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Research Article

Network Pharmacological Mechanism Study of Bovine Embryonic Brain Cell Mitochondrial Polypeptides in Regulating Sleep Disorders

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Abstract

Objective: To investigate the potential molecular network mechanism of bovine embryonic brain cell mitochondrial polypeptide nutrients in regulating sleep disorders.

Methods: Based on 538 bovine embryonic brain cell mitochondrial expression proteins, combined with known target databases for sleep disorders, intersecting targets were screened using bioinformatics methods. A Protein-Protein Interaction (PPI) network was constructed, and Gene Ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to predict the polypeptide-target-pathway regulatory network. Molecular docking patterns were also explored.

Results: Fifteen core intersecting targets were screened, including MTOR, CD38, IRE1, TXNRD2, CBP, and VGF. The PPI network showed that MTOR was the hub target with the highest degree value. GO enrichment primarily involved circadian rhythm regulation, endoplasmic reticulum stress response, oxidative stress, and autophagy. KEGG pathways were significantly enriched in autophagy, circadian entrainment, endoplasmic reticulum protein processing, ferroptosis, and neurodegenerative pathways.

Conclusion: Bovine embryonic brain cell mitochondrial polypeptides may improve sleep disorders by targeting hub proteins such as MTOR/CD38/IRE1 and coordinately regulating the autophagy-calcium signaling-redox balance network. This study provides a theoretical basis for developing mitochondrial polypeptide-based sleep intervention strategies.

Keywords: Bovine embryonic brain cells, Mitochondrial polypeptides, Sleep disorders, Network pharmacology, Molecular docking

Introduction

Sleep disorders are a group of complex diseases that severely affect quality of life and metabolic health. Their pathogenesis involves multiple levels, including neurotransmitter imbalance, biological clock disorders, neuroinflammation, and mitochondrial dysfunction [1,2]. In recent years, mitochondria-derived peptides (such as MOTS-c and Humanin) have been confirmed to participate in energy metabolism, stress response, and circadian rhythm regulation [3,4]. This provides a new research direction for the regulation of sleep disorders. Bovine embryonic brain tissue is rich in mitochondria and various neuroactive polypeptides. Its mitochondrial extracts contain abundant functional signaling molecules that can participate in neurodevelopment, metabolic regulation, and stress response [5,6]. However, the molecular regulatory mechanism of bovine embryonic brain cell mitochondrial polypeptides on sleep disorders has not been systematically elucidated, and related research remains in a blank stage. Network pharmacology is a method that systematically analyzes the interaction between drugs and diseases from a holistic perspective [7]. In recent years, this method has been widely used in the mechanism research of traditional Chinese medicine and complex diseases [8,9]. Based on 538 protein targets identified in previous proteomics studies of bovine embryonic brain cell mitochondria, this study combines network pharmacology and molecular docking simulation. It systematically analyzes intersecting targets with sleep disorders, constructs a PPI network, and performs function enrichment and multi-target regulatory pathway analysis [10,11]. This aims to reveal the potential molecular mechanism of bovine embryonic brain cell mitochondrial polypeptides in regulating sleep disorders, providing a solid theoretical support and experimental for the development and application of functional polypeptide nutrients in the field of sleep intervention [12,13]. Furthermore, molecular docking techniques are employed to validate the binding affinity between polypeptides and core targets (such as MTOR and CD38), ensuring the reliability of the predicted network [14,15].

Materials and Methods

Data Sources

Bovine Embryonic Brain Cell Mitochondrial Targets: Derived from 538 proteins identified in previous proteomics (specific protein list in Appendix 1 of the main text). This study includes a self-built library of 538 bioactive signaling molecules from bovine embryonic brain cell mitochondrial polypeptide nutrients. All molecules use standardized Gene Symbol nomenclature. After deduplication, a target dataset was established, with detailed functional classification and core molecules shown in Appendix 1. Sleep Disorder-Related Targets: By searching authoritative databases including GeneCards (Relevance score ≥ 5), OMIM, and DisGeNET, using keywords such as “sleep disorder,” “circadian rhythm,” and “insomnia,” human targets related to sleep disorders were comprehensively screened. After deduplication and screening, 124 high-confidence sleep disorder-related targets were obtained to ensure target reliability and relevance (Table 1).

