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The miRNA-106b-5p Target in Caspase 6 Induces Neuronal Cell Death in Epileptogenesis

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Abstract: ***Epilepsy is a chronic neurological disease that is often found and associated with specific neuropathology.[1] Epilepsy is a heterogeneous condition characterized by hyperexcitability of neurons and recurring, spontaneous seizures.[2,3] This disease is a common brain disorder and affects about 1% of the population. Epilepsy is the second highest number of health problems affecting 50-70 million people worldwide. As many as 2.4 million people have been diagnosed with epilepsy every year. In developing countries there are as many as 80% of epilepsy cases and three-quarters of them do not get proper therapy.[4] The diagnosis of epilepsy can be seen from clinical manifestations and is supported by the neurophysiological features typical of electroencephalogram for epilepsy, namely the presence of epileptic waves. Physicians often have difficulty in establishing the diagnosis of this disease, moreover clinical manifestations are similar to seizures and EEG images do not always show waves that are typical of an epileptic wave. There are no biomarkers, especially non-invasive ones to help make the diagnosis of this disease. The development of diagnostic biomarkers in epilepsy has added insight into epileptogenesis, so that therapeutic targets and adequate therapies can be developed in the future.[1] This biomarker also prevents misdiagnosis and increases the prevention of epilepsy. To achieve the success of this vision, evidence of the underlying mechanism of action of epileptogenesis is needed.[5] Experimental studies and clinical imaging of epileptics show that seizures result in neuronal death. Neuronal cell death as a basis for epileptogenesis is still controversial.[1] The apoptotic pathway may play a role in the pathogenesis of epilepsy.[6] The intrinsic apoptosis pathway is a relevant pathway in neuronal death induced by seizures. Induction of cell death in this pathway originates from disorders in the cell, the damage to deoxyribonucleic acid (DNA), endoplasmic reticulum stress and excess calcium.[1] Pathogenesis of various types of epilepsy is related to many important biological pathways, some of which are regulated by microRibonucleic Acid (microRNA) or abbreviated as miRNA, is a small non-coding RNA of 22 nucleotides, which can regulate the stability of some messengerRNA (mRNA) by binding to its complementary sequence in the 3' untranslated region (UTR) of mRNA coding. miRNA works in a certain way to regulate the stability of mRNA and translation.[4,7] Currently, various miRNA-based studies are being developed as biomarkers that can help provide clearer definitions in clinical trials. miRNA is known as a potential biomarker in several diseases such as cancer and cardiovascular disease, while its role in epileptogenesis is being developed.[5] Various types of miRNA contribute to epileptogenesis. One miRNA circulates with a high level of sensitivity and specificity, namely miR-106b-5p which is detected in the serum of epileptic patients.[4] miRNA has a target on neuron cells. Increased expression of one of the miRNAs, namely miR-106b-5p in the early phase of epileptogenesis, shows a potential role in the induction of cell cycle inhibition and neuronal apoptosis. The role of miR-106b-5p is predicted to regulate cysteinyl aspartate-specific protease (caspase) 6 which induces brain neuronal cell death.[4]***

Keywords: miR-106b-5p, caspase 6, Epileptogenesis

1. Epilepsy

Epilepsy is a common brain disorder that affects about 1% of the population. Epilepsy is the second highest number of health problems affecting 50-70 million people worldwide. As many as 2.4 million people have been diagnosed with epilepsy every year. In developing countries there are as many as 80% of epilepsy cases and three-quarters of them do not get proper therapy.[4] As many as 60% of the sufferers are focal epilepsy and most seizures originate from the temporal lobe.[8] The cardinal clinical picture of this disease is predisposing to recurring episodes of seizures due to abnormal and excessive electrical neurons triggers that interfere with brain function.[1] Based on the International League Against Epilepsy (ILAE) 2017 classifies seizures and epilepsy based on seizure type, epilepsy type and epilepsy syndrome and divides seizures into focal and general onset.[9] There are around 40 different epilepsy syndromes. Seizures can cause a different spectrum of symptoms from auras and déjà vu feelings, changes in autonomic function, to loss of consciousness and seizure movements.[1] Temporal lobe epilepsy is the most common type of epilepsy in adults and is associated with progressive hippocampal sclerosis, decreased cognitive function and treatment-resistant seizures.[3]

The diagnosis of epilepsy is traditionally based on EEG findings, clinical neurological history and neuroimaging findings, while also requiring clinical experience in the context of patient. Along with establishing the diagnosis of epilepsy provides an opportunity for patients to gain treatment, in this case long-term treatment, and the physicians' duty is to prevent psychological and social problems experienced by sufferers. Physicians' difficulty in establishing the diagnosis of epilepsy triggers the development of research on diagnosis biomarkers. This biomarker also prevents misdiagnosis and increases the prevention of epilepsy.

