NFAT, immunity and cancer: a transcription factor comes of age

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Abstract | Nuclear factor of activated T cells (NFAT) was first identified more than two decades ago as a major stimulation-responsive DNA-binding factor and transcriptional regulator in T cells. It is now clear that NFAT proteins have important functions in other cells of the immune system and regulate numerous developmental programmes in vertebrates. Dysregulation of these programmes can lead to malignant growth and cancer. This Review focuses on recent advances in our understanding of the transcriptional functions of NFAT proteins in the immune system and provides new insights into their potential roles in cancer development.

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Nuclear factor of activated T cells (NFAT) was identified more than 20 years ago in nuclear extracts of activated T cells as an inducible DNA-binding factor that binds to the interleukin-2 (*Il2*) promoter[1]. Interest in NFAT stemmed from the fact that its induction was inhibited by cyclosporin A (CsA), a drug that had revolutionized transplant medicine by blocking immune-mediated rejection of transplanted organs and tissues. It was soon recognized that NFAT contained both nuclear and cytoplasmic constituents[2]. The nuclear component was identified as the activator protein 1 (AP1) transcription factor, which is comprised of JUN homodimers and FOS–JUN heterodimers[3]. The realization that NFAT formed a complex with AP1 quickly led to the purification and molecular cloning of the founding member of the NFAT family NFAT1[4,5]; three other calcium-regulated NFAT proteins were subsequently identified[6,7]. Purified NFAT is a heavily phosphorylated protein that is dephosphorylated by the phosphatase calcineurin[4,5], which is the molecular target of CsA, and another potent immunosuppressive agent, FK506[8] (tacrolimus).

It is now clear that NFAT regulates not only T cell activation and differentiation[9,10] but also the function of other immune cells, including dendritic cells (DCs), B cells and megakaryocytes[11–13]. In addition, NFAT transcription factors have crucial roles in numerous developmental programmes in vertebrates, including those of the heart, skeletal muscle, smooth muscle, blood vessels, neurons, bone, pancreas and skin[9,14–17]. Moreover, dysregulation of NFAT signalling is now known to be associated with malignant transformation and the development of cancer[18,19].

In this Review we focus on recent insights into the roles of NFAT proteins in T cells and other components of the vertebrate immune system. In addition, we review recent studies that suggest an emerging role for NFAT in the pathogenesis of malignant diseases and tumour metastasis.

The NFAT family of transcription factors

The NFAT family consists of five proteins that are evolutionarily related to the REL (also known as c-Rel)–nuclear factor-κB (REL–NF-κB) family of transcription factors: NFAT1 (also known as NFATc2 or NFATp), NFAT2 (also known as NFATc1 or NFATc), NFAT3 (also known as NFATc4), NFAT4 (also known as NFATc3 or NFATx) and NFAT5 (also known as tonicity enhancer binding protein (TonEBP))[9,20]. NFAT proteins contain an amino-terminal transactivation domain (TAD), a regulatory domain (also known as the NFAT homology region (NHR)), a highly conserved DNA-binding domain (also known as the Rel-homology domain, (RHD)) and a carboxy-terminal domain (FIG. 1a). The regulatory domain, which is moderately conserved among NFAT proteins, contains multiple serine-rich regions (SRRs) that are phosphorylated by the NFAT kinases casein kinase 1 (CK1; also known as CSK1A1) and glycogen synthase kinase 3 (GSK3) and by the dual-specificity tyrosine-phosphorylation-regulated kinase (DYRK) under resting conditions. The regulatory domain also contains the docking sites for calcineurin and CK1, which controls NFAT activation by regulating the phosphorylation status of the SRR1 region (FIG. 1a).

NFAT1–NFAT4 are regulated by intracellular Ca^{2+} signalling[9,10], but NFAT5 — the primordial member of the NFAT family — is activated in response to osmotic