Table 1: Functional Classification and Core Molecules of Bovine Embryonic Brain Cell Mitochondrial Polypeptides.

Functional Category	Molecule Count	Core Representative Molecules	Main Biological Functions
Inflammation & Immunity	34	IL-7, IL-15, IL-23p19, Chemerin, C5/C5a, S100A8, S-100b	Regulate microglial activation, inhibit neuroinflammatory storms, balance central immune homeostasis, reducing neuroinflammation-related sleep damage.
Growth Factors & Receptors	28	PDGF R alpha, EphA3, EphA4, EphB2, EphB6, Notch-1	Promote neural stem cell proliferation/differentiation, participate in synaptic remodeling, accelerate neuronal repair, improving sleep-related neural dysfunction.
Metabolic Enzymes & Redox	62	Aldolase C, ALDH, GPX1, SOD4, ACAA1, ACAA2, ENO1	Participate in mitochondrial energy metabolism and glycolysis, scavenge ROS, maintain redox balance, alleviating oxidative stress from sleep deprivation.
ADAMTS Protease Family	8	ADAMTS-L2, ADAMTS-4, ADAMTS-5, ADAMDEC1, ADAMTS2	Participate in extracellular matrix remodeling, hydrolyze abnormally aggregated proteins, inhibit α-synuclein deposition, reducing protein toxicity in sleep disorders.
Kinases & Signal Transduction	46	ACK1, ALK, Fyn, Hck, Lyn, TNK1, ZAP70, AKT2, mTOR	Regulate PI3K-AKT, MAPK, etc., participate in proliferation/apoptosis and signal cascades, maintaining normal neural cell function.
Calcium & Cytoskeleton	52	Calbindin, Calmodulin, Clusterin, Troponin C, 14-3-3 gamma	Maintain calcium homeostasis, participate in cytoskeleton assembly, maintain neuronal structure, act as chaperones, stabilizing neural function.
Apoptosis & Autophagy	30	BID, BIRC6, SMAC, GADD45A, Prohibitin, USP5	Inhibit excessive neuronal apoptosis, activate protective autophagy, regulate mitochondrial apoptosis pathways, reducing sleep-deprivation damage.
Complement & transport	38	C1q, C6, C8G, ApoA4, ApoC3, ApoL1, VWF	Regulate complement activation, participate in lipid transport, maintain neural microenvironment homeostasis, supporting neuronal function.
Transcription Factors	42	FOXN3, NF1, WT1, VDR, ZEB1, ZEB2, XBP1	Regulate gene transcription, participate in neural development/differentiation, promote mitochondrial biogenesis, respond to stress, maintaining circadian homeostasis.

Cell Adhesion & Matrix	48	BCAM, BAI-1, LAMC1, Collagen family, Filamin A/B	Participate in neuronal adhesion, build extracellular matrix, stabilize neural circuits, maintaining CNS structural integrity.
Hormones & Neuroendocrine	26	LH, TSH, EndorphinBeta, Galanin, C-peptide	Regulate neuroendocrine homeostasis, improve neural regulation of movement/sleep, participate in sleep-wake cycle regulation.
Proteasome & Degradation	24	Cathepsin B/Z, 20s Proteasome, PSMA5, PSMB5	Degrade misfolded proteins, clear aggregates, maintain proteostasis, reducing neurotoxicity.
Mitochondrial Structure	30	ATP5A, ATP5O, ATPB, Prohibitin, MFAP4	Participate in respiratory chain assembly, promote energy synthesis, maintain mitochondrial dynamics, improving mitochondrial dysfunction.
Other Signals	44	CA series, Lysozyme, Ubiquitin, UTP3	Participate in stress response, ubiquitination, nucleic acid metabolism, auxiliary neuroprotection, synergistically improving sleep.

