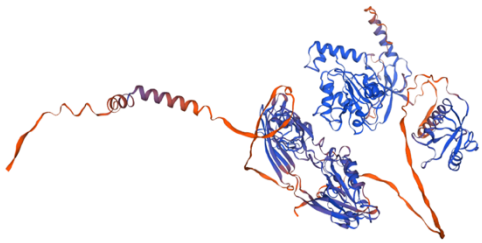


Figure 7. The structure of DLG-4 Protein

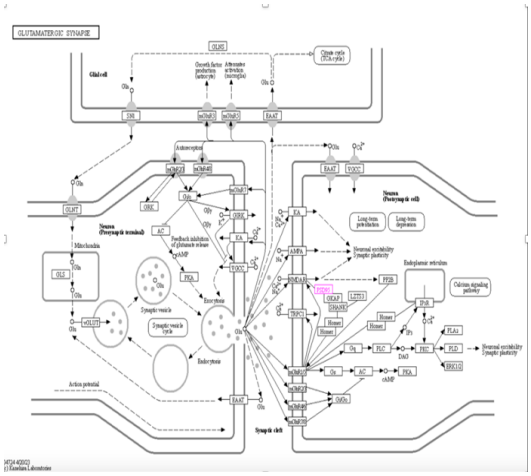


Figure 8. Glutamatergic system in postsynaptic neurons

The other protein kinases such as Protein Kinase C (PKC), PKA, tyrosine kinase, and Mitogen-activated protein kinase (MAPK) are also believed to be involved in the mechanisms of LTP.[17] The elevated levels of Ca2+ levels in the presynapse enhance the activity of PKC, leading to the phosphorylation of the GAP-43 protein and the mobilization of synaptic vesicles containing glutamate. Calcium in the postsynapse activates adenylate cyclase, initiating cAMP production. This secondary messenger pathway triggers PKA activation, which can regulate gene expression. PKA can modify transcription processes by phosphorylating various transcription factors, including cAMP response element-binding protein (CREB). CREB is a nuclear protein that functions to modulate the transcription of genes containing cAMP response elements (CRE) in their promoters. Phosphorylation of CREB initiates the transcription of genes associated with CRE. One of the proteins regulated by CREB is brain-derived neurotrophic factor (BDNF), a key regulator in the conversion of E-LTP to L-LTP. BDNF can bind to the specific tyrosine kinase receptor, TrkB, leading to receptor Trk autofosforilation and subsequent activation of tyrosine kinase. This activation further stimulates several signal transduction cascades such as the MAPK pathway, phosphatidylinositol 3-kinase (PI3K), and the phospholipase C-γ (PLC-γ) pathway. These signals are then transmitted to the nucleus, subsequently activating transcription factors and influencing gene expression.[18, 19]

BDNF, along with its specific receptor TrkB, is the primary neurotrophin that mediates the synapse plasticity process.[20] BDNF-TrkB plays a crucial role in the maturation and development of synapses, specifically by regulating the transport of DLG-4 to dendritic spines. BDNF activates the PI3K-AKT pathway, triggering the movement of DLG-4 to synapses through vesicle transport. The activation of BDNF-TrkB is also essential in the palmitoylation process of DLG-4, mediated by Phospholipase C γ (PLC γ) and protein kinase C (PKC). Palmitoylation of DLG-4 is involved in the process of tethering the protein to vesicle membranes and facilitating its integration into synapses. Another intracellular pathway activated by BDNF-TrkB is the Mitogen - activated protein kinases/ Extracellular signal - regulated kinases (MAPK/ERK) pathway. The MAPK/ERK pathway, along with the PI3K-AKT pathway, stimulates mTOR activation,

contributes to the increased expression of DLG-4 in dendritic spines by enhancing the phosphorylation of eukaryotic initiation factor 4E (eIF4E), 4E-binding protein 1 (4E-BP1), and ribosomal protein S6, thereby enhancing translational processes. Moreover, the MAPK/ERK pathway influences transcription processes by phosphorylating CREB, activating the BDNF gene, and amplifying synaptic maturation mediated by BDNF.[21,22,23]

5. Conclusion

In conclusion, DLG4/PSD95 is closely related to the glutamatergic system in postsynaptic neurons, especially long-term potentiation (LTP) which is a molecular mechanism of neuroplasticity. Bioinformatics analysis that has been described shows the characteristics of DLG4/PSD95 protein as a neuroplasticity parameter.

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