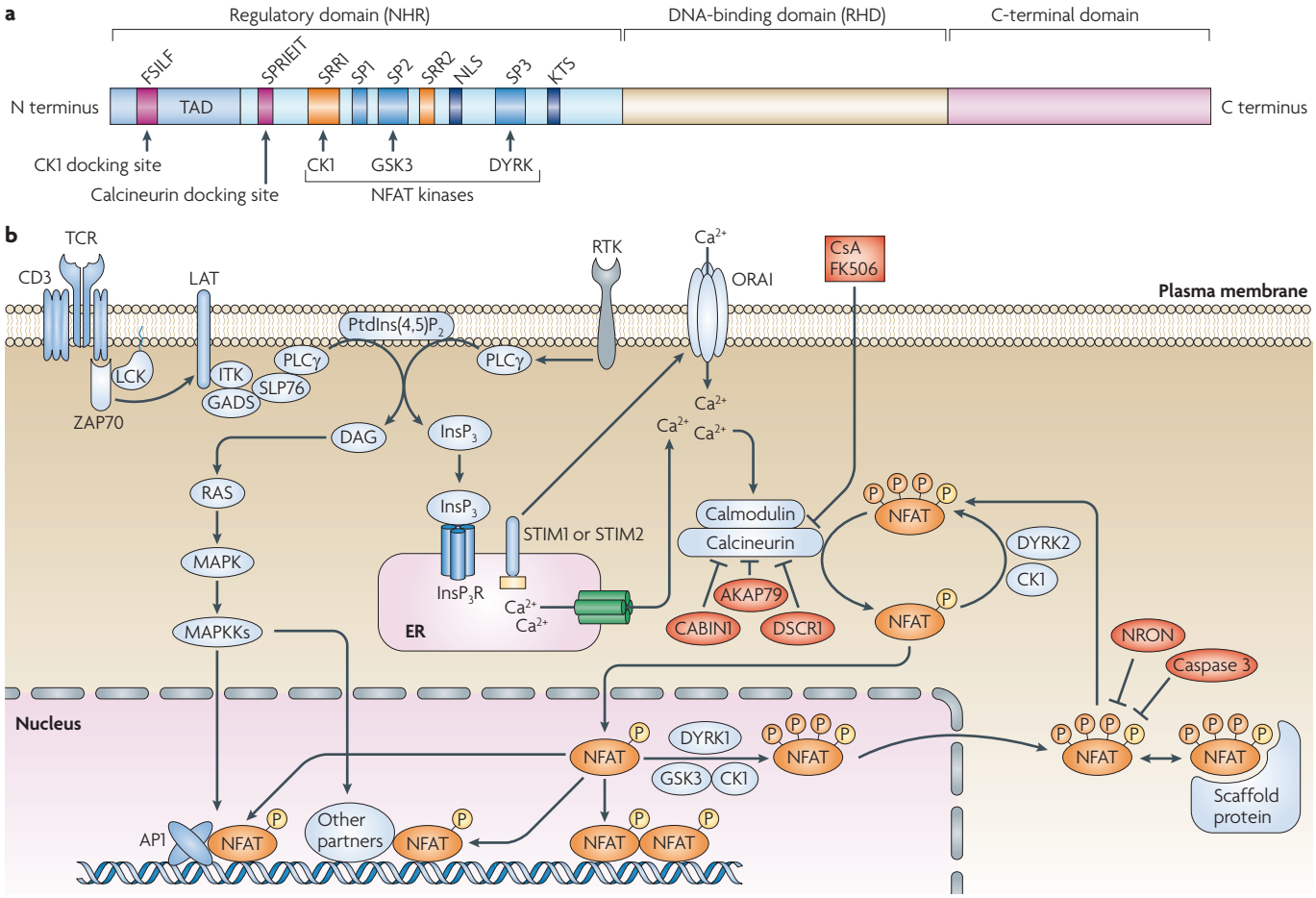

Figure 1 | **The Ca^{2+}–NFAT signalling pathway. a** | General structure of nuclear factor of activated T cells (NFAT) transcription factors. NFAT proteins consist of an amino-terminal regulatory domain (also known as an NFAT homology region (NHR)), a DNA-binding domain (also known as a REL-homology domain (RHD)) and a carboxy-terminal domain. The regulatory domain contains an N-terminal transactivation domain (TAD), as well as a docking site for for casein kinase 1 (CK1), termed FSILF, and for calcineurin, termed SPRIEIT. It also includes multiple serine-rich motifs (SRR1 , SP1, SP2, SRR2, SP3 and KTS) and a nuclear localization sequence (NLS). **b** | NFAT activation and regulation. Immunoreceptors and receptor tyrosine kinases (RTKs) bind to their ligands and activate phospholipase Cγ (PLCγ), which in turn hydrolyses phosphatidyl-4,5-bisphosphate ($PtdIns(4,5)P_2$) to diacylglecerol (DAG) and inositol-1,4,5-trisphosphate ($InsP_3$). $InsP_3$ binds to the $InsP_3$ receptor ($InsP_3R$) on the endoplasmic reticulum (ER) membrane and induces the efflux of Ca^{2+}. Stromal interaction molecule 1 (STIM1) and STIM2 subsequently detect the decrease of ER Ca^{2+} stores, form small clusters and communicate with the calcium-release-activated calcium (CRAC) channel protein ORAI in the plasma membrane to trigger store-operated Ca^{2+} entry. Ca^{2+} binds to the calcium sensor protein calmodulin, which in turn activates calcineurin. Calcineurin dephosphorylates and activates NFAT transcription factors, which then translocate to the nucleus, where they can cooperate with multiple transcriptional partners (for example, activator protein 1 (AP1)) to regulate gene expression. The activation of these partners is controlled by RAS–mitogen-activated protein kinase (MAPK) signalling and other pathways. NFAT proteins are rephosphorylated and inactivated by multiple NFAT kinases, such as glycogen-synthase kinase 3 (GSK3), CK1, and dual-specificity tyrosine-phosphorylation regulated kinase 1 (DYRK1) and DYRK2. The activity of calcineurin is regulated by multiple endogenous calcineurin inhibitors, such as calcineurin-binding protein 1 (CABIN1), A-kinase anchor protein 79 (AKAP79) and Down's syndrome critical region 1 (DSCR1). Cyclosporin A (CsA) and FK506 are potent pharmacological inhibitors of calcineurin. Different scaffold proteins have been shown to interact with NFAT proteins and to be involved in the regulation of their activity. Non-coding repressor of NFAT (NRON) and caspase 3 are negative regulators of NFAT activity. GADS, GRB2-related adaptor protein; ITK, IL-2-inducible T-cell kinase; LAT, linker for activation of T cells; MAPKK, MAPK kinase; SLP76, SH2-domain-containing leukocyte protein of 76 kDa; TCR, T cell receptor; ZAP70, ζ-chain-associated protein kinase of 70 kDa.

stress[21,22]. Predictably, mice that lack NFAT5 show marked deterioration of the kidney medulla, a region that is normally exposed to extreme hypertonic stress[23]. In lymphocytes NFAT5 controls the expression of several cytokines in response to osmotic stress, including tumour necrosis factor (TNF) and lymphotoxin[24].

Regulation of NFAT activity

The components of the Ca^{2+}–calcineurin–NFAT signalling pathway have been extensively reviewed[9,10,25,26]. Here we present a short comprehensive overview of the pathway and then focus on recent insights into the regulation of NFAT activation.

Table 1 | **NFAT kinases**

NFAT kinase	Kinase type	Substrate	Phosphorylation site	Refs
GSK3	Export	NFAT1	SP2	32
		NFAT2	SP2 and SP3	32
CK1	Export and maintenance	NFAT1	SRR1	33
DYRK1	Export	NFAT1 and NFAT2	SP3	30,31
DYRK2	Maintenance	NFAT1 and NFAT2	SP3	30,31

CK1, casein kinase 1; DYRK, dual-specificity tyrosine-phosphorylation regulated kinase; GSK3, glycogen synthase kinase 3; NFAT, nuclear factor of activated T cells; SP, Ser-Pro-X-X repeat motif; SRR, serine-rich region.

Activation of NFAT. In the cytoplasm NFAT is activated by cell surface receptors that are coupled to Ca^{2+} mobilization (FIG. 1b). Briefly, activation of phospholipase Cγ (PLCγ) by ligand binding to immunoreceptors, receptor tyrosine kinases and G-protein-coupled receptors leads to the production of inositol-1,4,5-trisphosphate ($InsP_3$), resulting in the release of Ca^{2+} from endoplasmic reticulum Ca^{2+} stores. This triggers a process known as store-operated Ca^{2+} entry (SOCE)[25,26], which leads to the activation of the Ca^{2+} sensor calmodulin and of diverse calmodulin-dependent enzymes, including calcineurin[9]. This phosphatase then dephosphorylates multiple phosphoserines in the regulatory domain of NFAT, leading to NFAT nuclear translocation. Once inside the nucleus NFAT can cooperate with multiple transcriptional partners, including AP1, forkhead box P-family proteins (such as FOXP2 and FOXP3) and proteins of the GATA family, to initiate and maintain specific transcriptional programmes that vary with cell type and the pattern of stimulation[9,10,20].

For calcineurin to dephosphorylate NFAT it must first dock with the DNA-binding factor at a specific motif in the regulatory domain. This motif possesses the consensus sequence Pro-X-Ile-X-Ile-Thr (in which X can be any amino acid)[27,28] and is highly conserved between different members of the NFAT family. A peptide with a high-affinity form of the Pro-X-Ile-X-Ile-Thr sequence, Pro-Val-Ile-Val-Ile-Thr (VIVIT), was selected from a randomized peptide library. This peptide binds calcineurin with high affinity and effectively competes with NFAT for calcineurin binding, thus blocking NFAT dephosphorylation[27]. Transgenic mice expressing a mutated form of NFAT1, in which the endogenous Pro-X-Ile-X-Ile-Thr sequence is replaced by the high-affinity VIVIT sequence, showed a hyperresponsive T cell phenotype that was characterized by increased production of cytokines and showed defects in embryonic and haematopoietic cell development[29].

NFAT kinases. Inside the nucleus NFAT is inactivated by the coordinated action of multiple NFAT kinases, resulting in NFAT rephosphorylation and relocation to the cytoplasm[30,31]. The phosphorylation sites of NFAT are located in multiple serine-rich motifs in the regulatory domain: the SRR1 and the Ser-Pro-X-X repeat motifs SP1, SP2 and SP3 (FIG. 1a). Recently, a more complete picture of NFAT kinases has started to emerge (TABLE 1). Export kinases are responsible for the rephosphorylation of NFAT inside the nucleus and induce its relocation to the cytoplasm. By contrast, maintenance kinases act in the cytosplasm, where they keep NFAT proteins in a fully phosphorylated state and prevent their translocation to the nucleus under resting conditions.

GSK3 is an export kinase that phosphorylates the SP2 motif of NFAT1 and both the SP2 and SP3 motifs of NFAT2 (REFS 31–33). The substrate sites for GSK3 in NFAT2 are created only after previous phosphorylation by a 'priming' kinase that can be either protein kinase A (PKA) or a DYRK[30,31] (DYRKs recently emerged as a new class of NFAT kinases[30,31]). DYRK1A and DYRK2 can directly phosphorylate the conserved SP3 motif of the NFAT1 regulatory domain and thus can prime for the subsequent phosphorylation of the SP2 and SRR1 motifs by GSK3 and CK1, respectively[31].

CK1, which phosphorylates the SRR1 motif, can operate both as an export and maintenance kinase[33] and docks at a conserved sequence motif near the N terminus of NFAT proteins (Phe-Ser-Ile-Leu-Phe in NFAT1)[33]. T cells from transgenic mice have been engineered to express a mutant version of NFAT1 that contains a low-affinity version of the CK1 docking site (Ala-Ser-Ile-Leu-Ala instead of Phe-Ser-Ile-Leu-Phe), as well as a high-affinity version of the calcineurin docking site (VIVIT). T cells from these mice have a hyperresponsive phenotype (characterized by increased production of the cytokines interferon-γ (IFNγ) and TNF upon stimulation) that is more pronounced than the phenotype seen if NFAT1 only contains the VIVIT mutation[29]. The defects in embryonic and haematopoietic cell development are also more severe in these mice than in mice bearing the VIVIT mutation alone, showing that CK1 has an important role in regulating the activity of NFAT proteins *in vivo*.

