DOWNLOAD PDF ACTIVITY-DEPENDENT SPINE REMODELING AND BRAIN DISORDERS SHU TAKIGAMI . [ET AL.]

Chapter 1 : - NLM Catalog Result

Dendritic spines are numerous small protrusions on the dendrites of most neurons in the central nervous system. Each spine is connected with an axon terminal of a glutamatergic neuron, and.

Received Jun 15; Accepted Jul This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. This article has been cited by other articles in PMC. Abstract Dendritic spines are the location of excitatory synapses in the mammalian nervous system and are neuron-specific subcellular structures essential for neural circuitry and function. Dendritic spine morphology is determined by the F-actin cytoskeleton. F-actin remodeling must coordinate with different stages of dendritic spinogenesis, starting from dendritic filopodia formation to the filopodia-spines transition and dendritic spine maturation and maintenance. Hundreds of genes, including F-actin cytoskeleton regulators, membrane proteins, adaptor proteins, and signaling molecules, are known to be involved in regulating synapse formation. Many of these genes are not neuron-specific, but how they specifically control dendritic spine formation in neurons is an intriguing question. Here, we summarize how ubiquitously expressed genes, including syndecan-2, NF1 encoding neurofibromin protein , VCP, and CASK, and the neuron-specific gene CTTNBP2 coordinate with neurotransmission, transsynaptic signaling, and cytoskeleton rearrangement to control dendritic filopodia formation, filopodia-spines transition, and dendritic spine maturation and maintenance. The aforementioned genes have been associated with neurological disorders, such as autism spectrum disorders ASDs , mental retardation, learning difficulty, and frontotemporal dementia. We also summarize the corresponding disorders in this report. Introduction The tiny protrusions emerging from dendrites known as dendritic spines are the primary subcellular locations of excitatory synapses in the mammalian central nervous system [1]. These structures are mainly supported by the F-actin cytoskeleton. Thus, F-actin cytoskeletal proteins and regulators are important factors for generating dendritic spines. Many membrane proteins and adaptor and signaling molecules are also involved in controlling dendritic spine formation and maintenance [2]. Several mechanisms have been described to form dendritic spines [3]. The most popular mechanism is that dendritic filopodia serve as precursors for dendritic spine formation. Interestingly, filopodia are ubiquitously found on various cell types. In contrast, dendritic spines are neuron-specific structures. Thus, the transition from filopodia to spines should be controlled by neuron-specific factors. Neuron-specific factors controlling dendritic spinogenesis fall into two categories. The first group is proteins specifically expressed in neurons. The second group is neuron-specific cellular responses or processes. These proteins or responses directly or indirectly regulate F-actin rearrangement and dynamics to promote dendritic spine formation. Studies of cytoskeleton-associated cortactin-binding protein 2 CTTNBP2 and heparan sulfate transmembrane proteoglycan HSPG syndecan-2 serve as examples for these two categories, respectively. CTTNBP2 is a neuron-specific cytoskeleton-associated protein and that is enriched at dendritic spines of mature neurons. Although syndecan-2 is widely expressed in many cell types, it is highly concentrated at synapses in neurons. Syndecan-2 cooperates with other proteins to trigger neurotransmission through a neuron-specific signal to induce dendritic spine formation. Additionally, neurofibromin, CASK, and VCP coordinate with syndecan-2 to control dendritic spinogenesis and were also associated with neurological disorders. These findings suggest that these genes are critical for neuronal function, likely through their regulation of dendritic spine formation. In this review, we will summarize the functions of these proteins in dendritic spinogenesis and use these proteins as examples to discuss how neuron-specific molecules coordinate with ubiquitously expressed proteins to generate neuron-specific signals for dendritic spine formation. In mammals, the syndecan protein family contains four members, syndecan-1, syndecan-2, syndecan-3, and syndecan-4 [7]. In rodent brains, syndecan-2 and syndecan-3 are the two major syndecans expressed in neurons with differential distribution; syndecan-2 is highly concentrated at synapses, while syndecan-3 is distributed along the axonal shaft [8].