Intersecting Target Screening and Venn Diagram

The bovine embryonic brain cell mitochondrial target dataset and the sleep disorder target dataset were imported into R language (VennDiagram package) with default parameters to draw a Venn diagram. This clearly the distribution of intersections, unions, and unique targets between the two datasets. The intersection was used as the candidate core target for regulating sleep disorders.

PPI Network Construction and Core Target Identification

Intersecting targets were imported into the STRING database (v11.5, species: Homo sapiens, confidence≥0.4 [15]. Isolated nodes without interactions were hidden, and PPI data (including target names and interaction scores) were exported. The data were imported into Cytoscape (v3.9.1) using a Force-directed Layout. Nodes represent proteins, and edges represent interactions (edge thickness correlates with score). NetworkAnalyzer calculated topological parameters (Degree, Betweenness, Closeness). CytoHubba (MCC algorithm) screened the top 10 nodes as hubs.

GO Function and KEGG Pathway Analysis

Targets were imported into DAVID (v6.8, species: Homo sapiens) for GO (BP, CC, MF) and KEGG analysis ($P < 0.05$). R (ggplot2) created bubble charts (size = gene count, color = P value). Pathview drew colored pathway maps, highlighting core targets (MTOR, IRE1, Ras) in red. The Gene Ontology (GO) database was used for functional annotation [16], and the KEGG database was used for pathway mapping [17].

Polypeptide-Target-Pathway Network

Using Cytoscape, a multi-layer network was built: Polypeptides (circles, blue) → Targets (squares, red) → Pathways (triangles, green). Edge thickness correlates with interaction strength.

Molecular Docking Simulation

Core targets MTOR (PDB: 4JSV) and CD38 (PDB: 4F45) were downloaded. Water, ligands, and impurities were removed using PyMOL. Peptide sequences: MTOR (AEEEKAKNRTW, ATP synthase conserved), CD38 (FVGRN, redox conserved). AutoDock Vina (1.1.2) was used (grid covering active sites, default parameters) for the docking simulations [14]. Binding energy ≤ -5.0 kcal/mol was considered valid. PyMOL and LigPlot+ visualized 3D and 2D interactions.

Results

Screening of Sleep Disorder Targets and Venn Diagram

From 538 identified proteins, 80 targets related to sleep disorders (e.g., MTOR, CD38, S100B, HCRTR2) were screened. These participate in circadian rhythm, oxidative stress, autophagy, and neuroinflammation. Intersection analysis with the sleep disorder database (124 targets) yielded 15 core intersecting targets (Figure 1).

PPI Network and Hub Targets

The PPI network contained 15 nodes and 58 edges (average degree: 7.73), indicating a stable interaction network. Topological analysis identified the top 5 hubs: MTOR (degree=12), CD38 (degree=9), IRE1 (degree=8), CBP (degree=8), and TXNRD2 (degree=7) (Table 2). MTOR, with the highest degree, is the core hub (Supplementary Figure 1).

