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Review

NFAT as cancer target: Mission possible?



Jiang-Jiang Qin^{a,1}, Subhasree Nag^{a,1}, Wei Wang^{a,b}, Jianwei Zhou^c, Wei-Dong Zhang^d,
Hui Wang^{e,f}, Ruiwen Zhang^{a,b,*}

^a Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA

^b Cancer Biology Center, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA

^c Department of Molecular Cell Biology and Toxicology, Cancer Center, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu 210029, PR China

^d School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, PR China

^e Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, PR China

^f Key Laboratory of Food Safety Risk Assessment, Ministry of Health, Beijing 100021, PR China

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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.

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Contents

1. Introduction	298
2. NFAT biology	298
3. Regulation of NFAT	299
3.1. Calcineurin–NFAT signaling pathway	299
3.2. Regulation of NFAT by kinases	299
3.3. NFAT2 auto-regulation	300
3.4. Post-translational modifications	300
3.5. Transcriptional and post-transcriptional control of NFAT	301

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, dichloroacetate; 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; GSK3 β , glycogen-synthase kinase 3 β ; 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- κ B, nuclear factor- κ B; NHD, NFAT homology domain; NLS, nuclear localization sequences; PARP, poly-ADP-ribose polymerase; PGE₂, prostaglandin E2; PI3K, phospho-inositol-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PLC γ , phospholipase C γ ; 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

* Corresponding author at: Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, 1300 S Coulter Street, Amarillo, TX 79106, USA. Tel.: +1 806 414 9248; fax: +1 806 356 5358.

E-mail address: ruiwen.zhang@ttuhsc.edu (R. Zhang).

¹ These authors contributed equally to this work.

4.	The NFAT signaling pathway in cancer development and progression	301
4.1.	Roles of NFAT in malignant transformation and cell proliferation	302
4.2.	Roles of NFAT in cell invasion and metastasis	302
4.3.	Roles of NFAT in angiogenesis	303
4.4.	Roles of NFAT in tumor microenvironment	304
4.5.	NFAT and epigenetic mechanisms	304
5.	Targeting NFAT for cancer prevention and therapy	304
5.1.	Targeting NFAT for cancer prevention	304
5.2.	Targeting NFAT for cancer therapy	304
6.	Future directions and conclusions	307
	Acknowledgments	307
	References	307

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 sub-cellular 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

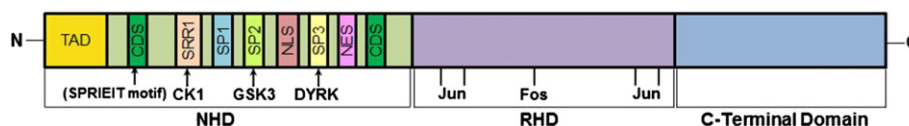


Fig. 1. Schematic structure of NFAT. The figure depicts domains common to NFAT isoforms 1–4. NFAT5 lacks the calcineurin-docking site (CDS) and is calcium unresponsive. The NFAT-homology domain (NHD) contains the transactivation domain (TAD), CDS with SPRIET motif, the serine-rich regions (SRR), the 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.

Table 1
NFAT proteins in the immune system and their roles in vertebrate development.

NFAT	Alternative names	Expression in immune cells	Expression features	Phenotype of knockout mice	Reference
NFAT1	NFATc2 or NFATp	Yes	Widely distributed in tissues; constitutes 80%–90% total NFAT in resting cells	Imbalance in IL-4 transcription; increased lymphocyte proliferation; splenomegaly	[33]
NFAT2	NFATc1 or NFATc	Yes	Upregulated mRNA level in activated T-cells and natural killer cells	Embryonic lethality (dE13.5–17.5); congestive cardiac failure; defects in valve formation; defective T-cell activation; reduction in IL-4 driven antibodies (IG1 and IGE); defective induction of CD40 and FasL (NFAT1/NFAT2 knockout); reduced B cell proliferation (NFAT1/NFAT2 knockout)	[11,31,32,34,35]
NFAT3	NFATc4	No	Implicated in cardiac hypertrophy	Affected viability and fertility	[8]
NFAT4	NFATc3 or NFATx	Yes	Highly expressed in thymocytes	Damaged myofibrils; enhanced lymphocyte proliferation and defects in T-cell positive selection (NFAT1/NFAT4 double knockout); highly increased lethal vascular defects (NFAT3/NFAT4 double knockout); axonal outgrowth defects (NFAT1/NFAT3/NFAT4 knockout)	[35,38]
NFAT5	TonEBP	Yes	Widely distributed in tissues; requires dimerization; maintains osmotic balance in cells	Hypernatremia	[14–16,39,40]

production [35]. Simultaneous NFAT3 and NFAT4 deletion produces lethal defects in embryonic vasculature formation, while deletion of three NFAT family members NFAT1, NFAT3, and NFAT4 causes drastic impairments in axonal outgrowth in the nervous system [38]. Several recent reviews comprehensively discuss the role of NFAT proteins in the immune system and in early embryonic development [5,24,36,37].

3. Regulation of NFAT

3.1. Calcineurin–NFAT signaling pathway

The regulation of the NFAT signaling pathway by calcium and calcineurin has been extensively reviewed [5,12,36]. We present here a brief overview of the calcineurin–NFAT signaling pathway, NFAT kinases, and other mechanisms for NFAT regulation, which have also been depicted in Fig. 2. Briefly, in normal, unstimulated cells, NFAT proteins are present in the cytosol in a hyperphosphorylated, inactive form [17,28,30]. They are activated by the engagement of cell surface receptors such as T-cell receptors (TCR), receptor tyrosine kinases (RTKs), and G-protein coupled receptors (GPCRs) with phospholipase C γ (PLC γ) activation [23,41]. The activation of PLC γ leads to the cleavage of membrane bound phosphatidylinositol 4,5-bisphosphate (PIP₂) and the release of diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP₃) [42]. IP₃ binds to IP₃ receptors on the endoplasmic reticulum (ER) and induces release of Ca²⁺ from intracellular storage sites, triggering the opening of specialized store-operated calcium channels (SOC) [42,43]. The intracellular free Ca²⁺ binds to calmodulin, which in turn, binds to the phosphatase calcineurin. Subsequently, calcineurin is activated, leading to the dephosphorylation and nuclear translocation of NFAT and the induction of NFAT-mediated gene transcription [42–44].

To effectively dephosphorylate NFAT, calcineurin must interact with NFAT at a specific motif in the NHD, which has the PXIXIT (X denotes any amino acid) consensus sequence (such as SPRIET in NFAT1 shown in Fig. 1) [28,30]. It has also been observed that NFAT nuclear localization is concomitant with nuclear relocalization of calcineurin [45]. In fact, NFAT and calcineurin are co-localized in the nucleus of pancreatic cancer cells [46]. Persistent calcineurin activity due to deregulation of upstream calcium signaling is also observed in cancer cells. For example, TRPV6-induced calcium influx in LNCaP cells activates calcineurin and promotes NFAT mediated cell survival and proliferation [47]. Under

normal physiological conditions, calcineurin activity can also be negatively regulated in a calcium independent manner by endogenous inhibitors such as A-kinase anchor protein 79 (AKAP79), calcineurin-binding protein 1 (CABIN1), and Down's syndrome critical region 1 (DSCR1) [48–50]. Deregulation of these negative regulators leads to constitutive calcineurin activation in cancer cells. Once inside the nucleus, the NFAT factors bind to the GGAAA consensus sequence in target gene promoter through homo- or heterodimerization, or co-operation with other transcription factors (Fig. 2) [4]. Depending on these partners and cofactors, NFAT transcription factors can either activate target gene promoters by enhancing local chromatin acetylation, or silence target genes by interacting with histone deacetylases [51].

3.2. Regulation of NFAT by kinases

An important mechanism for the regulation of NFAT transcriptional activity is the removal of NFAT from the nucleus or the NFAT translocation into the cytoplasm. The balance between nuclear import/export of NFAT proteins is tightly controlled by several priming, export, and maintenance NFAT kinases (Fig. 2), such as protein kinase A (PKA) [52], dual-specificity tyrosine-phosphorylation regulated kinase 1a (DYRK1a) [52], glycogen-synthase kinase 3 β (GSK3 β) [53], and casein kinase (CK1) [54], respectively. In cases of low intracellular calcium levels, nuclear import of NFAT proteins is abolished by a highly effective nuclear export mechanism, comprising nuclear priming kinases, such as DYRK1a and PKA [52]. DYRK1a phosphorylates nuclear NFAT(s), creating substrate sites (i.e. priming) for subsequent rephosphorylation by GSK3 β and nuclear export [52]. Export kinases facilitate nuclear translocation of the NFAT proteins while maintenance kinases retain NFAT proteins in the cytosol in a hyperphosphorylated state and prevent their nuclear translocation. GSK3 β rephosphorylation may not always result in negative regulation of NFAT transcriptional activity [55]. For example, GSK3 β mediated phosphorylation of the serine rich SP2 domain in NFAT1 protein seems to stabilize NFAT1 in cancer cells by protecting it from rapid ubiquitination and proteasomal degradation [55]. This may be a mechanism by which GSK3 β deregulation contributes to cancer development and progression [56].

NFAT retention in the cytosol is controlled via several maintenance kinases that phosphorylate the proteins at the N-terminus. These include CK1, mitogen activated protein kinases (MAPKs), c-JUN kinase (JNK), and extra-cellular signal related kinase (ERK) [57–63]. CK1

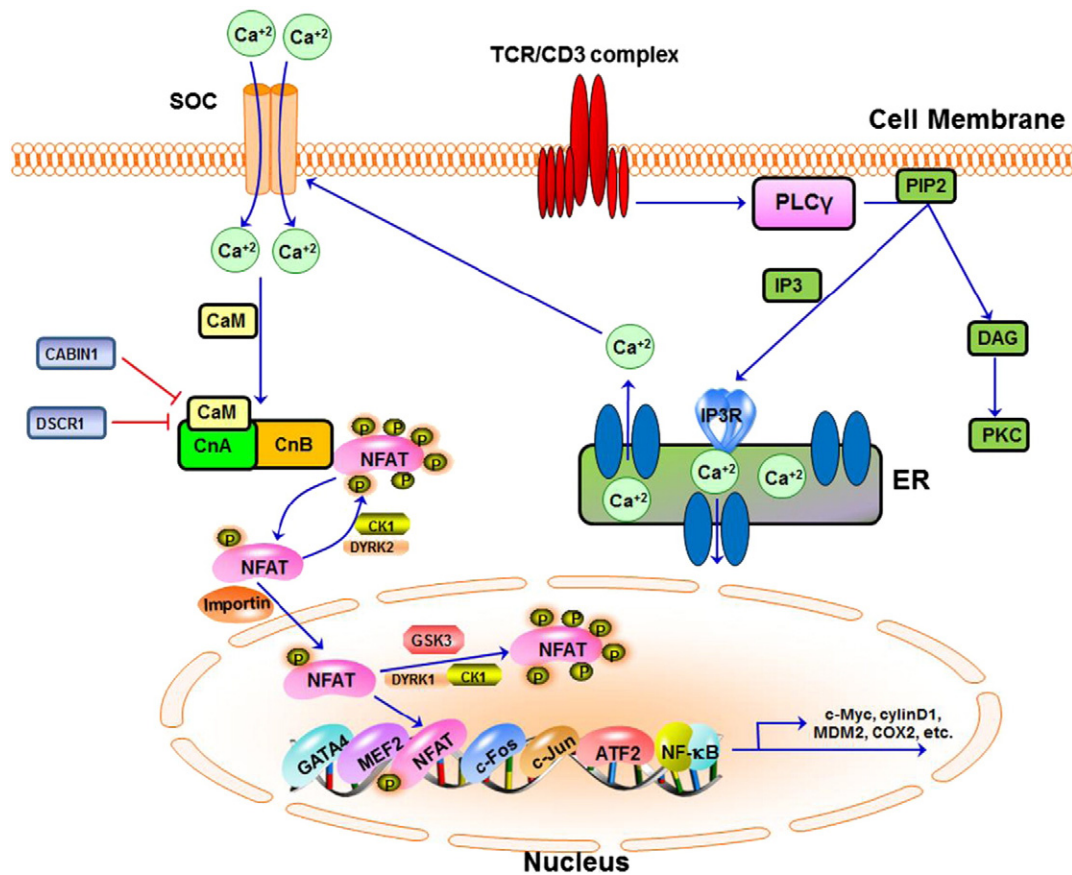


Fig. 2. The calcineurin–NFAT pathway: Activation and regulation. Inositol-1,4,5-trisphosphate (IP₃), generated by phospholipase C γ (PLC γ) via cleavage of phosphatidylinositol-4,5-bisphosphate (PIP₂), binds to the IP₃ receptor (IP₃R) and causes the release of Ca²⁺ from the endoplasmic reticulum (ER). This Ca²⁺ depletion is sensed by store-operated calcium channels (SOC). Influx of extracellular Ca²⁺ into the cytosol causes calmodulin (CaM) to bind to calcineurin (composed of calmodulin binding part Calcineurin A-CnA, and regulatory subunit Calcineurin B-CnB), causing its activation. Calcineurin is a phosphatase that dephosphorylates NFAT and leads to its nuclear translocation. The calcineurin-binding protein 1 (CABIN1) and Down's syndrome critical region 1 (DSCR1) protein are endogenous inhibitors of calcineurin. In the nucleus, the NFAT proteins interact with multiple transcriptional partners (such as GATA4, MEF2, c-Fos, c-Jun, etc.) to regulate gene expression. NFAT proteins undergo rephosphorylation and inactivation by multiple NFAT kinases, such as glycogen-synthase kinase 3 (GSK3), casein kinase 1 (CK1), and dual-specificity tyrosine-phosphorylation regulated kinase1/2 (DYRK1 and DYRK2). CK1 and DYRK2 also maintain NFATs in the cytoplasm in hyperphosphorylated state. ATF2: activating transcription factor 2; DAG: diacylglycerol; MEF2: myocyte enhancer factor-2; PKC: protein kinase C; TCR: T cell receptor.

phosphorylates the SRR1 motif of NFAT1 and serves as both an export and maintenance kinase [54,58]. CK1 docks at a conserved FSILF sequence motif near the N terminus [54]. Transgenic mice with a mutation at this CK1 docking site present several defects in embryonic and hematopoietic cell development, indicating the crucial role of CK1 in NFAT regulation [60]. The MAPKs also promote NFAT retention in the cytoplasm but positively affect NFAT transcriptional activity [61,62]. JNK, ERK, and p38 physically interact with the NFAT N-terminal region to phosphorylate conserved NFAT Ser-Pro motifs and Ser-172, thereby inhibiting NFAT nuclear import [62,63]. It is noteworthy that MAPK pathways are often activated in human cancers [64]. Thus, NFAT export to the cytosol may not limit NFAT signaling, but actually facilitates NFAT signaling [59,62].

