Brazilian Journal of Medical and Biological Research (2015) 48(1): 1-5, http://dx.doi.org/10.1590/1414-431X20144029
ISSN 1414-431X Overview

Myocardial ischemic preconditioning upregulated protein 1(*Mipu1*):zinc finger protein 667 – a multifunctional KRAB/C_2H_2 zinc finger protein

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Abstract

Myocardial ischemic preconditioning upregulated protein 1 (*Mipu1*) is a newly discovered upregulated gene produced in rats during the myocardial ischemic preconditioning process. *Mipu1* cDNA contains a 1824-base pair open reading frame and encodes a 608 amino acid protein with an N-terminal Krüppel-associated box (KRAB) domain and classical zinc finger C_2H_2 motifs in the C-terminus. *Mipu1* protein is located in the cell nucleus. Recent studies found that *Mipu1* has a protective effect on the ischemia-reperfusion injury of heart, brain, and other organs. As a nuclear factor, *Mipu1* may perform its protective function through directly transcribing and repressing the expression of proapoptotic genes to repress cell apoptosis. In addition, *Mipu1* also plays an important role in regulating the gene expression of downstream inflammatory mediators by inhibiting the activation of activator protein-1 and serum response element.

Key words: *Mipu1*; Zinc finger structure; Nuclear factor; Transcription and repression

The cDNA encoding early hematopoietic zinc finger protein

Myocardial ischemic preconditioning upregulated protein 1 (*Mipu1*) is upregulated during ischemic preconditioning by combining suppression subtractive hybridization and cDNA chip technology. It is currently designated as zinc finger protein 667 (ZNF667) by the Hugo Nomenclature committee and has GenBank accession number AY221750 (1,2). As a zinc finger nuclear transcriptional repressor, *Mipu1* inhibits oxidative stress-induced cell injury, which is due to downregulation of expression of the apoptosis-related genes *Fas* and *Bax* (2-4). Electrophoretic mobility shift assay (EMSA) and luciferase reporter gene assays showed that hypoxia inducible factor 1α (HIF-1α) and cAMP-response element binding protein (CREB) bound to the *Mipu1* promoter region and promoted its transcription during oxidative stress in cells (4,5).

The properties of *Mipu1*/ZNF667 are still only partially understood. However, its molecular features and expression profile as well as the biological functions so far identified suggest that it may play a role in the cardiovascular system. In this overview, we illustrate the data currently available on the structure, expression, interactions, and functional properties of this protein and discuss its possible significance in the cardiovascular field.

Biological characteristics of *Mipu1*

Mipu1, a typical N-terminal Krüppel-associated box (KRAB)/C_2H_2 zinc finger protein

A number of proteins with amino acid motifs capable of recognizing distinct DNA sequences via interaction with hydrogen donors and acceptors located in DNA major and minor grooves have been identified by bioinformatic analysis of DNA binding domains. The zinc finger domain can bind with DNA, the peptide, or histidine in the zinc finger protein and bind with divalent zinc ion to form a specific secondary structure. The zinc finger protein family has many subfamilies, among which C_2H_2 (or Kruppel) is the

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Received April 2, 2014. Accepted July 23, 2014. First published online October 31, 2014.

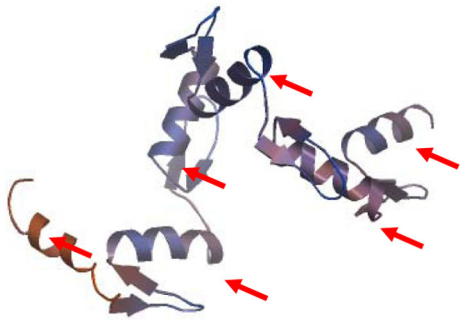

Figure 1. Structure of ZNF667. Arrows: zinc finger structure.

largest subfamily, in which the zinc finger sequence is CX_2CX_3FX_5LX_2HX_3H and the conserved sequence between the two zinc fingers is TGEKP(Y/F)X, where X represents any amino acid between conserved amino acids (6-9). The typical C_2H_2 zinc finger is a short protein motif with two histidine and two cysteine residues that hold a zinc ion with coordination bonds. It is obvious at present that they can also recognize various motifs in double-stranded DNA, single-stranded DNA, RNAs, and proteins (10-13). Depending on the domain at the N-terminal, C_2H_2 zinc finger proteins can be divided into four categories: FAX (finger-associated boxes), FAR (finger-associated repeats), POZ (pox virus and zinc fingers also known as Zin), and KRAB (Krüppel-associated box). The zinc finger proteins that contain KRAB, also called KRAB zinc finger proteins (KRAB-containing zinc finger proteins, KZNF), make up almost one-third (290 kinds) of all zinc finger proteins (799 kinds). They are the largest transcription repressor family in mammals and play an important role in embryonic development, cell differentiation, cell transformation, and cell cycle regulation (14-18) (Table 1; 19-27).

