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Review

Transcriptional Regulation by the NFAT Family in Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a hematological cancer with poor outcomes due to a lack of efficacious targeted therapies. The Nuclear Factor of Activated T Cells (NFAT) family of transcription factors is well characterized as a regulator of the cell cycle and differentiation in the myeloid lineage. Recent evidence has demonstrated that NFAT family members may have roles in regulating AML leukemogenesis and resistance to targeted therapy in myeloid leukemias. Furthermore gene expression data from patient samples show that some *NFAT*s are more highly expressed in poorly differentiated AML and after disease relapse, implying that the NFAT family may have roles in specific types of AML. This review outlines the evidence for the role of NFAT in healthy myeloid tissue and explores how NFAT might regulate AML pathogenesis, highlighting the potential to target specific NFAT proteins therapeutically in AML.

Keywords: Leukemia; NFAT, myeloid, cell cycle, differentiation, AML

1. Introduction

Acute myeloid leukemia (AML) is a hematopoietic malignancy of clonal origin with dismal survival outcomes[1]. AML results from an accumulation of mutations within cells of the myeloid lineage, leading to the expansion of immature and dysfunctional blasts, rapid clinical sequelae and often rapid death. Many of the genetic and epigenetic lesions responsible for driving AML pathogenesis are well-characterised, enabling sophisticated patient stratification into molecular subgroups and a shift towards targeted therapies for smaller strata of patients[2,3]. However, intra- and inter-patient molecular heterogeneity and the continuous mutational evolution of AML means that resistance to existing therapy is not uncommon[4,5]. Persistence of minimal residual disease after treatment is also attributed to therapy-resistant leukemic stem cells (LSCs), which exhibit distinct phenotypic and genomic properties to the bulk of AML blasts[6-8].

In addition to (cyto)genetic status the AML transcriptome has been described as a tool for patient prognostication, whereby expression profiles within circulating blasts and/or LSCs can aid in the risk stratification of patients and can reveal specific mechanisms of oncogenesis[9-12]. Transcriptional regulators could be ideal therapeutic targets for AML, such that the effectors of multiple signaling pathways could be targeted simultaneously[13]. In fact, a number of transcription factors with known roles in leukemia are under investigation as putative drug targets for AML, including RUNX1 and c-MYC[14].

The Nuclear Factor of Activated T Cells (NFAT) family of transcription factors has been demonstrated to have roles in the pathology of myeloid leukemias[15,16]. NFAT signaling has been well-characterised in various solid and lymphoid cancers, in addition to the mechanics of the innate immune system. Their role in pathology is often dependent on regulation of cell type-specific cytokine signaling networks, cell cycle progression and apoptosis[17-20]. This review outlines the functions of NFAT in myeloid tissues and examines current evidence supporting a role for NFAT in AML pathogenesis.

2. NFAT Proteins: Structure, Function and Regulation

The general structure and function of the NFAT family are reviewed extensively elsewhere[18,21-23]. To summarize, the NFAT family consists of five members, in which NFATc1-4 function downstream of calcium signaling – denoted as 'NFATc', although other nomenclature is often used - while NFAT5 is responsive to osmotic stress. This review will focus on NFATc1-4, henceforth referred to as 'NFAT' collectively.

NFAT proteins have high sequence homology in a conserved DNA-binding Rel homology domain, which is shared with the Rel superfamily of transcription factors (including NFκB)[24]. At the N-terminus is the NFAT homology domain (NHD), which contains phosphorylation sites that are targeted by upstream regulatory kinases. Critically this region possesses docking sites for regulatory phosphatase calcineurin, which dephosphorylates most of these phospho-sites. The N- and C- termini are flanked by transactivation domains (TADs) which are non-homologous between family members and are a key interaction point with transcriptional partner proteins[25,26].

Inactive NFAT proteins reside in the cytoplasm in a heavily phosphorylated state. Activation of calcium-coupled surface receptors (e.g. receptor tyrosine kinases) triggers a signaling cascade via phospholipase C (PLC), which promotes calcium influx in a process known as store operated calcium entry (SOCE). In response to elevated calcium the messenger calmodulin activates multiple target enzymes, which include calcineurin and calmodulin kinase (CAMK) isoforms. Activated calcineurin docks on NFAT at conserved PxIxIT peptide motifs and subsequently dephosphorylate at up to 14 known serine-rich motifs on the NHD[22,27].

The conformational change which follows NFAT dephosphorylation exposes a 'nuclear localization signal' enabling its nuclear import. The subcellular location of NFAT is carefully balanced by opposing calcineurin phosphatase activity and that of numerous kinases, which mask these localization signals to facilitate nuclear export in the absence of raised intracellular calcium. Examples of these kinases include GSK3, CK1 and JNK. Additionally, p38 MAPK has been found to regulate NFAT transactivation in the nucleus through phosphorylation at a motif separate to those regulated by calcineurin[25,28,29].

