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Research Article

The role of CITED2 in angiogenesis and neuroprotection after ischemic stroke

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ABSTRACT

CBP/P300-Interacting Transactivation with Glu/Asp-rich C-terminal domain 2（CITED2), a transcriptional co-activator, severs critical roles in cell development and metabolism. Currently, CITED2 involves in fundamental cell processes *via* interaction with several transcription factors or cofactors, including Hypoxia-Inducible Factor 1α (HIF-1α), Transcription Factor AP 2 (TFAP2), Pitx2c, ISL1, WT1, Oct4, Smad 2/3, Peroxisome Proliferator-Activated Receptor (PPAR), Ets-1, and E2F. The latest studies have reported that CITED2 appears to have negative regulation of angiogenesis, promotive neuronal apoptosis, and inhibiting inflammation. This mini-review aims to summarize the molecular mechanisms of CITED2 and how CITED2 plays a role in angiogenesis and neuroprotection after ischemic stroke, which may provide innovative therapeutic strategies.

Keywords: Ischemic stroke, Angiogenesis, Neuroprotection, CITED2

INTRODUCTION

Ischemic stroke is the result of cerebral artery occlusion and causes deficits in brain neural function, accounting for 80% of strokes and causing a significant economic burden to both society and patients [1,2]. Currently, recombinant Tissue Plasminogen Activator (rtPA) and endovascular intervention are the Food and Drug Administration (FDA)-approved treatments for ischemic stroke. However, this therapeutic application is restricted due to the limited time window [3]. Thus, there is an urgent need to search for a novel and promising approach. Effective therapies after ischemic stroke require protection or recovery of the neurovascular unit instead of neurons or vascular recanalization. The neurovascular unit is a conceptual anatomical framework comprising neurons, glial cells, and micro vessels [4]. Improving the neurovascular unit can increase the possibility of a good prognosis in ischemic stroke.

CITED2, also called MRG1/P35SRJ/MSG1, is a member of the CITED family, which also includes CITED1, CITED3, and CITED4. [5, 6] CITED2 is a nuclear protein widely expressed in mammalian cells and plays a significant role in the development and growth of cells [5-7]. Presently, most of the research has focused on the role of CITED2 in tumors, congenital heart disease, inflammation, and stem cells. As the role of CITED2 in ischemic stroke has not been extensively studied, reviewing the function of CITED2 in ischemic stroke may provide directions for future studies. In this mini-review, we discuss the molecular mechanisms of CITED2 and the role of CITED2 in angiogenesis and neuroprotection after ischemic stroke.

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MATERIALS AND METHODS

Molecular mechanisms of CITED2

CITED2 is a transcriptional co-regulator without a DNA binding domain, that can regulate the functions of several transcription factors, including TFAP2, PPAR, HIF-1α, Smad2/3, and modulates their target gene expression [8-11]. CITED2 function varies based on the interaction with different transcription factors. We briefly summarized the molecular mechanisms of CITED2 in this section (Table 1). CITED2 was originally found to bind with CBP/P300 instead of HIF-1α and inhibited the HIF-1α pathway. The downstream target of HIF-1α was found to be associated with angiogenesis and energy metabolism [12]. CITED2 activates TFAP2 mediated transcription which is essential for normal neural tube and cardiac development [13]. During cardiac development, CITED2 regulates the cardiac left-right patterning *via* the left-right patterning Nodal-Pitx2c pathway [14]. Through recruited to the ISL1 promoter, CITED2 enhanced Embryonic Stem Cell (ESC) cardiac differentiation [15]. Genetic evidence indicates that CITED2 is expressed in the developing adrenal and cooperates with WT1 to stimulate the transcription of Sf1, which shows that CITED2 participates in sex determination and early gonad development [16,17]. Notably, during early differentiation of ESC, CITED2 directly is recruited to Oct4 to regulate ESC pluripotency and differentiation [18]. In TGF β pathway, CITED2 binds with SMAD2/3 complex to upregulate the target gene expression, including Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinase 9 (MMP-9) [19]. CITED2 is also involved in cell metabolism *via* PPARα and PPARγ transcriptional activation [20,21]. CITED2 is considered to play a protective role in cartilage tissue by down-regulation of the Ets-1-MMP pathway [22]. A previous study demonstrated that CITED2 is involved in the E2F-mediated G1/S transition during the cell cycle [22].