Primary management of epilepsy by administering anti-epileptic drugs that work through mechanisms that influence excitatory and / or inhibitory neurotransmission.[1] Various types of interventions and antiepileptic drugs are not effective in 30% of patients. Most of the antiepileptic drugs target ion channels or receptors on neurons, such as gamma-aminobutyric acid (GABA) and glutamate receptors. Therefore, understanding of the mechanism behind the

disease is needed in an effort to identify the latest drug targets.[4]

2. Etiology

Most causes of epilepsy are unknown.[1] The epileptogenic process of most patients with temporal lobe epilepsy is triggered by brain damage caused by head trauma, stroke, tumor or status epilepticus. Brain damage is followed by a latent phase which is a time of extensive neurobiological changes. These changes include neuronal death, gliosis, axonal and dendritic plasticity, neurogenesis, inflammation, revascularization or vascular changes, changes in ion channel expression and molecular reorganization of cell membranes and extracellular matrix. This process is known as epileptogenesis. These changes cause recurrent spontaneous seizures called epilepsy.[1,8] A thorough discussion of the mechanisms that have implications for the pathogenesis of symptomatic epilepsy is still limited to literature review or theoretical review. There is an inherited form of epilepsy found in a small number of the population. This form is caused by gene mutations that encode ion channels, for example potassium or sodium channels. In addition to the inherited factors, it is thought that there is a combination of genetic and environmental factors which play a role in the pathogenesis of epilepsy in most sporadic epileptic populations.[1]

3. Neuronal Cell Death on Epilepsy

Cell death that is programmed or called apoptosis is a physiological process to eliminate unwanted cells during the development process and to maintain tissue homeostasis.[1,10] There are two apoptotic pathways namely the extrinsic and intrinsic pathways. The extrinsic pathway is initiated by cell death receptors on the cell surface of the tumor necrosis factor (TNF) family. Intrinsic pathway, which is a relevant pathway in neuronal death induced by seizures. Induction of cell death in this pathway comes from interference in cells, namely DNA damage, endoplasmic reticulum stress and excessive calcium.[1] The pathogenesis of epilepsy is thought to be related to changes in gene expression that regulate neurotransmitter signaling, ion channels, synaptic structures, neuronal cell death, gliosis and inflammation. Various defects in genes underlying the pathogenesis of epilepsy types have been successfully identified, where these genes are responsible for coding ion channel proteins. Familial epilepsy has a genetic and hereditary component. While the etiology of most non-familial epilepsy is no longer related to genetic mutations, although genetic linkages have identified several loci that are susceptible to epilepsy mutations. Analysis of genetic components in epilepsy can be identified using genome-wide analysis and single-point mutations can be identified through exome sequencing or whole-genome sequencing.[4]

Temporal lobe epilepsy is often associated with lesions called hippocampal sclerosis. This condition is characterized by loss of neuronal cells and brain atrophy, and is often asymmetric. The most frequently affected areas are cornu ammonis (CA) 1 and the hilar area of the dentate gear and CA3. Whereas CA2 and granular cells from the dentate gyrus are usually less likely to show neuronal cell loss. The growth of axons and the spread of neuron cells in the dentinal granular granular layer of the hippocampus are also seen in this condition.[1]

Neuronal cell death as the basis of epileptogenesis is still controversial. The primary cause of brain neuron cell death after a seizure may be due to excessive activation of the ion channel by glutamate, a major neurotransmitter in the brain. Neurons experience excessive sodium and calcium which causes edema, membrane rupture and cell lysis.[1] Experimental and clinical data suggest that neuronal cell death that occurs significantly after brain damage and the apoptotic pathway may play a role in addition to other mechanisms such as toxicity mediated by glutamic excitatory neurotransmitters.[6] In addition it also has energy failure, free radical production, activation of various proteases and DNA degradation. During the mid-19th century, research on cell death due to seizure induction succeeded in identifying gene-based mechanisms and programmed cell death.[1] There are two genes that play a role in apotosis namely the caspase family protein and B-cell lymphoma 2 (Bcl-2). In experimental models regarding epilepsy and epileptogenesis, researchers found that caspases 3 and 6 were actively expressed in the hippocampus. Whereas Bcl-2 family proteins such as Bax and Bcl-2 also contribute to the pathogenesis of temporal lobe epilepsy in humans. This suggests that the apoptotic pathway may play a role in the pathogenesis of epilepsy.[6] The statement also reinforces the hypothesis that apoptosis includes the processes involved in contributing to cell death in epilepsy.[1]