3.3. NFAT2 auto-regulation

In addition to modulation of NFAT turnover and cellular sublocalization via various NFAT modifying enzymes, regulation of individual NFAT isoform expression can also influence the physiological manifestations of NFAT transcriptional activity [5]. For example, NFAT2 is capable of existing as three distinct isoforms: NFAT2A, NFAT2B and NFAT2C [65]. The longer B and C isoforms are formed via alternative splicing and polyadenylation at the distal pA2 promoter

site, whereas the short isoform A arises from polyadenylation at the proximal pA1 site [66]. A positive autoregulatory loop regulates the differential expression of these isoforms. While NFAT2B and NFAT2C are expressed constitutively in naive T cells, NFAT2A (the shorter isoform) has a higher expression in effector T cells via the regulation by an NFAT-dependent inducible promoter [65]. The NFAT2 isoform is thus, preferentially, accumulated during cell lineage commitment and plays a key role in the differentiation of naive T cells to diverse effector T cell populations [66]. Inducible synthesis of NFAT2A is also crucial for osteoclast generation and for cardiac valve development in the maturing heart [67,68]. Thus, NFAT2A is an important orchestrator of cell fate determination and, consequently, deletion of NFAT2A is generally more harmful to development as compared to deletion of other NFAT family members.

3.4. Post-translational modifications

Apart from phosphorylation, various other post-translational modifications have been reported for NFAT proteins. Ubiquitination provides a mechanism for NFAT deactivation and turnover, while sumoylation of NFAT1 and NFAT2 isoforms results in their nuclear retention [69,70]. Small ubiquitin-like modifier 1 (SUMO1) targets the NFAT2C long isoform at two sites on its C-terminus, causing its nuclear translocation

and interaction with promyelocytic leukemia (PML) nuclear bodies [69]. The sumoylated NFAT2C then recruits histone deacetylases (HDACs) and deacetylates histones within the IL-2 promoter, thus suppressing IL-2 activity [69]. Thus, sumoylation transforms NFAT2C from a transcriptional activator to a repressor [69]. NFAT1 is ubiquitinated by the E3 ubiquitin ligase MDM2 in breast cancer cells [70]. Whether all NFAT isoforms are modified by ubiquitination and subsequently undergo proteasomal degradation remains to be clarified. Another post-translational modification that influences the mode and magnitude of NFAT activity is adenosine di-phosphate (ADP)-ribosylation. Poly-ADP-ribose polymerase (PARP) binds to NFAT proteins to induce ADP-ribosylation, increasing its DNA binding activity [71,72].

3.5. Transcriptional and post-transcriptional control of NFAT

NFAT transcription factors, due to their weak DNA binding capabilities, often partner with other factors to gain transcription regulation. For example, the transcription factor activator protein 1 (AP1) forms a quaternary complex with the NFAT and DNA to trigger T-cell activation [73,74], while NFAT partners with forkhead box P3 (FOXP3) for immunotolerance, and with GATA to control cell development [75,76]. Thus, the AP1 proteins (dimers of Fos and Jun) majorly partner with NFAT during T-cell activation to integrate the two signaling pathways induced in response to T-cell activation: calcium signaling and the RAS–MAPK pathway [73]. In addition to these, other cellular proteins have been identified that form stable nuclear complexes with NFAT. For example, the transcription factor Stat3 has been shown to be activated in PANC-1 cells through an NFAT2 induced autocrine factor [77]. Furthermore, shRNA depletion of Stat3 decreases the transformative capacity of NFAT2, suggesting that both factors act co-operatively to mediate malignant transformation [77]. It seems that the NFAT proteins act as signal integrators and detectors, integrating inflammatory, developmental, or oncogenic signals with Ca^{2+} -calcineurin pathway. Apart from transcriptional regulation of NFAT, proteolytic enzymes like caspase-3 and caspase-8 exert post-transcriptional control on NFAT activity [78]. NFAT1 and NFAT2 undergo rapid degradation in T-effector cells via cleavage by caspases. These effects can occur either via physical interaction (AP1, FOXP3, GATA, caspases) or through interaction with upstream components of calcineurin–NFAT pathway. Several of these “effectors” of NFAT activity are specific to cell type and physiological

and pathophysiological context. We summarize these various proteins that affect NFAT activity in Table 2.

In summary, the sensitivity to intracellular calcium flux, the control of nuclear export/import by NFAT kinases, the diverse post-translational modifications, and the transcriptional and post-transcriptional regulation fine-tune NFAT-mediated gene transcription.

4. The NFAT signaling pathway in cancer development and progression

The wide range of cellular processes controlled by the NFAT proteins and their crucial role in embryonic development, organogenesis, and cell fate determination indicates a strong oncogenic potential for this family of transcription factors. This oncogenic potential of NFAT proteins is further validated by their involvement in the regulation of genes that control cell cycle progression, cell development and differentiation, cell motility, tumorigenesis, and angiogenesis [22]. Moreover, it has been observed that the NFAT family members are constitutively activated and/or overexpressed in several cancer types, including breast cancer [79,80], pancreatic cancer [46], aggressive T cell lymphoma [94], Burkitt's lymphoma [95], and diffuse large B cell lymphoma [94,96,97]. For example, NFAT2 was shown to induce cell transformation and anchorage-independent cell growth in pancreatic cancers via its activation and overexpression [46]. Apart from the increased protein levels of NFAT family members, aberrations in the NFAT gene(s) have also been identified [98–100]. For example, an aggressive childhood sarcoma, Ewing sarcoma presents with chromosomal translocation in the NFAT1 gene and formation of a frequently amplified chimera gene by frame-fusion with the Ewing sarcoma breakpoint region 1 (EWSR1) gene [98–100]. Here, we have reviewed the studies of the NFAT expression in different cancers and presented a summary in Table 3.

Interestingly, the NFAT isoform NFAT1 has been shown to possess tumor suppressor activity in certain cellular contexts. The enforced expression of NFAT1 is seen to promote apoptosis of cell lines derived from Burkitt's lymphoma [115]. Similarly, NFAT1^{−/−} mice show increased vulnerability to chemically induced carcinogenesis than wild-type mice [108,116]. Though NFAT1^{−/−} mice are more susceptible to tumor growth, tumor progression is impeded in the absence of NFAT1 expression [116]. In fact, NFAT1 expression often correlates with aggressive invasive behavior in solid tumors [79,80,114]. This leads us to

Table 2
Transcriptional regulators of NFAT proteins and their biological effects.

Transcriptional regulator	NFAT isoforms	Effects on NFAT and/or biological consequences	Cell types	Reference
Akt	NFAT1	Inhibits NFAT1 nuclear localization and blocks breast cancer cell growth/migration; induces proteasomal degradation of NFAT1	MDA-MB-435	[79,80]
$\alpha\beta\gamma$	NFAT5	Activates NFAT5 transcription; increases cell invasion	MDA-MB-435, MDA-MB-231	[81]
AP1	NFAT1/2	Forms quaternary complex with NFAT and DNA to trigger T-cell activation	T-cells	[73,74]
Bcl-2	NFAT	Inhibits NFAT transcriptional activity and plays a pro-apoptotic role in the aged and oxidatively stressed central nervous system	PC12, HEK293, NIH-3T3	[82]
Caspase-3	NFAT1	Induces proteolytic cleavage of NFAT1	T-cells	[78]
EGFR	NFAT	Activates store-operated calcium entry into cells, leading to activation of NFAT and its downstream target COX-2	A431	[83]
FOXP3	NFAT1/2	Interacts with NFAT and causes immunotolerance; constitutively activates NFAT 1/2, independent of calcineurin activity	T regulatory cells	[75,76]
GATA	NFAT	Interacts with NFAT and increases cell growth and rate of proliferation (cardiac cell hypertrophy)	Cardiac myocytes	[84]
MDM2	NFAT1	Induces the ubiquitination and proteasomal degradation of NFAT1	MDA-MB-435, MDA-MB-231, SUM-159-PT	[55,79,80]
NF- κ B	NFAT	Interacts with NFAT and regulates its transcriptional activity and subcellular localization	T-cells, rat cardiomyocytes	[85–88]
Notch	NFAT4	Induces NFAT4 nuclear localization	Keratinocytes	[89]
p53-K120R mutant	NFAT	Activates NFAT	U-87, HepG2, YES-4	[90]
Stat3	NFAT2	Interacts with NFAT and induces malignant transformation	PANC-1	[77]
VEGF	NFAT1	Dephosphorylates and activates NFAT	HUVEC	[91–93]

Table 3
Epidemiological and clinical evidence connecting NFAT and cancer.

Cancer type	NFAT isoform	Proposed mechanism(s)	Clinical/biological outcomes	Reference
Ewing sarcoma	NFAT1	Amplified chimera due to chromosomal gene translocation	Not known	[98–100]
T-cell leukemia	NFAT	Calcineurin activation and NFAT nucleus translocation	Chemoresistance	[88,94,101]
Diffuse large B-cell lymphoma	NFAT2	Constitutively activated; Interacts with NF- κ B, binds to the CD154 promoter, and synergistically activates CD154 gene transcription	Increased tumor growth	[94,96,97]
Chronic lymphocytic leukemia	NFAT2	Overexpressed and constitutively activated	Increased cancer progression	[102,103]
Chronic myelogenous leukemia	NFAT2	Constitutively activated	Chemoresistance	[104]
Breast cancer	NFAT1, NFAT5	Overexpressed	Increased metastatic growth	[79,80]
Colon cancer	NFAT1	Constitutively activated	Induces tumor progression	[105]
Pancreatic cancer	NFAT2	Overexpressed	Increased tumor growth	[46]
Prostate cancer	NFAT	Activated NFAT promoter by TRPV6-mediated Ca^{+2} influx	Increased cell proliferation	[47]
Angiosarcoma	NFAT4	Activated by SFRP2	Increased angiogenesis	[106]
Melanoma	NFAT	Increased NFAT activity via BRAF-MEK-ERK pathway and a TGF- β dependent pathway	Increased migration and invasion	[107,108]
Endometrial cancer	NFAT	Regulation of IL11 and CXCL8 expression	Increased migration	[109,110]
Non-small cell lung cancer	NFAT	Overexpressed	Decreased post-operative survival	[111,112]
Glioblastoma	NFAT1	Overexpressed	Increased invasiveness	[113,114]

speculate whether the primary role of NFAT1 might be in the promotion of cell migration rather than tumor initiation. Although the molecular mechanisms for the oncogenic functions of NFAT proteins still remain to be elucidated, numerous important findings have been reported. In the later part of this section, we present a comprehensive overview of NFATs' roles in cancer development and progression, which is also depicted in Fig. 3.

4.1. Roles of NFAT in malignant transformation and cell proliferation

Several studies have investigated the role of the NFAT transcription factors in various aspects of malignant cell transformation and the tumorigenic process. NFAT2 mutants, which are constitutively localized to the nucleus, are reported to inhibit differentiation, induce malignant transformation, and increase cell proliferation in 3T3-L1 fibroblasts [117]. In contrast, nuclear localization of NFAT1 in fibroblasts leads to cell cycle arrest and apoptosis [115,116]. NFAT1 inhibits cyclin dependent kinase 4 (CDK4) and cyclin A2 expression, indicating an important role in the control of cell proliferation [118,119]. Mice deficient in NFAT1 and NFAT4 exhibit decreased activation-induced cell death (AICD), impaired Fas ligand (FasL) induction, and increased lymphoproliferation, thus providing evidence of their tumor suppressor activities [35,120]. However, in breast cancer cells, NFAT1 has been shown to induce MDM2 transcription and increases inactivation of p53, thus exhibiting pro-proliferation and anti-apoptotic properties [121]. In pancreatic cancer, NFAT1 has been shown to bind and silence the tumor suppressor gene p15^{INK4b} via the histone methyltransferase Suv39H1 [122]. Interestingly, NFAT1 is seen to be induced in advanced stages of pancreatic carcinoma, reinforcing the fact that it is central to tumor progression [122]. On the other hand, NFAT (especially NFAT2) and TGF- β act co-operatively to promote TGF- β driven cell proliferation and NFAT nuclear accumulation [123–126]. In pancreatic cancer cells, NFAT2 mediates the displacement of the Smad3 repressor from the c-Myc gene promoter and subsequent activation of c-Myc transcription [46,125]. The activated c-Myc, in turn, interacts with the NFAT complexes to transactivate several growth promoting elements, such as cyclin D1/D3, resulting in cell cycle progression [46]. NFAT2 also mediates the switch between stem cell dormancy and proliferation [127]. NFAT2 acts as the downstream of bone morphogenetic protein 4 (BMP4) in dormant stem cells, leading to the inhibition of check point kinases, such as CDK4. This process maintains the state of dormancy in the stem cell population [127]. It can also promote tumor progression

through the creation of a tumor cell population that possesses stem cell characteristics with self-renewal capacity [127,128].

4.2. Roles of NFAT in cell invasion and metastasis

Recent findings have established NFAT as a multifunctional and powerful regulator of the tumor progression and invasion process, particularly in breast cancer [79,80,129]. Constitutively active nuclear NFAT1 drives breast cancer cell migration and invasion through Matrigel in vitro, whereas NFAT5 expression promotes cell migration [79,80]. In an MMTV-neu breast cancer transgenic mouse model, treatment with tacrolimus (a calcineurin-NFAT signaling inhibitor, also known as FK506) results in the reduction of tumor microvascular density and tumor growth rate [130]. The effects of NFAT1 on breast cancer cell invasion are countered by Akt which induces MDM2 mediated proteasomal degradation of NFAT1 [80]. Interestingly, our group has recently demonstrated the presence of a consensus binding site for NFAT1 in the human *mdm2* P2 promoter [121]. High levels of both NFAT1 and MDM2 proteins were observed in human hepatocellular carcinoma tissues as compared to normal tissues, providing a basis for studying the NFAT-MDM2-p53 axis for cancer therapy [121]. Furthermore, it has been seen in breast cancer that a significant positive correlation exists between $\alpha 6 \beta 4$ integrin expression and that of NFAT1 and NFAT5 [81]. Enhanced expression of NFAT1 and NFAT5 along with $\alpha 6 \beta 4$ integrin is observed in both invasive breast cancer cells as also in patients with this disease [81]. The $\alpha 6 \beta 4$ integrin is released from hemidesmosomes in cancer cells and attaches to the actin cytoskeleton, activating NFAT5 transcription and facilitating cancer cell metastasis via activation of downstream targets such as COX-2 [129,131]. COX-2 catalyzes the synthesis of prostaglandin E2 (PGE₂), a potent mitogen that promotes cell invasion through the extra-cellular membrane (ECM) [132]. Further, the anti-metastatic Wnt ligand WNT5A is seen to block NFAT activation in human breast epithelial cells via binding to NFAT maintenance kinase, CK1 [133,134]. CK1, as discussed earlier, helps to keep the NFAT protein(s) in a hyperphosphorylated inactive form in the cytoplasm. NFAT1 can also bind to the promoter of glypican-6 (GPC6) and activate its transcription, increasing the invasiveness of breast cancer cells [135]. In addition to COX-2, NFATs also induce the transcription of pro-invasive genes such as autotaxin, in breast epithelial cells. Autotaxin mediates the conversion of lysophosphatidylcholine into lysophosphatidic acid (LPA) which promotes invasive and metastatic mammary carcinoma [136,137].