Once inside the nucleus NFAT binds DNA as a monomer, unlike other Rel superfamily members. The core NFAT DNA consensus binding sequence has been defined as 5'-GGAA(A)-3' in T cells, but variations have been described with differing binding affinities[23,25,30]. Lone NFAT DNA binding is often weak and it must bind in tandem with other factors at composite sequences to regulate transcription, as has been shown with AP-1 proteins Fos and Jun[31]. In the case of NFκB proteins, NFAT could either compete with them or bind cooperatively, depending on the DNA motifs[30]. One proteomics study described hundreds of putative NFAT interaction partners in T cells[32], raising the notion that NFAT proteins function as part of large transcriptional complexes and so are master integrators of upstream signaling pathways. A schematic diagram of NFAT function in the cell is shown in Figure 1.

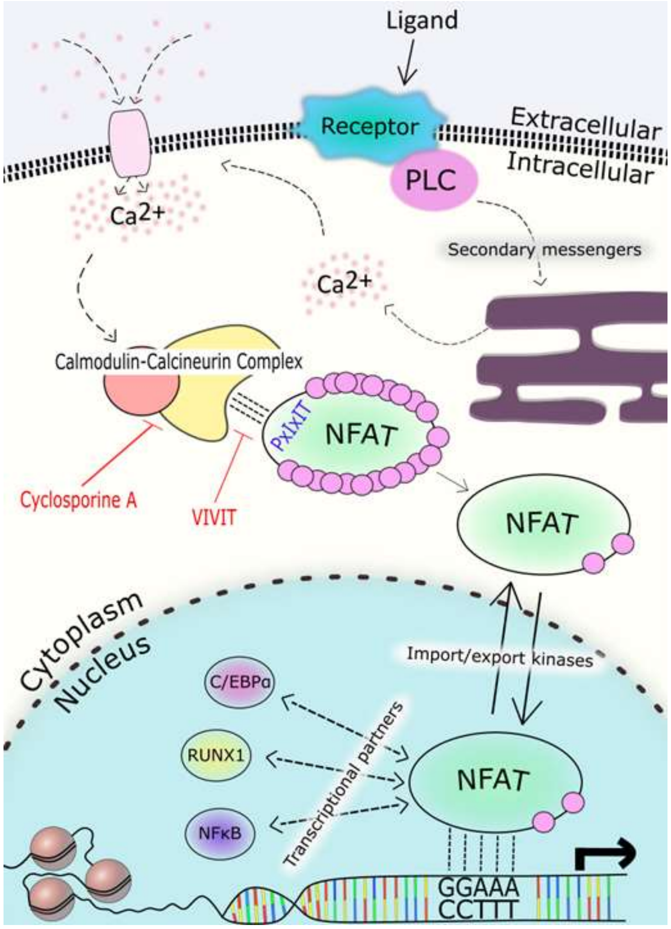

Figure 1. Schematic diagram of calcium-NFAT signaling. 1. Engagement of a calcium-coupled surface receptor by its ligand leads to activation of phospholipase C (PLC). 2. A cascade of signaling events is initiated by PLC which leads to the movement of calcium from the endoplasmic reticulum (ER) into the cytoplasm. 3. Following depletion of ER calcium stores surface calcium release-activated calcium (CRAC) channels are opened, enabling influx of calcium to the cell. 4. Raised calcium levels triggers activation of calmodulin, which binds calcineurin. 5. A conformational change in calcineurin allows it to bind to NFAT at the PxIxIT docking motif and dephosphorylate NFAT at ~14 phospho-sites. 6. Dephosphorylation of NFAT – with the exception of some residues not targeted by calcineurin – exposes a nuclear localization signal and enables its import to the nucleus. 7. Once in the nucleus NFAT can bind to consensus DNA sequences – including 5′-GGAAA-3′ – and activate (or inhibit) transcription. It may do so in cooperation with various transcriptional partners, which can include C/EBPα, RUNX1 and/or NFκB. NFAT's position in the nucleus is balanced by the activity of import and export kinases. Note that inhibitors cyclosporine A (CsA) and VIVIT peptide can inhibit activation of the calmodulin-calcineurin complex or binding of calcineurin to NFAT, respectively.

Inhibition of NFAT activation can be achieved by targeting calcineurin, using either of the small molecule inhibitors cyclosporine A (CsA) or tacrolimus. These are both used clinically to prevent graft rejection after organ transplantation, primarily due to the immunosuppressive effects on T and B cell activation secondary to inhibited NFAT-dependent cytokine transcription. While different in structure and target, they both form complexes with cellular immunophilins which can inhibit calcineurin phosphatase activity[33]. Calcineurin has numerous targets in addition to NFAT[34], making this a relatively non-specific means of inhibiting NFAT activity. Subsequently a more selective peptide inhibitor 'VIVIT peptide' was developed, which directly binds the calcineurin docking motif PxIxIT on NFAT, thus more selectively inhibiting NFAT activity[35]. These inhibitors are useful tool compounds to study NFAT function.