Syndecan-2 is involved in cell-cell and cell-matrix interactions through its heparan sulfate modification. It can also bind growth factors, such as fibroblast growth factor FGF and epidermal cell growth factor, and it acts as a coreceptor for these growth factors [7]. Syndecan-2 is broadly and dynamically expressed in several tissues and cell types [7 , 8]. During neural development, its expression gradually increases concurrent with synapse formation [8 , 9]. In mature neurons, such as cultured rat hippocampal neurons at 18 days after plating in vitro DIV or later, syndecan-2 is highly enriched at dendritic spines [9 , 10]. More importantly, overexpression of syndecan-2 in immature rat hippocampal cultured neurons, such as DIV, when endogenous syndecan-2 is not yet expressed, dendritic filopodia are massively induced at DIV and dendritic filopodia are then transformed to dendritic spines at DIV [9 , 11]. Those dendritic spines are expected to be functional, as they are adjacent to the presynaptic marker synaptophysin based on confocal microscopy [11 , 12]. Syndecaninduced dendritic spinogenesis serves as a model to explore the mechanisms underlying the initiation of dendritic spinogenesis namely, dendritic filopodia formation , the transition from filopodia to spines, and dendritic spine maturation and maintenance.

Chapter 2 : INSTITUTE OF NEUROSCIENCE

-- The actin cytoskeleton: central regulator of dendritic spine form and function / Andreas Birbach -- Pathological remodeling of dendritic spines / John N. Campbell, Jonathon E. Kurz and Severn B. Churn -- Spine plasticity in the rat medial amygdala / Alberto A. Rasia-Filho, JanaÃ-na Brusco, and Jorge E. Moreira -- Activity-dependent spine.

Function[edit] BDNF acts on certain neurons of the central nervous system and the peripheral nervous system , helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. Neurotrophins are proteins that help to stimulate and control neurogenesis, BDNF being one of the most active. BDNF is made in the endoplasmic reticulum and secreted from dense-core vesicles. The phenotype for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar abnormalities and an increase in the number of sympathetic neurons. TrkB autophosphorylation is dependent upon its ligand-specific association with BDNF, a widely expressed activity-dependent neurotic factor that regulates plasticity and is unregulated following hypoxic injury. The activation of the BDNF-TrkB pathway is important in the development of short term memory and the growth of neurons. While the TrkB receptor interacts with BDNF in a ligand-specific manner, all neurotrophins can interact with the p75 receptor. Recent studies have revealed a truncated isoform of the TrkB receptor t-TrkB may act as a dominant negative to the p75 neurotrophin receptor, inhibiting the activity of p75, and preventing BDNF-mediated cell death. AMPA and NMDA receptors are two ionotropic glutamate receptors involved in glutamatergic neurotransmission and essential to learning and memory via long-term potentiation. While AMPA receptor activation leads to depolarization via sodium influx, NMDA receptor activation by rapid successive firing allows calcium influx in addition to sodium. NMDA receptor activity[edit] NMDA receptor activation is essential to producing the activity-dependent molecular changes involved in the formation of new memories. Following exposure to an enriched environment, BDNF and NR1 phosphorylation levels are upregulated simultaneously, probably because BDNF is capable of phosphorylating NR1 subunits, in addition to its many other effects. This has been shown to be important for processes such as spatial memory in the hippocampus, demonstrating the therapeutic and functional relevance of BDNF-mediated NMDA receptor activation. This suggests BDNF is not only capable of initiating synapse formation through its effects on NMDA receptor activity, but it can also support the regular every-day signaling necessary for stable memory function. Blockading BDNF signaling with a tyrosine kinase inhibitor or a PKC inhibitor in wild type mice produced significant reductions in spontaneous action potential frequencies that were mediated by an increase in the amplitude of GABAergic inhibitory postsynaptic currents IPSC. In pre-synaptic neurons, actins are involved in synaptic vesicle recruitment and vesicle recovery following neurotransmitter release. Neurogenesis[edit] BDNF plays a significant role in neurogenesis. This becomes especially evident following suppression of TrkB activity. The increased visual, physical, and cognitive stimulation all translates into more neuronal activity and synaptic communication, which can produce structural or molecular activity-dependent alterations. Environmental enrichment enhances synaptogenesis, dendridogenesis, and neurogenesis, leading to improved performance on various learning and memory tasks. BDNF mediates more pathways involved in these enrichment-induced processes than any other molecule and is strongly regulated by calcium activity making it incredibly sensitive to neuronal activity.