Experimental studies and clinical imaging of epileptic sufferers show seizures that result in neuronal death. Post mortem research in the 19th century on brain tissue of epilepsy patients showed loss of neuron cells especially in an area of the brain called the hippocampus. This has the consequence of recurrent seizures in people with epilepsy. Research conducted on experimental animals induced by epilepticus or repetitive seizure stimulation shows the death of brain neuron cells in the hippocampus and associated brain areas. Before the study of brain neuron cell death after epilepticus status focused on caspase and Bcl-2, a pioneer from the John Olney and Brian Meldrum Laboratory found that brain neuron cell death was caused by over-activation of glutamate ionotropic receptors. In the past ten years, researchers have begun to discover that the multi-cell process of cell death programs is directly related to genes in brain samples of pharmacoresistent epilepsy patients who often experience seizures.[1] Research on cell death that occurs after seizures helps identify molecular mechanisms of apoptosis so that treatment strategies can be found for protection against brain cell damage due to seizures and epileptogenesis.[2]

Research in humans who suffer from temporal lobe epilepsy and temporal lobe epilepsy models in kainic acid-induced animals shows both mechanisms of excitotoxicity induced by excessive activation of the N-methyl-D-aspartate receptor (NMDA) and apoptotic mechanisms including protein-activation caspase family proteins that contribute to hippocampal atrophy occur immediately after the induction of chronic epileptic seizures. This finding is reinforced by

the evidence that NMDA receptor antagonists can reduce hippocampal damage after epilepticus.[1] While research on the identification of caspase substrates that contribute to seizures inducing neuronal cell death is still not widely known.[3]

The role of miRNA-106b-5p in Epileptogenesis

The development of epilepsy after epileptogenic events is called epileptogenesis. Epileptogenesis is divided into three phases, namely the acute phase (during and immediately after epileptogenic events), the latent phase (asymptomatic) and the chronic phase where seizures without provocation occur. The latent phase can last for days or years, where molecular and structural changes cause the brain to produce spontaneous repetitive seizures. These changes include loss of neurons, inflammation and synaptic changes. It is still not fully understood whether the process is a cause or consequence of epileptogenesis. What's more, epileptogenensis does not end in spontaneous seizures, but continues during the chronic phase of worsening the condition over time. There is no available therapy to stop this process.

Epigenetic mechanisms that are not related to changes in the DNA sequence involved in epileptogenesis can occur during the process of transcription or post-transcription settings. The pathogenesis of various types of epilepsy is related to many important biological pathways, some of which are regulated by miRNA, are small non-coding RNAs of 22 nucleotides, which can regulate the stability of several mRNAs by binding to the complementary sequence at 3 'UTR of mRNA coding. miRNA works in a certain way to regulate the stability of mRNA and translation.[4,7] miRNA is reported to reduce the stability of mRNA and translation to prevent expression of several proteins, thus potentially becoming a regulatory mechanism and therapeutic target for epilepsy. The miRNA represents the control of gene expression in epilepsy, so that it can be a potential target as a biomarker and therapeutic strategy. Changes have been observed in some miRNAs, such as serum miRNA-4521 in the hippocampus of patients with temporal lobe epilepsy and nerve tissue in animal models of status epilepticus. Changed levels of expression of some miRNAs, including transcription factors and neurotransmitter signaling components, in rat blood after seizures. The difference in the quantity of miRNA in the blood shows its role as a diagnostic biomarker.[5] Previous studies have focused on regulating miRNA in the target gene and several studies have analyzed the effect of changing expression on a single miRNA, increasing or decreasing miRNA levels in animal models of mice, and observing the development of processes pathological from epilepsy. Recent epigenetic profiles are investigating the possible role of miRNA in the pathogenesis of epilepsy.[4] Correlations have been found between methylation status and miRNA expression in hippocampal samples in humans and animal models.[5]

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miRNA sequences are transcribed by RNA polymerase as a long primary transcript (pri-miRNA). The majority of miRNA is located in the intron region of the mRNA coding protein. As soon as the mRNA is connected, pri-miRNA appears as a long structure. miRNA undergoes further processing into pre-miRNA and mature miRNA to subsequently form duplex miRNA. During the process of maturation, miRNA loses part of its sequence because it has been cut off by Drosha in the nucleus and DICER complex in the cytoplasm. A strand of duplex miRNA, called a guide strand, is known and enters the RNA-induced silencing complex (RISC) to produce a silencing effect. Another strand, the passenger strand (called miRNA), is usually degraded. Once miRNA recognizes the complementary sequence in the target mRNA (called the seed sequence), it binds to it, causing degradation or repression of mRNA, depending on the level of pairing with the seed sequence. In mammals, 60% of mRNAs have seed sequences that are known to bind to miRNA. miRNAs have an important role in regulating all cellular processes. In the brain, miRNAs are highly abundant and participate in regulating neurogenesis. In Dicer mice animal models, miRNA biogenesis is inhibited, causing neuronal death and premature death.[4]

