The Pathway in Acute Ischemic Stroke: A Review

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Abstract: Ischemic stroke is characterized by sudden loss of blood flow to specific regions of the brain and is a major cause of permanent disability and one of the leading causes of death worldwide. Sprouty-related EVH1 structural domain containing protein 1 (Spred1) is a recognized physiological inhibitor of the Ras/Raf-1/ERK pathway, and it has been shown that inhibition of Spred1 gene expression in ischemic mice contributes to cerebral angiogenesis and neurogenesis and further improves behavioral recovery through activation of the threonine kinase (AKT) and extracellular signal-related kinase (ERK) signaling pathways. We discuss the role of Spred1/ERK/VEGF signaling pathway activation in acute ischemic stroke, providing a positive panorama and suggesting that Spred1, vascular endothelial growth factor (VEGF) targets may be a promising pharmacological strategy for the treatment of ischemic stroke patients in the near future.

1. Introduction

Stroke is the main clinical type of cerebrovascular disease, which is a group of cerebrovascular diseases caused by organic brain injury, including ischemic stroke and hemorrhagic stroke, of which ischemic stroke accounts for a large proportion, and a study reported that ischemic stroke accounted for 81.9% of stroke inpatients in China in 2018 [1]. Characterized by high morbidity, mortality, disability and recurrence, it seriously endangers human life and health, and its makes stroke a global public health problem [2]. Globally, one in six people will have a stroke in their lifetime, and about 14 million people will have a stroke each year. Current treatments for the acute phase of ischemic stroke are effective, but the only approved pharmacological treatment for acute ischemic stroke (recombinant tissue fibrinogen activator intravenous thrombolysis) has significant clinical limitations - strict time window restrictions; anticoagulant and antiplatelet drug use may lead to bleeding risks [3], therefore Finding new targets for the treatment of acute ischemic stroke is of great interest. In this review, we summarize studies on the protective role of the Spred1/ERK/VEGF signaling pathway in acute ischemic stroke, highlighting the molecular events caused by ischemic stroke that are simultaneously regulated by Spred1/ERK/VEGF.

2. Study of the pathophysiological mechanism in the acute phase of ischemic stroke

The pathological process of ischemic stroke involves a complex temporal and spatial cascade of responses. There are many hypotheses about the pathogenesis, and according to current studies,

several influential theories are mainly as follows:

(1) Excitatory amino acid (EAA) damage theory: EAA is a substance that transmits excitatory information in the central nervous system and is also a neurotoxin. With a sustained decrease in cerebral blood flow and insufficient energy supply to brain tissue, EAA is released in large amounts from presynaptic nerve endings, and Osuga et al [4] confirmed that the concentration of EAA release is positively correlated with the duration of ischemia.

(2) Inflammatory response theory: The process of inflammatory response after cerebral ischemia can be broadly summarized as follows: ischemically damaged brain tissue cells will rapidly produce reactive oxygen species (ROS), pro-inflammatory cytokines and chemokines and release them extracellularly. These mediators will activate brain endothelial cells and induce the expression of adhesion molecules on the brain endothelium, which in turn will promote circulating leukocytes to cross the blood-brain barrier and finally enter the brain parenchyma. In turn, infiltrating leukocytes release a variety of pro-inflammatory mediators, including cytokines, chemokines, ROS, matrix metalloproteinases (MMPs) and other proteases, which further increase the inflammatory response in the brain and cause more extensive activation of brain-resident cells and infiltration of blood leukocytes, ultimately leading to blood-brain barrier (BBB) disruption, brain edema, neuroapoptosis and hemorrhagic transformation. In recent years, the role of microglia (MG) and astrocytes (Ast) in the inflammatory response has received increasing attention: 1). MG-mediated neuroinflammation plays a key role in the occurrence and development of ischemic stroke and has become an important target for the treatment of stroke. MG are immune cells in the nervous system, and after cerebral ischemia, MG can be activated within minutes after brain tissue injury and rapidly move to the After cerebral ischemia, MG is activated within minutes after brain tissue injury and rapidly migrates, proliferates, differentiates and produces both pro-inflammatory and anti-inflammatory effects at the site of injury [5]. In terms of pro-inflammation, MG ultimately promotes neuroapoptosis by producing a variety of neurotoxic factors, such as pro-inflammatory cytokines, chemokines, free radicals and other neurotoxic substances; in terms of anti-inflammation, the neuroprotective effects of MG are associated with an increase in neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF).2).Ast is also involved in Ast is the most widely distributed and abundant cell in the central nervous system and is an important cell that constitutes the neurovascular unit (NVU) in the post-ischemic inflammatory response of the brain. On the one hand, Ast releases pro-inflammatory factors such as TNF-α and IL-1 after proliferation, and forms glial restriction during the recovery period of cerebral ischemia, which damages neuronal connections with Ast and aggravates ischemic lesions; on the other hand, Ast can protect neurons by releasing a variety of anti-inflammatory factors such as TGF-β and IL-10, and forming glial scar during the subacute phase of cerebral ischemia.