Chapter 3 : Mr Adekunle Bademosi - Queensland Brain Institute - University of Queensland

Spine plasticity in the rat medial amygdala / Alberto A. Rasia-Filho, JanaÃ-na Brusco, and Jorge E. Moreira -- Activity-dependent spine remodeling and brain disorders / Shu Takigami [et al.]. Spines of the Ca1 pyramidal neurons exhibit species-related differences in the basal and the apical dendritic tree and along the septo-temporal axis.

There are currently publications in peer-reviewed journals with one or more of our students or alumni as co-authors. See here for a list of student authors. A list of all publications can be found here. Lists of publications by year can be found below. Birdsong decreases protein levels of FoxP2, a molecule required for human speech. Journal of neurophysiology , Determination of antibiotic hypersensitivity among 4, single-gene-knockout mutants of Escherichia coli. Journal of bacteriology , Immune blood biomarkers of Alzheimer disease patients. Journal of neuroimmunology , Nature methods 6, The Journal of biological chemistry , Internally mediated developmental desynchronization of neocortical network activity. The Journal of neuroscience: Mammillary body and fornix injury in congenital central hypoventilation syndrome. Pediatric research 66, The Trypanosoma brucei flagellum: Annual review of microbiology 63, Alzheimer disease macrophages shuttle amyloid-beta from neurons to vessels, contributing to amyloid angiopathy. Acta neuropathologica , Delayed stabilization of dendritic spines in fragile X mice. Cancer research 70, CMF70 is a subunit of the dynein regulatory complex. Journal of cell science , Genetic studies on the functional relevance of the protein prenyltransferases in skin keratinocytes. Human molecular genetics 19, Antibiotic sensitivity profiles determined with an Escherichia coli gene knockout collection: Antimicrobial agents and chemotherapy 54, Developmental biology , The sympathetic nervous system induces a metastatic switch in primary breast cancer. Female human iPSCs retain an inactive X chromosome. Cell stem cell 7, Role of lipid metabolism in smoothened derepression in hedgehog signaling. Developmental cell 19, Vascular remodeling of the vitelline artery initiates extravascular emergence of hematopoietic clusters. Phosphatase and tensin homolog regulates the pluripotent state and lineage fate choice in human embryonic stem cells. Stem cells Dayton, Ohio 29, Brain network local interconnectivity loss in aging APOE-4 allele carriers. Smad6 is essential to limit BMP signaling during cartilage development. Journal of bone and mineral research: Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. The Journal of experimental medicine , Cancer discovery 1, Target site recognition by a diversity-generating retroelement. PLoS genetics 7, e Isotope-reinforced polyunsaturated fatty acids protect yeast cells from oxidative stress. An integrated approach to elucidate the intra-viral and viral-cellular protein interaction networks of a gamma-herpesvirus. PLoS pathogens 7, e Selective advantage of resistant strains at trace levels of antibiotics: Antimicrobial agents and chemotherapy 55, Habituation of the C-start response in larval zebrafish exhibits several distinct phases and sensitivity to NMDA receptor blockade. PloS one 6, e Development of the Drosophila entero-endocrine lineage and its specification by the Notch signaling pathway. Osteoblast interactions within a biomimetic apatite microenvironment. Annals of biomedical engineering 39, Single cell analysis facilitates staging of Blimp1-dependent primordial germ cells derived from mouse embryonic stem cells. Further evidence of olfactory ensheathing glia facilitating axonal regeneration after a complete spinal cord transection. Experimental neurology , Nature genetics 44, Transcript dynamics of proinflammatory genes revealed by sequence analysis of subcellular RNA fractions. N-Glycans on the Nipah virus attachment glycoprotein modulate fusion and viral entry as they protect against antibody neutralization. Journal of virology 86, PloS one 7, e Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. Self-assembled polypeptide and polypeptide hybrid vesicles: Topics in current chemistry , Glutamate induces the elongation of early dendritic protrusions via mGluRs in wild type mice, but not in fragile X mice. Integrative survival-based molecular profiling of human pancreatic cancer. Delayed accumulation of intestinal coliform bacteria enhances life span and stress resistance in Caenorhabditis elegans fed respiratory deficient E. BMC microbiology 12, Inertial manipulation and transfer of microparticles across laminar fluid streams.