3. NFAT Expression in the Myeloid Lineage

NFAT expression in differentiated myeloid cells is generally lower than in T cells and CD34⁺ hematopoietic stem cells (HSCs). Kiani et al. demonstrated that NFATc1-3, but not NFATc4, are well expressed in CD34⁺ blood cells and altered within myeloid lineages. NFATc2 is downregulated in most differentiated myeloid cells, while NFATc1 is upregulated during the course of erythroid and megakaryocyte differentiation. NFATc3 is upregulated during erythroid but not megakaryocyte or eosinophil differentiation. Furthermore, inhibition of calcineurin-NFAT signaling with CsA was found to be permissive of CD34⁺ HSC differentiation into neutrophils[36,37]. These data suggest that NFATs are responsible for regulating differentiation in healthy cells and that the NFAT family members are non-redundant in determining cell fate. This also suggests that specific NFAT members could be more important in the development of some morphological subtypes of AML.

Supporting these *in vitro* findings, gene expression analysis of *NFATC1-4* from CD34⁺ and differentiated blood cells from healthy adults showed that mature granulocytes and monocytes exhibit lower expression of *NFATC1-3* than cells of a lymphoid origin or more primitive CD34⁺ cells (GSE51984 dataset; Figures 2(a)-(c)). *NFATC2* is poorly expressed in differentiated myeloid cells relative to *NFATC1* and *NFATC3*, suggesting that it has a diminished role in these mature cells. *NFATC4* expression was barely detectable in any lineage or CD34⁺ cells (RPKM<1; data not shown).

