ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

NFAT-5 / TonEBP and its Role in Physiological Regulation and Aging Process.

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ABSTRACT

The classical members of NFAT family proteins include NFATc1, NFATc2, NFATc3, NFATc4 and NFAT5. Amongst these proteins TonEBP/NFAT5 is the most extensively investigated member of Rel family of transcription factors including NF-κB and NFAT1-4, which have profound biological importance in normal physiology and disease. TonEBP/NFAT5 was initially identified as an osmosensitive transcription factor in tissues that experience large fluctuations in tonicity, such as the renal medulla. Now it is known that a variety of TonEBP/NFAT5 target genes have been identified during the recent years that are not directly involved in cellular osmoadaptation. These include genes involved in embryogenesis and development, inflammation, proliferation, hepatic detoxification, extracellular matrix production, and others. This review article examines the possible roles of NFAT 5/TonEBP in physiological regulation and aging process.

Keywords: NFAT5, Hypertonic stress, SIRT 1, Aging , TonEBP

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ISSN: 0975-8585

INTRODUCTION

The Nuclear Factor of Activated T Cells-5 (NFAT5), is also known as OREBP or TonEBP, is a member of the nuclear factors of the activated T cells family of transcription factors. It is also the only known tonicity-regulated transcription factor in most mammals. NFAT5 was initially known for its role in the hypertonic kidney inner medulla for orchestrating a genetic program to restore the cellular homeostasis. To restore biochemical homeostasis under hypertonic stress, cells elicit a genetic program of osmoadaptive responses in which intracellular electrolytes are gradually replaced by uncharged small organic osmolytes including sorbitol, betaine, myo-inositol, taurine and glycerophosphocholine [1,2]. These organic osmolytes play a key role in osmoadaptation because they can be accumulated to a high level without perturbing macromolecular structure and function. Specific enzymes and transporters are responsible for the accumulation of these organic osmolytes: sorbitol and glycerophosphocholine are synthesized by aldose reductase (AR) and neuropathy target esterase (NTE), respectively, whereas betaine, myo-inositol and taurine are taken up into cells via betaine transporter (BGT1), sodium/myo-inositol cotransporter (SMIT), and sodium/chloride-dependent taurine transporter (TAUT), respectively [3,4]. Gene transcription of these enzymes and transporters, collectively known as osmoprotective genes, is markedly upregulated by hypertonic challenge. Emerging evidence, however, suggests that NFAT5 might play a more diverse functional role, including a pivotal role in blood pressure regulation and the development of autoimmune diseases. Despite the growing significance of NFAT5 in physiology and diseases, our understanding of how its activity is regulated remains not well known. Furthermore, how changes in tonicities are converted into functional outputs via NFAT5 remains elusive. Therefore, this review aims to summarize our current knowledge on the functional roles of NFAT5 in osmotic stress adaptation and the signaling pathways that regulate its activity, apart from its role in thymocyte function, cardiovascular homeostasis and aging.

NFAT5 and Thymocyte function

Thymocytes are known to express the calcineurin-independent NFAT protein NFAT5, which has the hybrid features of both NF-κB and NFATc proteins [3]. NFAT5 protects the cells from osmotic stress [4], and NFAT5-deficient mice portray severe atrophy of the renal medulla, systemic hypernatremia, and a reduced thymocyte compartment and mature T-cell lymphopenia [5,6]. Whereas the thymocyte and T-lymphocyte deficiency of NFAT5-null mice can be explained by their systemic hypernatremia [7], mice expressing a T-lymphocyte–restricted dominant negative NFAT5 transgene were shown to have reduced thymic cellularity and peripheral T lymphopenia [8]. Because the tonicity of the thymus is not high enough to activate NFAT5 [9], this suggests an osmostress-independent role of NFAT5 in thymocyte development. Recent studies describe that NFAT5 expression is regulated by the IκB kinase β (IKKβ) pathway in thymocytes and acts as a survival factor downstream from the pre-TCR, independent of its osmoprotective function. Thus NFAT 5 depicts a distinct role in the thymocytes.