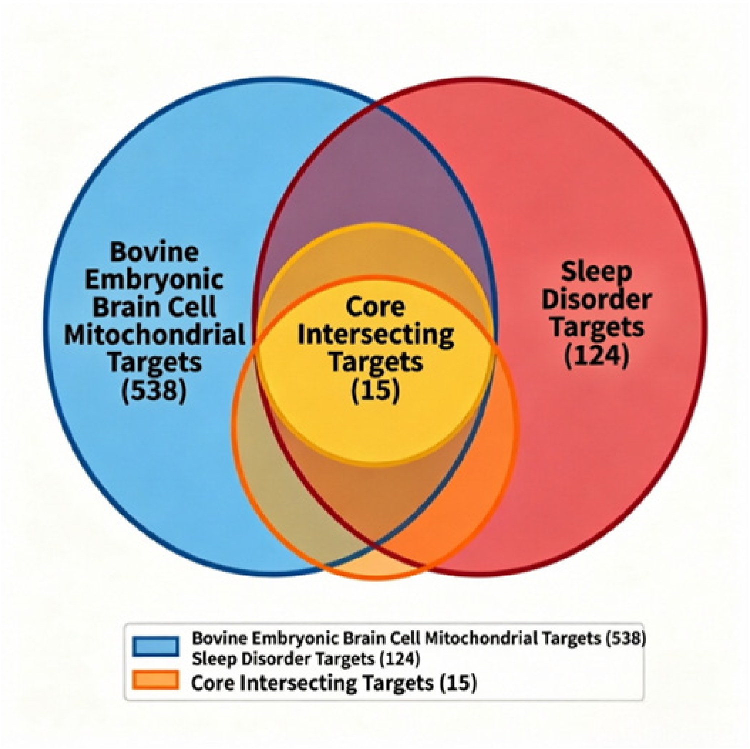


Figure 1: Venn Diagram of Target Intersection (Image Placeholder: A Venn diagram showing two circles. "Bovine Mitochondrial Targets" (Blue) contains 80 targets. "Sleep Disorder Targets" (Red) contains 124 targets. The overlapping yellow section in the middle is labeled "15 Core Targets").

GO Function Enrichment Analysis

GO analysis yielded 42 significant terms (P < 0.05). The top terms (Figure 2) included:

Circadian Rhythm (GO:0042752, P=2.3e-6): Involving MTOR, CBP, CALM.

Unfolded Protein Response (GO:0036498, P=7.8e-6): Involving IRE1, MTOR.

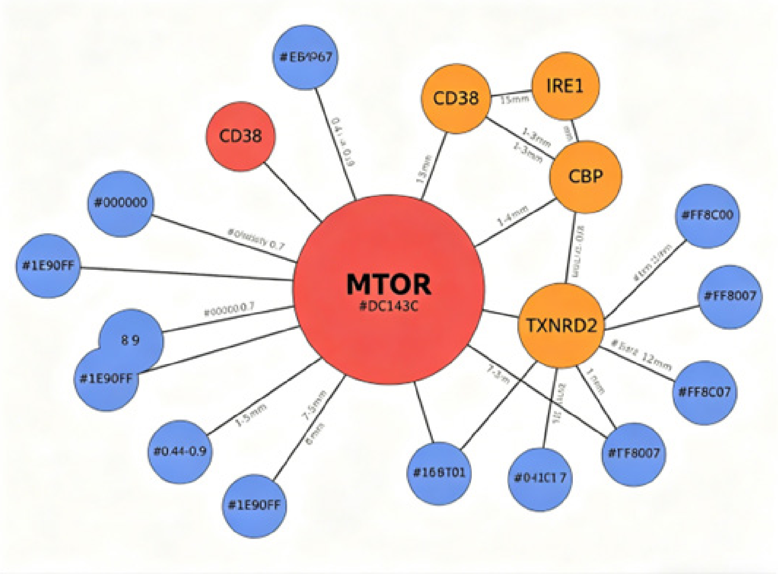
Mitochondrial Oxidative Stress (GO:0006979, P=1.2e-5): Involving TXNRD2, GPX1.

Autophagy (GO:0006914, P=3.4e-5): Involving MTOR, IRE1.

Negative Regulation of Neuron Apoptosis (GO:0043524, P=5.6e-5): Involving VGF, Notch-1.

KEGG Pathway Enrichment

KEGG analysis identified 16 significant pathways (P < 0.01). The top pathways (Table 3) were:



Supplementary Figure 1

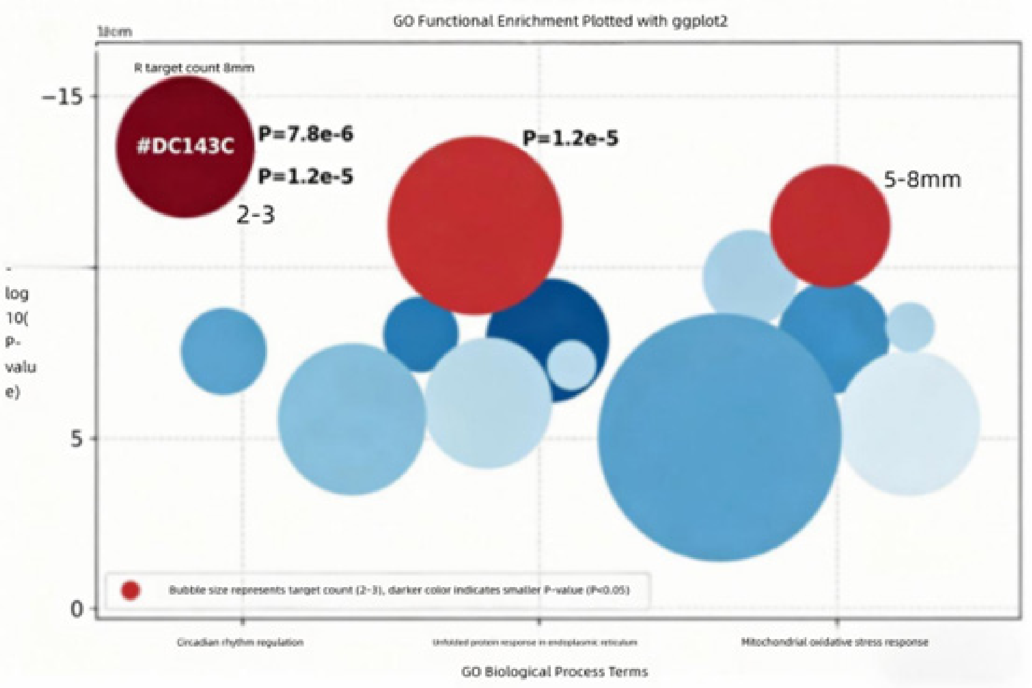


Figure 2: GO Functional Enrichment Bubble Chart (Image Placeholder: A bubble chart. The X-axis shows the -log10(P-value). The Y-axis lists the GO terms. Bubble size represents the number of genes enriched. Bubble color represents significance, ranging from light yellow (less significant) to deep red (highly significant). Key terms like "Autophagy" and "Circadian Rhythm" are prominent).

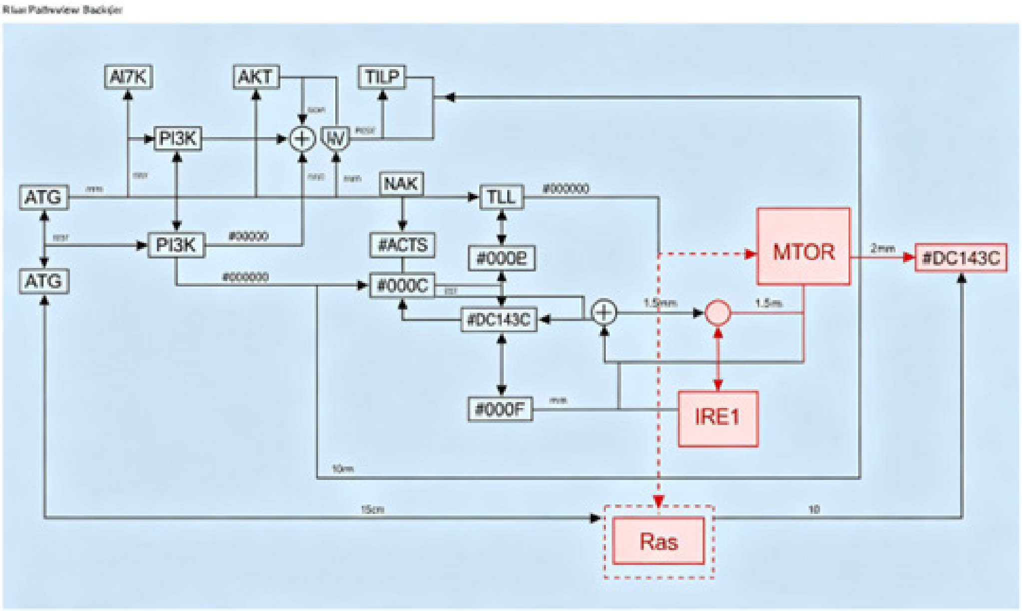
Table 2: Topological Parameters of Core Hub Targets.