(3) Apoptosis theory: as an important form of delayed neuronal death after cerebral ischemia, apoptosis is involved in ischemic cell damage, coexisting with neuronal cell necrosis, and has important pathophysiological significance in ischemic-hypoxic brain injury [6].

(4) Signal transduction pathway system theory: More and more studies show that hormones, cytokines, etc. all play a role through signal transduction pathway system, so the molecular mechanism of signal pathway in the pathogenesis and progression of ischemic stroke has become a hot spot, and the main signal pathways that have been studied in depth are: 1). MAPKs signaling pathway: The mitogen-activated protein kinase (MAPK) family regulates cell growth, differentiation, proliferation, apoptosis, inflammation and stress response to the external environment, and other important cellular physiopathological processes. 3), p38-MAPK and ERK5 [7]. JNK and p38 have similar functions and are associated with inflammation, apoptosis, and growth; ERK mainly governs cell growth and differentiation. There are many studies confirming

the important role of MAPK signaling pathway in the pathology of ischemic stroke, for example, a study showed that cerebral ischemia induces the expression of TNF-α and its receptors in the cerebral arterial wall and that this change is transcriptionally regulated through the MEK/ERK pathway [8]. Maddahi et al [9] demonstrated that after focal ischemia in rats TNF-α, IL-1β, IL-6 and iNOS microvascular expression were elevated and that these alterations were transcriptionally regulated by the MEK/ERK pathway. Wang Zhuo et al [10] found that p38MAPK activation is the main cause of Ast injury and consequently glial scar formation. 2). PI3K/Akt signaling pathway: the phosphatidylinositol trikinase/serine/threonine kinase Akt (PI3K/AKT) protein family is involved in a variety of cellular activities such as cell proliferation, tumors, and neurological diseases [11].The mechanisms of PI3K/Akt signaling pathway in ameliorating cerebral ischemic injury are mainly in four aspects: the PI3K/Akt signaling pathway can control apoptosis by regulating mTOR and Bcl-2 expression, control the synthesis of apoptotic and pro-apoptotic proteins, and then inhibit apoptosis; activation of VEGF-mediated PI3K/Akt signaling pathway can promote cerebral vascular neovascularization; nerve growth factor and tyrosine kinase binding activates PI3K/Akt signaling pathway and inhibits neuronal damage, which leads to increased release of neurotrophic factors; ischemic injury can lead to excessive activation of autophagy The activation of PI3K/Akt signaling pathway can inhibit the level of over-activated autophagy and reduce cellular damage. 3). Secretase/Notch signaling pathway: Secretase is a class of enzymes that cleaves the extracellular region of membrane proteins, and the Notch signaling pathway is widely present in vertebrates and nonvertebrates. Notch signaling pathway is an important pathway that communicates between adjacent cells and regulates cell development, mediating the exchange of information between brain cells after ischemic stroke [12]. The Notch signaling pathway affects neural differentiation and regeneration in stroke by regulating several downstream proteins, such as by increasing the expression of Notch1 and Jagged1 proteins and genes to promote the proliferation and differentiation of neural stem cells after ischemia.4). BDNF/TrkB signaling pathway: brain-derived neurotrophic factor (BDNF) is the second neurotrophic factor discovered after nerve growth factor (NGF). After the occurrence of ischemic stroke, brain ischemia and hypoxia lead to changes in cell membrane permeability and increased intracellular BDNF expression, which in turn protects neuronal cells and reduces the degree of injury, and the BNDF-TrkB pathway can also promote neurological recovery after cerebral infarction and improve functional deficits after stroke [13].

