Regulation of tonicity-dependent activation of NFAT5 by mitogen-activated protein kinases

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Introduction

NFAT5, nuclear factor of activation of T cell 5, is the newest member of the Rel transcription factor family. It shares about 40% homology in its DNA binding domain with other members of the family, NFAT1-4 and NF-κB [53]. Because of its indispensable role in protecting mammalian cells against hypertonic stress, NFAT5 is also called TonEBP, tonicity-responsive enhancer binding protein [58], and OREBP, osmolarity-responsive enhancer binding protein [42]. Kidney medulla is hypertonic, because of the urinary concentration mechanism. With ad libitum water intake and standard chow, the osmolarity in the papilla and inner medulla of male Sprague-Dawley rats is around 1100 and 780 mosmol/kg, respectively [18]. The osmolarity in the mouse inner medulla 2/3 is about 900 mosmol/kg [21]. Although hypertonicity in the renal medullary interstitial fluid provides an osmolar gradient driving water absorption, it is extremely stressful to the medullary cells and can kill them. Survival and function of the kidney medulla are dependent on NFAT5. In the kidney medulla, NFAT5 activates expression of two groups of genes that are essential for urinary concentration. The first group of the genes is called osmoprotective genes, including betaine/glycine...
transporter 1 (BGT1), sodium-dependent myo-inositol transporter (SMIT) and aldose reductase (AR) [12, 53]. Survived NFAT5 homozygous knockouts have profound renal medullary hypotrophy with reduced expression of the osmoprotective gene [53]. The second group of genes is directly involved in urinary concentration such as aquaporin-2 (AQP-2) [45, 46, 50] and possible aquaporin-1 (AQP-1) [47], which are necessary for maintaining adequate water permeability in the kidney medulla, and urea transporter 1 (UTA1) [45, 46, 61] for building hyperosmolarity in the renal medullary interstitium. Expression of a dominant negative mutant of NFAT5 in the kidney epithelial cells reduces expression of AQP-2 and UTA1, and impairs urinary concentration [46]. Thus, NFAT5 is tightly regulated in the kidney medulla to ensure normal process of urinary concentration. Hypokalemia, cyclosporine A and sepsis-induced urinary concentration defect is associated with reduced NFAT5 activity in the region [33, 45, 52].

Besides the exceptional hypertonicity in the kidney medulla, certain other tissues of body, including spleen, thymus and liver [24], human placenta [4, 74], intervertebral disc and articular cartilage [36, 82], are also normally exposed to the hypertonic environment. NFAT5 also activates expression of the osmoprotective genes, and dis-regulation of NFAT5 may involve in certain pathophysiological processes in these tissues [3, 4, 24, 36, 48, 46, 74, 75, 82]. Un-controlled type I diabetes mellitus has elevated blood glucose level. NFAT5 may play a role in type 1 diabetes-induced nephropathy [68] and disruption of transverse tubular network [28]. Moreover, hypertonicity is associated with certain inflammatory diseases and disorders [7, 62]. NFAT5 plays an important role in high salt diet-induced inflammation such as hypertension by mediating lymphatic expansion under the skin [55] and experimental autoimmune encephalomyelitis by activating expression of pro-inflammatory cytokines [29, 86].

In addition to its well-known osmoprotective function, NFAT5 also involves in biological processes and diseases that are not obviously associated with hypertonic stress, such as cardiomyocyte development and differentiation [1, 57], doxorubicin-induced cytotoxicity in cardiac myocytes [31], muscle migration, differentiation and atherosclerosis [25, 26, 65], intestinal goblet cell differentiation [98], brain injury [56, 90], cancer cell proliferation and migration [23, 44], T lymphocyte development [6], inflammatory arthritis [91], human immunodeficiency and autoimmune enterocolopathic [8], leishmania infection [13, 97] and proliferation of HIV virus [68].

Hypertonicity activates NFAT5 by increasing its transactivating activity, nuclear localization, DNA binding and protein abundance [12]. Hypertonicity up-regulates these mechanisms through phosphorylation of NFAT5 itself or signaling molecules, which is dependent on both increased kinase activities and reduced phosphatase activities [95, 96]. However, the present review only focuses on the mitogen activated protein kinases (MAPKs), because they are the most studied kinases in regulation of tonicity-dependent activation of NFAT5.

MAPKs have three major subcategories: p38, extracellular signal-regulated kinases (ERK) and c-Jun NH2-terminal protein kinases (JNK). Each family of MAPKs has multiple isoforms. MAPKs regulate a wide variety of cellular activities, including proliferation, apoptosis and differentiation. Some of these distinct functions are carried out by specific isoforms, whereas some of the specific even opposing functions are performed by the same isoform. Hypertonicity activates some isoforms in all three subcategories in cultured cells [32, 73, 76]. Similarly, water restriction increases the stimulatory phosphorylation of p38, ERK1/2 and JNK1/2 in the rat kidney [84]. MAPKs involve in regulation of hypertonicity-induced expression of an array of genes ranging from osmoprotective to inflammatory ones [15, 42, 73], some of which through NFAT5.