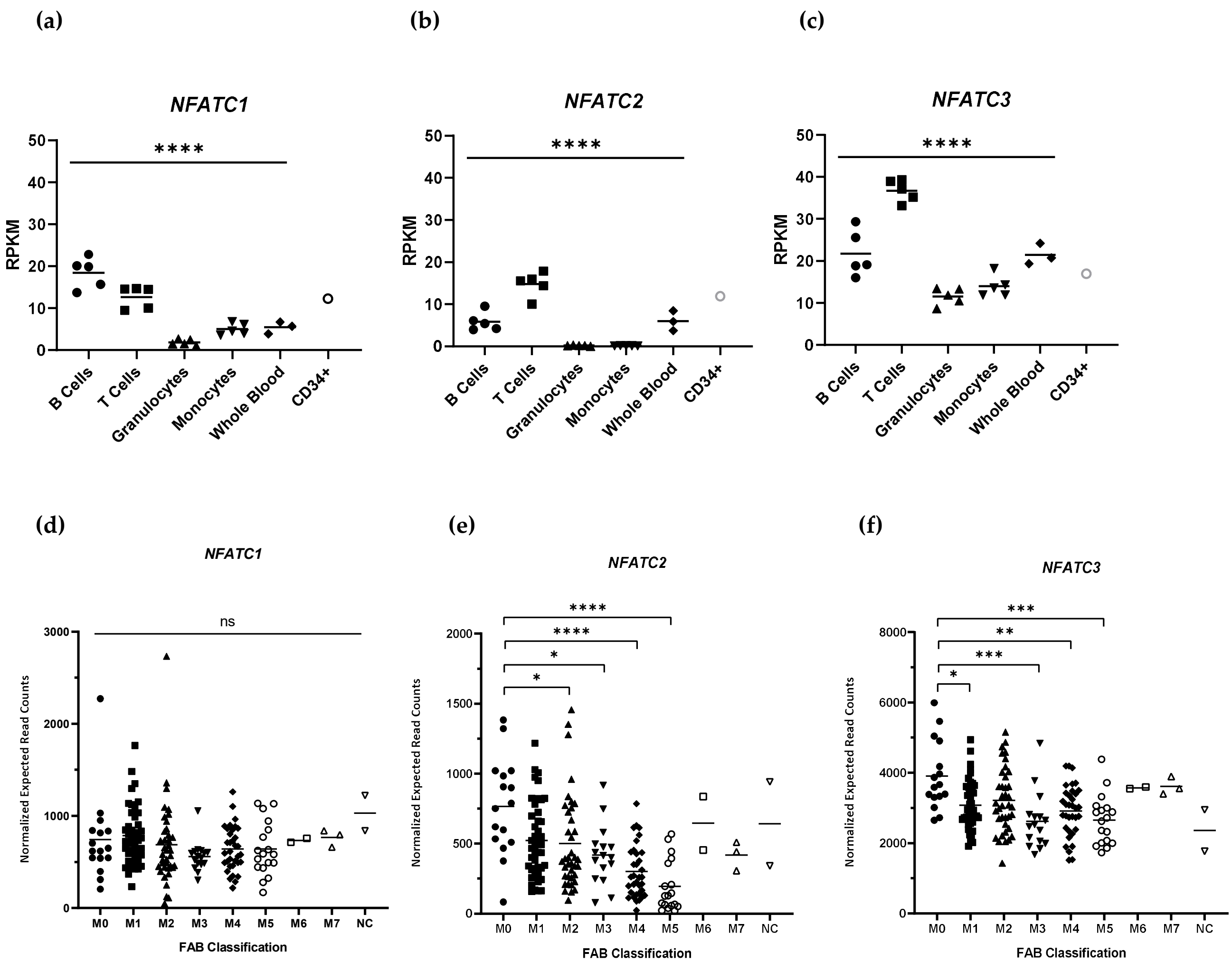

Figure 2. Differential expression patterns of NFAT family members in normal and leukemic myeloid cells. (a)-(c) RNA-seq data were generated by Pabst *et al*. from the peripheral blood of healthy volunteers for expression of *NFATC1-3* (GEO repository ID

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GSE51984[38]). Cells were sorted based on expression of the following surface markers: CD34; CD3 (T cells); CD19 (B cells); CD14 (monocytes); CD33 (granulocytes). Each data point represents an individual, except for CD34+ where the data point is an average of four individuals. Normalized expression values are presented as RPKM and *p* values are from a one-way ANOVA for a difference in mean (excluding CD34 data) are shown (****<0.0001). **(d)-(f).** RNA-seq data were obtained from the The Cancer Genome Atlas (TCGA) AML dataset[39]. Data were extracted using the *TCGAbiolinks* R package (R v4.0.0), for 173 adult patients with *de novo* AML for expression of *NFATC1-3* in AML tissue and annotated by disease FAB classification. NC = 'not classified'. Expression values are 'normalized expected read counts' derived from the RSEM method[40]. Dunnett's post-hoc test (following one-way ANOVA) *p* values for a difference in mean are shown (*<0.05; **<0.01; ***<0.001; ****<0.0001).

AML cells are characteristically poorly differentiated[41] and it is worth considering whether NFAT could have a role in maintaining the stem cell-like properties of blasts, given the observed gene expression profiles in healthy myeloid tissue. RNA-seq data for *NFAT* expression were extracted from the TCGA dataset of 173 adults with *de novo* AML[42] (Figures 2(d)-(f)). These were categorised according to the French-American-British (FAB) classification based on AML blast morphology[43], which gives a broad understanding of the differentiation status of blasts (classification described in Appendix A). These data show that poorly differentiated myeloid leukemia, particularly in type M0, have a significantly higher expression of *NFATC2* and *NFATC3* than more differentiated forms of AML. In contrast *NFATC1* expression appears consistent regardless of FAB subtype. It could be hypothesised that *NFATC2* and/or *NFATC3* negatively regulate differentiation pathways in AML and so warrant further investigation to determine the mechanism(s).

4. The role of NFAT in Myeloid Cells

NFAT proteins regulate genes which determine proliferation and lineage commitment in the myeloid lineage. In murine granulocyte-monocyte progenitor (GMP) cells NFAT was found to negatively regulate genes which determine cell cycle entry such as *Cdk4* and *Cdk6*. This activity was dependent on Flt3 ligand (Flt3-L) signaling and phospholipase PLCγ1-dependent calcium influx[44]. Another study found that CsA inhibition of Flt3-L stimulated murine dendritic cells (DCs) led to upregulation of genes which progress the cell cycle, suggesting that targets of calcineurin block Flt3 receptor-mediated cycling. Additionally, blockade of the calcineurin-NFAT interaction with VIVIT also led to the expansion of the myeloid compartment *in vivo*[45]. These studies suggest that the NFAT proteins inhibit proliferative signaling in myeloid development and interact with FLT3 receptor signaling.

In normal physiology the growth factors macrophage- and granulocyte- colony stimulating factor (M-CSF and G-CSF) trigger HSCs to differentiate into either macrophages/monocytes or granulocytes, respectively. *Nfatc1* expression was found to increase in murine bone marrow cultures stimulated with M-CSF, but not G-CSF. Differentiation triggered by M-CSF was partially blocked by VIVIT, suggesting that it is dependent on calcineurin-NFAT interaction. Furthermore, distinct from the Flt3-L-stimulated GMPs described above, stimulation with either M-CSF or G-CSF was found to induce PLCγ2 (but not PLCγ1) activity[46]. Therefore it appears that the regulatory function of NFAT in myelopoiesis, in the balance of proliferation and differentiation, is dependent on specific upstream signaling networks.