Autoregulation of NFAT2. NFAT2 can exist as three different isoforms, NFAT2A, NFAT2B and NFAT2C, which are related by alternative splicing and differ in their length and mechanism of expression. Of the five NFAT family members, NFAT2 is uniquely regulated by a positive autoregulatory loop[34]. Although the longer isoforms NFAT2B and NFAT2C are constitutively expressed in naive T cells, the shorter isoform NFAT2A, which is expressed preferentially in effector T cells, is under the control of an NFAT-dependent inducible promoter and is coupled to a different, more proximal polyadenylation site[35]. This regulatory strategy results in the accumulation of the short NFAT2A isoform during lineage commitment, which explains why deletion of NFAT2 is generally more deleterious to development than deletion of other NFAT family members.

Other mechanisms. In addition to reversible phosphorylation, several other mechanisms that control NFAT activation have been described in recent years. The cytoplasmic scaffold proteins HOMER2 and HOMER3 were reported to compete with calcineurin for NFAT binding and thus prevent NFAT dephosphorylation

Ubiquitylation
The attachment of the small protein ubiquitin to lysine residues that are present in other proteins; this often tags these proteins for rapid cellular degradation.

Sumoylation
The post-translational modification of proteins that involves the covalent attachment of small ubiquitin-related modifier (SUMO) and regulates the interactions of those proteins with other macromolecules.

Anergy
A state of unresponsiveness that is sometimes observed in T and B cells that are chronically stimulated or that are stimulated through the antigen receptor in the absence of co-stimulatory signals.

and activation[36]. Through the use of a library of short hairpin RNAs (shRNAs) directed against 512 evolutionarily conserved non-coding RNAs, a non-coding repressor of NFAT (NRON) was identified as a specific inhibitor of NFAT nuclear trafficking[37]. Depletion of NRON with shRNAs resulted in a substantial increase in NFAT activity in human embryonic kidney (HEK) 293 cells, as well as in murine 3T3 cells. The effect could be blocked using the calcineurin inhibitor CsA. Furthermore, knockdown of NRON with shRNAs in the T cell-derived Jurkat cell line resulted in increased NFAT activity after stimulation with phorbol 12-myristate 13 acetate (PMA) and ionomycin or following T cell receptor (TCR) engagement.

Caspase 3 has been reported to regulate the expression levels of NFAT1 in non-apoptotic effector T cells[38]. The study identified two potential caspase 3 cleavage sites in the transactivation domain of NFAT1 and showed that mutation of these cleavage sites resulted in significantly elevated NFAT activity. Ubiquitylation by the E3 ubiquitin ligase murine double minute 2 (MDM2) was also shown to control NFAT levels and activity in breast cancer cells[39,40]. Sumoylation was shown to be crucial for nuclear retention of NFAT in T cells[41,42]. Moreover, two studies identified ADP-ribosylation by poly-ADP-ribose polymerase 1 (PARP1) as a new mechanism for controlling NFAT activity[43,44]. Genetic ablation or pharmacological inhibition of PARP1 significantly compromised NFAT-dependent cytokine expression in T cells, suggesting that ADP-ribosylation contributes to the stability of NFAT and functions as a positive regulator of its activity in T cells.

Table 2 | **New roles of NFAT in the haematopoietic system**

Cell type	Family member	Function	Refs
T_{Reg} cells	NFAT1 and NFAT2	Regulation of *FOXP3* expression	60-62
		Regulation of *IL2*, *CD25* and *CTLA4* expression	65
T helper 17 cells	NFAT1 and NFAT2	Regulation of *IL17A*, *IL17F*, *IL21* and *IL22* expression	45-48
T follicular helper cells	NFAT2	Regulation of *IL4* expression through interaction with MAF	49,50
$CD8^+$ T cells	NFAT1	Tolerance induction	51
Dendritic cells	NFAT1	Regulation of *IL2*, *IL10* and *IL12* expression	11,68
		Induction of apoptosis	68
Mast cells	NFAT1 and NFAT2	Regulation of *IL13* and *TNF* expression	70,71
		Regulation of *HIF1a* expression	72
		Regulation of *A1* expression	73
B cells	NFAT2	B-1a cell development	75,76
		Signal transmission	76-78
NKT cells	NFAT2	NKT cell development	80
		Regulation of *EGR* expression	80
Megakaryocytes	NFAT2 and NFAT3	Regulation of CD40 ligand expression	13

CTLA4, cytotoxic lymphocyte antigen 4; *EGR*, epidermal growth response; *FOXP3*, forkhead box P3; *HIF1a*, hypoxia-inducible factor 1α; IL, interleukin; NFAT, nuclear factor of activated T cells; NKT cells, natural killer T cells; TNF, tumour necrosis factor; T_{Reg} cells, regulatory T cells.

In summary, the coordinated effects of calcineurin, NFAT kinases and an increasing number of post-translational mechanisms form a complex signalling network that can precisely and differentially regulate the activity of different NFAT family members in response to specific cellular requirements.

New roles for NFAT in immune cells

NFAT was originally identified as a major transcriptional regulator in naive T cells and differentiated effector T cells. These aspects of NFAT function have been covered extensively in previous reviews[10]. Additionally, the last few years have provided us with important new insights into the role of NFAT proteins in T cell tolerance — both in the cell-intrinsic mechanisms of T cell anergy and in the functions of regulatory T (T_{Reg}) cells. These recent studies of the molecular mechanisms of T cell tolerance have the potential to provide new strategies for the treatment of autoimmune disorders in the clinic and are discussed in more detail below.

In addition, new roles for NFAT have been suggested in T helper 17 (T_H17) cells[45–48], T follicular helper (T_{FH}) cells[49,50] and $CD8^+$ T cells[51]. It has also become apparent that NFAT transcription factors have important roles in various other cells of the haematopoietic system (TABLE 2). Although the picture is far from complete, numerous important observations have been made. In the latter part of this section we provide an overview of what is currently known about NFAT function in immune cells other than T cells (TABLE 2).

T cell anergy. TCR engagement in the absence of co-stimulatory signals leads to the activation of NFAT, but with poor concomitant activation of AP1. This results in the initiation of a transcriptional programme that culminates in T cell anergy[10,52]. Proteins that are upregulated in anergic T cells include: the E3 ubiquitin ligases itchy homologue E3 ubiquitin protein ligase (ITCH), Casitas B-lineage lymphoma B (CBL-B) and gene related to anergy in lymphocytes (GRAIL); the protease caspase 3; the transcriptional repressors Ikaros and groucho-related gene 4 (GRG4); and the protein tyrosine phosphatases receptor protein tyrosine phosphatase-κ (RPTPκ) and protein tyrosine phosphatase 1B (PTP1B) (FIG. 2). NFAT proteins have been shown to be crucial for the induction of these anergy-inducing genes in T cells; their expression is markedly reduced in T cells from NFAT1-deficient mice following stimulation with anergizing stimuli and can be quantitatively inhibited in wild-type T cells using CsA[53]. Biochemical analyses of cells that were made anergic by sustained calcium signalling have revealed that ITCH targets several signalling proteins, including PLCγ1 and PKCθ, for degradation in the lysosomal compartment by monoubiquitylation, thereby limiting T cell activation and promoting T cell unresponsiveness[54].