The full length of the *Mipu1* open reading frame is 1827 base pairs (bp), encoding 608 amino acids; it is composed of five exons and four introns, and maps to chromosome 1q12.1 (2). The N-terminal region of the encoded peptide chain has a KRAB domain, whereas the C-terminal region has 14 C_2H_2 zinc fingers; therefore, it is a typical KRAB/C_2H_2 zinc finger protein. The six zinc fingers at the C-terminus of *Mipu1* protein have been shown to combine with DNA, and *Mipu1* has been identified as a transcription repressor that binds to the specific DNA binding site 5′-TGTCTTATCGAA-3′, with CTTA as the key sequence of the binding site (3,25,28,29) (Figure 1).

Promoter region of *Mipu1* and its transcriptional regulation

Two different promoter prediction programs predicted two potential promoter regions for *Mipu1*: –104 to +146 bp, and –104 to +36 bp, with respect to the transcription start site. Both predicted *Mipu1* promoters include the region between –104 and +36 bp, proposed to be the core promoter or the minimal promoter. Seven different deletion constructs were transiently transfected into an H9c2 cardiomyocyte cell line, and showed the luciferase activity of the seven constructs relative to the promoter-less construct. The results mapped the minimal promoter of *Mipu1* to the region between –100 and +1 bp with respect to the transcription start site (30).

Lv et al. (30) showed that the GC box is essential for regulating the constitutive expression of *Mipu1*. However, the GC box has neither hypoxia-response nor stress-response elements, implying that other transcription factor binding sites within the *Mipu1* promoter region might be responsible for its upregulation during pathological stress (ischemic or hypoxic stress). One CREB binding site and one hypoxia response element (HRE) site were identified using the MatInspector software (http://www.genomatix.de/cgi-bin/matinspector_prof/mat_fam.pl). Our previous studies showed that hypoxia-reoxygenation or H_2O_2-mediated inducible expression of *Mipu1* is partially due to the activation of CREB (5,31). Recently using EMSA and luciferase reporter gene assays, Wang et al. (4) showed that HIF-1α bound to the HRE within the *Mipu1* promoter region and promoted its transcription.

Table 1. Role of C2H2 ZNF.

C2H2 ZNF	Role	Evidence for the role	Reference
ZNF139	Increased multi-drug resistance	Promoting the expression of *bcl-2* and inhibiting the expression of *Bax*	19
ZNF268	Contributes to cervical carcinogenesis	Enhancing NF-Kb signaling	20
ZNFD	Transcriptional activator in PKC signal pathway	Activates the transcriptional activities of AP1	21
	Development of mouse testis	Transcriptional regulation of HSE	22
HNF-4α	Regulation of fatty acid metabolism	Regulating human intestinal fatty acid binding protein (hFABP2) expression	23
	Regulation of bile acid synthesis in human liver	Regulation of expression of cholesterol 7α-hydroxylase (Cyp7a1)	24
ZNF667	Regulation of cell apoptosis	Repress expression of *Fas* and *Bax*	25-27

ZNF: zinc finger; HNF-4α: hepatocyte nuclear factor 4α.

Expression of *Mipu1*

Mipu1 mRNA is expressed in the heart, liver, spleen, lung, kidney, intestine, brain, and skeletal muscle of normal mice, with the highest level of expression in spleen and lung, a very high level of expression in heart and skeletal muscle, a very low level of expression in liver and brain, and the lowest level of expression in intestine. *Mipu1* protein has a very high level of expression in the heart and liver of normal rats and is mainly located in the nuclei of H9c2 myogenic cells, but it has a very low level of expression in liver, testis, kidney, and skeletal muscle and shows no signs of expression in spleen and lung (25,32,33). In studies of rat myocardial ischemia-reperfusion, *Mipu1* expression increased at 3 h of reperfusion, following 30 min of myocardial ischemia, reached its peak level 6 h later, and maintained that level until a further 12 h later. In addition, *Mipu1* expression in H9c2 cells could be induced by hydrogen peroxide (26), and it had an obviously higher expression in cerebral cortex and hippocampus after 12 and 24 h of reperfusion, after 3 min of ischemic preconditioning, than that of the sham surgery groups (32,34). Our results indicated that *Mipu1* mRNA expression was significantly increased during hypoxia-reoxygenation or H_2O_2 stimulation in H9c2 cells (5,31).