Table 1. Transcription factors interacting with CITED2.

Transcription factor (s)	Period or sites	Function	(Refs.)
HIF-1α	Hypoxia	Binds with CBP/P300 instead of HIF-1α to inhibit HIF-1α downstream targets expression	[10, 12]
TFAP2	Embryo development	Normal neural tube and cardiac development	[8, 13]
Pitx2c	Embryo development	Regulates the cardiac left-right patterning *via* Nodal- Pitx2c pathway	[13, 14]
ISL1	Embryo development	Enhances ESC cardiac differentiation	[15]
WT1	Embryo development	Regulates sex determination and early gonad development	[16, 17]
4-Oct	Embryo development	Regulates ESC pluripotency and differentiation	[18]
Smad 2/3	Tumor growth	Regulates the tumor growth by up regulating TGF-β downstream targets such as MMP9, VEGF	[11, 19]
PPARα, PPARγ	cell metabolism	Regulates cell metabolism	[9, 20, 21]
Ets-1	cartilage tissue	Protects cartilage tissue by down-regulation of the Ets-1-MMP pathway	[22]
E2F	cell cycle	Involves in the G1/S transition	[23]

TFAP2: Transcription Factor AP 2; Pitx2c: Paired like Homeodomain 2C; ISL1: ISL LIM Homeobox 1; ESC: Embryonic Stem Cell; WT1: Wilms Tumor 1 Transcription Factor; Oct4: Octamer-Binding Transcription Factor 4; Smad2/3: Small Mothers Against Decapentaplegic Homolog 2/3; TGF β: Transforming Growth Factor β; MMP9: Matrix Metalloproteinase 9; VEGF: Vascular Endothelial Growth Factor; PPARα: Peroxisome Proliferator-Activated Receptor α; PPARγ: Peroxisome Proliferator-Activated Receptor γ; Ets-1: Avian Erythroblastosis Virus E26 (V Ets) Oncogene Homolog 1; MMP: Matrix Metalloproteinase; E2F: E2 Promoter Binding Factor.

CITED2 and angiogenesis

Angiogenesis is the formation of new blood vessels that branch off from pre-existing blood vessels [23-25]. Angiogenesis is essential for brain repair after ischemic stroke as it can increase blood flow and metabolic nutrients in the damaged brain. We speculate that CITED2 may be involved in HIF-1α mediated VEGF angiogenesis pathway. It is generally believed that HIF-1α mediated VEGF angiogenesis pathway is activated in the acute stage after ischemic stroke. However, this self-protection pathway loses its function in the recovery period after ischemic stroke [26,27]. Under hypoxic conditions, the HIF-1α can translocate into the nucleus due to the inaction of oxygen-

dependent HIF Propyl Hydroxylase (PHD). In the nucleus, HIF-1α forms a heterodimer complex with HIF-1β and binds to CBP/P300, which can induce VEGF gene transcription by binding to the Hypoxia Response Element (HRE) in the VEGF promoter region [28,29]. Due to CITED2 being implicated in the modulating HIF-1α activity we speculate CITED2 may compete with HIF-1α that has translocate into the nucleus for binding to CBP/P300 in ischemic stroke. The CITED2-CBP/P300 complex inhibits the expression of VEGF gene encoding protein that can attenuate angiogenesis (Figure 1).