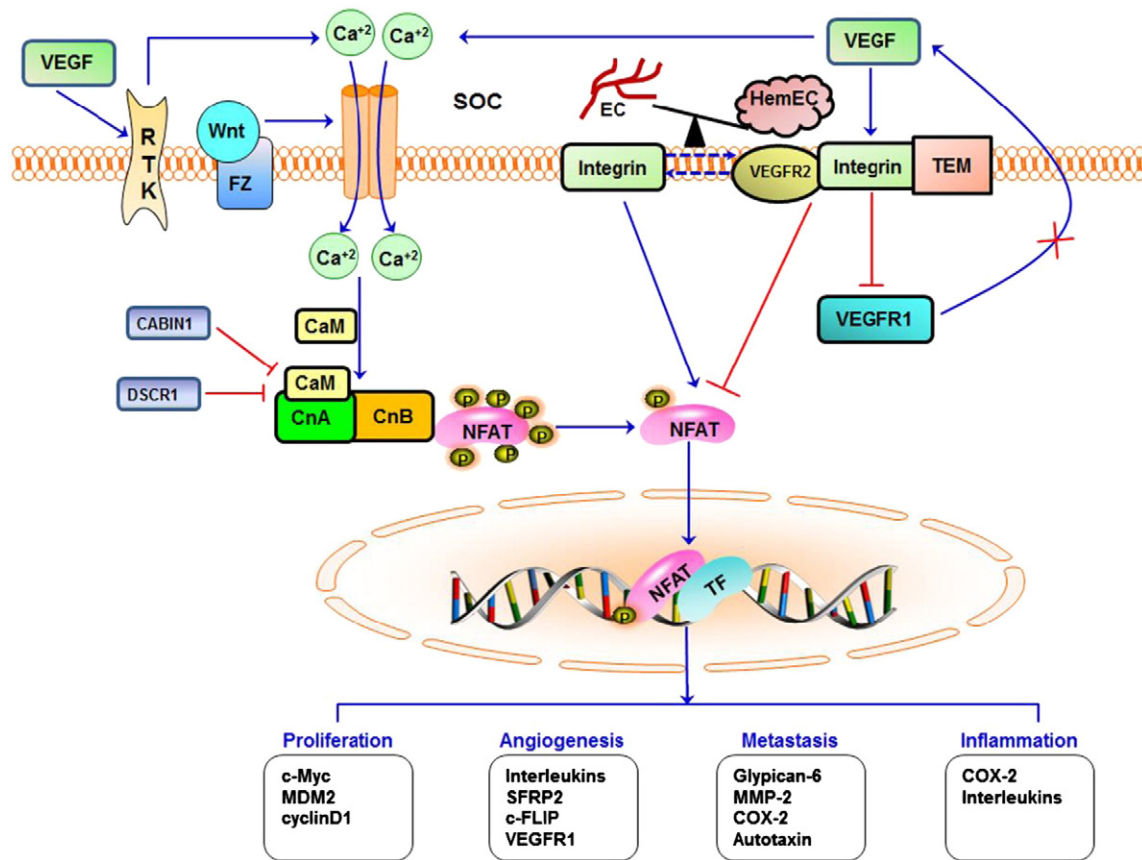


Fig. 3. Promotion of cancer development and progression by NFAT. In various cancers, NFAT can activate downstream targets to cause enhanced cell proliferation, inflammation, metastasis, and angiogenesis. However, the NFAT activity is cell-type and context dependent and is responsive to the external stimuli such as the activation of receptor tyrosine kinases (RTKs), integrin, and Wnt pathway. Endogenous inhibitors of calcineurin–NFAT, such as DSCR1 and CABIN1 also block activation of NFATs in endothelial cells and are potent inhibitors of tumor angiogenesis. NFAT controls angiogenesis via negative regulation of VEGF. In normal endothelial cells (ECs), activation of $\beta 1$ integrin leads to NFAT-dependent transcription of VEGFR1. VEGF levels are kept in check and normal angiogenesis takes place. This pathway can be inhibited by complex formation between $\beta 1$ integrin, VEGFR2, and tumor endothelial marker-8 (TEM8). In hemangioma endothelial cells (HemECs), VEGF signaling is constitutively activated due to enhanced complex formation in HemECs versus normal endothelial cells, which then leads to decreased VEGFR1 transcription. In the normal cell, VEGFR1 inhibits VEGF expression, normalizing vascular growth. TEM8 and VEGFR2 negatively regulate $\beta 1$ integrin activation and in turn suppress NFAT transcriptional activity. VEGF can activate NFAT signaling via increased Calcium influx and via activation of RTKs. TF, transcription factors; CABIN1, calcineurin-binding protein 1; DSCR1, Down's syndrome critical region 1; SOC, store-operated calcium channels; TEM8, tumor endothelial marker-8; VEGF, Vascular endothelial growth factor; VEGFR1, VEGF receptor 1; VEGFR2, VEGF receptor 2.

As a result of their invasion promoting characteristics, NFAT proteins are expected to regulate the transcription of matrix metalloproteinases (MMPs) that mediate the proteolytic degradation of basement membrane during tumor invasion and metastasis [138]. NFATs have been shown to be required for MMP activation in ECM remodeling activity of atrial myocytes and mesangial cells [138]. In an inbred genetic mouse model (Czech-II/Ei mouse) that produces tumors resembling human osteosarcoma metastasizing to the lungs, cell invasion is correlated with elevated levels of the MMP-2 and NFAT acts as an upstream regulator of this metalloprotease [139]. Recently, another new signaling axis involving NFAT, calcineurin–NFAT–angiopoietin-2 (Ang-2) signaling, has been demonstrated to be critical for the establishment of lung metastases [140]. Vascular endothelial growth factor (VEGF) levels in the lung trigger a threshold of calcineurin–NFAT signaling that transactivates Ang2 in lung endothelium, promoting angiogenesis and metastases [140].

4.3. Roles of NFAT in angiogenesis

The pro-angiogenic role of NFAT signaling was first demonstrated in *Nfat3/Nfat4* null mice and in the calcineurin B (*Cnb1*) knockout mice [8]. Mice lacking *Cnb1* or both *Nfat3/Nfat4* genes die at mid-gestation due to

disorganized vasculature and increased and deregulated expression of VEGFA [8,141]. NFAT appears to modulate the expression of VEGF by regulating the transcription of VEGF receptor 1 (VEGFR1). In infantile haemangiomas, absence of NFAT1 leads to decreased levels of VEGFR1, which leads to increased and aberrant expression of VEGF via a feedback mechanism [91–93]. VEGF stimulates PLC γ receptor-mediated activation, increasing intracellular calcium levels that activate calcineurin to cause NFAT nuclear translocation [142]. Nuclear NFAT switches on the transcription of pro-angiogenic genes such as COX-2, resulting in the synthesis of PGE₂ [129,131,136]. Though NFAT has an inhibitory effect on VEGF expression, VEGF can induce NFAT transcriptional activity by mediating its nuclear translocation [92,93]. NFAT activation by VEGF in endothelial cells also induces the pro-angiogenic factor granulocyte-macrophage colony-stimulating factor (GM-CSF) [143]. In fact, treatment with the calcineurin inhibitor cyclosporin A (CsA) leads to inhibition of VEGF-induced COX2 expression in endothelial cells [91]. Moreover, endogenous inhibitors of NFATs, such as DSCR1, are also potent inhibitors of tumor angiogenesis [144]. All these findings underscore the primary role played by NFAT proteins in the regulation of angiogenesis.

As discussed earlier, NFAT2 regulates lymphangiogenesis, especially the lymphatic patterning process and the subsequent valve formation

[4,8]. In this case, NFAT2 functions downstream of VEGFC, interacting with lymphangiogenesis promoting factors such as forkhead box C2 (FOXC2), VEGFR3, prospero-homeobox 1 (PROX1), and podoplanin [145]. This role of NFAT2 may contribute to its tumorigenic activity in hematological malignancies. Inhibition of NFAT4 reduces the SFRP2-stimulated angiogenesis in vitro, and inhibition of calcineurin with tacrolimus also blocks SFRP2-stimulated angiogenesis and angiosarcoma growth [130]. Zaichuk et al. propose that NFAT balances its effect on angiogenesis by inducing c-FLIP, a caspase 8 inhibitor, while concomitantly being sequestered in the cytoplasm by JNK [146].

4.4. Roles of NFAT in tumor microenvironment

Early studies on NFAT identified it as a transcriptional activator of chemokines in immune cells [21]. Inflammatory chemokines are, often, highly expressed in advanced forms of cancer and mediate metastatic invasion by promoting chemotaxis and migration of epithelial cells [147]. NFATs, due to their close association with chemokine activity, are expected to play an important role in tumor microenvironment modeling. Though NFAT isoforms (both mRNA and protein) have been detected in several cancer cell types, it is not clear if NFAT family members are endogenously expressed in fibroblasts in the tumor stroma, specifically those associated with carcinoma. Cytokine components of tumor-associated tissue possess the ability to direct the differentiation of infiltrating cells toward tumoristatic or tumor-promoting phenotypes [147]. NFAT transcription factors maintain a balance between the chemokine and cytokine factors via regulation of interleukin and IFN- γ expression by lymphocytes, and hence impact both pro- and anti-tumorigenic responses [108,116]. NFAT signaling in the tumor microenvironment probably impacts tumor progression and metastasis positively since several murine models of leukemia and lymphoma reveal hyperactivation of NFAT [94–97]. NFAT hyperactivation, likely, leads to tumor cell migration via a paracrine signaling loop involving infiltrating macrophages that secrete EGF and CSF1 (colony-stimulating factor-1) and tumor cells expressing EGFR [83]. EGFR activates store operated calcium entry into the cells thus setting in motion the calcineurin-NFAT signaling cascade [83]. Decreased IL-4 and TGF- β expression in the absence of NFAT1 also validate its ability to promote tumor progression via modulation of the tumor microenvironment [148].

4.5. NFAT and epigenetic mechanisms

As discussed earlier, NFAT proteins need other binding partners to activate gene transcription due to their imperfectly formed REL domain. Other than co-operation with their transcriptional partners, the NFAT proteins also increase chromatin acetylation to activate downstream targets or interact with histone deacetylases to silence target genes [51,96]. For example, NFAT2 regulates gene expression in diffuse B-lymphoma cells by conscripting the ATPase SMARCA4 (a chromatin remodeling complex enzyme) to NFAT2 targeted gene promoters [96]. This complex then employs additional factors to the active chromatin site to modulate gene transcription and transactivate proliferative and antiapoptotic downstream targets. Though the roles of NFAT in controlling miRNA in cardiac growth have been studied [149,150], few studies exist with regards to the regulation of NFAT by miRNAs in cancer. Recently, miR-1246 has been identified as a novel target of p53 and its homologues p63 and p73 [151]. MiR-1246 suppresses the expression of DYRK1A, decreases nuclear export of NFAT and activates NFAT [151]. Upon oncogenic stress, it was postulated that p53 activation might enhance the anticancer immune response by activating NFAT and preventing its nuclear export via DYRK1A. In this case, NFAT is expected to cause increased tumor surveillance effects, exerting antitumorigenic properties. Thus, we see that NFAT proteins employ both genetic as well as epigenetic means to affect various cellular signaling molecules, and this complex interplay is expected to regulate its

diverse roles in a wide range of functions from cell cycle control to cellular invasion.

5. Targeting NFAT for cancer prevention and therapy

Our discussion, so far, has highlighted the crucial importance of NFAT as a regulator of both tumor development as well as progression. Based on their oncogenic potential, the NFAT family seems to be an attractive target for both cancer prevention and therapy. We will discuss the validity of NFAT as a viable chemotherapeutic/chemopreventive target in the following paragraphs.

5.1. Targeting NFAT for cancer prevention

Although novel chemotherapeutic and surgical interventions have reduced cancer mortality over the years, several cancer types, often, are unresponsive to therapy or develop resistance quickly or present a high rate of relapse and metastasis [152]. Due to its multistep progression, prevention remains the most effective way to reduce cancer related morbidities [153]. Increasing evidences demonstrating the key role of NFAT in cancer development and progression suggest NFAT as a potential target for cancer chemoprevention [22]. Interestingly, the NFAT signaling axis is activated upon exposure to known environmental carcinogens such as arsenite [154], benzo[a]pyrene [155], nickel [156–158], and vanadium [159]. Arsenite and vanadium pentoxide cause induction of COX-2 expression in an NFAT-dependent manner, activating pro-survival pathways and mediating resistance to apoptosis in human bronchial epithelial Beas-2B cells [154,159]. It has been postulated that the carcinogenesis of vanadium to human bronchial cells may result from cell survival mediated by the NFAT-dependent induction of COX-2 [159]. Moreover, nickel compounds induce NFAT activation via generation of H₂O₂ [158]. These findings reveal the role of NFAT activation as a tumorigenesis and tumor progression mechanism. Thus, inhibition of NFAT activation and its downstream pro-inflammatory molecules might be an attractive and effective approach toward chemoprevention. For example, dietary components such as black raspberry extracts have been shown to block NFAT activation [160]. The flavonoids in blackberries inhibit NFAT activation downstream of the PI3K/Akt (phospho-inositol-3-kinase-Akt) pathway. These fractions also inhibit VEGF activation [160]. Interestingly, a phosphorylated derivative of the anti-inflammatory compound sulindac, phospho-sulindac has been recently identified as inducing NFAT2 in pancreatic cancer cell lines [161]. Exogenous knockdown of NFAT2 in pancreatic cancer cell lines increased their sensitivity to phospho-sulindac [161]. However, few studies have been performed yet to fully explore the validity of NFAT as a cancer target in vivo (especially clinical studies). Rationally developed combination treatments involving natural products along with chemotherapeutics seem to be a better choice for cancer chemoprevention. This strategy would improve the efficacy of cancer prevention while eliminating possible side effects. The key question unanswered is whether NFAT inhibition can decrease human cancer incidence in vivo and reduce tumor burden.

5.2. Targeting NFAT for cancer therapy

As already noted, the oncogenic potential of NFAT has led to speculation that pharmacological or genetic targeting of NFAT proteins would be an attractive approach in cancer therapy. Indeed, the two classical inhibitors of the calcineurin–NFAT signaling axis, CsA and tacrolimus, have shown significant anticancer activity [88,162–164]. Mechanistically, both CsA and tacrolimus bind to the immunophilin proteins and form a drug-immunophilin complex that directly binds to calcineurin, inhibiting calcineurin activity [45]. By interfering with calcineurin activity, both CsA and tacrolimus inhibit the dephosphorylation of numerous substrates, including NFAT proteins. Moreover, calcineurin also modulates other signaling pathways such as the

Table 4

Summary of NFAT inhibitors and their mechanisms of action.