Caspase 3 was recently shown to block TCR signalling by cleaving and inactivating proteins, including the GRB2-related adaptor protein downstream of SHC (GADS) and the guanine-nucleotide exchange factor

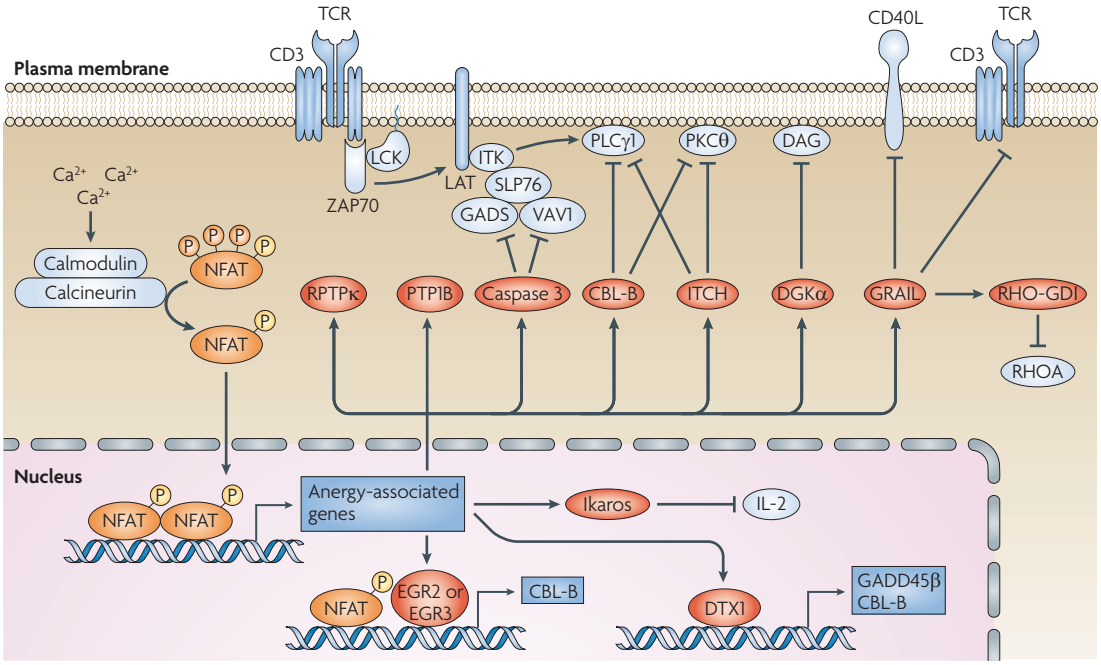

Figure 2 | **NFAT and T cell anergy.** In the absence of co-stimulatory signals, activation of nuclear factor of activated T cells (NFAT) induces the initiation of a transcriptional programme that results in a state of unresponsiveness. Following dephosphorylation, NFAT translocates to the nucleus,where it can form homodimers or heterodimers with other transcription factors. These transcriptional complexes then induce the expression of numerous anergy-associated genes. Among them are: E3 ubiquitin ligases, such as Casitas B-lineage lymphoma B (CBL-B), itchy homologue E3 ubiquitin protein ligase (ITCH), gene related to anergy in lymphocytes (GRAIL); proteases, such as caspase 3; protein tyrosine phosphatases, such as receptor-type protein tyrosine phosphatase-κ (RPTPκ) and protein tyrosine phosphatase 1B (PTP1B); transcriptional repressors, such as Ikaros; and transcriptional activators, such as early growth response 2 (EGR2) or EGR3, and protein deltex 1 (DTX1). Diacylglycerol kinase-α (DGKα) has also been shown to be upregulated in anergic T cells, where it depletes available diacylglycerol (DAG), thereby preventing downstream signal transduction. CBL-B and ITCH ubiquitylate phospholipase C-γ1 (PLCγ1) and protein kinase Cθ (PKCθ), thus initiating their degradation and destabilization of the immunological synapse. CBL-B is also known to directly target the T cell receptor (TCR) (not shown). Caspase 3 can block TCR signalling by cleaving and inactivating GRB2-related adaptor protein (GADS) and the guanine-nucleotide exchange factor VAV1. GRAIL has been shown to induce the ubiquitylation and degradation of CD40 ligand (CD40L) and to mediate degradation of the TCR–CD3 complex. It also stabilizes RHO GDP-dissociation inhibitor (RHO-GDI) by ubiquitylation, which subsequently sequesters Rho family GTPases such as RHOA. The transcriptional repressor Ikaros induces epigenetic changes in the interleukin-2 (*IL2*) promoter, resulting in stable silencing of *IL2* expression. EGR2 and EGR3 are upregulated in anergic T cells and cooperate with NFAT to activate the expression of other anergy-associated genes (such as CBL-B). DTX1 is a transcriptional target of NFAT and has been shown to induce the expression of growth arrest and DNA-damage-inducible 45β (GADD45β) and CBL-B.

VAV1, and to be crucially involved in the induction and maintenance of T cell tolerance[55]. In addition, deltex 1 (DTX1) was shown to be a transcriptional target of NFAT and a novel regulator of T cell anergy[56]. DTX1 is upregulated during the induction of anergy, and transgenic expression of DTX1 in murine T cells significantly attenuated their activation. DTX1 regulates the expression of two anergy-associated genes, growth arrest and DNA-damage-inducible 45β (GADD45β) and CBL-B. Mice with genetic ablation of DTX1 showed resistance to anergy induction and enhanced T cell activation. GRAIL, which is known to induce anergy by degradation of CD40 ligand (CD40L, also known as CD154), can also directly regulate T cell tolerance by mediating degradation of the TCR–CD3 complex (REF. 57). GRAIL-deficient mice were resistant to tolerance induction and more susceptible to autoimmune diseases compared with wild-type controls. GRAIL-deficient T cells showed delayed downregulation of TCR–CD3 complexes after activation; conversely, GRAIL expression promoted CD3 ubiquitylation and degradation.

In the absence of AP1, NFAT proteins can form dimers on DNA elements with appropriate palindromic or near-palindromic sequences[9]. A recent study showed that NFAT homodimers could directly activate the anergy-associated genes GRAIL and caspase 3 (REF. 58). GRAIL expression was activated by direct binding of NFAT homodimers to the GRAIL promoter at two distinct sites. Contrastingly, a mutant NFAT protein incapable of forming homodimers was unable to induce anergy in T cells.

***T_{Reg}* cells.** T_{Reg} cells are a distinct subpopulation of $CD4^+CD25^+$ T cells that are characterized by expression of the transcription factor FOXP3. There is clear genetic evidence that FOXP3 is crucial for preventing

IPEX syndrome
A disease caused by mutations in forkhead box P3 (FOXP3). It is characterized by refractory enteritis and in some patients autoimmune endocrinopathies, autoimmune diabetes and thyroiditis. Unlike *scurfy* mice, peripheral-blood mononuclear cells from IPEX patients fail to produce cytokines after *in vitro* stimulation.

autoimmunity. In humans, mutations in FOXP3 cause the so-called IPEX syndrome (immuno-dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome); *scurfy* mice, which carry a spontaneous null mutation in *Foxp3*, also develop an aggressive autoimmune disorder[59]. In mice two classes of *Foxp3*-expressing cells can be distinguished: 'natural' T_{Reg} (nT_{Reg}) cells (also known as thymic-derived T_{Reg} cells) and 'inducible' T_{Reg} (iT_{Reg}) cells, which differentiate into FOXP3$^+$ T_{Reg} cells in response to TCR activation in the presence of transforming growth factor-β (TGFβ) and retinoic acid *in vitro* or in gut-associated lymphoid tissue (GALT) *in vivo*.