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Chapter 4 : Brain-derived neurotrophic factor - Wikipedia

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 Abstract Dendritic spines are the location of excitatory synapses in the mammalian nervous system and are neuron-specific subcellular structures essential for neural circuitry and function. Dendritic spine morphology is determined by the F-actin cytoskeleton. F-actin remodeling must coordinate with different stages of dendritic spinogenesis, starting from dendritic filopodia formation to the filopodia-spines transition and dendritic spine maturation and maintenance. Hundreds of genes, including F-actin cytoskeleton regulators, membrane proteins, adaptor proteins, and signaling molecules, are known to be involved in regulating synapse formation. Many of these genes are not neuron-specific, but how they specifically control dendritic spine formation in neurons is an intriguing question. Here, we summarize how ubiquitously expressed genes, including syndecan-2, NF1 encoding neurofibromin protein , VCP, and CASK, and the neuron-specific gene CTTNBP2 coordinate with neurotransmission, transsynaptic signaling, and cytoskeleton rearrangement to control dendritic filopodia formation, filopodia-spines transition, and dendritic spine maturation and maintenance. The aforementioned genes have been associated with neurological disorders, such as autism spectrum disorders ASDs , mental retardation, learning difficulty, and frontotemporal dementia. We also summarize the corresponding disorders in this report. Introduction The tiny protrusions emerging from dendrites known as dendritic spines are the primary subcellular locations of excitatory synapses in the mammalian central nervous system [1]. These structures are mainly supported by the F-actin cytoskeleton. Thus, F-actin cytoskeletal proteins and regulators are important factors for generating dendritic spines. Many membrane proteins and adaptor and signaling molecules are also involved in controlling dendritic spine formation and maintenance [2]. Several mechanisms have been described to form dendritic spines [3]. The most popular mechanism is that dendritic filopodia serve as precursors for dendritic spine formation. Interestingly, filopodia are ubiquitously found on various cell types. In contrast, dendritic spines are neuron-specific structures. Thus, the transition from filopodia to spines should be controlled by neuron-specific factors. Neuron-specific factors controlling dendritic spinogenesis fall into two categories. The first group is proteins specifically expressed in neurons. The second group is neuron-specific cellular responses or processes. These proteins or responses directly or indirectly regulate F-actin rearrangement and dynamics to promote dendritic spine formation. Studies of cytoskeleton-associated cortactin-binding protein 2 CTTNBP2 and heparan sulfate transmembrane proteoglycan HSPG syndecan-2 serve as examples for these two categories, respectively. CTTNBP2 is a neuron-specific cytoskeleton-associated protein and that is enriched at dendritic spines of mature neurons. Although syndecan-2 is widely expressed in many cell types, it is highly concentrated at synapses in neurons. Syndecan-2 cooperates with other proteins to trigger neurotransmission through a neuron-specific signal to induce dendritic spine formation. Additionally, neurofibromin, CASK, and VCP coordinate with syndecan-2 to control dendritic spinogenesis and were also associated with neurological disorders. These findings suggest that these genes are critical for neuronal function, likely through their regulation of dendritic spine formation. In this review, we will summarize the functions of these proteins in dendritic spinogenesis and use these proteins as examples to discuss how neuron-specific molecules coordinate with ubiquitously expressed proteins to generate neuron-specific signals for dendritic spine formation. In mammals, the syndecan protein family contains four members, syndecan-1, syndecan-2, syndecan-3, and syndecan-4 [7]. In rodent brains, syndecan-2 and syndecan-3 are the two major syndecans expressed in neurons with differential distribution; syndecan-2 is highly concentrated at synapses,