Inhibitors	Mechanism(s) of action	Cancer models	Pharmacological effects	Reference
<i>Strategy 1: Target upstream regulators of NFAT</i>				
PxxIT peptides	Competes with NFAT and blocks its binding to calcineurin	NR	Inhibits NFAT driven gene expression in Jurkat T cells	[170]
LxVP peptide	Competes with NFAT and blocks its binding to calcineurin	NR	Exerts anti-inflammatory activity in macrophages; Inhibits p38 activation	[171]
VIVIT peptide	Blocks calcineurin–NFAT interaction	Chronic lymphocytic leukemia	Prevents IgM-induced cell survival; Exerts anti-inflammatory activity	[103,172]
11R VIVIT peptide	Blocks calcineurin–NFAT interaction.	NR	Inhibits macrophage cytokine expression; Attenuates colitis in experimental models	[173]
Cyclosporin A (CsA)	Binds to immunophilins and inhibit calcineurin activity.	T-cell leukemia and colorectal cancer	Regulates c-Myc, p21, and PCNA levels and then reduces cell proliferation; Inhibits multidrug resistance proteins	[88,162,174–176]
ISA247 (voclosporin)	Binds to immunophilins and inhibit calcineurin activity.	NR	Shows better bioavailability and efficacy than CsA	[177,178]
Tacrolimus (FK-506)	Binds to FK-506 Binding Protein (FKBP12) and inhibit calcineurin activity.	Chronic lymphocytic leukemia and prostate cancer	Promotes apoptosis	[163,164]
BTP2 (YM-58483)	Decreases SOC-channel-dependent Ca^{2+} influx via depolarization of cell membrane	NR	Inhibits proliferation and Ca^{2+} -dependent cytokine production in stimulated human $CD4^{+}$ T cells	[179,180]
McKeon compounds	Interferes with intracellular calcium mobilization involving store-operated calcium channels	NR	NR	[181]
Trifluoperazine	Binds to calmodulin and blocks its interaction with calcineurin	Gefitinib-resistant lung cancer	Suppresses IL-2 expression of α CD3/PMA-activated Jurkat T cells	[182]
Kaempferol	Inhibits phosphatase activity of calcineurin A by binding to its catalytic domain	Ovarian cancer	Suppresses IL-2 gene expression in Jurkat T cells; Inhibits TNF α -induced NF- κ B activation in HEK293 cells	[183–186]
Thiopental	Inhibits phosphatase activity of calcineurin	NR	Inhibits NF- κ B activation in Jurkat cells and in primary $CD3^{+}$ lymphocytes	[187]
INCA-1, 2, and 6	Block calcineurin–NFAT interaction via binding at residue Cys266 of calcineurin	NR	Inhibit the induction of downstream cytokine mRNAs	[188]
NCI3	Blocks calcineurin–NFAT interaction by binding to calcineurin and causing allosteric change	NR	Inhibits IL-2 secretion and cell proliferation upon stimulation of Jurkat or primary human T cells.	[189]
Dipyridamole	Blocks calcineurin–NFAT interaction	Breast and pancreatic cancer	Inhibits NFAT-dependent reporter gene and cytokine expression	[190,191]
Dichloroacetate (DCA)	Decreases intracellular Ca^{+2} via NFAT–Kv1.5 pathway	Glioblastoma, lung, breast, and endometrial cancer	Promotes apoptosis	[192,193]
Dehydroepiandrosterone (DHEA)	Inhibits Akt/GSK3- β /NFAT axis.	Breast cancer	Reverses systemic vascular remodeling following vascular injury	[194]
Curcumin	Inhibits Ca^{+2} mobilization	Various cancer	Suppresses T-cell activation; Inhibits IL-2 production	[195]
<i>Strategy 2: Directly target NFAT</i>				
ST-1959	Enhances NFAT1 nuclear export	NR	Inhibits T-cell activation, proliferation and cytokine production	[196]
Roc-1, 2 and 3	Inhibit NFAT2 nuclear translocation	NR	Reduce expression of IL-2, IL-4, IFN γ and TNF α ; Inhibit nuclear localization of c-jun	[197]
Helenalin	Inhibits NFAT1 nuclear translocation	Renal cell carcinoma	Induces G2/M cell cycle arrest via p21; Inhibits IL-2 production	[198]
Genistein	Reduces mRNA and protein expression of NFAT1	Liver cancer	Promotes apoptosis	[199,200]
Zoledronic acid	Induces NFAT1 ubiquitination and degradation	Breast and pancreatic cancer	Inhibits tumor cell growth by inducing G1 cell cycle arrest	[55]
<i>Strategy 3: Block NFAT–DNA binding</i>				
UR-1505	Blocks the binding of NFAT1 to DNA	NR	Inhibits T cell proliferation and IL-5 as well as IFN γ expression; exerts anti-inflammatory in rat colitis model	[201,202]
Triflusal	Inhibits NFAT1–DNA complex formation, and NF- κ B activation	NR	Inhibits expression of IL-2, IL-3, GM-CSF, TNF- α , TGF- β 1, lymphotactin, MIP-1 α , MIP-1 β , IFN- γ , and TNF- α , in Jurkat T cells	[203]
Caffeic acid phenethyl ester (CAPE)	Inhibits NFAT nuclear translocation and DNA binding	Prostate cancer	Inhibits IL-2 promoter activity and cytokine synthesis	[204]
Punicalagin	Inhibits NFAT nuclear translocation and DNA binding	Breast, lung, and cervical cancer	Inhibits IL-2 production of $CD4^{+}$ T cells	[205]
Imperatorin (furanocoumarin)	Inhibits NFAT transcriptional and DNA-binding activities.	Lung cancer	Inhibits the proliferation of SEB-stimulated T cells	[206]
Quinolone alkaloids	Inhibit NFAT transcriptional and DNA-binding activities.	NR	Inhibit NFAT and NF- κ B-dependent reporter gene expression in Jurkat T cells.	[207]
1,25-Dihydroxy-vitamin D3	Inhibits NFAT transcriptional activities	Various cancer	Inhibits GM-CSF transcription in Jurkat T cells; Inhibits IL-2 transcription	[208]
Digitoxin	Inhibits NFAT1 interaction with the proximal c-Myc promoter.	Cervical cancer	Suppresses c-Myc dependent cell proliferation and induces apoptosis	[209]
AM-404	Inhibits NFAT1–DNA binding and transcriptional activity	NR	Suppresses IL-2 and TNF α transcription, T cell proliferation and cytokine release in Jurkat T cells after α CD3/28 stimulation	[210]

NR, not reported.

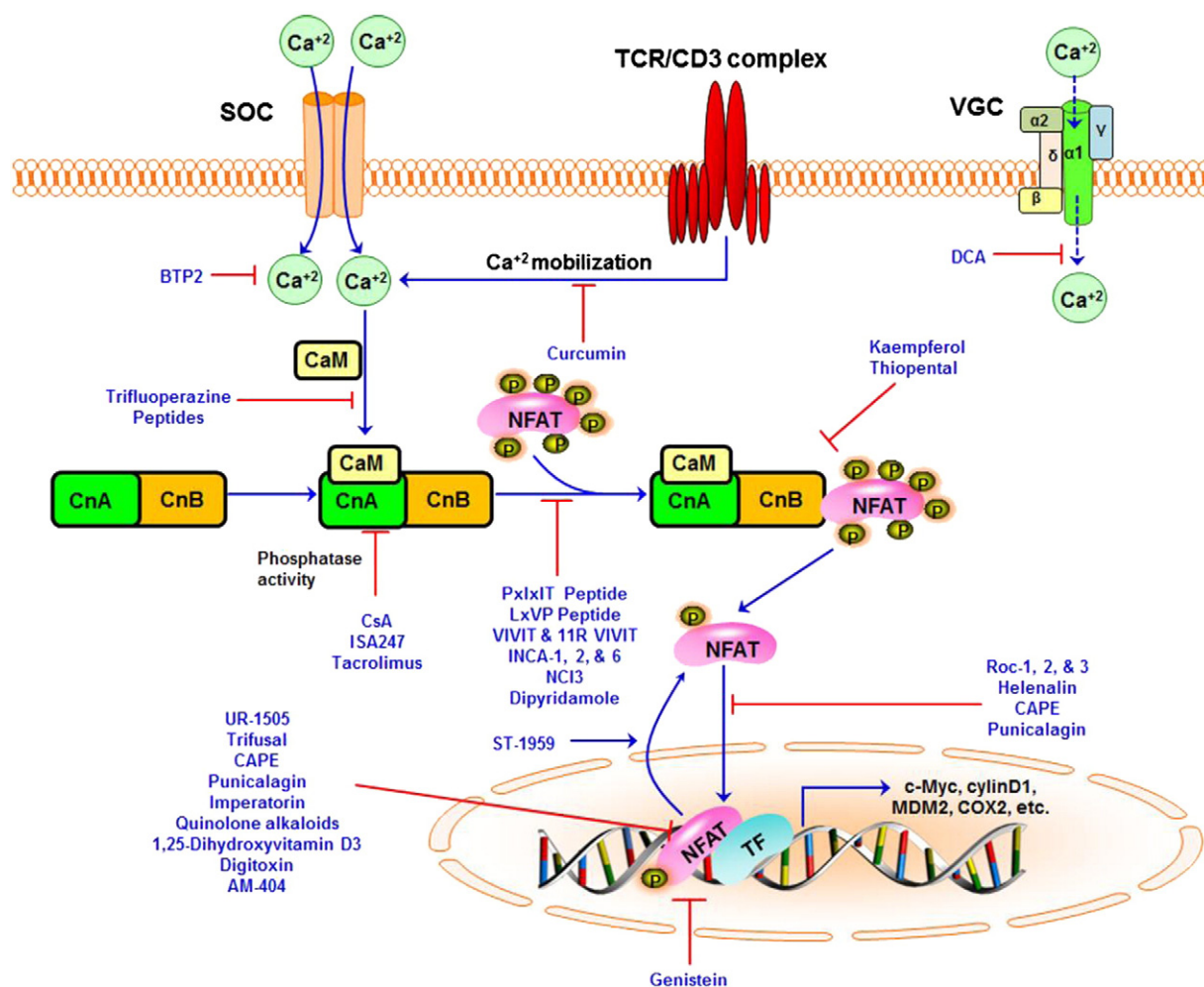


Fig. 4. Inhibition of the calcineurin–NFAT pathway at multiple levels. Increase in intra-cellular calcium levels causes calmodulin (CaM) and calcineurin B (CnB) to bind Ca^{2+} ions and activate calcineurin via a conformational change. Activated calcineurin binds to NFAT via the PxxIT and the LxxVP motifs of NFAT and dephosphorylates it. Dephosphorylation unmasks the nuclear localization sequence of NFAT. Then NFAT is translocated into the nucleus, where it is transactivated in co-operation with other transcription factors (TF). Different steps in this pathway are targeted by certain compounds to finally suppress NFAT-dependent gene expression. Some prototype inhibitors of calcineurin–NFAT signaling axis are depicted. Dichloroacetate (DCA) decreases mitochondrial membrane potential to cause translocation of reactive oxygen species (ROS) translocation to cytoplasm. Cytosolic ROS activates Kv1.5 potassium ion channels, preventing intracellular calcium entry through VGC. CsA, Tacrolimus, and ISA247 inhibit calcineurin phosphatase activity. ST-1959 increases nuclear export of NFAT. Genistein reduces mRNA and protein expressions of NFAT1. CnA, calcineurin A; SOC, store-operated calcium channels; TCR, T cell receptor; VGC, voltage gated calcium channels.

mitogenic RAS–MAP kinase cascade and the TGF- β /Smad pathway [165, 166], and regulates several pro-inflammatory molecules such as NF- κ B, Elk-1, and AP1 [44,45]. Thus, CsA or tacrolimus also targets NFAT-independent gene regulation in cancer. The lack of specificity may explain the neuro- and nephrotoxicity as well as cardiovascular and diabetic complications observed clinically with these drugs [167]. Nonetheless, one would predict that by virtue of the potent inhibition of the NFAT–calcineurin pathway, these drugs would work as effective anti-cancer therapeutics [22].

Though CsA and tacrolimus show effective anti-cancer activities, patients on long-term immunosuppressive treatments actually exhibit increased rate of cancer incidence [168]. This phenomenon might be explained by the fact that immunosuppressive agents likely suppress local tumor immunosurveillance. Moreover, it is expected that CsA and tacrolimus would affect the whole tumor microenvironment with multiple diverse effects on cellular pathophysiology. Therefore, new treatment strategies that specifically inhibit NFAT activity in the tumor endothelium or act specifically at the actual tumor site, without affecting the local immune response, are needed. Indeed, substantial efforts have been expended in the past decade to identify small molecule

inhibitors that work downstream of calcineurin to specifically inhibit NFAT activity. A summary of current NFAT inhibitors and their potential targets has been presented in Table 4 and Fig. 4. A peptide termed VIVIT has been developed that interferes with the calcineurin–NFAT interaction, and inhibits NFAT dephosphorylation and nuclear translocation [169,172,173]. Since peptides, as therapeutic entities, present several challenges with regards to delivery and stability, cell permeable varieties of VIVIT peptides (Table 4) have been developed [173].

Small molecule inhibitors (SMIs) of NFAT seem to be more promising therapeutic entities. SMIs similar in structure and function to CsA and tacrolimus but exhibiting fewer adverse effects, have been developed, e.g. ISA247 (voclosporin) [177,178]. Several compounds (Table 4) with diverse chemical structures have been synthesized and characterized as novel NFAT SMIs. Interestingly, classical calcium channel inhibitors such as diltiazem and penfluridol have been shown to have impressive anti-cancer activities [211,212]. Since inhibition of calcium channels would lead to decreased intracellular calcium levels, and consequently inhibition of NFAT activation, it may be worthwhile to develop calcium channel inhibitors as potential NFAT targeting anticancer therapeutics. Another class of drugs, originally introduced

for other applications, which has turned out to act as calcineurin/NFAT inhibitors includes the bisphosphonate zoledronic acid [55]. Recently, zoledronic acid was shown to induce NFAT1 ubiquitination in breast and pancreatic cancer in vitro and in vivo, through inhibition of GSK-3 β kinase activity and induction of MDM2 [55].

In summary, there are at least three strategies to develop NFAT SMLs: i) target the upstream regulators of NFAT (such as calmodulin, calcineurin, and GSK3) to inhibit NFAT protein dephosphorylation and nuclear translocation; ii) directly target NFAT to inhibit its expression, destabilize NFAT protein, inhibit NFAT nuclear translocation and/or increase NFAT nuclear export; and iii) block NFAT–DNA binding to inhibit NFAT transcriptional activity. In addition, exhaustive pre-clinical studies in validated animal models are required to determine if these novel calcineurin/NFAT inhibitors possess a capacity to prevent or reverse tumorigenesis in murine cancer models, beyond their well-established activities in immunosuppression. Although most of these inhibitors have not been tested in a cancer model, it seems that they may have potential uses in cancer therapy based on their antiproliferative and anti-inflammatory activities. However, we reiterate the necessity of stringent evaluation of their toxicities due to the pleiotropic functions of NFAT.

6. Future directions and conclusions

Accumulating evidence over the past decade indicates a key role for NFAT transcription factors in diverse pathophysiological states such as inflammation and cancer, apart from their seminal functions in immune surveillance. However, the common feature in all disease states is that the NFAT proteins must be activated in the nucleus and bind to the DNA to cause transcription of its downstream targets. NFAT activity has been shown to be crucial for cell survival and proliferation, invasive migration, and angiogenesis. Indeed, evidences from mouse models suggest that some NFAT isoforms (such as NFAT1) may be primarily involved in cell migration, invasion, and metastasis, instead of tumorigenesis. One must remember that the NFAT isoforms, though often performing redundant functions, affect the cancer development and progression process differently. Even their physiological effects seem cell type and context dependent. Therefore, there is an urgent need for developing targeted NFAT mouse models in which specific NFAT isoforms are either knocked down or activated in specific cell types or a particular cellular microenvironment. Though different NFAT isoforms perform different functions with regards to tumor proliferation and progression, the mechanisms driving these differences have yet to be deciphered. Similarly, up till now only few mediators (such as COX-2, glypican-6, MMP-2, c-Myc, and MDM2) of the NFAT signaling axis have been identified. It is likely that several other upstream/downstream targets remain to be discovered. Finally, we need to have a clearer idea of the processes that drive NFAT activation. Possible mutations and/or amplifications in NFAT binding partners and export/maintenance kinases are frequently seen in several human cancers that are associated with constitutive NFAT nuclear localization. For example, inhibition of GSK3 β activity has been shown to disrupt Stat3–NFAT1 interaction and NFAT transcriptional activity in both in vitro and in vivo pancreatic cancer models [77].