In addition to the *Foxp3* promoter, several distal regulatory regions control expression of the *Foxp3* gene. A 5′ regulatory region and three intronic regions — conserved non-coding sequence 1 (CNS1), CNS2 and CNS3 — have been defined[59–63]. CNS1 binds to SMAD3 and NFAT[61]. Both transcription factors are essential for chromatin modification and induction of *Foxp3* expression during iT_{Reg} cell differentiation and *Foxp3* induction is completely blocked by CsA or specific inhibitor of SMAD3 (SIS3). Surprisingly, deletion of the CNS1 regulatory region does not affect nT_{Reg} cell generation, but instead diminishes the number of FOXP3$^+$ cells in GALT, indicating that CNS1 is necessary for the development of iT_{Reg} but not nT_{Reg} cells[62]. The 5′ enhancer and CNS2 are both differentially methylated: these sites are demethylated in nT_{Reg} cells, which show stable *Foxp3* expression, but methylated in iT_{Reg} cells, which despite expressing equivalent levels of *Foxp3* to nT_{Reg} cells at the outset, lose *Foxp3* expression after adoptive transfer into recipient mice. CNS2 was reported to bind to STAT5 and cyclic-AMP-responsive-element-binding protein (CREB)–ATF, but *in vivo* it seems to be the focus of an autoregulatory loop, in which it is bound by FOXP3 and the runt-related transcription factor β (RUNXβ)–core binding factor β (CBFβ) complex in the demethylated state[62]. CNS3 binds to REL and is crucial for *Foxp3* expression by nT_{Reg} cells[62]. Thus, the different regulatory regions have distinct functional roles and *Foxp3* expression in nT_{Reg} cells and iT_{Reg} cells is under differential control.

In addition to regulating *Foxp3* expression in iT_{Reg} cells, several studies have documented a role for NFAT proteins in T_{Reg} cell function and as a transcriptional partner of FOXP3 (REFS 64–67). Genetic ablation of both NFAT1 and NFAT4 is compatible with T_{Reg} cell development, but is associated with the resistance of conventional CD4$^+$CD25$^-$ T cells to T_{Reg} cell-mediated suppression[64]. Although this study could not directly link this defect in suppression to either the regulatory or responder T cell population, it did show for the first time that NFAT transcription factors have an important role in this process. More recent work has provided clear biochemical and functional evidence for a cooperative interaction of NFAT and FOXP family members[65] by comparing the crystal structure of NFAT and FOXP2 (a close relative of FOXP3) with a previous crystal structure of NFAT and AP1 (FOS–JUN). It was shown that AP1 and FOXP2 bind to the same region of DNA adjacent to NFAT on a composite NFAT–AP1 element of the *Il2* promoter, but interact with non-overlapping residues of NFAT (FIG. 3b). Whereas NFAT–AP1 complexes induced *Il2* expression, NFAT–FOXP3 complexes inhibited this process, but induced the expression of two surface receptors expressed by T_{Reg} cells, cytotoxic T lymphocyte antigen 4 (CTLA4) and CD25. FOXP3 proteins with mutations that interfered with the FOXP3–NFAT interaction were shown to be less capable of inhibiting IL-2 production, upregulating CTLA4 and CD25 expression or suppressing peripheral T cell functions in a mouse model of type 1 diabetes. Taken together, these data suggest a crucial role for NFAT transcription factors in the differentiation and function of T_{Reg} cells. It should be noted, however, that T_{Reg} cells have an anergic phenotype and seem to be less capable than conventional T cells of translocating NFAT to the nucleus or of inducing NFAT2 expression in the autoregulatory loop described previously[42,67].

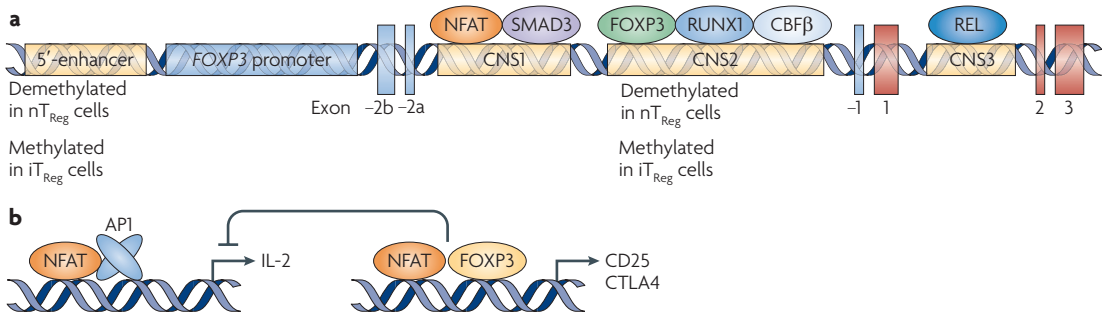

Figure 3 | **The role of NFAT in regulatory T cells. a** | Control of forkhead box P3 (FOXP3) expression by nuclear factor of activated T cells (NFAT). Expression of the *FOXP3* gene is controlled by several regulatory regions in addition to the *FOXP3* promoter. A 5′-enhancer and three conserved non-coding sequences (CNS1, CNS2 and CNS3) have been identified. CNS1 binds NFAT and SMAD3, which are both required for chromatin modification and FOXP3 induction during induced regulatory T (iT_{Reg}) cell differentiation. CNS2 is the focus of an autoregulatory loop by binding FOXP3 and the runt-related transcription factor 1–core binding factor-β complex (RUNX1–CBFβ). CNS3 was shown to bind REL (also known as c-Rel) and is essential for FOXP3 expression by natural T_{Reg} (nT_{Reg}) cells. The 5′-enhancer and CNS2 are differentially methylated: they are demethylated in nT_{Reg} cells, which have stable FOXP3 expression, but methylated in iT_{Reg} cells. **b** | Transcriptional regulation by NFAT and FOXP3 in T_{Reg} cells. NFAT–AP1 (activator protein 1) complexes induce interleukin-2 (*IL2*) expression. NFAT–FOXP3 complexes, however, inhibit *IL2* expression but induce the expression of *CD25* and cytotoxic T lymphocyte antigen 4 (*CTLA4*).

Dendritic cells. Dectin 1 (also known as CLEC7A) is a C-type lectin receptor with an intracellular immunoreceptor tyrosine-based activation motif (ITAM)-like motif that has been shown to have a crucial role in the detection of zymosan and pathogenic fungi by macrophages and DCs. Dectin 1 signalling was shown to modulate gene expression by activation of NFAT[68]; dectin 1-mediated NFAT activation regulated the expression of IL-2, IL-10 and IL-12 by zymosan-stimulated DCs. Another study reported that stimulation of murine DCs with lipopolysaccharide (LPS) induces Src-family kinases, PLCγ2 activation, influx of extracellular Ca^{2+} and the subsequent calcineurin-dependent nuclear translocation of NFAT1 (REF. 11). By using CD14-deficient bone marrow-derived DCs and stimulation with the Toll-like receptor 4 (TLR4)-specific stimulus paclitaxel (taxol), the authors showed that activation of the Ca^{2+}–NFAT signalling pathway in DCs is exclusively dependent on CD14 and is independent of TLR4-engagement. IL-2 production by DCs was further shown to be downregulated by inhibition of Src kinases, PLCγ2, calcineurin and NFAT proteins, providing evidence of an important role for Ca^{2+}–NFAT signalling in DC function. The study also indicated that LPS-induced NFAT activation is required for the induction of apoptotic cell death in terminally differentiated DCs, a process that is important for maintaining self-tolerance and preventing autoimmunity. The data provide evidence that the life cycle of DCs is regulated, at least in part, by Ca^{2+}–NFAT signalling that is activated by CD14. Taking into account the importance of CD14 in many types of disease, including heart failure and sepsis, this observation might eventually provide new treatment strategies.

Mast cells. Mast cells are resident in many different tissues and have a central role in the pathophysiology of asthma and other allergic diseases. Mast cells express IL-4 and IL-13 in addition to numerous other cytokines. IL-13 has been shown to be required for the induction of asthma-like disease in animal models[69]. One study suggested that NFAT2 is dispensable for *Il13* transcription in mast cells, whereas NFAT1 is a major transcriptional regulator of this cytokine. The authors used T cells deficient in either NFAT1 or NFAT2 or constitutively active variants of these proteins to show a synergistic interaction of NFAT1 with GATA proteins at the *Il13* promoter in mast cells[70]. Another group used small interfering RNA (siRNA)-mediated depletion to show that both NFAT1 and NFAT2 regulate IL-13 and TNF expression in mast cells, whereas degranulation and IL-6 expression are independent of NFAT activity[71].