Thus, we speculate that CITED2 exhibits a weaker negative regulation against angiogenesis in the acute stage after ischemic stroke, which is consistent with the angiogenesis mechanism in cerebral ischemia. During acute ischemic stroke, CITED2 expression may correlate with angiogenesis. Moreover, the degree of angiogenesis determines the establishment of collateral circulation, which means that CITED2 may have an association with the establishment of collateral circulation in the brain. Collateral circulation can improve perfusion and metabolism in the ischemic sites, and is beneficial for the recovery of neurological function. CITED2 may be a novel biomarker for collateral circulation to predict stroke prognosis. However, there is no direct evidence that the CITED2 negatively regulates the VEGF angiogenesis pathway in the acute stage during ischemic stroke. This mechanism still needs to be further verified. Overall, CITED2 may negatively regulate angiogenesis in ischemic stroke and may be a novel biomarker for collateral circulation, but this mechanism still needs to be verified.

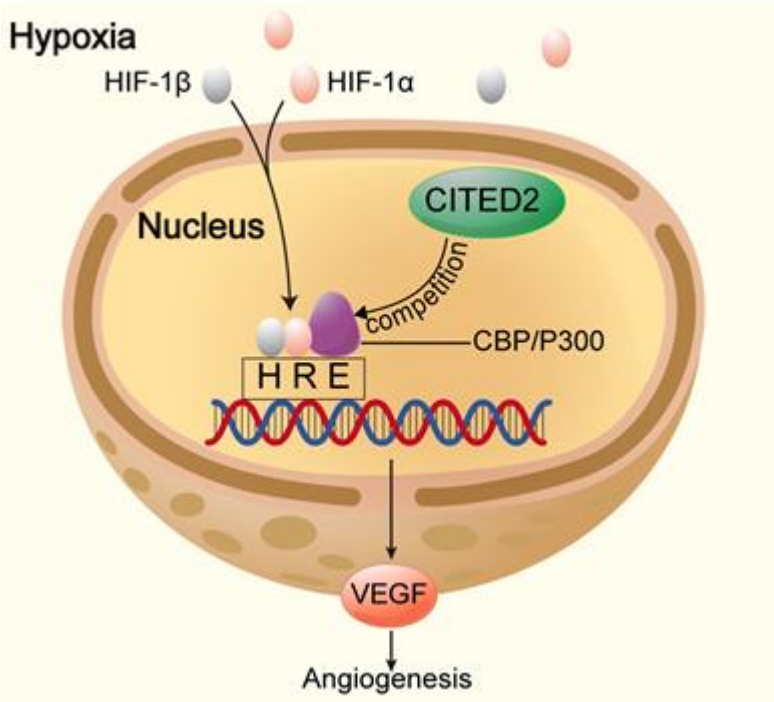

Figure 1. Regulation of CITED2 in angiogenesis. HIF-1α is stable in the hypoxia reaction and forms HIF with HIF-1β, which enters the nucleus. CITED2 competes with HIF-1α for binding to CBP/P300 to form a transcription complex. The transcriptional complexes combine with HRE sequences and regulate angiogenesis by inhibiting VEGF transcription.

CITED2 and neuroprotection

Ischemic brain damage can occur in a series of pathological changes in brain tissues such as excitotoxicity, reactive oxygen species, cellular apoptosis, and inflammation, which leads to neuroprotection becoming one of the therapeutic goals [30,31]. CITED2 plays a role in neuroprotection mainly by cellular apoptosis and inflammation.

RESULTS AND DISCUSSION

It was recently reported that CITED2 is a pro-apoptosis signal of stroke-induced cell death *via* activation of cycling-dependent kinase 4 (CDK4) mediated E2F transcription factor pathway. First, CDK4 is essential for delayed neuronal death and activated in neurons after cerebral ischemia. Then, CDK4 mediated the phosphorylated retinoblastoma protein (Rb) to activate E2F factors transcription. However, the role of each E2F family member is not clear. Currently, E2F1 is induced and activates CITED2 transcription after ischemia, which promotes neuronal death. In contrast, E2F4 is protective after cerebral ischemia by inhibiting CITED2 transcription [32-34].