The NFAT transcription factor family is also closely linked with inflammation. It is, therefore, expected that there will be significant cross-talk between NFAT and other pro-inflammatory signaling pathways, and these findings can facilitate development of better therapeutics with multi-modal mechanisms of action. Similarly, we need to understand if possible mutations or amplifications in NFAT isoforms exist that contribute to tumor development, progression, and possible chemoresistance. Finally, calcium signaling is known to affect cell cycle progression, cell survival, and angiogenesis. Since the calcineurin/NFAT pathway basically integrates cellular calcium flux with other signaling pathways, it will be interesting to note if compounds blocking intracellular calcium release can have inhibitory activities on NFAT

mediated cell migration and invasion. Indeed, a few calcium channel blockers have shown promising anti-cancer activity, and further insights into their anticancer mechanism of action may help repurpose these well-established drugs into novel therapeutics for cancer management. Answers to these important questions are necessary to unlock the full potential of NFAT as a valid target in human cancer.

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References

- [1] J.P. Shaw, P.J. Utz, D.B. Durand, J.J. Toole, E.A. Emmel, G.R. Crabtree, Identification of a putative regulator of early T cell activation genes, *Science* 241 (1988) 202–205.
- [2] D.B. Durand, J.P. Shaw, M.R. Bush, R.E. Replogle, R. Belagaje, G.R. Crabtree, Characterization of antigen receptor response elements within the interleukin-2 enhancer, *Mol. Cell. Biol.* 8 (1988) 1715–1724.
- [3] P.G. McCaffrey, A.E. Goldfeld, A. Rao, The role of NFATp in cyclosporin A-sensitive tumor necrosis factor- α gene transcription, *J. Biol. Chem.* 269 (1994) 30445–30450.
- [4] P.G. Hogan, L. Chen, J. Nardone, A. Rao, Transcriptional regulation by calcium, calcineurin, and NFAT, *Genes Dev.* 17 (2003) 2205–2232.
- [5] M.R. Müller, A. Rao, NFAT, immunity and cancer: a transcription factor comes of age, *Nat. Rev. Immunol.* 10 (2010) 645–656.
- [6] A. Rao, NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes, *Immunol. Today* 15 (1994) 274–281.
- [7] I.A. Graef, F. Chen, G.R. Crabtree, NFAT signaling in vertebrate development, *Curr. Opin. Genet. Dev.* 11 (2001) 505–512.
- [8] I.A. Graef, F. Chen, L. Chen, A. Kuo, G.R. Crabtree, Signals transduced by Ca(2+)/calcineurin and NFATc3/c4 pattern the developing vasculature, *Cell* 105 (2001) 863–875.
- [9] P.G. McCaffrey, C. Luo, T.K. Kerppola, J. Jain, T.M. Badalian, A.M. Ho, E. Burgeon, W.S. Lane, J.N. Lambert, T. Curran, Isolation of the cyclosporin-sensitive T cell transcription factor NFATp, *Science* 262 (1993) 750–754.
- [10] C. Luo, E. Burgeon, J.A. Carew, P.G. McCaffrey, T.M. Badalian, W.S. Lane, P.G. Hogan, A. Rao, Recombinant NFAT1 (NFATp) is regulated by calcineurin in T cells and mediates transcription of several cytokine genes, *Mol. Cell. Biol.* 16 (1996) 3955–3966.
- [11] J.P. Northrop, S.N. Ho, L. Chen, D.J. Thomas, L.A. Timmerman, G.P. Nolan, A. Admon, G.R. Crabtree, NF-AT components define a family of transcription factors targeted in T-cell activation, *Nature* 369 (1994) 497–502.
- [12] T. Hoey, Y.L. Sun, K. Williamson, X. Xu, Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins, *Immunity* 2 (1995) 461–472.
- [13] S.N. Ho, D.J. Thomas, L.A. Timmerman, X. Li, U. Francke, G.R. Crabtree, NFATc3, a lymphoid-specific NFATc family member that is calcium-regulated and exhibits distinct DNA binding specificity, *J. Biol. Chem.* 270 (1995) 19898–19907.
- [14] C. Lopez-Rodriguez, J. Aramburu, A.S. Rakeam, A. Rao, NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 7214–7219.
- [15] H. Miyakawa, S.K. Woo, S.C. Dahl, J.S. Handler, H.M. Kwon, Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 2538–2542.
- [16] J. Trama, W.Y. Go, S.N. Ho, The osmoprotective function of the NFAT5 transcription factor in T cell development and activation, *J. Immunol.* 169 (2002) 5477–5488.
- [17] C. Luo, K.T. Shaw, A. Raghavan, J. Aramburu, F. Garcia-Cozar, B.A. Perrino, P.G. Hogan, A. Rao, Interaction of calcineurin with a domain of the transcription factor NFAT1 that controls nuclear import, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 8907–8912.
- [18] S. Feske, J. Giltman, R. Dolmetsch, L.M. Staudt, A. Rao, Gene regulation mediated by calcium signals in T lymphocytes, *Nat. Immunol.* 2 (2001) 316–324.
- [19] S. Wesselborg, D.A. Fruman, J.K. Sagoo, B.E. Bierer, S.J. Burakoff, Identification of a physical interaction between calcineurin and nuclear factor of activated T cells (NFATp), *J. Biol. Chem.* 271 (1996) 1274–1277.
- [20] C. Loh, K.T. Shaw, J. Carew, J.P. Viola, C. Luo, B.A. Perrino, A. Rao, Calcineurin binds the transcription factor NFAT1 and reversibly regulates its activity, *J. Biol. Chem.* 271 (1996) 10884–10891.

- [21] A. Rao, C. Luo, P.G. Hogan, Transcription factors of the NFAT family: regulation and function, *Annu. Rev. Immunol.* 15 (1997) 707–747.
- [22] M. Mancini, A. Tokar, NFAT proteins: emerging roles in cancer progression, *Nat. Rev. Cancer* 9 (2009) 810–820.
- [23] G.R. Crabtree, E.N. Olson, NFAT signaling: choreographing the social lives of cells, *Cell* 109 (2002) S67–S79 (Suppl.).
- [24] M.G. Pan, Y. Xiong, F. Chen, NFAT gene family in inflammation and cancer, *Curr. Mol. Med.* 13 (2013) 543–554.
- [25] J. Jain, E. Burgeon, T.M. Badalian, P.G. Hogan, A. Rao, A similar DNA-binding motif in NFAT family proteins and the Rel homology region, *J. Biol. Chem.* 270 (1995) 4138–4145.
- [26] G.P. Nolan, NF-AT-AP-1 and Rel-bZIP: hybrid vigor and binding under the influence, *Cell* 77 (1994) 795–798.
- [27] M. Chytil, G.L. Verdine, The Rel family of eukaryotic transcription factors, *Curr. Opin. Struct. Biol.* 6 (1996) 91–100.
- [28] H. Okamura, J. Aramburu, C. García-Rodríguez, J.P. Viola, A. Raghavan, M. Tahiliani, X. Zhang, J. Qin, P.G. Hogan, A. Rao, Concerted dephosphorylation of the transcription factor NFAT1 induces a conformational switch that regulates transcriptional activity, *Mol. Cell* 6 (2000) 539–550.
- [29] C. Luo, E. Burgeon, A. Rao, Mechanisms of transactivation by nuclear factor of activated T cells-1, *J. Exp. Med.* 184 (1996) 141–147.
- [30] C.R. Beals, N.A. Clipstone, S.N. Ho, G.R. Crabtree, Nuclear localization of NF-ATc by a calcineurin-dependent, cyclosporin-sensitive intramolecular interaction, *Genes Dev.* 11 (1997) 824–834.
- [31] J.L. de la Pompa, L.A. Timmerman, H. Takimoto, H. Yoshida, A.J. Elia, E. Samper, J. Potter, A. Wakeham, L. Marengere, B.L. Langille, G.R. Crabtree, T.W. Mak, Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum, *Nature* 392 (1998) 182–186.
- [32] A.M. Ranger, M.J. Grusby, M.R. Hodge, E.M. Gravalles, F.C. de la Brousse, T. Hoey, C. Micanin, H.S. Baldwin, L.H. Glimcher, The transcription factor NF-ATc is essential for cardiac valve formation, *Nature* 392 (1998) 186–190.
- [33] A.V. Tsytsykova, A.E. Goldfeld, Nuclear factor of activated T cells transcription factor NFATp controls superantigen-induced lethal shock, *J. Exp. Med.* 192 (2000) 581–586.
- [34] S.L. Peng, A.J. Gerth, A.M. Ranger, L.H. Glimcher, NFATc1 and NFATc2 together control both T and B cell activation and differentiation, *Immunity* 14 (2001) 13–20.
- [35] A.M. Ranger, M. Oukka, J. Rengarajan, L.H. Glimcher, Inhibitory function of two NFAT family members in lymphoid homeostasis and Th2 development, *Immunity* 9 (1998) 627–635.
- [36] F. Macian, NFAT proteins: key regulators of T-cell development and function, *Nat. Rev. Immunol.* 5 (2005) 472–484.
- [37] J. Fric, T. Zelante, A.Y. Wong, A. Mertes, H.B. Yu, P. Ricciardi-Castagnoli, NFAT control of innate immunity, *Blood* 120 (2012) 1380–1389.
- [38] I.A. Graef, F. Wang, F. Charron, L. Chen, J. Neilson, M. Tessier-Lavigne, G.R. Crabtree, Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons, *Cell* 113 (2003) 657–670.
- [39] B. Eisenhaber, M. Sammer, W.H. Lua, W. Benetka, L.L. Liew, W. Yu, H.K. Lee, M. Koranda, F. Eisenhaber, S. Adhikari, Nuclear import of a lipid-modified transcription factor: mobilization of NFAT5 isoform a by osmotic stress, *Cell Cycle* 10 (2011) 3897–3911.
- [40] R. Berge-Bolaños, K. Drews-Elger, J. Aramburu, C. López-Rodríguez, NFAT5 regulates T lymphocyte homeostasis and CD24-dependent T cell expansion under pathologic hyponatremia, *J. Immunol.* 185 (2010) 6624–6635.
- [41] A.T. Serafini, R.S. Lewis, N.A. Clipstone, R.J. Bram, C. Fanger, S. Fiering, L.A. Herzenberg, G.R. Crabtree, Isolation of mutant T lymphocytes with defects in capacitative calcium entry, *Immunity* 3 (1995) 239–250.
- [42] Y. Gwack, S. Feske, S. Srikanth, P.G. Hogan, A. Rao, Signaling to transcription: store-operated Ca²⁺ entry and NFAT activation in lymphocytes, *Cell Calcium* 42 (2007) 145–156.
- [43] P. Kar, C. Nelson, A.B. Parekh, Selective activation of the transcription factor NFAT1 by calcium microdomains near Ca²⁺ release-activated Ca²⁺ (CRAC) channels, *J. Biol. Chem.* 286 (2011) 14795–14803.
- [44] H. Medyoun, J. Ghysdai, The calcineurin/NFAT signaling pathway: a novel therapeutic target in leukemia and solid tumors, *Cell Cycle* 7 (2008) 297–303.
- [45] J. Liu, J.D. Farmer Jr., W.S. Lane, J. Friedman, I. Weissman, S.L. Schreiber, Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP–FK506 complexes, *Cell* 66 (1991) 807–815.
- [46] M. Buchholz, A. Schatz, M. Wagner, P. Michl, T. Linhart, G. Adler, T.M. Gress, V. Ellenrieder, Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca²⁺/calcineurin signaling pathway, *EMBO J.* 25 (2006) 3714–3724.
- [47] V. Lehen'kyi, M. Flourakis, R. Skryma, N. Prevorskaya, TRPV6 channel controls prostate cancer cell proliferation via Ca(2+)/NFAT-dependent pathways, *Oncogene* 26 (2007) 7380–7385.
- [48] T.M. Klauk, M.C. Faux, K. Labudda, L.K. Langeberg, S. Jaken, J.D. Scott, Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein, *Science* 271 (1996) 1589–1592.
- [49] L. Sun, H.D. Youn, C. Loh, M. Stolow, W. He, J.O. Liu, Cabin 1, a negative regulator for calcineurin signaling in T lymphocytes, *Immunity* 8 (1998) 703–711.
- [50] J.J. Fuentes, L. Genesca, T.J. Kingsbury, K.W. Cunningham, M. Perez-Riba, X. Estivill, S. de la Luna, DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways, *Hum. Mol. Genet.* 9 (2000) 1681–1690.
- [51] M.K. Choo, H. Yeo, M. Zayzafoon, NFATc1 mediates HDAC-dependent transcriptional repression of osteocalcin expression during osteoblast differentiation, *Bone* 45 (2009) 579–589.
- [52] Y. Gwack, S. Sharma, J. Nardone, B. Tanasa, A. Iuga, S. Srikanth, H. Okamura, D. Bolton, S. Feske, P.G. Hogan, A. Rao, A genome-wide *Drosophila* RNAi screen identifies DYRK-family kinases as regulators of NFAT, *Nature* 441 (2006) 646–650.
- [53] C.R. Beals, C.M. Sheridan, C.W. Turck, P. Gardner, G.R. Crabtree, Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3, *Science* 275 (1997) 1930–1934.
- [54] H. Okamura, C. Garcia-Rodriguez, H. Martinson, J. Qin, D.M. Virshup, A. Rao, A conserved docking motif for CK1 binding controls the nuclear localization of NFAT1, *Mol. Cell. Biol.* 24 (2004) 4184–4195.
- [55] S.K. Singh, S. Baumgart, G. Singh, A.O. Konig, K. Reutlinger, L.C. Hofbauer, P. Barth, T.M. Gress, G. Lomberg, R. Urrutia, M.E. Fernandez-Zapico, V. Ellenrieder, Disruption of a nuclear NFATc2 protein stabilization loop confers breast and pancreatic cancer growth suppression by zoledronic acid, *J. Biol. Chem.* 286 (2011) 28761–28771.
- [56] R.J. Atkins, J. Dimou, L. Paradiso, A.P. Morokoff, A.H. Kaye, K.J. Drummond, C.M. Hovens, Regulation of glycogen synthase kinase-3 beta (GSK-3beta) by the Akt pathway in gliomas, *J. Clin. Neurosci.* 19 (2012) 1558–1563.
- [57] C.M. Porter, M.A. Havens, N.A. Clipstone, Identification of amino acid residues and protein kinases involved in the regulation of NFATc subcellular localization, *J. Biol. Chem.* 275 (2000) 3543–3551.
- [58] J. Zhu, F. Shibasaki, R. Price, J.C. Guillemot, T. Yano, V. Dötsch, G. Wagner, P. Ferrara, F. McKeon, Intramolecular masking of nuclear import signal on NF-AT4 by casein kinase I and MEKK1, *Cell* 93 (1998) 851–861.
- [59] T.T. Yang, Q. Xiong, H. Enslin, R.J. Davis, C.W. Chow, Phosphorylation of NFATc4 by p38 mitogen-activated protein kinases, *Mol. Cell. Biol.* 22 (2002) 3892–3904.
- [60] M.R. Müller, Y. Sasaki, I. Stevanovic, E.D. Lamperti, S. Ghosh, S. Sharma, C. Gelinas, D.J. Rossi, M.E. Pipkin, K. Rajewsky, P.G. Hogan, A. Rao, Requirement for balanced Ca/NFAT signaling in hematopoietic and embryonic development, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 7034–7039.
- [61] C.W. Chow, M. Rincón, J. Cavanagh, M. Dickens, R.J. Davis, Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway, *Science* 278 (1997) 1638–1641.
- [62] I. Ortega-Perez, E. Cano, F. Were, M. Villar, J. Vazquez, J.M. Redondo, c-Jun N-terminal kinase (JNK) positively regulates NFATc2 transactivation through phosphorylation within the N-terminal regulatory domain, *J. Biol. Chem.* 280 (2005) 20867–20878.
- [63] Y.C. Lee, A.Y. Chang, M.H. Lin-Feng, W.I. Tsou, I.H. Chiang, M.Z. Lai, Paxillin phosphorylation by JNK and p38 is required for NFAT activation, *Eur. J. Immunol.* 42 (2012) 2165–2175.
- [64] A.S. Dhillon, S. Hagan, O. Rath, W. Kolch, MAP kinase signalling pathways in cancer, *Oncogene* 26 (2007) 3279–3290.
- [65] S. Chuvpilo, E. Jankevics, D. Tyrsin, A. Akimzhanov, D. Moroz, M.K. Jha, J. Schulze-Luehrmann, B. Santner-Nanan, E. Feoktistova, T. Konig, A. Avots, E. Schmitt, F. Berberich-Siebert, A. Schimpl, E. Serfling, Autoregulation of NFATc1/A expression facilitates effector T cells to escape from rapid apoptosis, *Immunity* 16 (2002) 881–895.
- [66] E. Serfling, S. Chuvpilo, J. Liu, T. Höfer, A. Palmethofer, NFATc1 autoregulation: a crucial step for cell-fate determination, *Trends Immunol.* 27 (2006) 461–469.
- [67] H. Takayanagi, S. Kim, T. Koga, H. Nishina, M. Isshiki, H. Yoshida, A. Saiura, M. Isobe, T. Yokochi, J. Inoue, E.F. Wagner, T.W. Mak, T. Kodama, T. Taniguchi, Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts, *Dev. Cell* 3 (2002) 889–901.
- [68] B. Zhou, B. Wu, K.L. Tompkins, K.L. Boyer, J.C. Grindley, H.S. Baldwin, Characterization of Nfatc1 regulation identifies an enhancer required for gene expression that is specific to pro-valve endocardial cells in the developing heart, *Development* 132 (2005) 1137–1146.
- [69] A. Nayak, J. Glockner-Pagel, M. Vaeth, J.E. Schumann, M. Buttmann, T. Bopp, E. Schmitt, E. Serfling, F. Berberich-Siebert, Sumoylation of the transcription factor NFATc1 leads to its subnuclear relocalization and interleukin-2 repression by histone deacetylase, *J. Biol. Chem.* 284 (2009) 10935–10946.
- [70] S.L. Wyszomierski, D. Yu, A. Knotty turnaround? Akt1 as a metastasis suppressor, *Cancer Cell* 8 (2005) 437–439.
- [71] O.A. Olabisi, N. Soto-Nieves, E. Nieves, T.T. Yang, X. Yang, R.Y. Yu, H.Y. Suk, F. Macian, C.W. Chow, Regulation of transcription factor NFAT by ADP-ribosylation, *Mol. Cell. Biol.* 28 (2008) 2860–2871.
- [72] R. Valdor, V. Schreiber, L. Saenz, T. Martínez, A. Muñoz-Suano, M. Dominguez-Villar, P. Ramirez, P. Parrilla, E. Aguado, F. García-Cózar, J. Yélamos, Regulation of NFAT by poly(ADP-ribose) polymerase activity in T cells, *Mol. Immunol.* 45 (2008) 1863–1871.
- [73] L. Chen, J.N. Glover, P.G. Hogan, A. Rao, S.C. Harrison, Structure of the DNA-binding domains from NFAT, Fos and Jun bound specifically to DNA, *Nature* 392 (1998) 42–48.
- [74] P. Zhou, L.J. Sun, V. Dotsch, G. Wagner, G.L. Verdine, Solution structure of the core NFATC1/DNA complex, *Cell* 92 (1998) 687–696.
- [75] Q. Li, A. Shakya, X. Guo, H. Zhang, D. Tantin, P.E. Jensen, X. Chen, Constitutive nuclear localization of NFAT in Foxp3+ regulatory T cells independent of calcineurin activity, *J. Immunol.* 188 (2012) 4268–4277.
- [76] Y. Tone, K. Furuchi, Y. Kojima, M.L. Tykocinski, M.I. Greene, M. Tone, Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer, *Nat. Immunol.* 9 (2008) 194–202.
- [77] L. Lagunas, N.A. Clipstone, Deregulated NFATc1 activity transforms murine fibroblasts via an autocrine growth factor-mediated Stat3-dependent pathway, *J. Cell. Biochem.* 108 (2009) 237–248.