Because of their central role during inflammatory reactions, mast cell activation *in vivo* usually occurs in areas with insufficient oxygen supply. The expression of hypoxia-inducible factor 1α (HIF1α), a transcription factor that modulates gene expression in response to hypoxia in mast cells, has recently been shown to be regulated by Ca^{2+}–NFAT signalling[72]. Stimulation of human mast cells with the calcium ionophore ionomycin induced markedly elevated HIF1α expression, which was sensitive to calcineurin inhibition that was mediated by CsA or FK506. In addition, *in situ* mutagenesis experiments showed that ionomycin-induced *Hif1a* promoter activity is dependent on a conserved NFAT binding site.

Mast cell survival following stimulation of the high-affinity Fc receptor for IgE (FcεRI) is dependent on the expression and function of the prosurvival protein A1. Although A1 expression in monocytes and lymphocytes was previously shown to be regulated by NF-κB, its expression in mast cells was recently shown to be controlled by NFAT1 (REF. 73). FcεRI-mediated A1 expression remained unaffected in mast cells expressing a super-repressor, which potently inhibited the activity of all NF-κB subunits, or after genetic ablation of the NF-κB subunits RelA and c-Rel, but could be completely inhibited by treatment with CsA. Sequence analysis of the A1 promoter revealed a putative NFAT binding site and FcεRI engagement or stimulation with ionomycin resulted in induction of A1 protein expression. In summary, the available studies show that NFAT transcription factors have an important role in mast cell function and survival by controlling the expression of several crucial proteins, including IL-13, TNF, HIF1α and A1.

B cells. B-1 cells are a subclass of B cells that can be further divided into $CD5^+$ B-1a and $CD5^-$ B-1b subtypes. B-1a cells are a phenotypically and functionally distinct population of B cells that are long-lived and typically express CD5, CD43 and high levels of surface IgM together with low surface IgD and CD45 (also known as B220)[74]. In mice, these cells were shown to have an important role in the response to T cell-independent type 2 antigens and to be the main producers of natural serum IgM. Studies using mice deficient in different NFAT family members to generate bone marrow chimaeras showed that Ca^{2+}–NFAT signalling has an important role in B-1a cell development[75]. Although the B-1a cell compartment is normal in mice that lack NFAT1, both splenic and peritoneal B-1a cells are essentially absent in NFAT2-deficient mice. Another study provided indirect evidence for NFAT involvement in B cell function by using mice with B cell-specific deletion of the regulatory b1 subunit of calcineurin (Cnb1)[76]. They showed that follicular and marginal zone B cells develop normally in these mice, but that the numbers of B-1 cells are markedly reduced. They also reported that these mice show reduced plasma cell development and antibody production and that their B cells have a cell-intrinsic proliferation defect downstream of the B cell receptor (BCR). Several other recent studies have shown that engagement of the BCR and MHC class II molecules on B cells can activate NFAT and induce NFAT-dependent gene transcription[12,77,78].

NKT cells. Natural killer T (NKT) cells are a distinct subset of lymphocytes that co-express TCR αβ and markers of the natural killer (NK) cell lineage[79]. These cells constitute a crucial first line of defence against infectious agents, influence the maintenance of immunological tolerance and are essential for the rejection of transplanted tumours. Furthermore, NKT cells are involved in the pathogenesis of allergic asthma and chronic

Small interfering RNA
(siRNA). Double-stranded RNAs (dsRNAs) with sequences that precisely match a given gene and that are able to 'knock down' the expression of that gene by directing RNA-degrading enzymes to destroy the encoded mRNA transcript. The two most common forms of dsRNAs used for gene silencing are short — usually 21 nucleotides long — siRNAs or the plasmid-delivered short hairpin RNAs (shRNAs).

T cell-independent type 2 antigens
Antigens that directly activate B cells. These antigens often contain multiple identical epitopes, which can crosslink B cell receptors.

Natural serum IgM
Antibodies that normally circulate in the blood of non-immunized mice. They are highly crossreactive and bind with low affinity to both microbial and self-antigens. A large proportion of natural IgM is derived from peritoneal B-1 cells.

obstructive pulmonary disease (COPD). A recent study showed that calcineurin–NFAT signalling is essential for the development of NKT cells. Selective ablation of this signalling cascade by targeted deletion of *Cnb1* in the thymus resulted in significantly compromised NKT cell populations[80]. The authors further showed that the gene that encodes the transcription factor early growth response 2 (EGR2), a target of NFAT, is specifically required for the development of mouse NKT cells, but not for the development of conventional $CD4^+$ and $CD8^+$ T cells. NKT cells developed normally in the absence of EGR1 or EGR3, indicating that EGR2 is a specific regulator of NKT cell differentiation. These findings indicate an important role for the Ca^{2+}–NFAT signalling pathway in the ontogeny of NKT cells.

Megakaryocytes. Megakaryocytes are the precursors of blood platelets in mammalian bone marrow. In addition to activated T cells, platelets are an abundant source of CD40L, which is a member of the TNF ligand superfamily and has an important role in the initiation and regulation of cellular and humoral immune responses[81]. Megakaryocyte development is unaffected in mice in which *Cnb1* has been deleted[82], suggesting that calcineurin–NFAT signalling is not required; however, NFAT contributes to megakaryocyte function by regulating CD40L expression[13]. CD40L has been implicated in the pathogenesis of systemic lupus erythematosus (SLE), type 1 diabetes and cardiovascular disease. Experiments using human and mouse bone marrow have shown that CD40L is abundantly expressed in primary human $CD34^+$ and mouse haematopoietic progenitor cells following cytokine-driven differentiation into the megakaryocyte lineage[13]. Furthermore, in several established megakaryocyte-like cell lines NFAT2 is crucial for the megakaryocyte-specific expression of CD40L and its expression can be suppressed by inhibition of calcineurin. Another study demonstrated that the expression of NFAT3 is significantly upregulated during megakaryocytic differentiation of human $CD34^+$ haematopoietic progenitor cells[83]. Although the true role of Ca^{2+}–NFAT signalling in megakaryocytes *in vivo* is still under debate, these studies provide the first evidence that NFAT proteins might have an important role in this process.

The studies cited above show that the Ca^{2+}–NFAT signalling pathway has a multitude of different functions in a wide array of cells of the vertebrate immune system. The existence of different NFAT family members and the complex nature of its regulatory pathways make NFAT an ideal transcription factor for performing different tasks in a variety of cells at the same time.

NFAT and cancer

As noted above it is well-established that Ca^{2+}–NFAT signalling regulates cell differentiation and development in many different cell types and organ systems. It would therefore be unsurprising if dysregulation of this pathway was associated with malignant growth and the development of cancer. Although mutations of NFAT proteins have not been associated with human cancers, numerous studies over the last few years document aberrant NFAT signalling — usually involving overexpression and/or hyperactivity — in tumour development and metastasis (TABLE 3).

Regulation of cell homeostasis and proliferation. Several studies have addressed the role of Ca^{2+}–NFAT signalling in the regulation of the mammalian cell cycle (FIG. 4). NFAT1 has been shown to repress the expression of the G_0–G_1 checkpoint kinase cyclin-dependent kinase 4 (CDK4) and of cyclin A2, indicating an important role in the control of cell-cycle progression and cell proliferation[84,85]. Mice deficient in NFAT1 and NFAT4 have been reported to show increased lymphoproliferation, decreased activation-induced cell death (AICD) and impaired Fas ligand (FasL) induction[35,86]. It was further shown that NFAT1 and NFAT4 have a direct pro-apototic activity, whereas no pro-apoptotic activity was observed for NFAT2 (REFS 35,87). Expression of a constitutively active version of NFAT2 in fibroblasts induces cell transformation and dysregulation of contact inhibition and colony formation[88]. NFAT1 and NFAT2 have distinct and opposing effects in tumorigenesis. Expression of constitutively active NFAT1 in fibroblasts resulted in cell-cycle arrest, apoptosis and inhibition of RasV12-mediated malignant transformation, whereas expression of constitutively active NFAT2 induced uninhibited growth and colony formation in soft-agar medium[18]; these results were reproducible in a mouse xenotransplant model. Furthermore, NFAT1-deficient mice spontaneously developed lymphoma with long latency and were more susceptible to chemical-induced carcinogenesis. In addition, NFAT2 has recently been shown to have an important role in regulating the quiescence and proliferation of stem cells[17].

