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Review

NFAT as cancer target: Mission possible?

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ABSTRACT

The NFAT signaling pathway regulates various aspects of cellular functions; NFAT acts as a calcium sensor, integrating calcium signaling with other pathways involved in development and growth, immune response, and inflammatory response. The NFAT family of transcription factors regulates diverse cellular functions such as cell survival, proliferation, migration, invasion, and angiogenesis. The NFAT isoforms are constitutively activated and overexpressed in several cancer types wherein they transactivate downstream targets that play important roles in cancer development and progression. Though the NFAT family has been conclusively proved to be pivotal in cancer progression, the different isoforms play distinct roles in different cellular contexts. In this review, our discussion is focused on the mechanisms that drive the activation of various NFAT isoforms in cancer. Additionally, we analyze the potential of NFAT as a valid target for cancer prevention and therapy.

Contents

Abbreviations: ADP, adenosine di-phosphate; AICD, activation-induced cell death; AKAP79, A-kinase anchor protein 79; Ang-2, angiopoietin-2; AP1, activator protein 1; ARRE-2, antigen receptor response element-2; BMP4, bone morphogenetic protein 4; CABIN1, calcineurin-binding protein 1; CaM, calmodulin; CDK4, cyclin dependent kinase 4; CDS, calcineurin docking site; CK1, casein kinase 1; CnA, calcineurin A; CnB, calcineurin B; CsA, cyclosporin A; CSF1, colony-stimulating factor-1; DAG, diacylglycerol; DCA, dicholoroacetate; DSCR1, Down's syndrome critical region 1; DYRK, dual-specificity tyrosine-phosphorylation regulated kinase; ECM, extra-cellular membrane; ECs, endothelial cells; ER, endoplasmic reticulum; ERK, extra-cellular signal related kinase; EWSR1, Ewing sarcoma breakpoint region 1; FasL, Fas ligand; FOXP3, forkhead box P3; FOXC2, forkhead box C2; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPC6, glypican-6; GPCRs, G-protein coupled receptors; GSK3β, glycogen-synthase kinase 3β; HDACs, histone deacetylases; HemECs, hemangioma endothelial cells; IL-2, interleukin-2; IP3, inositol-1,4,5-triphosphate; IP3R, IP3 receptor; JNK, c-JUN kinase; LPA, lysophosphatidic acid; MAPKs, mitogen activated protein kinases; MMPs, matrix metalloproteinases; NES, nuclear export signal; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; NHD, NFAT homology domain; NLS, nuclear localization sequences; PARP, poly-ADP-ribose polymerase; PGE_2, prostaglandin E2; PI3K, phospho-inositol-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PLCγ, phospholipase Cγ; PML, promyelocytic leukemia; PROX1, prospero homeobox 1; RHD, Rel-homology domain; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; SMIs, small molecule inhibitors; SOC, store-operated calcium channel; SRR, serine rich regions; SUMO1, Small ubiquitin-like modifier 1; TAD, transactivation domain; TCR, T-cell receptors; TEM8, tumor endothelial marker-8; TF, transcription factors; TonEBP, tonicity-responsive enhancer-binding protein; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor

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1. Introduction

The nuclear factor of activated T cells (NFAT) was first described as an inducible nuclear factor binding to the antigen receptor response element-2 (ARRE-2) of the interleukin-2 (IL-2) promoter in human T cells [1,2]. Subsequent studies revealed that NFAT was not only expressed in T cells, but also ubiquitously expressed in various immune and non-immune cells in the vertebrate systems [3–5]. Recent studies have further indicated that NFAT plays multiple regulatory roles in cell fate determination, embryonic development, and organogenesis (especially the cardiac, hematopoietic, skeletal, and neuronal systems) [6–8].

The NFAT family contains five members, including four calcium-responsive isoforms named NFAT1 (NFATc2 or NFATp) [9,10], NFAT2 (NFATc1 or NFATc) [11], NFAT3 (NFATc4) [12], and NFAT4 (NFATc3 or NFATx) [13], and a tonicity-responsive enhancer-binding protein (TonEBP, also known as NFAT5) [14–16]. Except for NFAT5, the other members are activated by Ca^{2+} influx in the cell, either via the PLC-γ pathway or via store-operated Ca^{2+} entry, typically in T lymphoid cells [17]. The calcium-responsive NFAT isoforms (NFAT1–NFAT4) exist in a hyperphosphorylated state in the cytoplasm [17]. They are usually activated by increased intracellular calcium levels, via dephosphorylation by calcineurin and subsequent nuclear translocation [18–20]. Once in the nucleus, NFAT1–NFAT4 activate transcription of downstream gene targets, thus directly linking calcium signaling to gene expression [21–23].