- [78] W. Wu, R.S. Misra, J.Q. Russell, R.A. Flavell, M. Rincon, R.C. Budd, Proteolytic regulation of nuclear factor of activated T (NFAT) c2 cells and NFAT activity by caspase-3, *J. Biol. Chem.* 281 (2006) 10682–10690.
- [79] M. Yoeli-Lerner, G.K. Yiu, I. Rabinovitz, P. Erhardt, S. Jauliac, A. Toker, Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT, *Mol. Cell* 20 (2005) 539–550.
- [80] M. Yoeli-Lerner, Y.R. Chin, C.K. Hansen, A. Toker, Akt/protein kinase b and glycogen synthase kinase-3 β signaling pathway regulates cell migration through the NFAT1 transcription factor, *Mol. Cancer Res.* 7 (2009) 425–432.
- [81] S. Jauliac, C. Lopez-Rodriguez, L.M. Shaw, L.F. Brown, A. Rao, A. Toker, The role of NFAT transcription factors in integrin-mediated carcinoma invasion, *Nat. Cell Biol.* 4 (2002) 540–544.
- [82] C.A. Massaad, B.P. Portier, G. Tagliatella, Inhibition of transcription factor activity by nuclear compartment-associated Bcl-2, *J. Biol. Chem.* 279 (2004) 54470–54478.
- [83] J.Y. Wang, B.K. Chen, Y.S. Wang, Y.T. Tsai, W.C. Chen, W.C. Chang, M.F. Hou, Y.C. Wu, Involvement of store-operated calcium signaling in EGF-mediated COX-2 gene activation in cancer cells, *Cell. Signal.* 24 (2012) 162–169.
- [84] S. Pikkariainen, H. Tokola, R. Kerkela, H. Ruskoaho, GATA transcription factors in the developing and adult heart, *Cardiovasc. Res.* 63 (2004) 196–207.
- [85] I. Matsuda, E.S. Masuda, A. Tsuboi, S. Behnam, N. Arai, K. Arai, Characterization of NF(P), the nuclear factor that interacts with the regulatory P sequence (5'-CGAA AATTTC-3') of the human interleukin-4 gene: relationship to NF-kappa B and NF-AT, *Biochem. Biophys. Res. Commun.* 199 (1994) 439–446.
- [86] P.G. McCaffrey, J. Jain, C. Jamieson, R. Sen, A. Rao, A T cell nuclear factor resembling NF-AT binds to an NF-kappa B site and to the conserved lymphokine promoter sequence "cytokine-1", *J. Biol. Chem.* 267 (1992) 1864–1871.
- [87] Q. Liu, Y. Chen, M. Auger-Messier, J.D. Molkentin, Interaction between NFkB and NFAT coordinates cardiac hypertrophy and pathological remodeling, *Circ. Res.* 110 (2012) 1077–1086.
- [88] H. Medyouf, H. Alcalde, C. Berthier, M.C. Guillemin, N.R. dos Santos, A. Janin, D. Decaudin, H. de Thé, J. Ghysdail, Targeting calcineurin activation as a therapeutic strategy for T-cell acute lymphoblastic leukemia, *Nat. Med.* 13 (2007) 736–741.
- [89] C. Mammucari, A. Tommasi di Vignano, A.A. Sharov, J. Neilson, M.C. Havrda, D.R. Roop, V.A. Botchkarev, G.R. Crabtree, G.P. Dotto, Integration of Notch 1 and calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation control, *Dev. Cell* 8 (2005) 665–676.
- [90] N. Shinmen, T. Koshida, T. Kumazawa, K. Sato, H. Shimada, T. Matsutani, Y. Iwadate, M. Takiguchi, T. Hiwasa, Activation of NFAT signal by p53-K120R mutant, *FEBS Lett.* 583 (2009) 1916–1922.
- [91] G.L. Hernández, O.V. Volpert, M.A. Iñiguez, E. Lorenzo, S. Martínez-Martínez, R. Grau, M. Fresno, J.M. Redondo, Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2, *J. Exp. Med.* 193 (2001) 607–620.
- [92] M. Jinnin, D. Medici, L. Park, N. Limaye, Y. Liu, E. Boscolo, J. Bischoff, M. Vikkula, E. Boye, B.R. Olsen, Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma, *Nat. Med.* 14 (2008) 1236–1246.
- [93] B.A. Hesser, X.H. Liang, G. Camenisch, S. Yang, D.A. Lewin, R. Scheller, N. Ferrara, H. P. Gerber, Down syndrome critical region protein 1 (DSCR1), a novel VEGF target gene that regulates expression of inflammatory markers on activated endothelial cells, *Blood* 104 (2004) 149–158.
- [94] L.V. Pham, A.T. Tamayo, L.C. Yoshimura, Y.C. Lin-Lee, R.J. Ford, Constitutive NF-kappaB and NFAT activation in aggressive B-cell lymphomas synergistically activates the CD154 gene and maintains lymphoma cell survival, *Blood* 106 (2005) 3940–3947.
- [95] T. Marafioti, M. Pozzobon, M.L. Hansmann, R. Ventura, S.A. Pileri, H. Robertson, S. Gesk, P. Gaulard, T.F. Barth, M.Q. Du, L. Leoncini, P. Moller, Y. Natkunam, R. Siebert, D.Y. Mason, The NFATc1 transcription factor is widely expressed in white cells and translocates from the cytoplasm to the nucleus in a subset of human lymphomas, *Br. J. Haematol.* 128 (2005) 333–342.
- [96] L.V. Pham, A.T. Tamayo, C. Li, C. Bueso-Ramos, R.J. Ford, An epigenetic chromatin remodeling role for NFATc1 in transcriptional regulation of growth and survival genes in diffuse large B-cell lymphomas, *Blood* 116 (2010) 3899–3906.
- [97] L. Yu, F.C. Lin-Lee, L.V. Pham, A. Tamayo, L. Yoshimura, R.J. Ford, Constitutive NF-kappaB and NFAT activation leads to stimulation of the BlyS survival pathway in aggressive B-cell lymphomas, *Blood* 107 (2006) 4540–4548.
- [98] K. Szuhai, M. Ijszenga, D. de Jong, A. Karseladze, H.J. Tanke, P.C. Hogendoorn, The NFATc2 gene is involved in a novel cloned translocation in a Ewing sarcoma variant that couples its function in immunology to oncology, *Clin. Cancer Res.* 15 (2009) 2259–2268.
- [99] E. Arbajian, L. Magnusson, O. Brosjö, J. Wejde, A.L. Folpe, K.H. Nord, F. Mertens, A benign vascular tumor with a new fusion gene: EWSR1–NFATC1 in hemangioma of the bone, *Am. J. Surg. Pathol.* 37 (2013) 613–616.
- [100] S. Sankar, S.L. Lessnick, Promiscuous partnerships in Ewing's sarcoma, *Cancer Genet.* 204 (2011) 351–365.
- [101] S.Z. Glud, A.B. Sørensen, M. Andrusis, B. Wang, E. Kondo, R. Jessen, L. Krenacs, E. Stelkovic, M. Wabl, E. Serfling, A. Palmethofer, F.S. Pedersen, A tumor-suppressor function for NFATc3 in T-cell lymphomagenesis by murine leukemia virus, *Blood* 106 (2005) 3546–3552.
- [102] L. Pei, J.H. Choi, J. Liu, E.J. Lee, B. McCarthy, J.M. Wilson, E. Speir, F. Awan, H. Tae, G. Arthur, J.L. Schnabel, K.H. Taylor, X. Wang, D. Xu, H.F. Ding, D.H. Munn, C. Caldwell, H. Shi, Genome-wide DNA methylation analysis reveals novel epigenetic changes in chronic lymphocytic leukemia, *Epigenetics* 7 (2012) 567–578.
- [103] C. Le Roy, P.A. Deglesne, N. Chevallier, T. Beitar, V. Eclache, M. Quettier, M. Boubaya, R. Letestu, V. Lévy, F. Ajchenbaum-Cymbalista, N. Varin-Blank, The degree of BCR and NFAT activation predicts clinical outcomes in chronic lymphocytic leukemia, *Blood* 120 (2012) 356–365.
- [104] M.A. Gregory, T.L. Phang, P. Neviani, F. Alvarez-Calderon, C.A. Eide, T. O'Hare, V. Zaberezhnyy, R.T. Williams, B.J. Drucker, D. Perrotti, J. Degregori, Wnt/Ca2+/NFAT signaling maintains survival of Ph+ leukemia cells upon inhibition of Bcr–Abl, *Cancer Cell* 18 (2010) 74–87.
- [105] K. Gerlach, C. Daniel, H.A. Lehr, A. Nikolaev, T. Gerlach, R. Atreya, S. Rose-John, M.F. Neurath, B. Weigmann, Transcription factor NFATc2 controls the emergence of colon cancer associated with IL-6-dependent colitis, *Cancer Res.* 72 (2012) 4340–4350.
- [106] A. Courtwright, S. Siamakpour-Reihani, J.L. Arbiser, N. Banet, E. Hilliard, L. Fried, C. Livasy, D. Ketelsen, D.B. Nepal, C.M. Perou, C. Patterson, N. Klauber-Demore, Secreted frizzled-related protein 2 stimulates angiogenesis via a calcineurin/NFAT signaling pathway, *Cancer Res.* 69 (2009) 4621–4628.
- [107] R.J. Flockhart, J.L. Armstrong, N.J. Reynolds, P.E. Lovat, NFAT signalling is a novel target of oncogenic BRAF in metastatic melanoma, *Br. J. Cancer* 101 (2009) 1448–1455.
- [108] M.B. Werneck, A. Vieira-de-Abreu, R. Chammas, J.P. Viola, NFAT1 transcription factor is central in the regulation of tissue microenvironment for tumor metastasis, *Cancer Immunol. Immunother.* 60 (2011) 537–546.
- [109] K.J. Sales, D. Maldonado-Perez, V. Grant, R.D. Catalano, M.R. Wilson, P. Brown, A.R. Williams, R.A. Anderson, E.A. Thompson, H.N. Jabbar, Prostaglandin F(2alpha)-F-prostanoid receptor regulates CXCL8 expression in endometrial adenocarcinoma cells via the calcium–calcineurin–NFAT pathway, *Biochim. Biophys. Acta* 1793 (2009) 1917–1928.
- [110] K.J. Sales, V. Grant, I.H. Cook, D. Maldonado-Perez, R.A. Anderson, A.R. Williams, H. N. Jabbar, Interleukin-11 in endometrial adenocarcinoma is regulated by prostaglandin F2alpha-F-prostanoid receptor interaction via the calcium–calcineurin–nuclear factor of activated T cells pathway and negatively regulated by the regulator of calcineurin-1, *Am. J. Pathol.* 176 (2010) 435–445.
- [111] K. Zhang, N. Li, Z. Chen, K. Shao, F. Zhou, C. Zhang, X. Mu, J. Wan, B. Li, X. Feng, S. Shi, M. Xiong, K. Cao, X. Wang, C. Huang, J. He, High expression of nuclear factor of activated T cells in Chinese primary non-small cell lung cancer tissues, *Int. J. Biol. Markers* 22 (2007) 221–225.
- [112] Z.L. Chen, S.H. Zhao, Z. Wang, B. Qiu, B.Z. Li, F. Zhou, X.G. Tan, J. He, Expression and unique functions of four nuclear factor of activated T cells isoforms in non-small cell lung cancer, *Chin. J. Cancer* 30 (2011) 62–68.
- [113] A. Wu, K. Ericson, W. Chao, W.C. Low, NFAT and AP1 are essential for the expression of a glioblastoma multiforme related IL-13Ra2 transcript, *Cell. Oncol.* 32 (2010) 313–329.
- [114] X. Tie, S. Han, L. Meng, Y. Wang, A. Wu, NFAT1 is highly expressed in, and regulates the invasion of, glioblastoma multiforme cells, *PLoS One* 8 (2013) e66008.
- [115] B.K. Robbs, P.I. Lucena, J.P. Viola, The transcription factor NFAT1 induces apoptosis through cooperation with Ras/Raf/MEK/ERK pathway and upregulation of TNF- α expression, *Biochim. Biophys. Acta* 1833 (2013) 2016–2028.
- [116] B.K. Robbs, A.L. Cruz, M.B. Werneck, G.P. Mognol, J.P. Viola, Dual roles for NFAT transcription factor genes as oncogenes and tumor suppressors, *Mol. Cell. Biol.* 28 (2008) 7168–7181.
- [117] J.W. Neal, N.A. Clipstone, A constitutively active NFATc1 mutant induces a transformed phenotype in 3T3-L1 fibroblasts, *J. Biol. Chem.* 278 (2003) 17246–17254.
- [118] S. Baksh, H.R. Widlund, A.A. Frazer-Abel, J. Du, S. Fosmire, D.E. Fisher, J.A. DeCaprio, J.F. Modiano, S.J. Burakoff, NFATc2-mediated repression of cyclin-dependent kinase 4 expression, *Mol. Cell* 10 (2002) 1071–1081.
- [119] L.D. Carvalho, L.K. Teixeira, N. Carrossini, A.T. Almeida, K.M. Ansel, A. Rao, J.P. Viola, The NFAT1 transcription factor is a repressor of cyclin A2 gene expression, *Cell Cycle* 6 (2007) 1789–1795.
- [120] M. Oukka, I.C. Ho, F.C. de la Brousse, T. Hoey, M.J. Grusby, L.H. Glimcher, The transcription factor NFAT4 is involved in the generation and survival of T cells, *Immunity* 9 (1998) 295–304.
- [121] X. Zhang, Z. Zhang, J. Cheng, M. Li, W. Wang, W. Xu, H. Wang, R. Zhang, Transcription factor NFAT1 activates the mdm2 oncogene independent of p53, *J. Biol. Chem.* 287 (2012) 30468–30476.
- [122] S. Baumgart, E. Glesel, G. Singh, N.M. Chen, K. Reutlinger, J. Zhang, D.D. Billadeau, M. E. Fernandez-Zapico, T.M. Gress, S.K. Singh, V. Ellenrieder, Restricted heterochromatin formation links NFATc2 repressor activity with growth promotion in pancreatic cancer, *Gastroenterology* 142 (2012) 388–398.
- [123] S. Sengupta, S. Jana, S. Biswas, P.K. Mandal, A. Bhattacharyya, Cooperative involvement of NFAT and SnoN mediates transforming growth factor- β (TGF- β) induced EMT in metastatic breast cancer (MDA-MB 231) cells, *Clin. Exp. Metastasis* 30 (2013) 1019–1031.
- [124] S. Sengupta, S. Jana, A. Bhattacharyya, TGF- β –Smad2 dependent activation of CDC 25A plays an important role in cell proliferation through NFAT activation in metastatic breast cancer cells, *Cell. Signal.* 26 (2014) 240–252.
- [125] G. Singh, S.K. Singh, A. König, K. Reutlinger, M.D. Nye, T. Adhikary, M. Eilers, T.M. Gress, M.E. Fernandez-Zapico, V. Ellenrieder, Sequential activation of NFAT and c-Myc transcription factors mediates the TGF-beta switch from a suppressor to a promoter of cancer cell proliferation, *J. Biol. Chem.* 285 (2010) 27241–27250.
- [126] M.E. Fernandez-Zapico, V. Ellenrieder, NFAT transcription factors, the potent mediating "Dr. Jekyll–Mr. Hyde" transformation of the TGF β pathway in cancer cells, *Cell Cycle* 9 (2010) 3838–3839.
- [127] V. Horsley, A.O. Aliprantis, L. Polak, L.H. Glimcher, E. Fuchs, NFATc1 balances quiescence and proliferation of skin stem cells, *Cell* 132 (2008) 299–310.
- [128] S.A. Mani, W. Guo, M.J. Liao, E.N. Eaton, A. Ayyanan, A.Y. Zhou, M. Brooks, F. Reinhard, C.C. Zhang, M. Shiptitsin, L.L. Campbell, K. Polyak, C. Briskin, J. Yang, R.