while syndecan-3 is distributed along the axonal shaft [8]. Syndecan-2 is involved in cell-cell and cell-matrix interactions through its heparan sulfate modification. It can also bind growth factors, such as fibroblast growth factor FGF and epidermal cell growth factor, and it acts as a coreceptor for these growth factors [7]. Syndecan-2 is broadly and dynamically expressed in several tissues and cell types [7 , 8]. During neural development, its expression gradually increases concurrent with synapse formation [8 , 9]. In mature neurons, such as cultured rat hippocampal neurons at 18 days after plating in vitro DIV or later, syndecan-2 is highly enriched at dendritic spines [9 , 10]. More importantly, overexpression of syndecan-2 in immature rat hippocampal cultured neurons, such as DIV, when endogenous syndecan-2 is not yet expressed, dendritic filopodia are massively induced at DIV and dendritic filopodia are then transformed to dendritic spines at DIV [9 , 11]. Those dendritic spines are expected to be functional, as they are adjacent to the presynaptic marker synaptophysin based on confocal microscopy [11 , 12]. Syndecaninduced dendritic spinogenesis serves as a model to explore the mechanisms underlying the initiation of dendritic spinogenesis namely, dendritic filopodia formation , the transition from filopodia to spines, and dendritic spine maturation and maintenance. Schematic structure and amino acid sequences of syndecans. The C1 and C2 Motifs of Syndecan-2 Work Sequentially to Promote Dendritic Spinogenesis The ectodomain of syndecan-2 heparan sulfate modification is involved in cell-cell and cell-matrix interactions [7]. Its transmembrane domain is required for homodimerization or oligomerization [13], which is critical for the protein-protein interactions of syndecan-2 [14]. The cytoplasmic domain of syndecan-2 contains only 32 amino acid residues Figure 1. Although it is short, it is divided into three motifs, conserved domain 1 C1 , the variable region V , and conserved domain 2 C2. The C1 and C2 motifs are conserved among different syndecans, while the sequences of the V regions vary Figure 1. The C2 is required for the dendritic filopodia-spines transition and dendritic spine maintenance [15 , 16]. However, those filopodia are unable to transform into dendritic spine at 9 DIV [11 , 15 , 16]. These analyses indicate that the function of syndecan-2 in dendritic spinogenesis can be separated into two sequential steps, namely, filopodia and spine formation, which are controlled by two distinct motifs in syndecan Because both C1 and C2 motifs are short and lack recognizable enzymatic domains, syndecan-2 binding partners have been identified to determine its molecular mechanism underlying dendritic spine formation. Several direct binding partners summarized in Table 1 have been identified for the C1 domains of syndecan-2, including neurofibromin [17] and ezrin [18]. Among these, the interactions between syndecan-2 and neurofibromin and CASK have been shown to be relevant in dendritic spine formation. Because the cytoplasmic tail of syndecan-2 is very short, it is unlikely that a single syndecan-2 molecule can simultaneously interact with all of its binding partners. Because the C1 and C2 motifs are involved in two sequential processes, it is likely that neurofibromin and CASK sequentially interact with syndecan Alternatively, it is possible that because syndecan-2 forms at least a dimer through its transmembrane domain, different syndecan-2 molecules in dimers or oligomers separately interact with neurofibromin and CASK. This would suggest that syndecan-2, neurofibromin, and CASK form a single large complex. Further investigation, including coimmunoprecipitation experiments, is required to address this question. Similar to syndecan-2, neurofibromin is widely expressed in different cell types, though its expression level is much higher in the nervous system [25]. NF1 is one of the most common human inherited disorders featured by changes in skin pigmentation, benign tumor growth, and learning difficulty [26 , 27]. Neurofibromin suppresses tumor growth through its ability to downregulate the RAS pathway [28]. In addition to its RAS activity, neurofibromin can increase cAMP concentration by activating adenylate cyclase [29]. Although the molecular mechanisms are less clear, the GRD and C-terminal region of neurofibromin are required for cAMP pathway activation Figure 2 a [30]. Both Gs-dependent and Gs-independent pathways are involved in neurofibromin-regulated adenylate cyclase activation [30]. The cAMP pathway has been shown to be involved in learning and memory in Drosophila [31] and dendritic spine formation in the mammalian nervous system [11]. Function of neurofibromin in neurons. The Jn and Pn fragments interact with syndecan Both GRD and the C-terminal half of neurofibromin are involved in adenylate cyclase activity regulation. In a