Epileptogenesis and temporal lobe epilepsy in mice can be modeled by inducing epilepticus status through proconvulsant injection, which activates neuronal transmission of excitation or reduces transmission of inhibition, such as pilocarpine (muscarinic acetaminch receptor agonist), clothic acid (glutamate receptor agonist), or activating neuronal transmission of excitation or reducing transmission of inhibition, such as pilocarpine (muscarinic receptor agonist receptors), clothic acid (glutamate receptor agonist), or pentylenetrazole (GABA)). This experimental animal model represents the acute, latent and chronic phases of epilepsy and has been used extensively to investigate the molecular and cellular mechanisms underlying epilepsy, especially those that developing today are the contribution of miRNA. The dysregulation of miRNA contributes to all three phases of epileptogenesis and provides hope for new discoveries for therapeutic targets in epilepsy.[11] Anti-epileptic drugs are found to be effective in less than half of patients, and specific antiepileptic drugs are not available. The current focus on miRNA targets is a new challenge in the pathogenesis, diagnosis and treatment of epilepsy and its transparency in clinical practice. Potential targets for miRNA-based therapy in epilepsy are being developed. As a primary target, miRNA has been found to be able to control excitability and suppress seizures in eilepsy and other conditions. This pathway changes in the brain tissue of epileptic patients, and experimental epileptogenic influences are found in experimental animal models.[5]

Several animal models of epilepsy are being developed in an effort to understand the onset and development of the pathology process. Research on epilepsy that is currently developing, evolving animal models with simple acute seizure manifestations by giving pentylenetetrazol or volatile chemoconvulsant flurothyl to induce seizures, or seizure models with maximal electric shocks, but these models fail to create spontaneous seizures that resemble epilepsy. Previous studies on epilepsy used several animal models, ranging from *Drosophila melanogaster*, *Caenorhabditis elegans* and *Danio rerio* to primates. Nowadays, researchers often use *Rattus norvegicus* and *Mus musculus* as experimental animal models of epilepsy. The use of these animals helps to develop a genetic epilepsy model. This model was developed starting from the observation of single gene mutations that can cause epileptic diseases, such as mutations of ion channels (voltage-gated sodium channels, potassium channels or calcium channels) and neurotransmitter receptors.[4]

Recently animal models of epilepsy that resemble temporal lobe epilepsy have been developed to examine the status epilepticus, a condition characterized by episodes of prolonged seizures that develop by brain damage as a precipitating factor, which affects the hippocampus and/or temporal lobes. This experimental animal model was developed to resemble temporal lobe epilepsy, which is the most common form of epilepsy which shows a clinical history including seizures, pre-epileptic latent period before the onset of spontaneous seizure manifestations, chronic clinical manifestations and histopathological changes typical of a temporal lobe epilepsy in human. Until now there has never been proposed research using experimental animals with non-focal epilepsy models, possibly due to the origin of this form of epilepsy can not be known completely. In making animal models of the status epilepticus in temporal lobe epilepsy, the primary model in acquired epilepsy is caused by intracerebral or systemic injection using drugs (such as pilocarpine, clothic acid, or bicuculine) or toxins (tetanus toxin) or resembling etiology due to febrile seizures using treatment (such as pilocarpine, clothic acid, or bicuculine) or toxins (tetanus toxin) or resembling etiology due to febrile seizures using treatment (such as pilocarpine, clothic acid, or bicuculine) or toxins (tetanus toxin) hyperthermia, continuous electrical stimulation, or ischemia. Hyperthermia is a good model to resemble temporal lobe epilepsy, whereas treatment using kainic acid (glutamate receptor agonist) or pilocarpine (muscarinic cholinergic receptor agonist) is a frequently used model. Both of these methods cause the same histopathological profile as observed in patients with mesial temporal lobe epilepsy.[4]

MiRNA research on body fluids in experimental animal models of epilepsy can be useful for identifying new, sensitive, specific, easily produced, predictive, and accurate biomarkers and for developing new, non-invasive, standardized, affordable, and easy and easy tests for shows the detection of testing of the differential diagnosis of temporal lobe epilepsy. Several single miRNAs demonstrate diagnostic ability. Various studies have looked at miRNA in the hippocampus, dentus dentatus, plasma, and blood and show a picture of increased or decreased expression of certain miRNA in the acute, latent or chronic phase after induction of epilepticus status. One interesting thing about miRNA is that it can be secreted and found in several human body fluids including tears, milk, serum, plasma, saliva and urine both in physiological and pathological conditions. This causes miRNA to be a promising molecule for diagnosis, prognosis or developing new therapies. Although the expression of miRNA levels in body fluids is not too large, some inspection techniques can carry out isolation, identification and amplification using (quantitative Polymerase Chain Reactio (qPCR) or next-generation sequencing (NGS) approaches at more affordable prices. MiRNA can be detected at blood serum. This causes miRNA to be used as a potential biomarker for establishing diagnosis, therapy, assessing risk and therapeutic response, stable miRNA in serum and miRNA examination in blood can be accessed easily, quickly, noninvasively and at the inexpensive price. A change in miRNA profile in body fluids is a potential biomarker that is very useful in epileptogenesis.[4]