3. SPRED1/ERK/VEGF signaling pathway and ischemic stroke

3.1. Review of SPRED1/ERK/VEGF signaling pathway

MAPK, the mitogen-activated protein kinase, has a signaling pathway that transduces extracellular signals into cells and conducts cellular signals through a three-level kinase cascade (MAPKKK→MAPKK→MAPK), thereby regulating biological functions such as cell proliferation, differentiation, apoptosis, inflammatory responses, and vascular development. There are different subfamilies of MAPK, of which, for Ras-Raf- MEK-ERK is the most intensively studied, and this pathway is associated with several neurodegenerative diseases [14]. In the ERK signaling pathway, the GTPase-active Ras protein is activated by the upstream receptor tyrosine kinase (RTK), and the activated Ras activates Raf by binding to the N-terminal structural domain of Raf, which can further activate MEK by binding to the downstream mitogen-activated protein (MEK), which in turn activates the downstream sole The activated MEK then activates the downstream sole substrate, extracellular regulatory protein kinase (ERK), and finally the activated ERK enters the nucleus and causes a series of physiological and biochemical reactions, as shown in Figure 1.
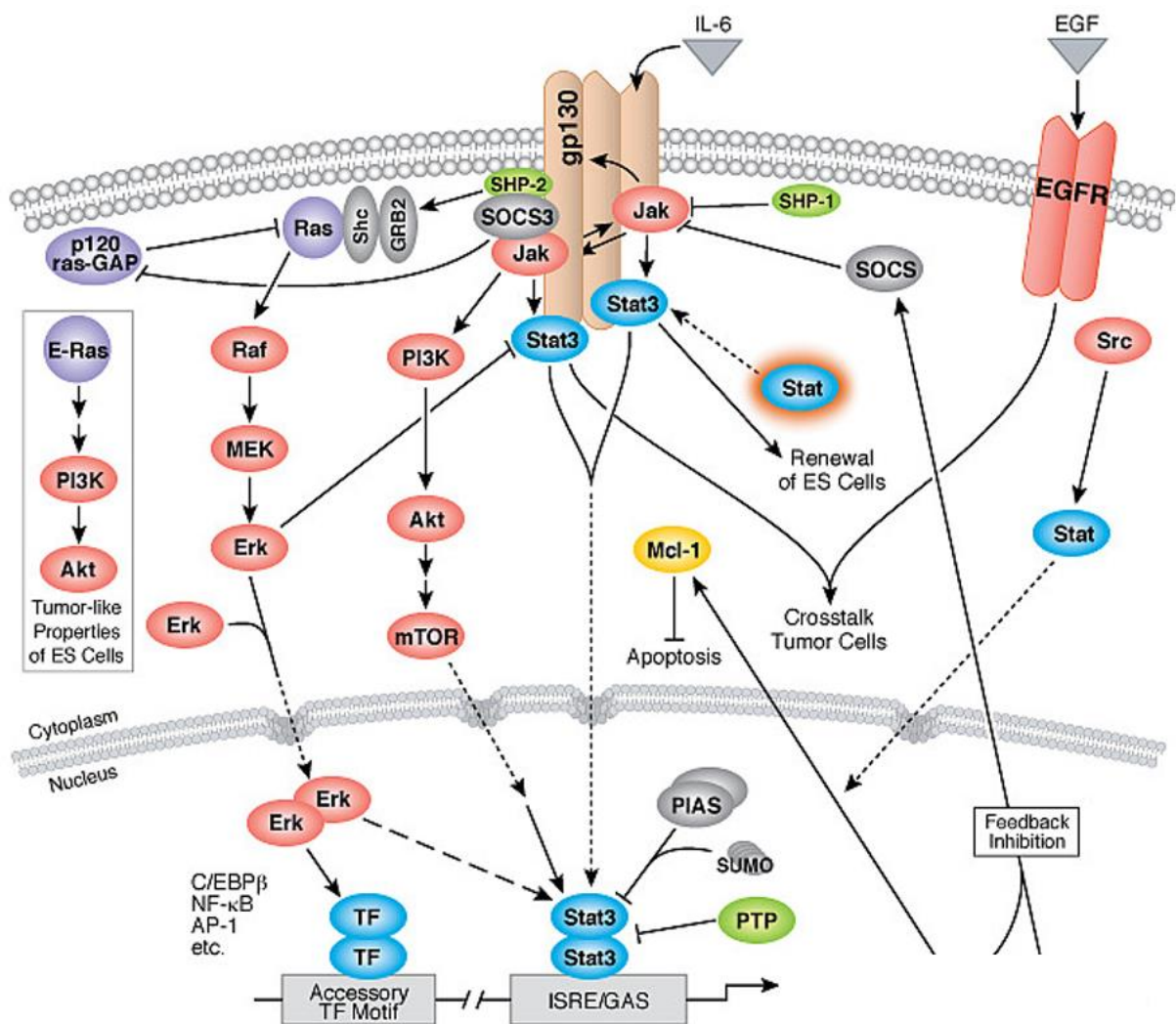
Figure 1 Overview of the Ras-Raf-MEK-ERK pathway

In the ERK signaling pathway, Ras protein with GTPase activity is activated by the upstream receptor tyrosine kinase (RTK), etc. The activated Ras activates Raf by binding to the N-terminal domain of Raf, and the activated Raf can further bind to the downstream mitogen-activated protein (MEK) to activate MEK, and the activated MEK further activates the downstream only The activated MEK then activates the downstream sole substrate, extracellular regulatory protein kinase (ERK), and finally the activated ERK enters the nucleus and causes a series of physiological and biochemical reactions.