NFAT proteins are well characterised in T cell effector function and also play a role in the myeloid cell response to pathogens. Pattern recognition receptors (PRRs), such as TLR4, respond to structural elements of invading microbes to trigger an immune response. Engagement of PRRs in a number of differentiated myeloid cell types can stimulate the calcineurin-NFAT interaction via calcium influx initiated by Syk and PLCγ[47]. NFAT can also bind the canonical 5'-GGAAA-3' DNA motif and regulate the expression of various cytokines in dendritic cells and macrophages, including

IL-2, IL-10 and IL-12, which influence immune responses[48,49]. There is limited evidence suggesting that systemic CsA treatment in transplant patients could worsen outcomes due to a greater risk of fungal infection, secondary to inhibition of myeloid effector cell function specifically[47]. Some of these roles of NFATs are shown schematically in Figure 3.

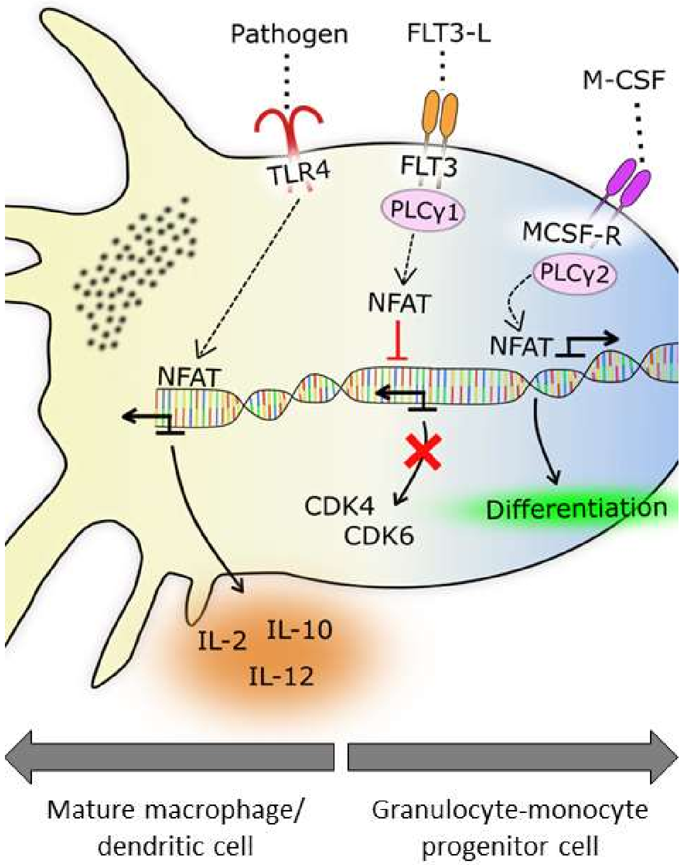

Figure 3. Schematic diagram of putative roles for NFAT in myeloid lineage cells. Roles for NFAT inferred from aforementioned studies[44-49] are shown as putative roles in human myeloid cells, schematically as a myeloid cell in different 'stages' of differentiation. Left: in mature dendritic cells pathogens trigger pattern recognition receptors (PRRs) such as TLR4, which is thought to be upstream of NFAT-driven cytokine transcription. Middle: in progenitor cells NFAT is downstream of a FLT3-PLCγ1 axis, whereby it inhibits regulators of the cell cycle. NFAT may also act downstream of FLT3 in dendritic cells. Right: in granulocyte-monocyte progenitors (GMPs) NFAT activates myeloid differentiation in response to M-CSF receptor engagement, which also signals via PLCγ2.

The evidence discussed highlights that NFAT activity can direct myeloid progenitors towards quiescence by inhibiting the cell cycle or favour differentiation, depending on the specific upstream pathways activated. Leukaemic transformation to AML is dependent on deregulation of these processes in steady state myelopoiesis and these changes are often promoted through transcription factors[41]. As such, NFAT proteins could influence AML initiation or maintenance downstream of mutated signaling proteins. For example, the FLT3 receptor is commonly mutated in AML, leading to enhanced proliferation. Understanding the relationship between FLT3-L and NFAT activity in healthy myeloid cells could therefore provide insight into this relationship in leukemia. In parallel, TLR4 participates in HSC regulation and is overexpressed in some types of AML[50,51] and so it is worth considering whether a TLR-NFAT axis is as important in oncogenesis as in mature myeloid cell function.

Broadly speaking, NFAT proteins have a greater role in less differentiated myeloid cells (Figure 2) and might also be important in the differentiation status of AML. It could also be inferred that each family member is non-redundant and so further investigation into individual roles is warranted. Ultimately, given the distorted nature of the hematopoietic hierarchy in AML[7] these are only inferences from healthy cells and should be examined more closely in leukemia tissue.

5. NFAT Signaling in AML

There is growing evidence that NFAT signaling cooperates with mutations of the Fms related tyrosine kinase receptor 3 (FLT3) receptor in AML. Internal tandem duplication of the FLT3 receptor ($FLT3^{ITD}$) is present in around 25% of AML cases and confers particularly poor outcomes for patients compared to other AML subtypes[52]. This is a gain-of-function mutation which causes ligand-independent proliferative signaling[53].