Tumour cell migration and metastasis. During the process of malignant transformation, epithelial cells on the basement membrane fundamentally change their morphology, adopting a mesenchymal phenotype and acquiring the ability to transmigrate through connective tissues[89].

Table 3 | **Roles of Ca^{2+}–NFAT signalling in different types of cancer**

Cancer type	Family member	Function	Refs
B cell lymphomas	NFAT2	Maintaining lymphoma cell survival and counteracting apoptosis by induction of survival factors such as CD40 ligand and BLYS	107,108
T-ALL	NFAT1–NFAT4	Constitutive activation of calcineurin leads to NFAT activation	106,109
CML	NFAT2	Development of resistance to TKI treatment	110
Breast cancer	NFAT1 and NFAT5	Control of cancer cell migration and invasion by induction of COX2	39,40
Pancreatic cancer	NFAT2	Induction of MYC expression	111,112
Prostate cancer	ND	Regulation of cancer cell proliferation	113
Melanoma	NFAT2 and NFAT4	Induction of COX2	114
Endometrial cancer	ND	Regulation of *IL11* and *CXCL8* expression	115,116

BLYS, B lymphocyte stimulator; CML, chronic myeloid leukaemia; COX2, cyclooxygenase 2; CXCL8, CXC-chemokine ligand 8; IL, interleukin; NFAT, nuclear factor of activated T cells; ND, not determined; T-ALL, T cell acute lymphoblastic leukaemia; TKI, tyrosine kinase inhibitor.

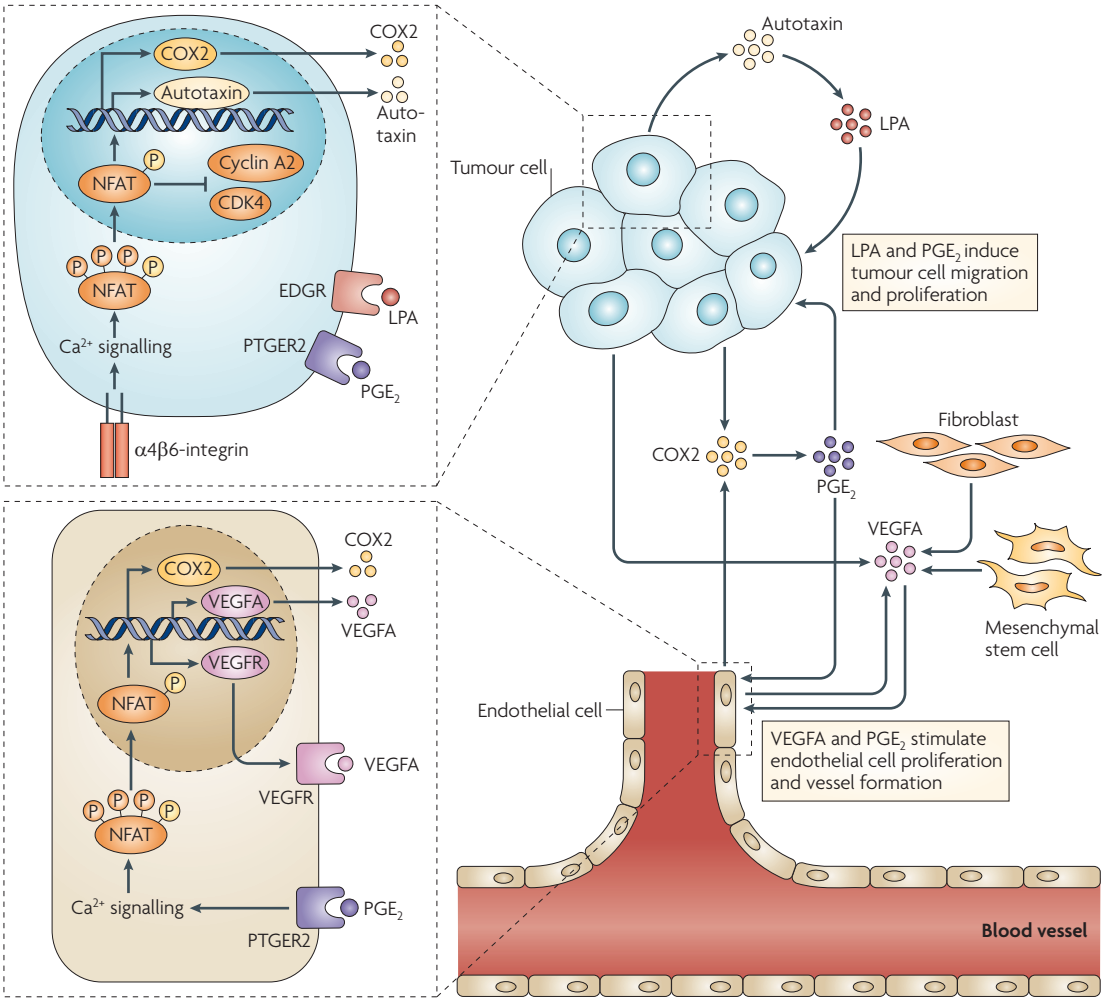

Figure 4 | **The multiple roles of NFAT in the pathogenesis of cancer.** Engagement of integrins such as α4β6-integrin on tumour cells induces Ca^{2+} entry and, subsequently, the activation and nuclear translocation of nuclear factor of activated T cells (NFAT) transcription factors. NFAT then activates the expression of various genes, including autotaxin and cyclooxygenase 2 (*COX2*). Autotaxin and COX2 are secreted into the extracellular matrix, where they catalyse the synthesis of lysophosphatidic acid (LPA) and prostaglandin E_2 (PGE_2), respectively. Both LPA and PGE_2 are potent inducers of tumour cell migration and proliferation. They act in an autocrine and paracrine manner by binding to endothelial differentiation gene receptor (EDGR; also known as LPAR) and the PGE_2 receptor prostanoid EP2 receptor (PTGER2), respectively. PGE_2 also binds to *PTGER2* on endothelial cells, stimulating their proliferation and migration. Vascular endothelial growth factor A (VEGFA) is secreted into the extracellular matrix by multiple cell types, including mesenchymal stem cells, fibroblasts, endothelial cells and the tumour cells themselves. VEGFA subsequently binds to VEGF receptors (VEGFRs) on endothelial cells, which leads to the activation of the Ca^{2+}–NFAT signalling cascade. Activated NFAT induces the expression of VEGFA, VEGFR and COX2 (which catalyses the synthesis of PGE_2 in the extracellular matrix) by endothelial cells. VEGFA and PGE_2 are potent stimulators of endothelial cell proliferation and vessel formation. NFAT proteins have also been shown to repress the expression of the G_0–G_1 checkpoint kinase cyclin-dependent kinase 4 (CDK4) and of cyclin A2 in cancer cells, indicating that they have an important role in cell cycle control.

Epithelial-to-mesenchymal transition
(EMT). A cell developmental programme that is characterized by decreased expression of E cadherin, loss of cell adhesion and increased cell motility.

Metastasize
To spread from one part of the body to another.

This process has been called epithelial-to-mesenchymal transition (EMT) and has been shown to be crucial for the ability of tumour cells to metastasize. Expression of constitutively active NFAT1 in breast cancer cells has been shown to promote their migration and invasion *in vitro*, whereas NFAT5 promotes migration but not invasion[90]. In line with these observations, overexpression of NFAT1 and NFAT5 was observed in human breast cancer cell lines and in tumour biopsies from patients with invasive ductal breast carcinoma and correlated with concomitant expression of α4β6 integrin. Indeed, engagement of integrins such as α4β6 on tumour cells can induce Ca^{2+} influx, thereby resulting in the activation and nuclear translocation of NFAT transcription factors (FIG. 4). Inside the nucleus, NFAT induces the expression of numerous genes, including cyclooxygenase 2 (*COX2*) and autotaxin, which in turn catalyse the synthesis of lysophosphatidic acid (LPA) and prostaglandin E_2 (PGE_2)[91–94]. LPA and PGE_2 have growth-factor-like properties, including the stimulation of cell proliferation and chemotaxis. LPA and PGE_2 act in both an autocrine and paracrine manner by binding endothelial differentiation gene (EDG) and PGE_2

receptors, respectively. This process has the potential to efficiently induce migration of tumour cells through the extracellular matrix of connective tissue and to promote tumour metastasis.