NFAT 5 and signal transduction

The transcription factor, NFAT5 is rapidly activated by hypertonicity. In cells cultured at 300 mOsm, NFAT5 is present in the nucleus and cytoplasm, but a change to hypertonic medium causes NFAT5 translocation to the nucleus and upregulation of NFAT5 gene expression [10]. In the rat kidney, nuclear localization of NFAT5 reduces after water loading and increases after dehydration [11]. The activation of NFAT5 by hypertonic stress results in the induction of several genes implicated in osmotic tolerance, such as the aldose reductase (AR) [12]. The NFAT5 target genes contain at least one osmotic response element (ORE) consensus [13,14] and AP-1 site [15].

There are several positive and negative upstream molecular regulators of the tonicity-dependent activation of NFAT5 transactivating activity. For e.g the RNA helicase A [16]; epidermal growth factor receptor (EGFR) [17]; cAMP-dependent kinase (PKA) [18]; p38 mitogen-activated protein kinase (MAPK) [19]; Fyn, a member of the Src family of non-receptor, cytoplasmic protein tyrosine kinases [19]; Ataxia Telangiectasia Mutated (ATM) [20]; phosphatidyl 3-kinase Class IA (PI3K-IA) [21]; eNOS-NO system and PLCγ1 [22]. Experiments conducted on HEK293 and Jurkat cells suggest that PI3K class IA is upstream of ATM in high NaCl-induced activation of NFAT5 [23].

NFAT 5 in vascular smooth muscle cells

Recent experimental studies auggest that NFAT5 was able to modulate the phenotype of these vascular smooth muscle cells upon angiotensin II or PDGF-BB stimulation [24]. In fact, hypertonicity was originally described as the prototypic mechanism of NFAT5 activation. This microenvironmental stimulus induces phosphorylation of the carboxy-terminal transactivation domain of NFAT5, which subsequently results in its nuclear translocation and thus activation. Similarly, likewise, angiotensin II (a G-protein-coupled receptor agonist that induces VSMC contraction) stimulates the entry of NFAT5 into the nucleus without altering its protein or mRNA expression [25,26]. By expanding these findings, our study identified biomechanical stretch as a novel regulatory determinant of NFAT5 activity in VSMCs, which increases its mRNA expression, elicits its transient translocation to the nucleus, and like angiotensin II increases its protein abundance. In this context, it is reasonable to assume that AP-1 controls the mRNA expression of NFAT-5 in stretch-stimulated VSMCs as it is a crucial regulator of stretch-dependent gene expression and may control NFAT5 expression through binding to multiple AP-1 binding sites located in the NFAT5 promoter. The orchestration of NFAT5 activity becomes even more complex when considering that nucleocytoplasmic trafficking of NFAT5 which may also be influenced by palmitoylation and/or myristoylation as has been observed upon changes in osmolarity [27]. Along these data, it was found that palmitoylation of NFAT5 is required to enter the nucleus in stretch-stimulated VSMCs and is enhanced further upon inhibition of depalmitoylation. Palmitoylation is a highly relevant and reversible post-translational modification of proteins regulating their activity, localization, and trafficking. The human genome is known to encode 23 protein palmitoyl acyltransferases that are all capable to link palmitate to cysteine residues acting as putative palmitoylation sites in many proteins such as Src family of kinases, G-protein-coupled receptors, Ras GTPases as well as NFAT5.

ISSN: 0975-8585

Western Immunoblot Analysis of NFAT 5

Western blot analysis of NFAT 5 can be conducted by using adherent cells which are washed with ice-cold PBS, and lysed with cell lysis buffer (20 mM HEPES [pH 7.2], 10% glycerol, 10 mM Na_3VO_4, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM dithiothreitol, 1 µg/mL leupeptin, 1 µg/mL pepstatin, and 1% Triton X-100) on ice for 30 minutes. The lysates are than sonicated, the cell homogenates centrifuged at 15,000*g* for 10 minutes at 4°C, and the supernatant fractions stored at −70°C before use.