Exogenous expression of *$Flt3^{ITD}$*/*$FLT3^{ITD}$* in hematopoietic cells has been shown to induce a myeloproliferative disease and it is understood to require other driver mutations to induce overt AML[54,55]. In one of these models co-expression of *$Flt3^{ITD}$* with a constitutively active form of human *NFATC1* led to the rapid development of myeloid leukemia and expansion of immature blasts *in vivo*. Interestingly the expression of constitutive *NFATC1* alone inhibited the colony forming capacity of sorted $Lin^-Sca1^+c\text{-}kit^+$ (LSK) bone marrow cells, but *$Flt3^{ITD}$* co-expression increased colony formation dramatically, more than *$Flt3^{ITD}$* alone[54].

The observed phenotypes imply that NFATc1 has an inhibitory effect on expansion of primitive LSK cells, in parallel to the studies conducted in GMPs[44,45]. Constitutively active FLT3 signaling appears to supersede this and the cooperativity with NFAT induces a distinct transcriptional program permissive of AML development[54]. Signaling downstream of $FLT3^{ITD}$ is different from that of the normal FLT3 receptor, as has been demonstrated in murine hematopoietic cells with aberrant activation of STAT5[56]. One possibility is that engagement of pathological signaling by $FLT3^{ITD}$ may influence the recruitment of other factors to transcriptional complexes containing NFATc1. However, this model is an artificial representation of AML and does not reflect on the true ontogeny of leukemia. Evidence from relapsed AML patients suggests that $FLT3^{ITD}$ often arises as a later event and is not consistently found in the founding LSC clone[57], which should be borne in mind when considering NFAT as an effective therapeutic target.

NFATc1 activity can also mediate resistance to tyrosine kinase inhibitors (TKIs). $FLT3^{ITD}$ AML can be treated with TKIs such as sorafenib and quizartinib but point mutations and/or ‘escape’ signaling pathways often lead to resistance and relapse[58]. Metzelder *et al*. demonstrated that depletion of *NFATC1* by shRNA or NFATc1 functional inhibition with CsA or VIVIT treatment could increase sensitivity of $FLT3^{ITD}$ AML cells to sorafenib. Expression of a constitutively nuclear NFATc1 with $FLT3^{ITD}$ in myeloid progenitor cells increased resistance to sorafenib and also induced morphological signs of de-differentiation[15]. It is not clear whether NFATc1 and $FLT3^{ITD}$ cooperativity activate a specific resistance mechanism, which permit $FLT3^{ITD}$ blasts to escape sorafenib-mediated cell death. The reversal of cell maturation may also highlight a pathogenic role of NFATc1 in maintaining stem cell-like properties, akin to the high expression of NFATc1-3 observed in in normal HSCs[36,37].

Resistance to TKIs in chronic myeloid leukemia (CML) was also found to be linked to NFAT activity. CML is characterised by the *BCR/ABL* fusion oncogene, which is effectively targeted by the TKI imatinib. As with FLT3 inhibitors resistance to imatinib can arise through a number of mechanisms, including *BCR/ABL* mutations and receptor-independent means[59]. Gregory *et al*. identified NFAT-stimulated autocrine IL-4 signaling as a mechanism of imatinib resistance and the effect was modulated primarily by NFATc1[16]. IL-4 is an established regulatory target of NFAT in the function of various immune cells[17]. Sung *et al*. found that AML cells can increase resistance to FLT3^{ITD} inhibition by autocrine stimulation with various cytokines, including IL-6 and GM-CSF, which are also targets of NFAT in some myeloid lineage cells[17,60]. Based on the evidence available investigation into the role of NFAT in autocrine cytokine signaling in AML may yield further insight into the mechanism(s) of resistance to FLT3 inhibitors.

Resistance to therapy, be it FLT3 inhibitors or otherwise, is a common cause of relapse in AML[61], with evidence so far focusing on TKIs and FLT3^{ITD} AML. To investigate whether NFAT might be important in AML relapse following chemotherapy, RNA-seq data were extracted from the GSE83533 dataset, which is derived from paired patient samples (n=19) at diagnosis and at relapse. All patients were initially treated with combination chemotherapy, in some cases followed by stem cell transplantation[62]. The expression of *NFATC2* and *NFATC3* is significantly higher in relapse samples, while *NFATC1* expression is unchanged (Figure 4). As these were paired samples this suggests there could be outgrowth of *NFATC2/3*^{high} chemoresistant clone(s) following initial therapy, or that NFATc2/3-driven signaling is recruited secondary to acquired resistance mechanisms.