Various types of miRNA have been identified that are expressed in epileptic conditions. One example is miR-146a which is increasing in mice with epilepsy and humans with temporal lobe epilepsy. Several types of miRNA have been investigated and have a functional role in epilepsy, mainly through the manipulation of single miRNA in mouse animal models that reduce neuronal cell death or seizure severity. The function of miRNA can be easily changed by changes in sense and antisense oligonucleotides (agomirs (a.k.a. mimics) and antagomirs). This causes changes in gene expression, so that candidates can be used as new therapeutic targets in epilepsy. Here are seven microRNAs with strong therapeutic potential based on evidence of dysregulation and their function in epilepsy.[11]

Several studies on epilepsy, showed changes in the profile of miRNA expression globally in various models of experimental animals and humans. Specific miRNA in brain tissue is associated with seizures that induce neuronal cell death or neuroprotection. It is known that there are changes in components of the miRNA biogenesis pathway in brain tissue of epilepsy patients resulting in changes in the regulatory system by miRNA in terms of neuron microstructures, cell death, inflammation and apoptosis. Several studies of the genome-wide miRNA expression-profiling over the past 5 years have identified more than 100 different miRNAs in patients with epilepsy or animal models, and produce interesting evidence that epilepsy is associated with extensive changes in miRNA expression. Research on miRNA in humans suffering from epilepsy was first published in 2010 and reported that there was an increase in miR-146a, a miRNA related to the regulation of the inflammatory response, in the hippocampus.[4]

Various types of miRNA contribute to epileptogenesis, including miR-146a, miR-134, miR-132, miR-128, miR-34a, miR-324-5p, miR-124, miR-155, miR-142-5p, miR-142 21-5p, miR-187, miR-106b-5p.[11] In epileptic conditions, this miRNA has increased expression. The expression of circulating miRNA shows dynamic changes in relation to the pathology process that occurs. Some miRNAs can be expressed immediately after the onset of a seizure, while others are expressed only after the disease has become

chronic.[4] In the past 5 years, several target studies and studies related to the genome miRNA expression identified 100 different miRNAs in epilepsy patients and animal models, and showed convincing evidence that epilepsy is associated with massive changes in miRNA expression. Although many miRNAs are reported to be related to epilepsy, whether deregulated miRNA is suitable for the prediction of epilepsy risk, diagnosis or outcome prediction, requires further verification. Recently, Wang conducted a study of selected miRNA, which was then validated in 117 epilepsy patients and 112 controls using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The sample used was the serum of epilepsy sufferers. It was found that Let-7d-5p, miR-106b-5p, miR-130a-3p and miR-146a-5p had increased expression, while miR-15a-5p and mIR-194-5p had decreased expression in epilepsy patients compared to controls ($p < 0.0001$). Among these miRNAs, serum miR-106b-5p has the best diagnostic value for epilepsy with a sensitivity of 80.3% and specificity of 81.2%.[12] Other studies have found 12 miRNAs have decreased expression and 3 were found to be increased in drugs-resistant patients to groups that are responsive to therapy. Several confirmed miRNAs based on qRT-PCR, including miR-194-5p, miR-301a-3p, miR-30b-5p, miR-342-5p, and miR-4446-3p were statistically disregulated in the drug-resistant group when compared with treatment-resistant and control groups. Of the 5 miRNAs, miR-301a-3p was reported as the best diagnostic biomarker for drug-resistant epilepsy because of its high sensitivity and specificity. It was noted that miR-301a-3p and miR-106b-5p were also reported to have decreased expression of the hippocampus tissue in patients with temporal lobe epilepsy.[13]

Colleagues verified the severity of epilepsy in deregulated miRNA, and assessed whether miRNA could be a useful clinical tool in predicting outcomes, and correlation analysis was performed. An found that miR-106b correlated closely with the National Hospital Seizure Severity Scale (NHS3) score with $r = 0.5896$, but serum miR-194-5p and serum miR-301a did not show significant results. There was no significant relationship between the four miRNAs and duration of illness and frequency of seizures ($p > 0.05$). These findings indicate that the expression levels of miR-106b and miR-146a are positively correlated with seizure severity. The combination of the two is said to have a much better sensitivity / specificity (AUC = 0.887, 95% confidence interval 0.788-0.927, $p < 0.001$), which is 14.6% higher than miR-146a and 12.8% higher than miR-106b only.[14]