Protein concentrations in the resultant supernatant fractions are determined by using the Bradford reagent. Equal amounts of protein (30 µg) are boiled in Laemmli sample buffer and resolved by 8% SDS-PAGE. The proteins are transferred to polyvinylidene fluoride (PVDF) membranes (BioTrace, Pall Corporation, USA) and probed overnight with the primary antibodies to NFAT5 (dilution, 1:2000). Immunoreactive bands are detected with horseradish peroxidase–conjugated secondary antibodies and can be visualized by enhanced chemiluminescence.

Figure 1: Western Blot of human NFAT5/TonEBP from transfected cells (Courtesy Pierce antibody products, Rockford, IL, USA)

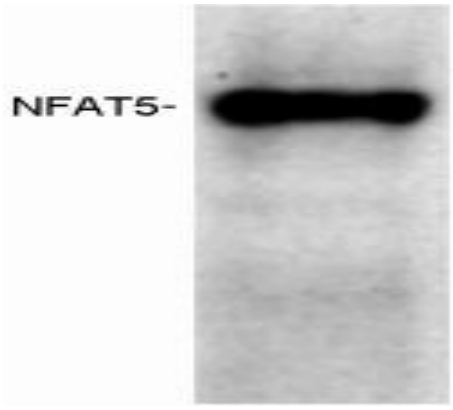
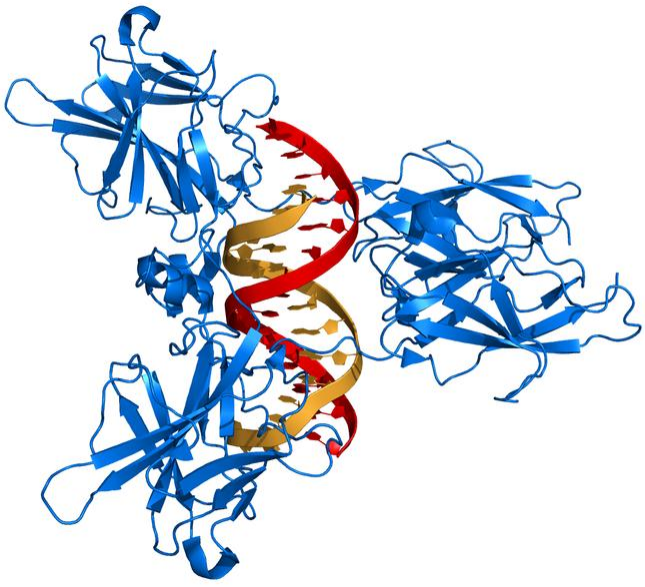


Figure 2: Ribbons diagram of the complex between NFAT5 and DNA.



NFAT 5 and SIRT 1

The silent information regulator 2 (Sir2) protein family (sirtuins or SIRTs) belongs to class III histone/protein deacetylases (HDACs). SIRT1, the mammalian homolog of Sir2 also mediates a variety of physiological processes such as life span and fat mobilization. SIRT1 has been reported to deacetylate a broad array of targets including histones (H3, H4), transcription factors (p53, FOXO, NFkB) and transcriptional cofactors (p300, NcoR, PGC-1α). In addition, recent studies point to SIRTl as a key regulator of vascular endothelial homeostasis controlling angiogenesis, vascular tone and endothelial dysfunction, especially probably for anti-inflammation. Recent studies demonstrate for the first time that NFAT is a new target of SIRT1. SIRT1 inhibits NFATc3 transcriptional activity by deacetylating NFATc3. And, SIRT1 suppresses PMA/Io induced NFATc3 dependent inflammatory genes expression in endothelial cells, leading to the inhibition of inflammation. Thus it is speculated that NFAT 5 may play a role in the process of aging and metabolic regulation [28,29].