yeast two-hybrid screen using different fragments of neurofibromin as baits, syndecan-2 was identified as a neurofibromin binding partner [17]. Notably, neurofibromin has two independent interacting domains for the C1 motif of syndecan One is the Jn fragment corresponding to amino acid residues â€” in the GRD of human neurofibromin; the other is the Pn fragment containing amino acid residues â€” Figure 2 a [17]. The Jn and Pn compete for binding to the C1 motif of syndecan In addition to biochemical studies demonstrating the direct interaction between syndecan-2 and neurofibromin, fluorescence immunostaining further demonstrated the colocalization of syndecan-2 and neurofibromin at synapses in cultured hippocampal neurons [17]. Moreover, both Nf1 knockdown and haploinsufficiency reduce the density of dendritic spines in both rat hippocampal and mouse cortical cultured neurons and in brains [11 , 32], consistent with a function of neurofibromin in regulating dendritic spine formation. The next question is how the syndecanneurofibromin complex regulates dendritic spine formation. One study examined syndecan-2 downstream signaling for triggering filopodia formation. Using a panel of inhibitors to suppress various kinase activities, protein kinase A PKA was identified to be required for syndecaninduced filopodia formation [11]. Combined with the analysis using different motif deletion mutants of syndecan-2, we found that the C1 motif of syndecan-2 is essential for PKA-dependent filopodia formation [11]. Because neurofibromin interacts with the C1 motif and also activates the cAMP pathway, cultured hippocampal neurons were then used to investigate whether neurofibromin mediates syndecaninduced filopodia formation. Both Nf1 knockdown and Jn fragment expression, which acts as a dominant-negative to disrupt the interaction between endogenous neurofibromin and syndecan-2, suppress syndecaninduced dendritic filopodia formation of rat hippocampal cultured neurons at 5 DIV [11]. Thus, neurofibromin mediates the signal from syndecan-2 to the cAMP pathway to initiate dendritic spinogenesis. Because filopodia are supported by F-actin bundles, the syndecanneurofibromin-cAMP pathway has to induce F-actin polymerization and bundle formation to promote dendritic filopodia formation. Although the PKA pathway is required for dendritic filopodia formation, increased intracellular cAMP concentrations alone cannot induce dendritic filopodia formation [11], suggesting that other factor s are involved. From an immunoprecipitation-mass spectrometry study, valosin-containing protein VCP, also known as P97 was identified as a neurofibromin-binding protein [32]. These evidences suggest that VCP mutations impair brain function. A combination of human genetic studies, mouse genetic models, and cultured hippocampal and cortical neurons have indicated that neurofibromin interacts with VCP and guides VCP to promote dendritic spinogenesis [32]. The roles of VCP and neurofibromin in dendritic spine formation may account for the neural phenotypes in patients with mutations in the NF1 and VCP genes. However, it is still unclear how VCP regulates dendritic spine formation. To fully address the molecular regulation of neurofibromin and VCP in dendritic spinogenesis, further studies are required. The function of the syndecanneurofibromin interaction in dendritic spine formation is summarized in Figure 2 b. Mutations in the human CASK gene result in X-linked mental retardation and microcephaly with pontine and cerebellar hypoplasia [39 â€” 43]. CASK belongs to the membrane-associated guanylate kinase MAGUK family and functions as a scaffold protein to interact with more than two dozen cellular proteins [44]. It is widely distributed in neurons, including synapses, dendrites, axons, and soma [10]. At synapses, it localizes to both pre- and postsynaptic sites [10]. In mouse pontine explants and rat hippocampal cultured neurons, CASK knockdown impairs synapse formation at the pre- and postsynapse, respectively [16 , 45]. At presynaptic sites, it binds the membrane protein neurexin and other scaffold proteins, such as Mint1, mLin7, and liprin, to control presynaptic button formation [45 â€” 48]. In cultured hippocampal neurons, expression of the PDZ alone of CASK or the C-terminal tail of syndecan-2 that disrupts the interaction between endogenous CASK and syndecan-2 reduces dendritic spine density, narrows spine heads, and shortens spine length at 18 DIV, suggesting that the CASK-syndecan-2 interaction is critical for dendritic spine formation [16]. To investigate whether CASK is involved in dendritic spinogenesis initiation or dendritic spine stabilization, a time course study using a knockdown approach in cultured hippocampal neurons has been performed [16]. The time window of 15â€”18 DIV covering the initiation toward maturation of dendritic spinogenesis was used for