- A. Weinberg, The epithelial–mesenchymal transition generates cells with properties of stem cells, *Cell* 133 (2008) 704–715.
- [129] G.K. Yiu, A. Tokor, NFAT induces breast cancer cell invasion by promoting the induction of cyclooxygenase-2, *J. Biol. Chem.* 281 (2006) 12210–12217.
- [130] S. Siamakpour-Reihani, J. Caster, D. Bandhu Nepal, A. Courtwright, E. Hilliard, J. Usary, D. Ketelsen, D. Darr, X.J. Shen, C. Patterson, N. Klauber-Demore, The role of calcineurin/NFAT in SFRP2 induced angiogenesis—a rationale for breast cancer treatment with the calcineurin inhibitor tacrolimus, *PLoS One* 6 (2011) e20412.
- [131] M. Vázquez-Cedeira, P.A. Lazo, Human VRK2 (vaccinia-related kinase 2) modulates tumor cell invasion by hyperactivation of NFAT1 and expression of cyclooxygenase-2, *J. Biol. Chem.* 287 (2012) 42739–42750.
- [132] A. Greenhough, H.J. Smartt, A.E. Moore, H.R. Roberts, A.C. Williams, C. Paraskeva, A. Kaidi, The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment, *Carcinogenesis* 30 (2009) 377–386.
- [133] J. Dejmek, A. Säfholm, C. Kamp Nielsen, T. Andersson, K. Leandersson, Wnt-5a/Ca2+–induced NFAT activity is counteracted by Wnt-5a/Yes-Cdc42-casein kinase 1α signaling in human mammary epithelial cells, *Mol. Cell. Biol.* 26 (2006) 6024–6036.
- [134] S. Foldynová-Trantírková, P. Sekyrová, K. Tmejová, E. Brumovská, O. Bernatík, W. Blankenfeldt, P. Krejčí, A. Kozubík, T. Dolezal, L. Trantírek, V. Bryja, Breast cancer-specific mutations in CK1ε/p53 inhibit Wnt/β-catenin and activate the Wnt/Rac1/JNK and NFAT pathways to decrease cell adhesion and promote cell migration, *Breast Cancer Res.* 12 (2010) R30.
- [135] G.K. Yiu, A. Kaunisto, Y.R. Chin, A. Tokor, NFAT promotes carcinoma invasive migration through glypican-6, *Biochem. J.* 440 (2011) 157–166.
- [136] M. Chen, K.L. O'Connor, Integrin α6β4 promotes expression of autotaxin/ENPP2 autocrine motility factor in breast carcinoma cells, *Oncogene* 24 (2005) 5125–5130.
- [137] A. Seifert, S. Rau, G. Kullertz, B. Fischer, A.N. Santos, TCDD induces cell migration via NFATc1/ATX-signaling in MCF-7 cells, *Toxicol. Lett.* 184 (2009) 26–32.
- [138] E. Saygili, O.R. Rana, C. Meyer, C. Gemein, M.G. Andrzejewski, A. Ludwig, C. Weber, U. Schotten, A. Kruttgen, J. Weis, R.H. Schwinger, K. Mischke, T. Rassaf, M. Kelm, P. Schauer, The angiotensin–calcineurin–NFAT pathway mediates stretch-induced up-regulation of matrix metalloproteinases-2/-9 in atrial myocytes, *Basic Res. Cardiol.* 104 (2009) 435–448.
- [139] P. Velupillai, C.K. Sung, Y. Tian, J. Dahl, J. Carroll, R. Bronson, T. Benjamin, Polyoma virus-induced osteosarcomas in inbred strains of mice: host determinants of metastasis, *PLoS Pathog.* 6 (2010) e1000733.
- [140] T. Minami, S. Jiang, K. Schadler, J. Suehiro, T. Osawa, Y. Oike, M. Miura, M. Naito, T. Kodama, S. Ryeom, The calcineurin–NFAT–angiopoietin-2 signaling axis in lung endothelium is critical for the establishment of lung metastases, *Cell Rep.* 4 (2013) 709–723.
- [141] M. Maillet, J. Davis, M. Auger-Messier, A. York, H. Osinska, J. Piquereau, J.N. Lorenz, J. Robbins, R. Ventura-Clapier, J.D. Molkentin, Heart-specific deletion of CnB1 reveals multiple mechanisms whereby calcineurin regulates cardiac growth and function, *J. Biol. Chem.* 285 (2010) 6716–6724.
- [142] R.A. Schulz, K.E. Yutzy, Calcineurin signaling and NFAT activation in cardiovascular and skeletal muscle development, *Dev. Biol.* 266 (2004) 1–16.
- [143] G.W. Cockerill, A.G. Bert, G.R. Ryan, J.R. Gamble, M.A. Vadas, P.N. Cockerill, Regulation of granulocyte-macrophage colony-stimulating factor and E-selectin expression in endothelial cells by cyclosporin A and the T-cell transcription factor NFAT, *Blood* 86 (1995) 2689–2698.
- [144] K.H. Baek, A. Zaslavsky, R.C. Lynch, C. Britt, Y. Okada, R.J. Siarey, M.W. Lensch, I.H. Park, S.S. Yoon, T. Minami, J.R. Korenberg, J. Folkman, G.Q. Daley, W.C. Aird, Z. Galdzicki, S. Ryeom, Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1, *Nature* 459 (2009) 1126–1130.
- [145] R.M. Kulkarni, J.M. Greenberg, A.L. Akeson, NFATc1 regulates lymphatic endothelial development, *Mech. Dev.* 126 (2009) 350–365.
- [146] T.A. Zaichuk, E.H. Shroff, R. Emmanuel, S. Filleur, T. Nelius, O.V. Volpert, Nuclear factor of activated T cells balances angiogenesis activation and inhibition, *J. Exp. Med.* 199 (2004) 1513–1522.
- [147] P. Allavena, A. Sica, G. Solinas, C. Porta, A. Mantovani, The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages, *Crit. Rev. Oncol. Hematol.* 66 (2008) 1–9.
- [148] M.R. Hodge, A.M. Ranger, F. Charles de la Brousse, T. Hoey, M.J. Grusby, L.H. Glimcher, Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice, *Immunity* 4 (1996) 397–405.
- [149] R. Kumarswamy, T. Thum, Non-coding RNAs in cardiac remodeling and heart failure, *Circ. Res.* 113 (2013) 676–689.
- [150] P.A. da Costa Martins, C. Salic, M.M. Gladka, A.S. Armand, S. Leptidis, H. el Azzouzi, A. Hansen, C.J. Coenen-de Roo, M.F. Bierhuizen, R. van der Nagel, J. van Kuik, R. de Weger, A. de Bruin, G. Condorelli, M.L. Arbones, T. Eschenhagen, L.J. De Windt, MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signaling, *Nat. Cell Biol.* 12 (2010) 1220–1227.
- [151] J.M. Liao, X. Zhou, Y. Zhang, H. Lu, MiR-1246: a new link of the p53 family with cancer and Down syndrome, *Cell Cycle* 11 (2012) 2624–2630.
- [152] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (2011) 646–674.
- [153] W.P. Steward, K. Brown, Cancer chemoprevention: a rapidly evolving field, *Br. J. Cancer* 109 (2013) 1–7.
- [154] J. Ding, J. Li, C. Xue, K. Wu, W. Ouyang, D. Zhang, Y. Yan, C. Huang, Cyclooxygenase-2 induction by arsenite is through a nuclear factor of activated T-cell-dependent pathway and plays an antiapoptotic role in Beas-2B cells, *J. Biol. Chem.* 281 (2006) 24405–24413.
- [155] W. Ouyang, Y. Hu, J. Li, M. Ding, Y. Lu, D. Zhang, Y. Yan, L. Song, Q. Qu, D. Desai, S. Amin, C. Huang, Direct evidence for the critical role of NFAT3 in benzo[a]pyrene diol-epoxide-induced cell transformation through mediation of inflammatory cytokine TNF induction in mouse epidermal C141 cells, *Carcinogenesis* 28 (2007) 2218–2226.
- [156] J. Ding, Y. Huang, B. Ning, W. Gong, J. Li, H. Wang, C.Y. Chen, C. Huang, TNF-α induction by nickel compounds is specific through ERKs/AP-1-dependent pathway in human bronchial epithelial cells, *Curr. Cancer Drug Targets* 9 (2009) 81–90.
- [157] T. Cai, X. Li, J. Ding, W. Luo, J. Li, C.A. Huang, Cross-talk between NFAT and NF-κappaB pathways is crucial for nickel-induced COX-2 expression in Beas-2B cells, *Curr. Cancer Drug Targets* 11 (2011) 548–559.
- [158] C. Huang, J. Li, M. Costa, Z. Zhang, S.S. Leonard, V. Castranova, V. Vallyathan, G. Ju, X. Shi, Hydrogen peroxide mediates activation of nuclear factor of activated T cells (NFAT) by nickel subsulfide, *Cancer Res.* 61 (2001) 8051–8057.
- [159] H. Tang, Y. Sun, Q. Xiu, H. Lu, H. Han, Cyclooxygenase-2 induction requires activation of nuclear factor of activated T-cells in Beas-2B cells after vanadium exposure and plays an anti-apoptotic role, *Arch. Biochem. Biophys.* 468 (2007) 92–99.
- [160] J. Li, D. Zhang, G.D. Stoner, C. Huang, Differential effects of black raspberry and strawberry extracts on BaPDE-induced activation of transcription factors and their target genes, *Mol. Carcinog.* 47 (2008) 286–294.
- [161] O.T. Murray, C.C. Wong, K. Vrankova, B. Rigas, Phospho-sulindac inhibits pancreatic cancer growth: NFATc1 as a drug resistance candidate, *Int. J. Oncol.* 44 (2014) 521–529.
- [162] C.R. Lee, J.N. Chun, S.Y. Kim, S. Park, S.H. Kim, E.J. Park, I.S. Kim, N.H. Cho, I.G. Kim, I. So, T.W. Kim, J.H. Jeon, Cyclosporin A suppresses prostate cancer cell growth through CaMKKβ/AMPK-mediated inhibition of mTORC1 signaling, *Biochem. Pharmacol.* 84 (2012) 425–431.
- [163] W. Garrido, M. Munoz, R. San Martin, C. Quezada, FK506 confers chemosensitivity to anticancer drugs in glioblastoma multiforme cells by decreasing the expression of the multiple resistance-associated protein-1, *Biochem. Biophys. Res. Commun.* 411 (2011) 62–68.
- [164] S. Romano, A. Di Pace, A. Sorrentino, R. Bisogni, L. Sivero, M.F. Romano, FK506 binding proteins as targets in anticancer therapy, *Anticancer Agents Med. Chem.* 10 (2010) 651–656.
- [165] J.L. Gooch, Y. Gorin, B.X. Zhang, H.E. Abboud, Involvement of calcineurin in transforming growth factor-β-mediated regulation of extracellular matrix accumulation, *J. Biol. Chem.* 279 (2004) 15561–15570.
- [166] A. Manninen, G.H. Renkema, K. Saksela, Synergistic activation of NFAT by HIV-1 nef and the Ras/MAPK pathway, *J. Biol. Chem.* 275 (2000) 16513–16517.
- [167] J.R. Azzi, M.H. Sayegh, S.G. Mallat, Calcineurin inhibitors: 40 years later, can't live without ..., *J. Immunol.* 191 (2013) 5785–5791.
- [168] J.M. Durnian, R.M. Stewart, R. Tatham, M. Batterbury, S.B. Kaye, Cyclosporin-A associated malignancy, *Clin. Ophthalmol.* 1 (2007) 421–430.
- [169] J. Zheng, F. Fang, X. Zeng, T.R. Medler, A.A. Fiorillo, C.V. Clevenger, Negative cross talk between NFAT1 and Stat5 signaling in breast cancer, *Mol. Endocrinol.* 25 (2011) 2054–2064.
- [170] J. Aramburu, M.B. Yaffe, C. López-Rodríguez, L.C. Cantley, P.G. Hogan, A. Rao, Affinity-driven peptide selection of an NFAT inhibitor more selective than cyclosporin A, *Science* 285 (1999) 2129–2133.
- [171] A. Escolano, S. Martinez-Martinez, A. Alfranca, K. Urso, H.M. Izquierdo, M. Delgado, F. Martin, G. Sabio, D. Sancho, P. Gomez-Del Arco, J.M. Redondo, Specific calcineurin targeting in macrophages confers resistance to inflammation via MKP-1 and p38, *EMBO J.* 33 (2014) 1117–1133.
- [172] H. Yu, T.J. van Berkel, E.A. Biessen, Therapeutic potential of VIVIT, a selective peptide inhibitor of nuclear factor of activated T cells, in cardiovascular disorders, *Cardiovasc. Drug Rev.* 25 (2007) 175–187.
- [173] H.Z. Elloumi, N. Maharsakh, K.N. Rao, T. Kobayashi, H.S. Ryu, M. Muhlbauer, F. Li, C. Jobin, S.E. Plevy, A cell permeable peptide inhibitor of NFAT inhibits macrophage cytokine expression and ameliorates experimental colitis, *PLoS One* 7 (2012) e34172.
- [174] H. Wang, B. Grzywacz, D. Sukovich, V. McCullar, Q. Cao, A.B. Lee, B.R. Blazar, D.N. Cornfield, J.S. Miller, M.R. Verneris, The unexpected effect of cyclosporin A on CD56+CD16– and CD56+CD16+ natural killer cell subpopulations, *Blood* 110 (2007) 1530–1539.
- [175] T. Masuo, S. Okamura, Y. Zhang, M. Mori, Cyclosporine A inhibits colorectal cancer proliferation probably by regulating expression levels of c-Myc, p21(WAF1/CIP1) and proliferating cell nuclear antigen, *Cancer Lett.* 285 (2009) 66–72.
- [176] H. Morjani, C. Madoulet, Immunosuppressors as multidrug resistance reversal agents, *Methods Mol. Biol.* 596 (2010) 433–446.
- [177] M. Sieber, R. Baumgrass, Novel inhibitors of the calcineurin/NFATc hub – alternatives to CsA and FK506? *Cell Commun. Signal.* 7 (2009) 25.
- [178] ISA 247: trans-ISA 247, trans-R 1524, ISA(TX)247, ISATx 247, ISATx247, LX 211, LX211, R 1524, R-1524, Drugs R D 8 (2007) 103–112.
- [179] K. Ohga, R. Takekawa, Y. Arakida, Y. Shimizu, J. Ishikawa, Characterization of YM-58483/BTP2, a novel store-operated Ca2+ entry blocker, on T cell-mediated immune responses in vivo, *Int. Immunopharmacol.* 8 (2008) 1787–1792.
- [180] C. Zitt, B. Strauss, E.C. Schwarz, N. Spaeth, G. Rast, A. Hatzelmann, M. Hoth, Potent inhibition of Ca2+ release-activated Ca2+ channels and T-lymphocyte activation by the pyrazole derivative BTP2, *J. Biol. Chem.* 279 (2004) 12427–12437.
- [181] N. Venkatesh, Y. Feng, B. DeDecker, P. Yacono, D. Golani, T. Mitchison, F. McKeon, Chemical genetics to identify NFAT inhibitors: potential of targeting calcium mobilization in immunosuppression, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 8969–8974.
- [182] C.T. Yeh, A.T. Wu, P.M. Chang, K.Y. Chen, C.N. Yang, S.C. Yang, C.C. Ho, C.C. Chen, Y.L. Kuo, P.Y. Lee, Y.W. Liu, C.C. Yen, M. Hsiao, P.J. Lu, J.M. Lai, L.S. Wang, C.H. Wu, J.F. Chiou, P.C. Yang, C.Y. Huang, Trifluoperazine, an antipsychotic agent, inhibits