Besides cellular apoptosis, CITED2 also participates in inflammation. Inflammation is initiated by vessel occlusion, and pro-inflammatory cytokines are released from intravascular leukocytes, ischemic endothelium, and

brain parenchyma. The existence of anti-inflammatory cytokines restricts pro-inflammatory pathways that prevent excessive inflammatory damage [35]. In inflammation, CITED2 plays an anti-inflammatory role. Following ischemia, microglia, the resident macrophages in the brain, and astrocytes are immediately activated. Then activated cells secrete cytokines and chemokine's, which induced the migration of intravascular inflammatory cells to ischemic brain tissue [36]. CITED2, a negative regulatory factor of macrophage pro-inflammatory activation, is mainly expressed in macrophages and alleviates inflammatory insults. During inflammation, CITED2 cooperates with PPARγ to promote anti-inflammatory gene expression. Simultaneously, HIF-1α acts as a pro-inflammatory cytokine and is inhibited by CITED2 to protect brain tissue from further inflammatory insults. However, CITED2 expression is low, when macrophages are exposed to pro-inflammatory cytokines, which points to a weak anti-inflammatory role of CITED2 in the early stages.

Taken together, this seems to be a contradiction that CITED2 is associated with neuronal apoptosis and inhibiting inflammation. For this, we hypothesized that: the level of CITED2 expression exists in homeostasis in neuroprotection (Figure 2). It is generally believed that cell death in the penumbra is mainly apoptosis and develops within several hours or days. When activated CITED2 is abundantly expressed and plays a pro-apoptosis role, and a significant number of neurons are induced apoptosis. It is well known that brain injury initiates a cascade of inflammatory responses to further aggravate ischemic brain injury. By this time, abundant CITED2 exhibits anti-inflammatory effects and restricts inflammation aggravation, which plays a neuroprotective role. When the brain restores homeostasis, the level of CITED2 expression may decrease. Thus, CITED2 may be a pro-apoptosis role in the early stage and an anti-inflammatory role during the late period. This hypothesis remains to be fully validated.

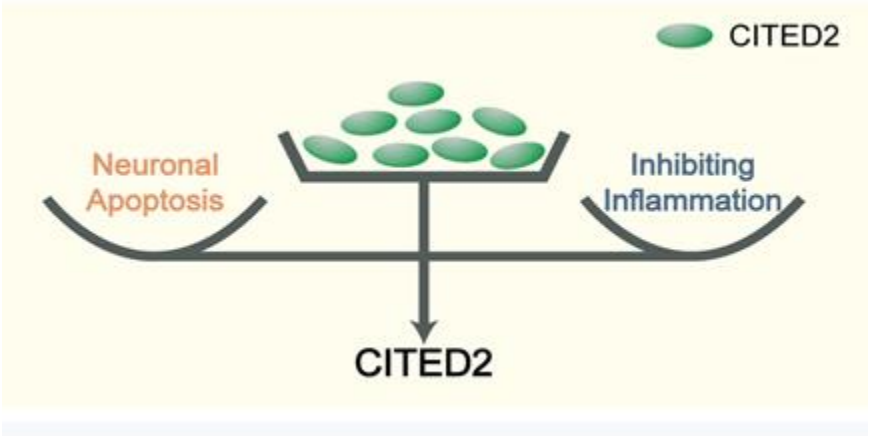

Figure 2. Role of CITED2 in homeostasis. The level of CITED2 expression exists in homeostasis in neuroprotection, abrogation of CITED2 homeostasis may lead to neuronal apoptosis or inhibiting inflammation.

CONCLUSION

Several published studies have described that CITED2 plays different roles in ischemic stroke, including negative regulation of angiogenesis, promotive neuronal apoptosis, and inhibiting inflammation. Among these, promotive neuronal apoptosis and inhibiting inflammation seem to be a contradiction of CITED2 roles. Based on the CITED2 mechanism, we hypothesized that CITED2 may be a pro-apoptosis role in the early stage and an anti-inflammatory role during the late period. This hypothesis still needs to be verified and may provide a novel direction for the treatment, which also is our subject of future study. CITED2 may be a novel therapeutic target in promoting neurovascular unit by angiogenesis and neuroprotection. Thus, intervening CITED2 expression may be more beneficial for functional recovery of ischemic stroke. Moreover, CITED2 may be a novel biomarker to predict stroke prognosis. A better understanding of CITED2 mechanisms can guide the development of innovative treatment.