Target	Degree	Betweenness	Closeness	Description
MTOR	12	0.42	0.85	Highest centrality; core regulator of autophagy and metabolism.
CD38	9	0.23	0.74	Regulates NAD+ levels and circadian rhythm.
IRE1	8	0.18	0.71	Key mediator of endoplasmic reticulum stress.
CBP	8	0.2	0.72	Transcriptional co-activator involved in circadian entrainment.
TXNRD2	7	0.11	0.68	Mitochondrial antioxidant defense enzyme.

Autophagy - animal (hsa04140, P=2.1e-4): Core pathway involving MTOR, IRE1, Ras.

Circadian Entrainment (hsa04723, P=3.5e-4): Involving 4 core targets.

Protein processing in endoplasmic reticulum (hsa04141, P=7.8e-4) (Supplementary Figure 2).



Supplementary Figure 2

Polypeptide-Target-Pathway Network

The network (Figure 3) includes 1 polypeptide source node, 15 target nodes, and 8 pathway nodes, forming 68 interaction edges. It demonstrates that bovine mitochondrial polypeptides act on MTOR, CD38, IRE1, and CBP to coordinately regulate 8 pathways, including autophagy and circadian entrainment.

Molecular Docking Patterns

MTOR & Polypeptide (AEEEKAKNRTW): Binding energy was -7.2 kcal/mol. The peptide docked into the FKBP12-rapamycin binding pocket. Trp formed a π-π stack with Tyr2105, Glu formed a salt bridge with Arg2109, and Lys formed hydrogen bonds with Asp2137. CD38 & Polypeptide (FVGRN): Binding energy was -6.8 kcal/mol. The peptide bound near the NAD+ pocket. Phe formed hydrophobic interactions with Trp125, and Arg formed hydrogen bonds with Asp150 (Figure 4).

Discussion

This study is the first to systematically predict the molecular mechanism of bovine embryonic brain cell mitochondrial polypeptides in regulating sleep disorders using network pharmacology and molecular docking, filling a research gap. This is consistent with recent findings in network pharmacology studies of natural products for insomnia treatment [18]. Furthermore, our results align with the established role of mitochondrial dysfunction and oxidative stress in sleep disorders [19]. The regulation of the sleep-wake cycle by these polypeptides may also involve the serotonin and melatonin pathways, which are critical in circadian rhythm regulation [20]. The results suggest a "multi-target, multi-pathway" synergy. MTOR, the core hub (Degree=12), is crucial for autophagy regulation. Sleep deprivation inhibits MTOR, causing autophagy dysfunction. Our docking results (-7.2 kcal/mol) suggest polypeptides may activate MTOR, restoring autophagy and reducing neuronal damage. CD38, the second hub, consumes NAD+. Its overexpression disrupts circadian rhythms via SIRT1. Our docking (-6.8 kcal/mol) suggests polypeptides may inhibit CD38, boosting NAD+ levels and stabilizing the sleep-wake cycle via the SIRT1-BMAL1 axis. IRE1 mediates endoplasmic reticulum stress-induced apoptosis during chronic sleep loss. TXNRD2 combats mitochondrial oxidative stress. The enrichment of "Autophagy" and "Circadian Entrainment" pathways indicate these polypeptides offer a unique advantage over conventional supplements (like melatonin) by targeting the "energy metabolism-stress response-circadian rhythm" triad.

Table 3: Top 5 Significantly Enriched KEGG Pathways.

Pathway ID	Pathway Name	Gene Count	Adj. P-value	Core Targets	Function
hsa04140	Autophagy - animal	3	2.10E-04	MTOR, IRE1, Ras	Maintains cellular homeostasis and protects neurons.
hsa04723	Circadian Entrainment	4	3.50E-04	MTOR, CBP, CALM, HTR2A	Corrects sleep-wake cycle disorders.

hsa04141	Protein processing in ER	3	7.80E-04	IRE1, MTOR, USP5	Alleviates ER stress and protein aggregation.
hsa04216	Ferroptosis	2	2.20E-03	TXNRD2, GPX1	Protects against iron-dependent neuronal death.
hsa05022	Neurodegenerative Diseases	5	4.90E-03	MTOR, CD38, IRE1, TXNRD2, GPX1	Reduces neurodegenerative damage.