analysis. At 15 DIV, wild-type dendritic spines are immature, long, and thin, and they are present at a low density. As they mature at 18 DIV, dendritic spine density increases, spine length decreases, and spine width increases.

Chapter 5 : Minor in Biomedical Research â€“ Student Publications | Biomedical Research Minor

Book Description: A dendritic spine (or spine) is a small membranous protrusion from a neuron's dendrite that typically receives input from a single synapse of an axon. Dendritic spines serve as a storage site for synaptic strength and help transmit electrical signals to the neuron's cell body.

This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Increasing evidence suggests that epilepsy is the result of synaptic reorganization and pathological excitatory loop formation in the central nervous system; however, the mechanisms that regulate this process are not well understood. We tested this hypothesis using a magnesium-free medium-induced epileptic model of cultured hippocampal neurons. Silencing miR inhibited the electrical excitability level of cultured epileptic neurons, whereas silencing pGAP had an opposite effect. In addition, we verified the effect of miR in vivo and found that silencing miR inhibited the aberrant formation of dendritic spines and chronic spontaneous seizure in a lithium-pilocarpine-induced epileptic mouse model. Finally, we confirmed that silencing miR has a neuroprotective effect on cultured epileptic neurons; however, this effect did not occur through the pGAP pathway. Introduction Epilepsy is a neurological disorder that is characterized by recurrent seizures that result from abnormal and synchronous firing of neurons in the brain. Approximately one-third of the patients with epilepsy do not respond to drugs and are said to have intractable epilepsy. Although the precise mechanism of seizure recurrence remains elusive, elucidation of the mechanisms involved in the transformation of a normal brain into one capable of producing recurrent seizures and of maintaining an epileptic state is essential for understanding epileptogenesis and for developing new treatments for epilepsy. MicroRNA miR is significantly upregulated during active synaptogenesis and plays important roles in spine formation and maturation [2 â€” 5]. Several studies have shown that miR is persistently upregulated during epileptogenesis after acute brain injury [9 â€” 13]. Because synaptic dysfunction and reorganization are the most important histopathological changes in epileptic foci [14], we aimed to investigate whether miR plays a role in epileptogenesis by regulating synaptic reorganization. It is an important cytoskeletal regulator that is regulated by neuronal activity-related signaling pathways that result in the depolymerization of the cytoskeleton and a reduction in the density and volume of dendritic spines. This study aimed to explore the possible molecular mechanisms of miR and its target, pGAP, during epileptogenesis. Materials and Methods 2. All experimental procedures were performed in accordance with the international guidelines for the use of animals and the guidelines of the Animal Care Committee of Chongqing Medical University, China. Hippocampal Neuron Culture Hippocampal neurons from to day-old embryonic mice were cultured cells per square centimeter on plates coated with poly-L-lysine Catalog number P, Sigma, USA as described previously [17]. The neurons were then maintained in neurobasal medium Catalog number , Gibco, USA supplemented with B27 Catalog number , Gibco and 0. SREDs are typically observed within 12â€”24 h using patch clamp recordings and can last for the life of the neurons in culture. This hippocampal neuronal culture model of status epilepticus SE has been well characterized as a useful in vitro model of refractory SE [18]. Cell Transfection A miR antagomir ant was used to silence the expression level of miR Patch Clamp Recordings The membrane potentials of neurons were measured with whole-cell current-clamp recordings using a patch clamp amplifier. The pipette resistance and capacitance were compensated electronically after the establishment of a gigaseal. To optimize the success of recording from pyramidal neurons, phase-bright cells were selected based on both size and pyramidal soma. Whole-cell resistance and resting membrane potential were also monitored before and during the experiments, and a cell was accepted for study only if these parameters remained stable. Data were collected and analyzed using Clamp-fit The PCR mixture contained Real-time PCR was performed under the following conditions: WB analysis was performed as described previously [8]. The primary antibodies used were goat anti-pGAP 1: After the proteins were precipitated, an immunoblot was performed, and the activated