1. p38

p38 has four isoforms: p38α [27], p38β [34], p38γ [51] and p38δ [35]. Deletion of p38α leads to death during embryogenesis, whereas single knockout of p38β, γ or δ or double knockout of γ and δ leads to fertile and viable mice without apparent phenotype [2]. Hypertonicity activates all these four isoforms [27, 34, 35, 51]. p38α is the first isoform cloned and also called p38. Once it was cloned, it was realized that it shared 52% homology in deduced amino acid with HOG-1, high osmolarity glycerol response 1, a kinase essential for yeast to adapt to hyperosmotic stress [27]. The osmoprotective role of p38α was convincingly demonstrated by the observation that it complemented HOG-1 mutations in yeast [27]. The imidazole derivatives such as SB203580 inhibit p38α and also p38β, but not p38γ [5, 35]. Since SB203580 and its analog reduce hypertonicity-induced NFAT5 transcriptional activity [22, 50, 51, 60, 79], it is concluded that p38 contributes to tonicity-dependent activation of NFAT5 without specifying p38α, since p38α is also named as p38 [27]. However, these nomenclatures add confusion for interpretation of other results. For example, inhibition of MKK3 by its dominant negative mutant [43] and over expression of a MAP kinase phosphatase, MKP-1 [93], both inhibit p38, but don’t significantly inhibit NFAT5 transcriptional activity, raising a question concerning the role of p38 in activation of NFAT5. The observation that p38α mediates hypertonicity-induced activation of NFAT5, whereas p38δ does the opposite may explain this discrepancy [93]. It is interpreted that inhibition of the positive effect of...
p38α by SB203580 or by a dominant negative mutant of p38α [40] unmasks an inhibitory effect of p38δ. Further, the dichotomy of p38α and p38δ explains why over expression of a dominant negative MKK3 or MKP-1 prevents high NaCl-induced phosphorylation of p38, but has little effect on NFAT5 transcriptional activity [43]. Since MKK3 activates both p38α and p38δ [20, 35], and MKP-1 inhibits both p38α and p38δ [93], the dominant negative mutant of MKK3 and over expression of MKP-1 reduce both of their activities, therefore, should not cause any net change in NFAT5 activity [43, 93].

Hypertonicity activates p38 by dual-phosphorylation of Thr and Tyr within the motif Thr-Gly-Tyr located in kinase subdomain VIII through a protein kinase cascade, including MKK3 and MKK6, which are activated by their upstream kinases including MEKK3 [17, 80]. Accordingly, it is not surprising that MKK6 [14, 94] and MEKK3 [66] have been demonstrated to contribute to toxicity-dependent activation of NFAT5. The different effects of MKK3 and MKK6 on the NFAT5 activity may lie in their preferential effects on p38α and p38δ. Both of them activate p38α [17, 80], but MKK3 strongly activates p38δ in response to hypertonicity, whereas MKK6 does not [69].

p38 contributes to toxicity-dependent activation of NFAT5 by increasing its transactivating activity without significantly affecting its nuclear localization [40, 94], so does MKK6 [94]. p38 only transiently contributes to high NaCl-induced NFAT5 protein abundance in HEK293 cells [94]. Although hypertonicity potently increases the stimulatory phosphorylation of p38, and p38 is the most studied MAPK in the context of toxicity-dependent regulation of NFAT5, the exact mechanisms underlying the effects are far from clear. In addition to the molecular mechanism by which p38 regulates NFAT5 transactivating activity remains to be elucidated, whether p38 is critical for toxicity-dependent activation of the transcription factor is questionable. Knockdown of Rac1 or OSM by its siRNA reduces high NaCl-induced NFAT5 transcriptional activity, but increases phosphorylation of p38 at both basal and hypertonic levels in HEK293 cells [94]. It should be noted that an opposite effect of knockdown of Rac1 or OSM on phosphorylation of p38 in the same type of cells was reported [80]. We recently found that siRNA-mediated knockdown PKCα has the opposite effects on NFAT5 activity and phosphorylation of p38 similarly as knockdown of Rac1 and OSM (data not shown). Although whether activation of p38 is regulated by cell volume or intracellular ionic strength remains unclear, hypertonicity, which reduces NFAT5 activity [85, 87], also activates p38 in various types of cells [38, 64, 75, 77]. These observations call for more attention to which isoform of p38 when the effect of p38 on NFAT5 is examined, since each isoform is known to have different biological effects [2, 54, 93].