Previous studies have shown that vascular endothelial growth factor (VEGF) can activate the intracellular Ras/Raf-1/ERK signaling pathway [15], and at the same time, this pathway can stimulate the transcription of genes involved in angiogenesis, thus promoting angiogenesis.The Ras/Raf-1/ERK signaling pathway can enhance the transcription of VEGF by activating transcription factors, hypoxia-inducible factor-1 and transcriptional activator proteins, etc. transcription and translation of VEGF and improve the stability of VEGF mRNA [16]. It is well known that VEGF is the most specific and pro-vascular building regulatory growth factor. Many modern scholars have found that VEGF has certain cerebral protective effects during ischemic brain injury [17], which can promote the proliferation and migration of vascular endothelial cells, induce neoangiogenesis, and protect neurons by directly or indirectly inhibiting neuronal apoptosis [18]. After the occurrence of cerebral ischemia, VEGF/VEGFR activation, endothelial progenitor cell

mobilization and proliferation are accelerated, which can promote the formation of neovascularization in the semidark zone [19].Yano et al [20] demonstrated that continuous low-dose release of VEGF has neuroprotective and pro-angiogenic effects.

MicroRNAs (microRNAs) are a class of evolutionarily highly conserved, endogenous single-stranded noncoding RNAs consisting of 18-25 nucleotides. miR-126 was first identified in vascular endothelial cells and is expressed relatively specifically in endothelial cells, and Sprouty-related EVH1 structural domain containing protein 1 (Spred1) is a downstream gene of miR-126. Engelhardt et al [21] found that in adult mouse tissues, Spred1 mRNA was most strongly expressed in the brain and Spred1 protein was most strongly expressed in whole brain and cerebellar homogenates; in human tissues, Spred1 was mainly expressed in adult brain and fetal tissues, and the results of this study suggest that Spred1 may be a key target in neurological-related diseases.

3.2. Role of Spred1/ERK/VEGF signaling pathway in ischemic stroke

Spred1 is a recognized physiological inhibitor of the Ras/Raf-1/ERK pathway [22, 23], and in recent years it has been found that Spred1 inhibits endothelial cell proliferation and migration by suppressing growth factor-induced ERK activation [24]. shusheng Wang et al [25] investigated miR-126 to control vascular integrity and angiogenesis by mechanism, they demonstrated the role of the SPRED1/ERK/VEGF pathway in angiogenesis: the binding of VEGF and fibroblast growth factor (FGF) to their receptors on endothelial cells activates the Ras-MAPK-ERK signaling pathway, which stimulates the transcription of genes involved in angiogenesis; and they, by further measuring the expression of Spred1 protein demonstrated that Spred1 is a negative regulator of the Ras-MAPK-ERK signaling pathway, which attenuates MAPK responses to VEGF and FGF, as shown in Figure 2.
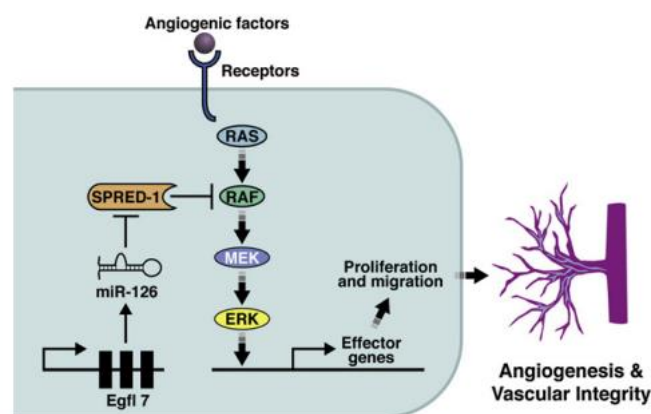

Figure 2 Role of SPRED1/ERK/VEGF pathway in angiogenesis

Although Spred1 is strongly expressed in brain tissue, its mechanism of action is more commonly seen in studies of cardiovascular-related diseases, for example, DA Silva et al [26] demonstrated that miR-126-3p promotes cardiac angiogenesis by directly downregulating Spred1 and indirectly regulating the VEGF pathway. In recent years, Spred1 started to receive attention in studies related to ischemic stroke, and it was demonstrated that miR-126 overexpression suppresses Spred1 gene expression, which contributes to cerebral angiogenesis and neurogenesis in ischemic mice and further improves behavioral recovery by activating AKT and ERK signaling pathways [27].

In conclusion, the literature reviewed herein clearly shows the role of SPRED1/ERK/VEGF activation in the acute phase of ischemic stroke in angiogenesis and cerebral protection in ischemic

stroke, providing a positive panorama and suggesting that Spred1, VEGF targets may be promising pharmacological strategies for the treatment of patients with ischemic stroke in the near future.

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