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Not applicable.

CONFLICT OF INTEREST

The authors declare the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Hu X, Silva TMD, Chen J, Faraci FM (2017). Cerebral Vascular Disease and Neurovascular Injury in Ischemic Stroke. Circ Res. 120: 449-471.

2. Beal CC (2010). Gender and stroke symptoms: a review of the current literature. J Neurosci Nurs. 42: 80-87.

3. Tan Z, Li X, Turner RC, Logsdon AF, Lucke-Wold B, DiPasquale K, Jeong SS, Chen R, et al. (2014).

Combination treatment of r-tPA and an optimized human apyrase reduces mortality rate and hemorrhagic transformation 6h after ischemic stroke in aged female rats. Eur J Pharmacol. 738: 368-373.

4. Del Zoppo GJ (2006). Stroke and neurovascular protection. N Engl J Med. 354: 553-555

5. Bhattacharya S, Michels CL, Leung MK, Arany ZP, Kung AL, Livingston DM (1999). Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes Dev. 13: 64-75.

6. Shioda T, Fenner MH, Isselbacher K (1997). MSG1 and its related protein MRG1 share a transcription acti vating domain. Gene. 204: 235-241.

7. Shing Ng PK, Yu RMK, Kwong TFN, Wong MML, K ong RYC (2010). Transcriptional regulation and functional implication of the grass carp CITED1 (gcCITED1) in the negative regulation of HIF-1. Int J Biochem Cell Biol. 42: 1544-1552.

8. Braganca J, Eloranta JJ, Bamforth SD, Ibbitt JC, Hurst HC, Bhattacharya S (2003). Physical and functional interactions among AP 2 transcription factors, p300/C REB-binding
protein, and CITED2. J Biol Chem. 278:16021-9.

9. Tien ES, Davis JW, Vanden Heuvel JP (2004). Identifi cation of the CREB-binding Protein/p300-interacting Protein CITED2 as a Peroxisome Proliferator activated Receptor α Coregulator. J Biol Chem. 279: 24053-24063.

10. Chou YT, Wang H, Chen Y, Danielpour D, Yang YC (2006). Cited2 modulates TGF-beta-
mediated upregulation of MMP9. Oncogene. 25: 5547-5560.

11. Leu T, Schützhold V, Fandrey J, Ferenz KB (2019). When the Brain Yearns for Oxygen. Neurosignals. 27: 50-61.

12. Bamforth SD, Bragança J, Eloranta JJ, Murdoch JN, Marques FI, Kranc KR, Farza H et al. (2001). Cardiac malformations, adrenal agenesis, neural crest defects and exencephaly in mice lacking Cited2, a new Tfap2 co-activator. Nat Genet. 29: 469-474.

13. Weninger WJ, Lopes Floro K, Bennett MB, Withington SL, Preis JI, Barbera JP, Mohun TJ et al. (2005). Cited2 is required both for heart morphogenesis and establishment of the left-right axis in mouse development. Development. 132: 1337-1348.

14. Pacheco-Leyva I, Matias AC, Oliveira DV, Santos JM, Nascimento R, Guerreiro E, Michell AC et al. (2016). CITED2 Cooperates with ISL1 and Promotes Cardiac Differentiation of Mouse Embryonic Stem Cells. Stem cell reports. 7: 1037-1049.

15. Val P, Martinez-Barbera JP, Swain A (2007). Adrenal development is initiated by Cited2 and Wt1 through modulation of Sf-1 dosage. Development. 134: 2349-2358.

16. Buaas FW, Val P, Swain A (2009). The transcription co-factor CITED2 functions during sex determination and early gonad development. Hum Mol Genet. 18: 2989-3001.

17. Li Q, Ramírez-Bergeron DL, Dunwoodie SL, Yang YC (2012). Cited2 Gene Controls Pluripotency and Cardiomyocyte Differentiation of Murine Embryonic Stem Cells through Oct4 Gene. J Biol Chem. 287: 29088-29100.