Dysregulation of NFAT signaling is associated with malignant phenotypes and tumor progression [22]. It has been observed that NFAT isoforms are overexpressed and/or constitutively activated in both human solid tumors and hematological malignancies [5,22,24]. Indeed, the NFAT transcription factors have been shown to regulate cell survival, differentiation, angiogenesis, invasive migration, and the tumor microenvironment, which will be discussed in the subsequent sections. Therefore, a thorough understanding of NFAT's roles in tumor development and progression will facilitate the development of safe and effective treatment modalities targeting the NFAT pathway in cancer.

In this review, we focus on the recent findings related to the NFAT regulation and their roles in tumor development and progression. In addition, we review various inhibitors of NFAT and the current strategies for targeting the NFAT signaling in cancers.

2. NFAT biology

All NFAT proteins share a highly conserved Rel-homology domain (RHD) (Fig. 1) [25]. This domain is structurally similar to the DNA binding domain of the nuclear factor-κB (NF-κB) family [26,27]. As a unifying characteristic in all NFAT proteins, RHD endows the NFAT members with a common DNA-binding specificity [25]. In addition, the calcium-responsive NFAT isoforms (NFAT1–NFAT4) typically have another moderately conserved domain, NFAT homology domain (NHD) (Fig. 1) that binds to promoter elements, initiating gene transcription [10]. The NHD, located at N terminus, possesses several serine rich regions (SRR), providing around fourteen phosphorylation sites to the various kinases that target NFAT [28]. When these sites are heavily phosphorylated, the NFAT proteins are confined to the cytoplasm [28]. The N terminus also contains several other regulatory domains, including a transactivation domain (TAD) [29], and a calcineurin docking site (CDS) [17]. The nuclear localization sequences (NLS1 and 2) and the nuclear export signal (NES), also present in this domain, control the subcellular localization of NFAT [28,30]. Dephosphorylation of the serine residues by calcineurin unmasks the NLS, while rephosphorylation of the serine residues masks the NLS, exposing the NES and shuttling the NFAT proteins out of the nucleus [28,30]. However, NFAT5 retains only the RHD and is devoid of the CDS, thus being insensitive to calcium and calcineurin [14–16]. Instead, its transcriptional activity is dependent on extracellular tonicity [15].

NFAT proteins often perform redundant functions in cells [4]. Although no significant phenotypic abnormalities were found in mice lacking individual NFAT proteins (Table 1), a few notable exceptions are observed. For example, NFAT2 deletion causes defective cardiac valve formation leading to embryonic lethality [31,32], while NFAT1 deletion reduces mast cell cytokine production [33]. In most cases, however, pronounced physiological defects will not occur unless at least two NFAT proteins are absent (Table 1). For instance, concomitant deletion of NFAT1 and NFAT2 abolishes cytokine production in T cells [34], while deletion of both NFAT1 and NFAT4 increases Th2 cytokine

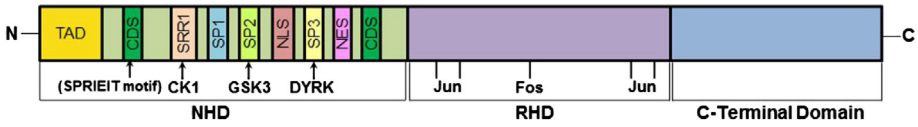

Fig. 1. Schematic structure of NFAT. The figure depicts domains common to NFAT isoforms 1–4. NFAT5 lacks the calcineurin-docking site (CDS) and is calcium unresponsive. The NFAT-homology domain (NHD) contains the transactivation domain (TAD), CDS with SPRIEIT motif, the serine-rich regions (SRR), the serine–proline rich motifs (SP1–SP3), the nuclear localization sequence (NLS), and the nuclear export signal (NES). The export and maintenance kinases, casein kinase 1 (CK1), glycogen synthase kinase 3 (GSK3), and dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) bind to the SRR1, SP2, and SP3 domains, respectively. The Rel-homology domain (RHD) comprises the DNA binding domain and is similar to that present in the NF-κB transcription factor family. The RHD also contains the recognition sites for transcriptional binding partners such as Fos and Jun.