NFAT5 and DNA complex

NFAT 5 belongs to the NFAT family of transcription factors and its DNA binding domain shares a high sequence identity with the NFAT1−4 proteins. However, unlike other NFAT1−4 proteins which can bind DNA as monomers, NFAT5 binds to DNA as a dimer. Several experimental data suggest that it likely forms a dimer in solution even in the absence of DNA and dimerization is necessary for DNA binding and transcriptional activity. The DNA affinity of NFAT5 is much lower than that of NFAT1− 4, however, it has a slower dissociation rate than another Rel dimeric transcription factor, NF-kB. The NFAT 5 gene encodes multiple isoforms of which NFAT5 a and c have been most extensively studied. NFAT a and c have identical sequences, but NFATc has an additional 76 amino acids at the N-terminus. In this work, we use the amino acid numbering of the NFAT5 isoform a but include notation of the corresponding amino acids in isoform c. The crystal structure of an NFAT5 region containing amino acids 170−470 (246−546 isoform c) in complex with DNA was resolved about 10 years ago. This region of NFAT5 has two domains: an N-terminal Rel homology domain (RHD) which makes most of the contacts with DNA and a C-terminal IPT domain which mediates interactions between the two monomers and has limited contacts with DNA. The crystal structure of the NFAT5 complex with DNA remains the only available structure of this protein. According to this structure, one monomer of NFAT5 makes specifically contacts with the conserved DNA “consensus” nucleotides (TGGAAA) of the NFAT5 cognate DNA element, ORE . The other monomer binds to other, nonconserved nucleotides of OREs which differ among NFAT5 target genes [30].

NFAT5/TonEBP and biochemical parameters

In our continuing studies on the role of this transcription factor, we have found that modulation of NFAT5/TonEBP by drugs and chemicals like the Doxorubicin, Nitrates and Rottlerin can contribute to specific changes in the activity of enzymes like the Aldose reductase, Malondialdehyde, and various amino acids and blood gases. Apart from this the effects on the histopathology in animal tissues have also been explored [31,32].

ISSN: 0975-8585

SUMMARY AND CONCLUSION

Nuclear factor of activated T cells 5 (NFAT5) is the most extensively researched member of the Rel family of transcription factors, including NF-κB and NFAT1-4, which plays a central role in inducible gene expression during the immune response. NFAT5 was initially described to mediate osmoprotective gene expression in renal medullary cells, which are routinely faced by high extracellular osmolalities. Recent results however indicate profound biological importance of the mammalian osmotic stress response in view of NFAT5 dependent gene regulation in non-renal tissues. For e.g in mononuclear and epithelial cells, NFAT5 stimulates the expression of various pro-inflammatory cytokines during elevated ambient tonicity. This review article summarizes the current knowledge regarding the role of NFAT 5 in various systemic and local processes in view of NFAT5 activation and regulation, and NFAT5 dependent intracellular signal transduction. Future aspects of NFAT5/TonEBP are already exploring the role in plasma, the interstitial tonicity of lymphoid organs like spleen and thymus and that of liver in substantially hypertonic physiological conditions. In addition, anisotonic disorders (hypernatremia, diabetes mellitus, dehydration) entail systemic hyperosmolality, and, in inflammatory disorders, the skin, intestine, and cornea are sites of local hyperosmolality and NFAT-5 role in these disorders apart from aging needs to be probed.

ACKNOWLEDGEMENTS

The author acknowledges the useful discussion and encouragement by Dr.Joan Ferraris, National Institute of Health, Bethesda, USA

REFERENCES

[1] López-Rodríguez C, Aramburu J, Rakeman AS, Rao A. Proc Natl Acad Sci USA 1999; 96 (13): 7214–7219.
[2] López-Rodríguez C, Aramburu J, Jin L, Rakeman AS, Michino M, Rao A. Immunity 2001; 15(1):47–58.
[3] Aramburu J, Drews-Elger K, Estrada-Gelonch A, Minguillón J, Morancho B, Santiago V, López-Rodríguez C. Biochem Pharmacol 2006;72 (11):1597–1604.
[4] López-Rodríguez C, Antos CL, Shelton JM, Richardson JA, Lin F, Novobrantseva TI, Bronson RT, Igarashi P, Rao A, Olson EN. Proc Natl Acad Sci USA 2004; 101(8):2392–2397.
[5] Berga-Bolaños R, Drews-Elger K, Aramburu J, López-Rodríguez C. J Immunol 2010; 185(11): 6624–6635.
[6] Drews-Elger K, Ortells MC, Rao A, López-Rodríguez C, Aramburu J. PLoS ONE 2009; 4(4):e5245.
[7] Trama J, Go WY, Ho SN. J Immunol 2002;169(10); 5477–5488.
[8] Go WY, Liu X, Roti MA, Liu F, Ho SN. Proc Natl Acad Sci USA 2004;101(29):10673–10678.
[9] Morancho B, Minguillón J, Molkentin JD, LópezRodríguez C, Aramburu J. BMC Mol Biol 2008;9:13.
[10] Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. Science 1982;217:1214-1222.