2. ERK1/2

ERKs, with at least 8 isoforms, plays a critical role in signaling mitogenic effects triggered by growth factors, serum, phorbol esters and etc [70]. Only ERK1/2 has been studied for its role in regulation of NFAT5. ERK1 and ERK2, 44 and 42 kDa, share 83% identical in amino acid sequence with most differences outside the kinase core [9, 10]. They often have similar biological activities. High NaCl activates ERK1/2 by increasing its phosphorylation [79, 83] and nuclear accumulation [83]. ERK1/2 activation is necessary for high NaCl-enhanced expression of TNF-α in LPS- or PMA-activated THP-1 cells [15], monocyte chemoattractant protein-1 (MCP1) in NRK52E rat renal proximal tubular cells [42], cyclooxygenase-2 expression in mIMCD-K2 cells [89]. Although NFAT5 is a major transcription factor activated by high NaCl and is responsible for high NaCl-dependent activation of gene expression, it is not clear whether ERK1/2 is involved in high NaCl-induced activation of NFAT5. Using chemical inhibitors PD98059 and U-0126, ERK1/2 has been demonstrated to contribute to high NaCl-induced activation of NFAT5 in nucleus pulposus [59] and possibly in mIMCD3 cells [81], but apparently not in primary splenocytes [59]. Presumably, the differences come from the different model systems that were studied. On the other hand, ERK2 siRNA and a dominant-negative mutant of ERK2 and PD98059 reduce high NaCl-dependent NFAT5 transcriptional activity in nucleus pulposus [79], and ERK2 siRNAs inhibit high NaCl-induced NFAT5 activity in HEK293 cells [83]. ERK2 siRNAs exert this effect through inhibiting NFAT5 transactivating activity [79, 83] without significantly affecting high NaCl-induced NFAT5 nuclear accumulation [83].

High NaCl phosphorylates ERK1/2 through MEK1/2 [79, 99]. MEK1/2 is activated by pro-oncogene serine/threonine kinases Ras and Raf, and PKCs including PKCα [16, 83]. MEK1/2 contributes to toxicity-dependent activation of NFAT5 in nucleus pulposus [79], so does PKCα in HEK293 cells [83]. More interestingly, knockout of PKCα decreases NFAT5 protein abundance and expression of its targets in the kidney inner medulla, associated with reduced phosphorylation of ERK1/2 [83]. Like the effect on p38, hypertonicity also increases phosphorylation of ERK1/2 in human keratinocytes [38], mIMCD3 cells [92], renal epithelial A6 cells [73], although hypotonic stress-induced inhibition of ERK in A6 cells is also reported [63]. Therefore, the mechanisms for how ERK1/2 contributes to toxicity-dependent activation of NFAT5 remain to be elucidated.
3. JNKs

JNKs were initially identified in the early 1990s as the protein kinases that bind to a specific region of c-Jun and are involved in the phosphorylation of c-Jun at the N-terminal Ser-63 and Ser-73 sites in the transcriptional activation domain [19, 29]. This family has 10 isoforms encoded by 3 distinct genes: JNK1, JNK2, and JNK3 with 4 isoforms of JNK1, 4 isoforms of JNK2, and 2 isoforms of JNK3. Of these, JNK1 and JNK2 are found everywhere whereas JNK3 is expressed mainly in the heart, testes, and brain [71]. Despite the fact that JNK is the largest family of MAPKs, the effect of JNK on toxicity-dependent activation of NFAT5 is elusive and also the least studied. A dominant negative mutant of JNK1 and JNK2 all reduce protein abundance of aquaporin-1 [81], a target of NFAT5 [47].

Conclusions and Perspectives

It is concluded that p38α, possible p38β, but not p38δ, and ERK1/2 contribute to toxicity dependent activation of NFAT5 by increasing its transactivating activity without significantly affecting its nuclear localization in cell culture. However, the exact molecular mechanisms underlying these effects remain unknown, since hypotonicity, which reduces NFAT5 activity [85, 87], also increases the stimulatory phosphorylation of these MAPKs [38, 64, 75, 77], and knockdown of Ral1 reduces NFAT5 activity with increased phosphorylation of p38 [84]. Identification of a specific isoform in regulation of NFAT5 may cast some light on this mystery. It is also certainly possible that the final function output of activation of these MAPKs by different toxicities depends on duration and strength of the activation, interactions with specific scaffolds, changes in subcellular localization and network with other signaling molecules [11, 72]. Further, whether MAPKs regulate NFAT5 activity in the tissues exposed to hypertonicity under either physiological or pathophysiological conditions awaits for being demonstrated. Since the MAPKs regulate a wide variety of biological activities, and knockout of p38α and ERK2 results in embryonic lethality [2], tissue-specific knockout of the MAPKs will generate more useful information than the general knockout. Advances and maturation in the technologies generating tissue-specific and conditional knockouts will allow ordinary laboratories to perform such studies.

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