Colleagues focused on 8 epilepsy-related miRNAs circulating in humans, and analyzed their role in cellular functions related to epilepsy. The first is neuroinflammation with microglia involvement, which is called the “immune system” in *in silico* analysis. This pathway includes several genes that are regulated by miR-15a-5p (decreased expression), miR-106b-5p, miR-146 and miR-451. There is an increased expression of miR-106b in epilepsy, said to also be involved in inflammation related to Alzheimer's disease. Simvastatin was proposed as a possible anti-inflammatory and anti-apoptotic agent in Alzheimer's disease, and recently statins have also been tested as potential anti-seizure drugs, due to their anti-inflammatory mechanisms and protective power. Second, neurogenesis, cell cycle control, and cell proliferation, called the “cell cycle” in *in silico* analysis. This pathway states that all of these genes are involved in the differentiation and proliferation of neuronal cells, which are controlled by miR-15a-5p, miR-34a, miR-106b-5p, and miR-146. In epilepsy mouse models induced by electrical stimulation, miR-106b-5p is reported to have increased expression in the initial phase, indicating the potential role of this miRNA in inducing neuronal cell cycle blockade and neuronal apoptosis. Third, validated targets of miR-15a-5p (decreased expression), miR-106b-5p, miR-146 and miR-45, are involved in the pathways involved in pro- and anti-apoptotic signaling.[4]

The challenge in miRNA research in relation to the diagnosis of epilepsy is that there are some inconsistencies in the results of circulating miRNA that can be identified and miRNA with unique characteristics related to epilepsy is not detected. The differences in the pathological processes in animal models (rats and mice) and humans cause differences in circulating miRNA profiles. In addition, the circulating miRNA profile is also influenced by the technique of making epileptic animal models (electrical stimulus, pilocarpine injection or kainic acid) and the differences in methods in miRNA analysis (NGS or RT-PCR). The difficulty in identifying major miRNAs in human body fluids (such as cerebrospinal fluid, serum and plasma) that play a role in the process of epileptic pathology also adds to the challenges for researchers.[4]

miRNA circulating in epilepsy affects cell function. Changes in physiological processes in temporal lobe epilepsy include neuroinflamation, neurogenesis and apoptosis. miR-106b-5p plays a role in the process of neuroinflamation by regulating several genes involving microglia. In addition, miR-106b-5p also plays a role in neurogenesis in cell cycle regulation and cell proliferation. In animal models of epilepsy mice induced by electrical stimulation, miR-106b-5p is reported to have increased expression in the initial phase and this shows a potential role in the induction of cell cycle inhibition and neuronal apoptosis. miR-106b-5p is predicted to regulate caspase 6 and MAPK-binding protein 1 (MAPKBP1).[4]

Increased Caspase 6 Expression Induces Brain Neuron Cell Death

Temporal lobe epilepsy in humans can be made in rat model by making epileptogenic processes with epilepticus status. Acute neurodegeneration can be detected within hours to 3 days after epilepticus status due to necrosis. More than a few years ago, caspase was identified as a candidate for a group of proteolytic enzymes that contribute to neurodegeneration after epileptogenic events, including status epilepticus, stroke and head trauma.[8]

Caspase belongs to the family of cysteine-aspartic proteases, which are the main mediators in apoptosis and inflammation.[15] During post-translation, caspase modifies its substrate through the process of breaking down at specific locations, which causes inactivation of the substrate or increases its function through the growth of active fragments. Caspase is categorized as an apoptosis initiator or an end-stage excecutionary.[17] Caspase consists of 14 types

and based on its function is divided into 3, namely caspase initiator (apoptotic initiators) namely caspase 2, 8, 9, 10), exclusives (apoptotic effectors) namely caspase 3, 6, 7 and caspases that participate in the process of cytokine formation (inflammatory caspases) namely caspase 1, 4, 5, 11, 12, 13, 14. All caspases are produced as catalytically inactive zymogens in cells and carry out proteolytic activation during apoptosis. Apoptotic initiators induce activation at the proximal, then the process and activation of apoptotic effectors occur. Apoptotic effectors are responsible for the breakdown process of cellular substrates thereby triggering cell death.[15] According to Mendez-Armenta caspase is divided into procaspase (2,6,7,8, and 9) and caspase (3,7,8, and 9).[10] Most studies examine the increased expression and activity of caspase 3 after epileptogenic events. However, inhibition of caspase 3 does not save all neurons and does not prevent epileptogenesis. Contrary to caspase 7 which is not activated by epilepticus status and does not induce neuronal and glia cell death. Expression and activation of caspase 6 has been reported after the occurrence of status epilepticus. Even transient caspase 6 expression can induce neuronal death.[8]

The intrinsic apotosis pathway, that is the mitochondrial dysfunction through cytochrome c release and caspase induction, is a sign of molecular cell death. This cytochrome c release was seen in the hippocampus of mice at 2 hours post epilepticus status using kainic acid. Another group showed the release of cytochrome c immediately after injection of fabric acid was given.[1]