[11] Garcia-Perez A, Burg MB. Physiol Rev 1991; 71:1081-1115.
[12] Ferraris JD, Williams CK, Martin BM, Burg MB, García-Pérez A. Proc Natl Acad Sci USA 1994;91: 10742–6.
[13] Ferraris JD, Williams CK, Ohtaka A, García-Pérez A. Am J Physiol1999; 276: C667–73.
[14] Ferraris JD, Williams CK, Persaud P, Zhang Z, Chen Y, Burg MB. Proc Natl Acad Sci USA 2002;99: 739–44.
[15] Irarrazabal CE, Williams CK, Ely MA, Birrer MJ, Garcia-Perez A. J Biol Chem 2008;283: 2554–63.
[16] Colla E, Lee SD, Sheen MR, Woo SK, Kwon HM. Biochem J 2006; 393: 411–9.
[17] Küper C, Steinert D, Fraek ML, Beck FX, Neuhofer W. Am J Physiol Renal Physiol 2009;296: F1100–8.
[18] Ferraris JD, Persaud P, Williams CK, Chen Y, Burg MB. Proc Natl Acad Sci USA 2002;99: 16800–5.
[19] Ko BC, Lam AK, Kapus A, Fan L, Chung SK, Chung SS. J Biol Chem 2002;277: 46085–92.
[20] Irarrazabal CE, Liu JC, Burg MB, Ferraris JD. Proc Natl Acad Sci USA 2004;101: 8809–14.
[21] Irarrazabal CE, Burg MB, Ward SG, Ferraris JD.2006; Proc Natl Acad Sci USA 2006;103: 8882–7.
[22] Yuan K, Kim SY, Oh YB, Yu J, Shah A, Park BH and Kim SH.Peptides 2010;31: 1319–25.
[23] Irarrazabal CE, Gallazzini M, Schnetz MP, Kunin M, Simons BL. Proc Natl Acad Sci USA 2010;107: 906–11.
[24] Halterman JA, Kwon HM, Zargham R, Bortz PD, Wamhoff BR. Arterioscler Thromb Vasc Biol 1; 31:2287-2296.
[25] Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM. Proc Natl Acad Sci USA 1999; 96:2538-2542.
[26] Dahl SC, Handler JS, Kwon HM. Am J Physiol Cell Physiol 2001; 280:C248-C253.
[27] Eisenhaber B, Sammer M, Lua WH, Benetka W, Liew LL, Yu W, Lee HK,Koranda M, Eis enhaber F, Adhikari S. Cell Cycle 2011; 10:3897-3911.
[28] Jia Yu Yan, Liu De Pei, Chen Hou Zao. PhD Thesis. Beijing Union Medical College.Biochemistry and Molecular Biology
[29] Lee SD, Colla E, Sheen MR, Na KY, Kwon HM. J Biol Chem 2003; 278:47571-47577.
[30] Minghui Li, Benjamin A. Shoemaker, Ratna R. Thangudu, Joan D. Ferraris, Maurice B. Burg, and Anna R. Panchenko. J Phys Chem B 2013 117, 13226−13234
[31] T. Pradhap, M. Ashokan and Manoj G. Tyagi. J Med Med Sci 2012;3(6), 409-414
[32] Aniket Kumar , Winston A B, Mahalakshmi C , Soosai Manickam Amirtham , Manoj G. Tyagi . Biomed Res 2014; 25 (3) (In Press)