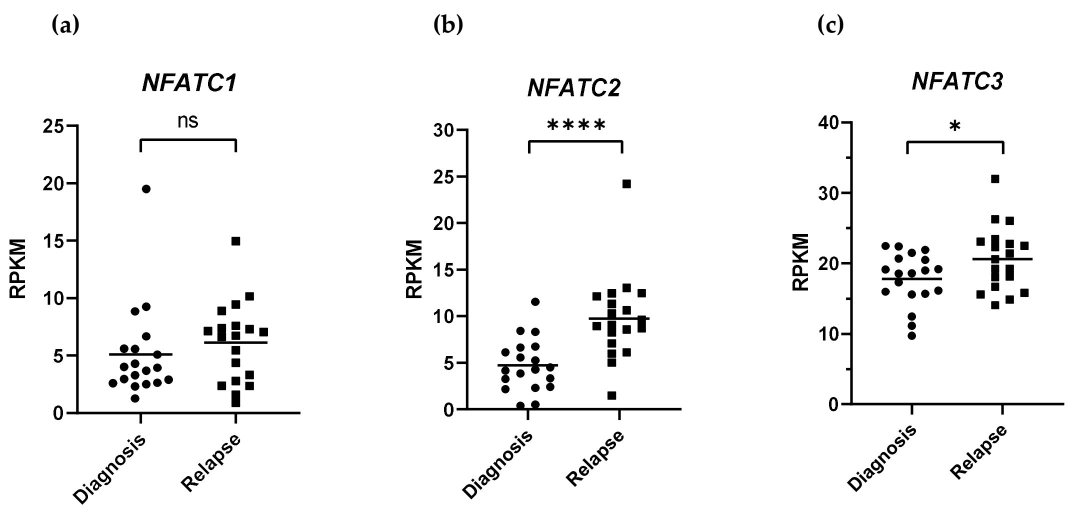

Figure 4. *NFAT* expression in AML patients at diagnosis and relapse. (a)-(c) RNA-seq data were generated by Li *et al*. from samples derived from patients with AML (n=19) at diagnosis and relapse, following a standard treatment protocol. Normalized expression data for *NFATC1-3* are shown as RPKM from diagnosis and relapsed samples. Wilcoxon matched pairs signed rank test *p* values, for a difference in medians, are shown (*<0.05; ****<0.0001).

Chemotherapy primarily targets cycling cells and so is often evaded in AML by subclones that are more quiescent and/or plastic in their state of differentiation, like the LSC population. However, the ability of the LSCs to persist through treatment and regenerate AML blasts is highly multi-dimensional, depending on the interaction of epigenetic and transcriptional regulators, evasion of the immune response and interaction with bone marrow microenvironment[61,63]. The evidence discussed shows that NFAT can regulate cycle genes and stem cell properties in myeloid physiology and pathology, and so could plausibly have roles in mediating chemotherapy resistance or LSC development, but its precise role in this complex interplay is not yet clear.

The role of NFAT transcriptional partners may aid the generation of a more complete picture of the active regulatory networks in AML. For example, *RUNX1* may cooperate with FLT3^{ITD} in the development of AML[64] and is also known

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to regulate key oncogenes, such as p53[14]. *RUNX1* somatic mutations and chromosomal translocations are well characterised in AML[65]. Masuda *et al.* demonstrated that RUNX proteins regulate *NFATC2* transcription and this was inhibited by the RUNX inhibitor Chb-M' in their models of AML[66], suggesting that *NFATC2* could play a role in the mechanism of RUNX-driven oncogenesis. Various other transcriptional partners of NFAT are known to be deregulated in AML, such as AP-1 proteins[67,68] and C/EBPα[69], although their intrinsic involvement with NFAT has not been demonstrated in this context. A focused investigation into their relationship with NFAT in AML may yield novel mechanisms of action and/or means of targeting NFAT activity.

At present there is a lack of evidence around whether NFAT proteins mediate leukemia initiation or participate in maintenance in tandem with other mutational drivers. Existing models focus on $FLT3^{ITD}$-driven signaling and are based primarily on synthetic models of AML, which do not necessarily reflect the complex clonal architecture or molecular heterogeneity of *de novo* leukemogenesis. Additionally, most studies present evidence for NFATc1 activity or are based on inhibition of all NFAT or calcineurin activity, in the absence of more specific compounds. Evidence from solid tumours suggests that individual members of the NFAT family have distinct and sometimes opposing roles in regulation of the cell cycle[20]. Together with the differential expression profiles of *NFATC1-3* presented in this review it is reasonable to postulate that each NFAT family member may contribute differentially to AML pathogenesis, and should be investigated as such. It should also be noted that functional variants of *NFAT* genes are not commonly found, although not absent, as shown by mutational profiling of large AML patient datasets[5,42]. In light of the evidence available, there are some considerations for future therapeutic strategies to target NFAT signaling in AML.