Cytoprotection effects of *Mipu1*

It has been demonstrated that *Mipu1* has a high expression in rat heart and is mainly located in the nuclei of H9c2 myogenic cells (25). The expression pattern and nuclear localization suggest that *Mipu1* plays a role in the regulation of gene transcription in the cardiovascular system. Upregulation of *Mipu1* is induced after myocardial infarction mainly in the infarcted area, and to some extent in the remote noninfarcted myocardium, suggesting that it may play an important role in myocardial infarction; however, further studies are needed to identify the mechanism (26). Overexpression of *Mipu1* can reduce H9c2 cell injury caused by $CoCl_2$-serum-free culture (1). At the same time, promoter activity and expression of *Mipu1* increased significantly during the hypoxia-reoxygenation process, which suggests that it may be involved in the injury of H9c2 cells (1). Being a zinc finger nuclear transcriptional repressor, its DNA binding sequence is 5-TGTCTTATCGAA-3, within which CTTA is the core sequence binding site (25). Recent studies have also shown that *Mipu1* can reduce apoptosis of H9c2 induced by H_2O_2 and tumor necrosis factor alpha (TNF-α), and can repress the expression of the apoptosis-related genes *Fas* and *Bax* (25-27). Overexpression of *Mipu1* represses transcriptional activity of serum response element (SRE) and activator protein-l (AP-1), and inhibition of *Mipu1* expression by RNAi can increase the transcriptional activity of SRE and AP-1; that is, *Mipu1* may be involved in the function of SRE and AP-1 during the transcriptional regulation process and plays an important role in the pathological process of heart and vascular diseases through regulating the mitogen-activated protein kinase (MAPK) signaling pathway (35).

HIF-1 serves as an important endogenous cytoprotective gene that maintains oxygen homeostasis by inducing the expression of cluster genes, such as *EPO*, *HO-1*, and *iNOS*, at the transcriptional level (36-40). Recently, Wang et al. (4) reported that HIF-1α bound to the HRE within the *Mipu1* promoter region and promoted its transcription, leading to cytoprotection of HIF-1 against H_2O_2-mediated injury in H9c2 cells partly through regulation of *Mipu1* expression. Our previous studies also found that hypoxia-reoxygenation or H_2O_2-induced upregulation of *Mipu1* in H9c2 cardiomyocytes was mediated by cAMP/protein kinase A (PKA)-dependent CREB activation, and that the cytoprotection of CREB against hypoxia-reoxygenation or H_2O_2-mediated injury in H9c2 cells occurs partly through regulation of *Mipu1* expression (5).

Expression of *Mipu1* is markedly increased in endotoxemia, which may have an important role in the inflammatory reaction process induced by lipopolysaccharide (LPS) (41). Further analysis of the role of *Mipu1* and its mechanism in the inflammatory process caused by LPS may provide new ideas and experimental clues for the prevention and cure of sepsis and other related diseases.

Perspectives

In summary, *Mipu1* is a nuclear factor with a variety of biological functions, such as participation in the process of myocardial ischemic preconditioning, protection of the myocardium from ischemic disease, and inflammation. Analysis of the function of *Mipu1* in ischemic heart disease is beneficial because it may provide new ideas for clinical treatment and prevention of ischemic heart disease. However, further development of related technologies is needed to obtain a comprehensive and detailed understanding of the function of *Mipu1* and its role in ischemic-related diseases.

Acknowledgments

Research supported by the National Natural Science Foundation of China (#81100212, #81170277, and #81100106), the PhD Programs Foundation of the Ministry of Education of the People's Republic of China (#20114324120004 and #20124324110003), the China Postdoctoral Science Foundation (#2012 M511383), the Scientific Research Fund of Hunan Provincial Education Department (#11C1094 and #11C1095), the Science and Technology Project of Hunan Province (#2014FJ3014), the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province (#2008-244), and the Construct Program of the Key Discipline in Human Hunan Province (#2011-76).

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