Rac1 and Cdc42 were detected with specific monoclonal antibodies followed by an HRP-conjugated secondary antibody. To detect the nuclei, the slides were incubated with DAPI for 5 min at room temperature in the dark and observed with a fluorescence microscope. The apoptotic index was expressed as the ratio of the number of TUNEL-positive neurons to the total number of neurons. For SE induction, all the mice were intraperitoneally i. All of the mice were allowed to recover for 48 h after SE and were then intracerebroventricularly i. The dose of ant was 1 nmol, which was achieved by dilution in double-distilled water. Analysis of Spontaneous Seizures by Continuous Video Monitoring Animals in the chronic period with spontaneous recurrent seizures SRS associated with pilocarpine epilepsy were recorded every day for 24 h using a closed circuit video system to detect the class 4 and class 5 seizures at the 6th week. An observer who was blinded to the study reviewed the videos. Seizures were counted using a modified six-point Racine scale. Clinical events with a score below 2 were excluded. To assess the reliability of the counting of dendritic protrusions, a blind study was initially performed [20]. Each experiment was repeated at least three times using independent preparations. Expression of miR and pGAP Is Associated with Synaptogenesis and Electrophysiological Activity It has been reported that the expression level of mature miR is low during the first week in the neonatal rat hippocampus, with a significant increase in miR levels during weeks 2â€”4, which is also a critical period for the development and maturation of spines in rodents [15]. We evaluated the chronological expression level of miR and pGAP during the maturation process of cultured hippocampal neurons. This result indicated that the expression of miR and pGAP might correlate with the process of physiological spine maturation and synaptogenesis. The expression levels of miR and pGAP at 6 h, 3 d, 5 d, and 7 d after MGF treatment were evaluated to determine whether the levels of miR and pGAP were influenced by the electrophysiological activity of cultured neurons. The results showed that miR was upregulated 6 h to 7 d following magnesium-free medium treatment, with statistical significance at 6 h, 3 d, and 7 d, while pGAP protein was downregulated from 6 h to 7 d following magnesium-free medium treatment, with statistical significance at 3 d, 5 d, and 7 d Figures 1 c and 1 d. The results suggest that miR expression is increased during the active synaptogenesis periods of the immature brain. Moreover, the upregulation of miR in the SRED model of hippocampal neurons suggests that the pathological electrical excitability may also influence miR expression, which may correlate with the pathological synaptogenesis of the CNS during epileptogenesis. The expression level of pGAP has significantly upregulated after ant treatment which indicated that the expression level of pGAP was negatively regulated by miR in our cultured epileptic hippocampal neurons Figure 2 e. Knockdown of miR and pGAP in cultured neurons. Transfection efficiency was measured by RFP fluorescence. Transfection efficiency was measured by GFP fluorescence. Rac1 and Cdc42 are considered two important promotors of dendritic branching and synaptic plasticity, while RhoA acts in the opposite manner [16]. The activation level of Rac1 and Cdc42 was tested. First, we found that the activation levels of both Rac1 and Cdc42 were significantly elevated in MGF-treated hippocampal neurons compared to control hippocampal neurons Figures 3 a1 and 3 a2. The results showed that transfection of ant or LV-shpGAP did not significantly affect the activity of Rac1 Figures 3 b1 and 3 b2 , while the activity of Cdc42 was significantly inhibited after ant transfection and elevated after LV-shpGAP transfection Figures 3 c1 and 3 c2. The level of active Rac1 was detected at DIV Our experiment showed that ant treatment significantly decreased the AP frequency, suggesting that ant can significantly inhibited neuronal electrical excitability in our SRED model of cultured hippocampal neurons Figures 4 a2 â€” 4 a4. Representative trace of AP in cultured hippocampal neurons. AP frequency was tested 24 h after the MGF treatment.