6. Therapeutic Targeting of NFAT Proteins

Cyclosporine A, tacrolimus and VIVIT peptide have served as key inhibitors for experimental research into NFAT but their clinical application is quite limited. Calcineurin, the target of CsA, has a number of targets other than NFAT which are less well characterised[34]. Clinical use of CsA in organ transplant patients is associated with significant nephrotoxicity and neurotoxicity, due to some of these other targets and the role of NFAT proteins in the nervous and cardiovascular systems[70]. Tacrolimus has even higher toxicity but some evidence suggests that lower doses could be well-tolerated by patients[71,72], though this would still carry the issue of non-specificity towards NFAT. CsA is also known to inhibit P-glycoprotein (Pgp), which can increase cellular efflux of some chemotherapeutics and reduce their efficacy. One randomised controlled trial of patients with poor risk AML (n=226) found that intravenous CsA treatment improved overall survival, although this was linked with inhibition of Pgp and so potentially not connected to NFAT activity[73]. However, highly toxic chemotherapy regimens are not well tolerated by cohorts of older AML patients and so more targeted drugs would be advantageous.

VIVIT has the advantage of targeting the calcineurin-NFAT interaction specifically. It has been developed to be cell permeable, stable in the circulation (half-life = 30 hours) and is capable of inhibiting T cell function in mice[74]. There is currently no clinical data regarding VIVIT, however some *in vivo* data in cardiovascular disease models suggest that its pharmacological properties are undesirable for application to patients[75]. There are various experimental compounds which target other elements of NFAT function. The salicylic acid derivative UR-1505 specifically blocks NFAT binding to DNA and was found to be an effective immunosuppressant[76], but its efficacy translated poorly to the clinic as a dermatitis therapy, particularly when compared with tacrolimus[77]. Based on extremely limited clinical information there is clearly a need to develop NFAT-targeted therapy further in order to progress research into its viability.

Additionally, recent research has identified novel means of targeting specific NFAT family members. A novel calcineurin-binding region (CNBR) is present only in some NFATs, while other binding regions have variable binding affinities for calcineurin between NFATc1-4, meaning that it could be possible to preferentially target some of the NFAT family members therapeutically. For example, it may be possible to specifically inhibit the interaction of calcineurin with either NFATc1 and NFATc4 by targeting CNBR3, but no such inhibitor exists presently[78]. More broadly, therapeutic targeting of transcription factors has been shown to be challenging, although inhibitors of the related Rel protein NF-κB are in early clinical trials for the treatment of AML and other cancers[79]. Some of these inhibitors target nuclear shuttling, DNA binding and downstream targets of NF-κB, which could be applied similarly for inhibition of NFAT in a clinical setting. It may therefore be possible to target nuclear import/export kinases, targets of NFAT and/or transcriptional partners of NFAT, but further evidence is needed.

7. Conclusions

At present there is intriguing evidence to implicate NFAT proteins in AML. By looking at their roles in normal myeloid physiology and in other types of cancer it is conceivable that NFAT regulates the transcription of key cell cycle and/or differentiation programs in leukemogenesis. Furthermore, NFAT has been observed to play a role in resistance to TKIs in myeloid leukemias and may mediate patient relapse. However, it is still to be ascertained whether NFAT is important in the context of some mutational profiles – as with $FLT3^{ITD}$ – or if its oncogenic properties are applicable across numerous AML subtypes. Further characterisation of individual NFATs is essential to understanding these roles and may elucidate more specific targets. If NFAT inhibitors continue to be developed for more clinical applications the NFAT family of transcription factors may become a viable treatment target in AML.

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Gene Expression Omnibus [GSE83533] at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE83533 ;

The Cancer Genome Atlas [TCGA-LAML] at https://portal.gdc.cancer.gov/repository

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Appendix A: FAB Classification of AML

FAB Classification	Description
M0	Minimally differentiated AML
M1	Myeloid leukaemia (without maturation)
M2	Myeloid leukaemia (with maturation)
M3	Acute progranulocytic leukaemia
M4	Myelomonocytic leukaemia
M5	Monocytic leukaemia
M6	Erythroid leukaemia
M7	Megakaryocytic leukaemia

Table showing the description of each FAB category[43].

Abbreviations

AML	Acute Myeloid Leukemia
CAMK	Calmodulin Kinase
CML	Chronic Myeloid Leukemia
CNBR	Calcineurin-binding Region
CsA	Cyclosporine A
DC	Dendritic Cell
FAB Classification	French-American-British Classification
FLT3	Fms related tyrosine kinase receptor 3
$FLT3^{ITD}$	FLT3 Internal Tandem Duplication
FLT3-L	FLT3 Ligand
G-CSF	Granulocyte Colony Stimulating Factor
GMP	Granulocyte-monocyte Progenitor
HSC	Hematopoietic Stem Cell
LSC	Leukemic Stem Cell
M-CSF	Macrophage Colony Stimulating Factor
NFAT	Nuclear Factor of Activated T Cells
NHD	NFAT Homology Domain
Pgp	P-glycoprotein
PLC	Phospholipase C
PRR	Pattern Recognition Receptor
SOCE	Store Operated Calcium Entry
TAD	Transactivation Domain
TKI	Tyrosine Kinase Inhibitor

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