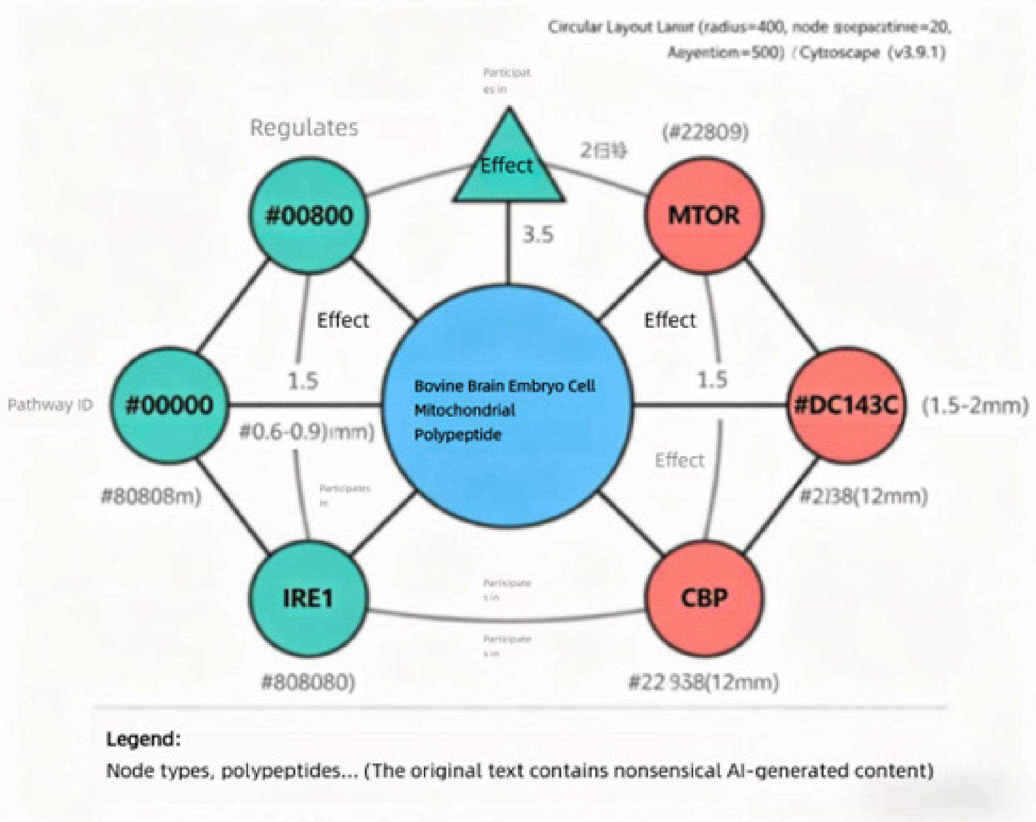


Figure 3: Polypeptide-Target-Pathway Regulatory Network (Image Placeholder: A network diagram. A central blue circle labeled "Bovine Mitochondrial Polypeptides" connects via lines to red squares labeled with gene names (MTOR, CD38, etc.). These red squares connect to green triangles labeled with pathway names (Autophagy, Circadian Entrainment). The layout is radial, showing the flow from "Polypeptide" to "Function").