- cancer stem cell growth and overcomes drug resistance of lung cancer, *Am. J. Respir. Crit. Care Med.* 186 (2012) 1180–1188.
- [183] H. Luo, G.O. Rankin, L. Liu, M.K. Daddysman, B.H. Jiang, Y.C. Chen, Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells, *Nutr. Cancer* 61 (2009) 554–563.
- [184] A.Y. Chen, Y.C. Chen, A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention, *Food Chem.* 138 (2013) 2099–2107.
- [185] H. Wang, C.L. Zhou, H. Lei, S.D. Zhang, J. Zheng, Q. Wei, Kaempferol: a new immunosuppressant of calcineurin, *IUBMB Life* 60 (2008) 549–554.
- [186] H. Lei, Y. Qi, Z.G. Jia, W.L. Lin, Q. Wei, Studies on the interactions of kaempferol to calcineurin by spectroscopic methods and docking, *Biochim. Biophys. Acta* 1794 (2009) 1269–1275.
- [187] M. Humar, S.E. Pischke, T. Loop, A. Hoetzel, R. Schmidt, C. Klaas, H.L. Pahl, K.K. Geiger, B.H. Pannen, Barbiturates directly inhibit the calmodulin/calcineurin complex: a novel mechanism of inhibition of nuclear factor of activated T cells, *Mol. Pharmacol.* 65 (2004) 350–361.
- [188] M.H. Roehrl, S. Kang, J. Aramburu, G. Wagner, A. Rao, P.G. Hogan, Selective inhibition of calcineurin–NFAT signaling by blocking protein–protein interaction with small organic molecules, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 7554–7559.
- [189] M. Sieber, M. Karanik, C. Brandt, C. Blex, M. Podtschaske, F. Erdmann, R. Rost, E. Serfling, J. Liebscher, M. Patzel, A. Radbruch, G. Fischer, R. Baumgrass, Inhibition of calcineurin–NFAT signaling by the pyrazolopyrimidine compound NC13, *Eur. J. Immunol.* 37 (2007) 2617–2626.
- [190] D. Spano, J.C. Marshall, N. Marino, D. De Martino, A. Romano, M.N. Scoppettuolo, A.M. Bello, V. Di Dato, L. Navas, G. De Vita, C. Medaglia, P.S. Steeg, M. Zollo, Dipyrindamole prevents triple-negative breast-cancer progression, *Clin. Exp. Metastasis* 30 (2013) 47–68.
- [191] W.H. Isacoff, J.K. Bendetti, J.J. Barstis, A.R. Jazieh, J.S. Macdonald, P.A. Philip, Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyrindamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700, *J. Clin. Oncol.* 25 (2007) 1665–1669.
- [192] S. Bonnet, S.L. Archer, J. Allalunis-Turner, A. Haromy, C. Beaulieu, R. Thompson, C.T. Lee, G.D. Lopaschuk, L. Puttagunta, G. Harry, K. Hashimoto, C.J. Porter, M.A. Andrade, B. Thebaud, E.D. Michelakis, A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth, *Cancer Cell* 11 (2007) 37–51.
- [193] J.Y. Wong, G.S. Huggins, M. Debidia, N.C. Munshi, I. De Vivo, Dichloroacetate induces apoptosis in endometrial cancer cells, *Gynecol. Oncol.* 109 (2008) 394–402.
- [194] V. Gayosso, L.F. Montano, R. Lopez-Marure, DHEA-induced antiproliferative effect in MCF-7 cells is androgen- and estrogen receptor-independent, *Cancer J.* 12 (2006) 160–165.
- [195] C. Kliem, A. Merling, M. Gaiasi, R. Kohler, P.H. Krammer, M. Li-Weber, Curcumin suppresses T cell activation by blocking Ca²⁺ mobilization and nuclear factor of activated T cells (NFAT) activation, *J. Biol. Chem.* 287 (2012) 10200–10209.
- [196] R. Lindstedt, V. Ruggiero, V. D' Alessio, S. Manganello, F. Petronzelli, M.A. Stasi, S. Vendetti, A. Assandri, P. Carminati, R. De Santis, The immunosuppressor st1959, a 3,5-diaryl-s-triazole derivative, inhibits T cell activation by reducing NFAT nuclear residency, *Int. J. Immunopathol. Pharmacol.* 22 (2009) 29–42.
- [197] P. Proksch, M. Gaiasi, M.K. Treiber, K. Palfi, A. Merling, H. Spring, P.H. Krammer, M. Li-Weber, Rocaglamide derivatives are immunosuppressive phytochemicals that target NF-AT activity in T cells, *J. Immunol.* 174 (2005) 7075–7084.
- [198] J.H. Jang, T. Iqbal, K.J. Min, S. Kim, J.W. Park, E.I. Son, T.J. Lee, T.K. Kwon, Helenalin-induced apoptosis is dependent on production of reactive oxygen species and independent of induction of endoplasmic reticulum stress in renal cell carcinoma, *Toxicol. In Vitro* 27 (2013) 588–596.
- [199] W. Dai, F. Wang, L. He, C. Lin, S. Wu, P. Chen, Y. Zhang, M. Shen, D. Wu, C. Wang, J. Lu, Y. Zhou, X. Xu, L. Xu, C. Guo, Genistein inhibits hepatocellular carcinoma cell migration by reversing the epithelial–mesenchymal transition: partial mediation by the transcription factor NFAT1, *Mol. Carcinog.* (2013) (Electronic publication ahead of print).
- [200] M. Li, Z. Zhang, D.L. Hill, X. Chen, H. Wang, R. Zhang, Genistein, a dietary isoflavone, down-regulates the MDM2 oncogene at both transcriptional and posttranslational levels, *Cancer Res.* 65 (2005) 8200–8208.
- [201] J. Roman, A.F. de Arriba, S. Barron, P. Michelena, M. Giral, M. Merlos, E. Bailon, M. Comalada, J. Galvez, A. Zarzuelo, I. Ramis, UR-1505, a new salicylate, blocks T cell activation through nuclear factor of activated T cells, *Mol. Pharmacol.* 72 (2007) 269–279.
- [202] E. Bailon, J. Roman, I. Ramis, P. Michelena, D. Balsa, M. Merlos, A. Zarzuelo, J. Galvez, M. Comalada, The new salicylate derivative UR-1505 modulates the Th2/humoral response in a dextran sodium sulphate model of colitis that resembles ulcerative colitis, *J. Pharmacol. Sci.* 109 (2009) 315–318.
- [203] M. Aceves, A. Dueñas, C. Gómez, E. San Vicente, M.S. Crespo, C. García-Rodríguez, A new pharmacological effect of salicylates: inhibition of NFAT-dependent transcription, *J. Immunol.* 173 (2004) 5721–5729.
- [204] J.T. Sanderson, H. Clabault, C. Patton, G. Lassalle-Claux, J. Jean-Francois, A.F. Pare, M. J. Hebert, M.E. Surette, M. Touaibia, Antiproliferative, antiandrogenic and cytotoxic effects of novel caffeic acid derivatives in LNCaP human androgen-dependent prostate cancer cells, *Bioorg. Med. Chem.* 21 (2013) 7182–7193.
- [205] F. Aqil, R. Munagala, M.V. Vadhanam, H. Kausar, J. Jeyabalan, D.J. Schultz, R.C. Gupta, Anti-proliferative activity and protection against oxidative DNA damage by punicalagin isolated from pomegranate husk, *Food Res. Int.* 49 (2012) 345–353.
- [206] K. Choochuay, P. Chunhacha, V. Pongrakhananon, R. Luechapudiporn, P. Chanvorachote, Imperatorin sensitizes anoikis and inhibits anchorage-independent growth of lung cancer cells, *J. Nat. Med.* 67 (2013) 599–606.
- [207] H.Z. Jin, J.H. Lee, D. Lee, H.S. Lee, Y.S. Hong, Y.H. Kim, J.J. Lee, Quinolone alkaloids with inhibitory activity against nuclear factor of activated T cells from the fruits of *Evodia rutaecarpa*, *Biol. Pharm. Bull.* 27 (2004) 926–928.
- [208] T.L. Towers, L.P. Freedman, Granulocyte-macrophage colony-stimulating factor gene transcription is directly repressed by the vitamin D3 receptor. Implications for allosteric influences on nuclear receptor structure and function by a DNA element, *J. Biol. Chem.* 273 (1998) 10338–10348.
- [209] Q.F. Yang, C.L. Dalgard, O. Eidelman, C. Jozwik, B.S. Pollard, M. Srivastava, H.B. Pollard, Digitoxin induces apoptosis in cancer cells by inhibiting nuclear factor of activated T-cells-driven c-MYC expression, *J. Carcinog.* 12 (2013) 8.
- [210] F.J. Caballero, C.M. Navarrete, S. Hess, B.L. Fiebich, G. Appendino, A. Macho, E. Munoz, R. Sancho, The acetaminophen-derived bioactive N-acylphenolamine AM404 inhibits NFAT by targeting nuclear regulatory events, *Biochem. Pharmacol.* 73 (2007) 1013–1023.
- [211] I. Kaddour-Djebbar, V. Choudhary, V. Lakshminathan, R. Shirley, M. El Gaish, M. Al-Shabrawey, B. Al-Husein, R. Zhong, M. Davis, Z. Dong, W.B. Bollag, M.V. Kumar, Diltiazem enhances the apoptotic effects of proteasome inhibitors to induce prostate cancer cell death, *J. Pharmacol. Exp. Ther.* 341 (2012) 646–655.
- [212] L. Wu, Y.Y. Liu, Z.X. Li, Q. Zhao, X. Wang, Y. Yu, Y.Y. Wang, Y.Q. Wang, F. Luo, Antitumor effects of penfluridol through dysregulation of cholesterol homeostasis, *Asian Pac. J. Cancer Prev.* 15 (2014) 489–494.