The gene that codes for caspase 6 in humans is Mch2. Caspase 6 expression is very high in the brain (localized in the cytosol and nerve terminals) and peripheral tissue. In addition to playing a role in apoptosis, caspase 6 also participates in axon pruning during the development process, pathological axonal degeneration and neurodegeneration disease.[15] Caspase 6 changes in the brains of patients with temporal lobe epilepsy are found in both zymogenic and cleaved subunits forms. Caspase 6 expression in hippocampus of patients with intractable temporal lobe epilepsy examined using Western blotting obtained higher pro-form levels than cleaved subunits.[1] Guo and friends found the effect of caspase 1 activation on the induction of neuronal cell death in humans mediated by caspase 6. Caspase 6 can break caspase 2 and 8 so that it induces mitochondrial permeability and causes cytochrome c release and activation of the excitatory caspase.[16] Caspase 6 can also break caspase 3 either *in vitro* or *ex vivo*. Even caspase 6 activation has been discovered before observing the excitotoxic caspase 3 model in vivo and neurodegeneration brain tissue.[17] Caspase 3, 7, 8, 10 are able to activate caspase 6 in vitro.[8] Caspase 6 activation is caused by the absence of caspase 3 and caspase 6 brain tissue to perform autoactivation *in vitro* and *in vivo*.[15] Caspase 6 activity is inhibited by the caspase 8 inhibitor after the occurrence of epilepticus status in vivo. In neuronal culture, caspase 6 activates caspase 3. The known substrate for caspase 6 is lamin A, a protein structure in the cell nucleus (nuclear lamins). Caspase 6 also breaks down the cytoskeleton and structural proteins, proteins associated with cell adhesion. Aside from this, caspase is also suspected of having a non-lethal function, for example caspase 6 is thought to contribute to other functions relevant to epileptogenesis such as axon guidance systems, synaptic plasticity and migration of newly formed neurons. Caspase 6 plays a number of functions in epileptogenic tissue, which contributes to the reorganization and maturation of the epileptogenic network.[8] The status epilepticus results in cell damage caused by oxidative stress by involving calcium overload and induction of apoptosis. Differences in expression of several types of caspases such as caspase 2, 3, 6, 7, and 9 can be detected through immunohistochemical methods in brain samples of patients with temporal lobe epilepsy. Caspase appears to be localized to both the cell body and dendrites so that it supports the breakdown of intracellular structures or synaptic proteins. It has been reported that there is a change in gene expression in the caspase and procaspase families in the hippocampus with intractable temporal lobe epilepsy.[10]

During the process of apoptosis, caspase 6 is localized within the nucleus, breaking down the nucleus structural protein called the nuclear mitotic apparatus protein (NuMA), structural nucleus protein and lamin protein, and inducing contraction and fragmentation of the nucleus. In addition, caspase 6 breaks down many transcription factors, such as nuclear factor (NF)-κB, special AT-rich sequence-binding protein 1 (SATB1), activator protein 2α (AP-2α) and cAMP response element-binding (CREB)-binding protein (CBP), which all combine to break down nucleus substrates.[15] More than 60 proteins have been identified as caspase 6 substrates and / or casapse 6-interacting proteins. The breakdown of caspase 6 substrate causes neurodegeneration. Increased glutamate neurotransmitters and decreased glutamate transporter activity cause extrasynaptic NMDAR signaling and increased intracellular calcium levels, induction of p53, caspase 6 activation and the breakdown of caspase 6 substrates. Caspase 6 selectively breaks down CBP, where it is suspected that caspase 6 activation contributes to changes in transcription of genes which can be observed in neurodegenerative diseases. NF-κB is also broken down by caspase 6, which gives rise to an inactive p65 molecule during the transcription process which acts as an inhibitor of NF-κB and causes apoptosis.[17]

Research on caspase 6 was conducted by Narkilahti and Pichen in 2005 on the rats model which were induced with kainic acid which was divided into 5 treatment groups namely 8 hours, 24 hours, 48 hours, 1 week and 4 weeks after induction of status epilepticus compared to the control group injected with 0.9% saline solution. The activity and cleavage examination of caspase 6 was carried out with an enzyme assay of caspase 6 and Western blot, whereas neuronal damage was examined using caspase 6 immunohistochemistry. From this study it was found that caspase 6 expression in the hippocampus looked different between groups ($p < 0.005$, Kruskall-Wallis). Post-hoc analysis showed an increase in caspase 6 for 18 times in the 48-hour group after induction of status epilepticus. All hippocampus of experimental animals with epileptic status showed damage, especially in CA3a and CA1 pyramidal cell layers. The highest severity of damage was seen in the group 1 week after induction of epilepticus status. The duration and severity of epileptic status determine the presence of neurodegeneration.[8]

Henshall and colleagues reported that increased caspase 6 activity was seen in the hippocampus 24 hours after the induction of epilepticus status using intra-amygdala kainic acid injection and 48-72 hours later its activity decreased with the control group. In line with the results of this study, the increase in caspase 6 activity is temporary. Increased caspase 6 activity 48 h after induction of epilepticus status is induced by systemic kainic acid injection. The time difference in achieving the activity at its peak also depends on the model used. The in vivo neurodegeneration model reports that a temporary increase in caspase 6 activity can be detected in human neuron culture after a decrease in serum expression.[18] Caspase 6 transient activity in neurodegeneration induced by epilepticus status occurs in the initial phase of epileptogenesis.[8]