18. Jayaraman S, Doucet M, Kominsky SL (2017). Down-regulation of CITED2 attenuates breast tumor growth, vessel formation and TGF-β-induced expression of VEGFA. Oncotarget. 8: 6169-6178.

19. Gonzalez YR, Zhang Y, Behzadpoor D, Cregan S, Bamforth S, Slack RS, Park DS (2008). CITED2 Signals through Peroxisome Proliferator-Activated Receptor- to Regulate Death of Cortical Neurons after DNA Damage. J Neurosci. 28: 5559-5569.

20. Liu Z, Wang Y, Dou C, Sun L, Li Q, Wang L, Xu Q, et al. (2018). MicroRNA-1468 promotes tumor progression by activating PPAR-γ-mediated AKT signaling in human hepatocellular carcinoma. J Exp Clin Cancer Res. 37.

21. Yokota H, Goldring MB, Sun HB (2003). CITED2-mediated Regulation of MMP-1 and MMP-13 in Human Chondrocytes under Flow Shear. J Biol Chem. 278: 47275-47280.

22. Chou YT, Hsieh CH, Chiou SH, Hsu CF, Kao YR, Lee CC, Chung CH et al. (2012). CITED2 functions as a molecular switch of cytokine-induced proliferation and quiescence. Cell Death Differ. 19: 2015-2028.

23. Risau W (1997). Mechanisms of angiogenesis. Nature. 386: 671-674.

24. Carmeliet P (2000). Mechanisms of angiogenesis and arteriogenesis. Nat Med. 6: 389-395.

25. Wang H, Xu X, Yin Y, Yu S, Ren H, Xue Q, Xu X (2020). Catalpol protects vascular structure and promotes angiogenesis in cerebral ischemic rats by targeting HIF-1alpha/VEGF. Phytomedicine. 78:153300.

26. Storkebaum E, Lambrechts D, Carmeliet P (2004). VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection. Bioessays. 26: 943-954.

27. Semenza GL (2004). Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology (Bethesda).

19:176-182.

28. Semenza GL (2009). Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology (Bethesda). 24: 97-106.

29. Chamorro Á, Dirnagl U, Urra X, Planas AM (2016). Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. Lancet Neurol. 1: 869-881.

30. Wang L, Liu H, Zhang L, Wang G, Zhang M, Yu Y (2017). Neuroprotection of Dexmedetomidine against Cerebral Ischemia-Reperfusion Injury in Rats: Involved in Inhibition of NF-κB and Inflammation Response. Biomol Ther. 25: 383-389.

31. Huang T, González YR, Qu D, Huang E, Safarpour F, Wang E, Joselin A et al. (2019). The pro-death role of Cited2 in stroke is regulated by E2F1/4 transcription factors. J Biol Chem. 294: 8617-8629.

32. Kruman II, Wersto RP, Cardozo-Pelaez F, Smilenov L, Chan SL, Chrest FJ, Emokpae R Jr, et al. (2004). Cell cycle activation linked to neuronal cell death initiated by DNA damage. Neuron. 41: 549-561.

33. Giovanni A, Wirtz-Brugger F, Keramaris E, Slack R, Park DS (1999). Involvement of cell cycle elements, cyclin-dependent kinases, pRb, and E2F x DP, in B-amyloid-induced neuronal death. J Biol Chem. 274: 19011-9016.

34. Anrather J, Iadecola C (2016). Inflammation and Strok e: An Overview. Neurotherapeutics. 13: 661-670.

35. Taylor RA, Sansing LH (2013). Microglial Responses after Ischemic Stroke and Intracerebral Hemorrhage. Clin Dev Immunol. 2013: 1-10.

36. Kim GD, Das R, Rao X, Zhong J, Deiuliis JA, Ramirez-Bergeron DL, Rajagopalan S Rajagopalan S et al. (2018). CITED2 Restrains Proinflammatory Macr ophage Activation and Response. Mol Cell Biol. 38: 452-517.