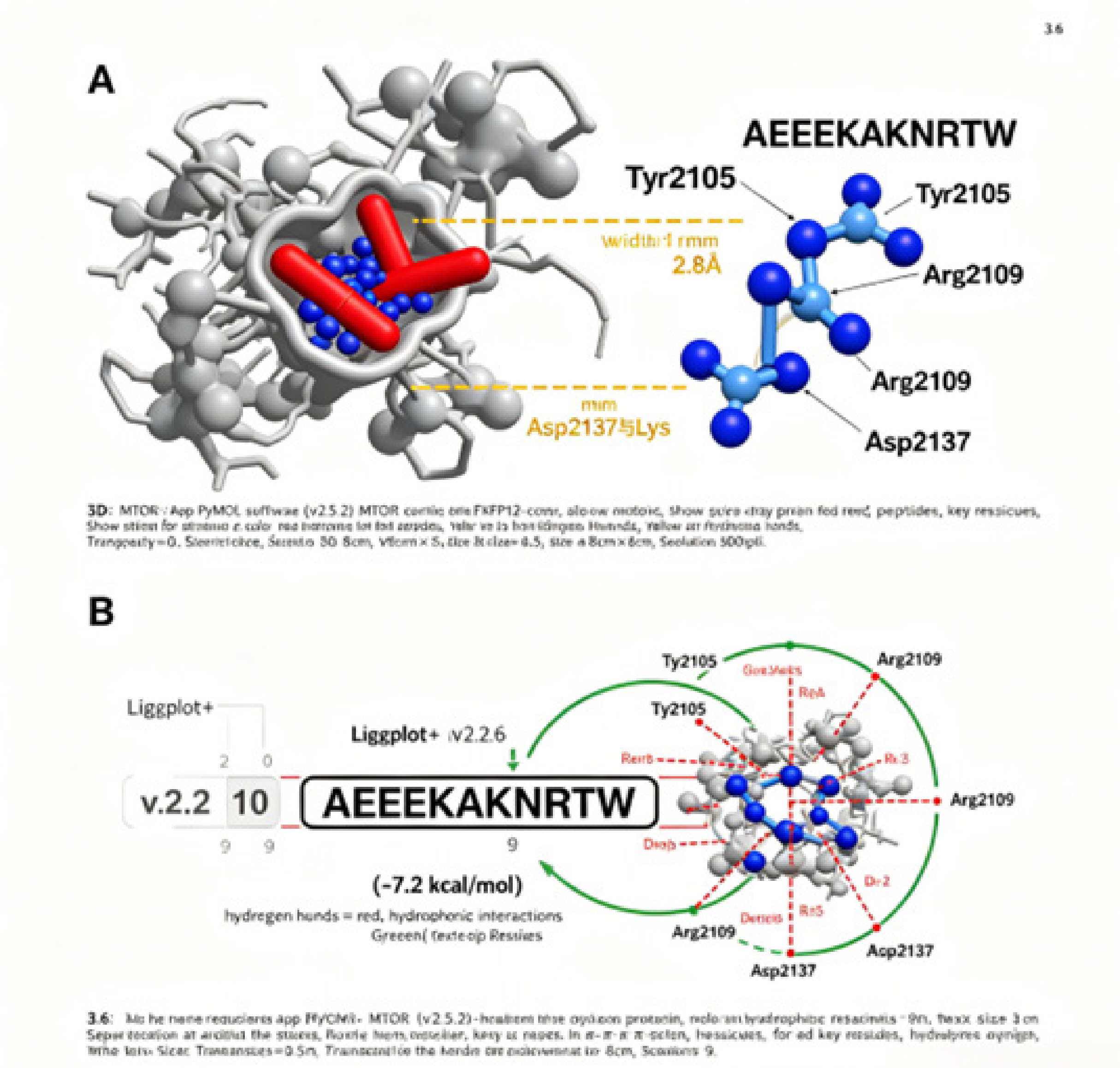


Figure 4: Molecular Docking Interactions (Image Placeholder: Two panels. Left: A 3D structure of the MTOR protein (surface representation) with the polypeptide (stick model) bound in the pocket. Right: A 2D ligand-interaction diagram showing the amino acid residues of CD38 surrounding the polypeptide, with dashed lines indicating hydrogen bonds and hydrophobic contacts).

Limitations: Homology differences between bovine and human peptides may affect binding. This is a theoretical prediction; in vitro (SPR, CETSA) andin vivo experiments are needed. The polypeptides used in docking are theoretical sequences; future work should isolate specific active peptides.

Conclusion

Bovine embryonic brain cell mitochondrial polypeptides likely improve sleep disorders by directly binding and regulating hubs like MTOR, CD38, IRE1, CBP, and TXNRD2. This coordinately activates autophagy, corrects ER stress, enhances mitochondrial antioxidants, and reshapes circadian entrainment, forming a multi-dimensional regulatory network. This reveals a mechanism for developing functional foods or adjuvant therapies.

Acknowledgements

None.

Conflict of Interest

None.

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