Caspase 6 is expressed in the hippocampus of the control group and its expression increases after status epilepticus. This shows that caspase 6 is mainly localized to neurons in the hippocampus. That expression is also detected in the dendrites. The results of research conducted by Narkilahti and Pitanen in 2005 showed that there was an expression of cleaved caspase 6 in the hippocampus of mice. The entire cell layer to the septotemporal axis of the hippocampus is stained by caspase 6. This increase in caspase 6 begins 24 hours after the status epilepticus and continues to increase for a period of 4 weeks. There is an intense increase in caspase 6 in some regions such as in septal CA3a indicating the presence of prominent neuronal cell death. In contrast, caspase 6 painting in the hilum appears the most intense at 24 and 48 hours and shows hilar cell death after epilepticus status, and then decreases.[8] Research conducted by Ferrer and colleagues in 2000 reported that caspase 6 expression appears in the hilum and The hippocampal CA1 24 hours after systemic kainic acid injection in female mice.[19] In line with the results of this study, Troy and colleagues also obtained results that revealed caspase 6 expression in the CA1 hippocampus 24 hours after induction of status using pilocarpine. From these results it can be concluded that caspase 6 expression from immunohistochemical data in regions of the hippocampus contributes to cell death that is acute after status epilepticus.[20]

In addition to neuron cells, caspase 6 is also localized in dendrites. Research conducted by Henshall et al. showed that there was cleaved caspase 6 at the dendritic end of the pyramidal cells in the hippocampus CA3a and CA1.[18] In a study conducted by Narkilahti and Pitanen in 2005 it was found that there was a time-dependent expression of caspase 6 in CA3a and CA1a dendritic end of pyramidal cells in septal CA3a and temporal CA1. In CA3a, caspase 6 expression was seen in the control group dendrites. At 24 hours there was an increase in expression in the area. At 48 hours and 1 week only the proximal dendrites remain labeled. Caspase 6 expression in the more distal branches reappears within 4 weeks. At temporal CA1, the expression of dendritic caspase 6 was most evident in the control group. Status epilepticus causes a decrease in caspase 6 expression in dendrites CA1 within 24 hours to 1 week. The dendritic expression of CA1 returned within 4 weeks. Caspase 6 expression in the supragranular layer and the inner molecular layer of the dentate gyrus occurs 24 hours after epilepticus status and is maintained for up to 1 week after status epilepticus. The results of the staining appear diffuse and are not localized in the intradendritic compartment. It is still a question whether caspase 6 had a role in the initial reorganization of the dendritic region proximal to the dentate gyrus after status epilepticus. This data shows that the expression of caspase 6 in the principal cell layers does not specifically appear in the cell body. From these results, it can be concluded that the expression of caspase 6 in cell bodies and dendrites depends on time differences. It is still questionable whether this is related to hippocampal damage that occurs slowly. The function of caspase 6 still needs to be further investigated.[8]

Caspase 6 appears first in the hippocampal dendrites and pyramidal cells that are sensitive to damage induced by the status epilepticus. In the later stages, caspase 6 moves to the cell bodies of these neurons. This shows that the cell death signal is obtained in the cell body and completes the process of cell death. Further research is still needed on the effect of caspase 6 inhibitors on neuronal cell death due to status epilepticus and subsequently improving functional outcomes after status epilepticus.[8]

Prominent caspase 6 expression in dendrites after epilepticus status events when neurobiological and neurodegeneration changes associated with epileptogenesis are in process. During epileptogenesis, axonal reorganization of the hippocampus occurs in the mossy fiber pathway. Mossy fiber terminates in the dendritic region of CA3 as well as the inner molecular layer of the dentus dentus that is rich in caspase 6. Previous research has shown that caspase 6 participates in post-synaptic signaling pathways through the β-amyloid precursor protein which is one of its substrates. At present, substrates of caspase 6 include cytoskeletal and structural proteins, like the protein associated with cell adhesion. The contribution of delayed caspase 6 expression in the dendritic region of CA3 and dentate gyrus to the reorganization of Mossy Fiber Pathway is still remaining as a hypothesis.[8]

4. Conclusion

The pathogenesis of various types of epilepsy is related to many important biological pathways. The apoptotic pathway played by miR-106b-5p is likely to play a role in the pathogenesis of epilepsy. miR-106b-5p induces brain neuron cell death through its regulation of caspase 6. Caspase 6 plays an important role in the process of axonal degeneration (neurodegeneration) and contributes to neuronal cell death in epileptic conditions by breaking down the nucleus structural proteins.

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