Angiogenesis. Tumours depend on the vasculature to receive the necessary nutrients and oxygen that are required for survival. Furthermore, blood vessels and lymphatic vessels are used by cancer cells to disseminate and metastasize to regional lymph nodes and distal organs. It was initially shown using mice deficient in NFAT3 and NFAT4 that Ca^{2+}–NFAT signalling was required for the development of an intact vascular system[95]. Vascular endothelial growth factor A (VEGFA) has been shown to be crucial for efficient tumour angiogenesis[96]. VEGFA is secreted into the microenvironment by endothelial cells, fibroblasts and tumour cells (FIG. 4) and can act as a potent endothelial cell permeability factor[97]. Engagement of VEGFA receptors (VEGFR1 and VEGFR2) on endothelial cells by VEGFA induces the activation of PLCγ, leading to Ca^{2+} influx and the nuclear translocation of NFAT, which in an autocrine loop induces the expression of additional VEGFA and VEGFR[98] (FIG. 4). Both VEGFA and PGE_2 (the expression of which is also controlled by NFAT) stimulate endothelial cell proliferation, migration and, eventually, vessel formation[99]. Activation of NFAT by VEGFA also induces expression of tissue factor, which has been shown to be an important initiator of blood coagulation and angiogenesis, and of colony stimulating factor (CSF), which is required for endothelial cell differentiation and survival. Members of the RCAN family, which are endogenous inhibitors of calcineurin, have been shown to block NFAT activation in endothelial cells and to be potent inhibitors of tumour angiogenesis[100,101]. Furthermore, NFAT2 has been shown to be an important regulator of lymphangiogenesis by interacting with promoting factors such as forkhead box protein C2 (FOXC2), podoplanin, prospero-related homeobox gene 1 (PROX1) and VEGFR3 (REFS 102,103).

Cancers of the haematopoietic system. A comprehensive review of ~300 biopsies of human lymphomas revealed that overexpression and aberrant nuclear translocation of NFAT2 can be routinely detected in diffuse large B cell lymphomas (DLBCLs), Burkitt's and Burkitt's-like lymphomas, and certain T cell lymphomas[104]. NFAT2 was also consistently overexpressed and translocated to the nucleus in chronic lymphocytic leukaemia (CLL) and in the lymphocyte predominant subtype of Hodgkin's disease. Another laboratory has expanded these observations by analysing DNA methylation and chromatin modifications of the NFAT2 promoter in Hodgkin's lymphoma cells[105]. NFAT4-deficient mice were shown to be more susceptible to the development of T cell lymphomas following infection with murine leukaemia virus SL3-3 than wild-type mice, providing the first piece of evidence that NFAT transcription factors can also act as tumour suppressors[106]. Other groups have reported that NFAT2 regulates the expression of B cell survival factors, such as CD40L and B lymphocyte stimulator (BLYS), in DLBCLs and mantle cell lymphomas[107,108]. In another study it was found that treatment of mice with calcineurin inhibitors in two mouse models of acute T lymphoblastic leukaemia resulted in rapid tumour clearance and apoptosis of leukaemic blast cells[109]. A recent paper has reported that activation of the Ca^{2+}–NFAT signalling pathway has an important role in the development of resistance to treatment with tyrosine kinase inhibitors in chronic myeloid leukaemia (CML)[110]. Taken together, the available data strongly suggest an important role for Ca^{2+}–NFAT signalling in the pathogenesis of haematological malignancies. Nevertheless, the exact genetic and epigenetic mechanisms that induce aberrant nuclear translocation of NFAT and drive transformation remain elusive and require further investigation.

Solid tumours. The bulk of the evidence implicating the Ca^{2+}–NFAT signalling pathway in the pathogenesis of solid tumours comes from studies of breast cancer and pancreatic ductal carcinoma[39,111,112]. As mentioned above, NFAT1 is important in regulating tumour cell migration and metastasis in breast cancer. Another study has reported constitutive overexpression and aberrant nuclear translocation of NFAT2 in biopsies from patients with pancreatic cancers[111]. Furthermore, it was shown that NFAT2 can directly induce transcriptional activation of the MYC oncogene by binding to the proximal *MYC* promoter[111,112]. Other studies have shown that NFAT transcription factors are involved in cancer cell proliferation in prostate tumours[113] and can also be important in malignant melanoma and endometrial carcinoma[114–116].

Therefore, in addition to their well-defined role in the immune system, NFAT transcription factors have been shown in recent years to be of crucial importance for tumour cell proliferation, migration and angiogenesis.

Therapeutic implications

As a consequence of its established role in T-cell-mediated immunity, NFAT has long been considered an attractive target for the therapeutic modulation of immune responses. The studies summarized above indicate an additional role for NFAT in carcinogenesis and tumour progression, implying that components of the Ca^{2+}–calcineurin–NFAT signalling pathway might also be promising targets for cancer therapy. The calcineurin inhibitors CsA and FK506 are routinely administered in the clinic to treat autoimmune diseases and to prevent graft rejection. These inhibitors function by blocking the enzymatic activity of calcineurin and, therefore, also affect the numerous other targets of this phosphatase, which can potentially account for the nephro- and neurotoxicities observed during their clinical use[117]. The development of inhibitors of NFAT activity with increased specificity is therefore desirable, and considerable progress has been achieved during the last few years.

A cell-permeable version of the VIVIT peptide has been successfully used to prolong graft survival after islet cell transplantation in mice[118]. Because the therapeutic use of peptide inhibitors is limited by the issues of delivery and product stability, small molecules that inhibit NFAT activation are preferred for clinical use. Several compounds that blocked the NFAT–calcineurin

Angiogenesis
The development of new blood vessels from existing blood vessels. It is frequently associated with tumour development and inflammation.

Tyrosine kinase inhibitors
Drugs that specifically inhibit tyrosine kinases, which are important for cancer development and progression.

Oncogene
A gene that when overexpressed or when incorporating a gain-of-function mutation contributes to oncogenesis.

interaction and inhibited NFAT-dependent cytokine production in T cells were identified using a fluorescence polarization assay, in which a library of small organic molecules was screened for their ability to block the binding of labelled VIVIT peptide to calcineurin[119]. These compounds still need to be analysed in suitable animal models of autoimmune disease and cancer to investigate their potential to specifically inhibit NFAT function and ameliorate disease. In addition, because NFAT activation in non-excitable cells depends on SOCE, the endoplasmic reticulum calcium sensor stromal cell interaction molecules (STIM1 and STIM2) and the calcium release-activated calcium channel protein 1 (ORAI1) are also promising pharmacological targets.

Concluding remarks

More than 20 years after its original description as a transcription factor in T cells, NFAT has well-established roles in a number of different cell types and developmental programmes. In particular, new information on the emerging role of Ca^{2+}–NFAT signalling in tumorigenesis and cancer progression has the potential to substantially increase our understanding of the molecular basis of certain types of cancer. Analysis of the Ca^{2+}–NFAT signalling pathway in conditionally gene-disrupted mice, which allow lineage-specific deletion of pathway components in animal cancer models, will enable us to investigate the role of NFAT in tumorigenesis *in vivo*.

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Competing interests statement
A.R. declares competing financial interests: see Web version for details.

DATABASES
UniProtKB: http://www.uniprot.org
NFAT1 | NFAT2 | NFAT3 | NFAT4 | NFAT5

FURTHER INFORMATION
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