An epigenetic chromatin remodeling role for NFATc1 in transcriptional regulation of growth and survival genes in diffuse large B-cell lymphomas

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The nuclear factor of activated T cells (NFAT) family of transcription factors functions as integrators of multiple signaling pathways by binding to chromatin in combination with other transcription factors and coactivators to regulate genes central for cell growth and survival in hematopoietic cells. Recent experimental evidence has implicated the calcineurin/NFAT signaling pathway in the pathogenesis of various malignancies, including diffuse large B-cell lymphoma (DLBCL). However, the molecular mechanism(s) underlying NFATc1 regulation of genes controlling lymphoma cell growth and survival is still unclear. In this study, we demonstrate that the transcription factor NFATc1 regulates gene expression in DLBCL cells through a chromatin remodeling mechanism that involves recruitment of the SWItch/Sucrose NonFermentable chromatin remodeling complex ATPase enzyme SMARCA4 (also known as Brahma-related gene 1) to NFATc1 targeted gene promoters. The NFATc1/Brahma-related gene 1 complex induces promoter DNase I hypersensitive sites and recruits other transcription factors to the active chromatin site to regulate gene transcription. Targeting NFATc1 with specific small hairpin RNA inhibits DNase I hypersensitive site formation and down-regulates target gene expression. Our data support a novel epigenetic control mechanism for the transcriptional regulation of growth and survival genes by NFATc1 in the pathophysiology of DLBCL and suggests that targeting NFATc1 could potentially have therapeutic value. (Blood. 2010; 116(19):3899-3906)

Introduction

Diffuse large B-cell lymphoma (DLBCL), an aggressive form of non-Hodgkin B-cell lymphoma (NHL-B), is the most common subtype of aggressive NHL-B, accounting for more than 30% of all NHL-B cases.1-3 The pathophysiology of DLBCL appears to depend on several growth and survival signaling pathways,4-6 including nuclear factor of activated T cells (NFAT),7,8 a well-known family of transcription factors that play important roles in regulation of the immune system, best known for their critical roles in T-cell activation and cytokine transcriptional regulation.9,10

Our understanding of the molecular mechanism(s) controlling B-cell lymphoma cell growth and survival mediated through the NFAT pathway is still incomplete however and requires further elucidation.

Activation of the NFAT signaling pathway has been implicated recently in both hematologic and solid tumors in neoplastic development.11,12 NFATc2/NFAT5 expression and transcriptional activation are induced downstream of integrin signaling, promoting carcinoma cell migration and invasion in a mouse carcinoma model.13 Constitutive activation of NFATc1 has been found in approximately 70% of pancreatic carcinomas, and blocking NFATc1 activation with cyclosporin A inhibited both cell growth and survival in a pancreatic tumor cell line.14 A recent immunohistologic study showed nuclear expression of NFATc1 in some cases of DLBCL,3 but a more recent study indicated that NFATc1 is overexpressed in a subset of DLBCL due to genomic amplification.7 This is probably not surprising, because NFATc1 and NFATc2 have long been described as also functional in the B-cell lineage, but their roles and importance in normal and neoplastic B-cell biology have not been substantively pursued or elucidated. The significance of these findings poses important translational research questions regarding the molecular and cell biology of NFAT proteins and their key roles in controlling cell proliferation, survival, and other biologic functions in neoplastic as well as normal B-lymphoid cells. It has become increasingly apparent that, in addition to its role in T-lymphocyte activation, NFAT is also involved in critical aspects of malignant cell transformation and tumorigenic processes.15,16

Although we have shown previously that NFAT family member NFATc1 is constitutively activated and can maintain cell growth and survival in DLBCL cell lines and primary cells,3 the molecular mechanism(s) underlying NFATc1 regulation of cell growth and survival in DLBCL is still unclear. Studies in other lymphoid and hematopoietic cell types (eg, T cells, mast cells) by Cockerill17 and Goldfeld’s group18 have indicated that, in closely linked genes such as GM-CSF and IL3, NFAT plays a major role in chromatin remodeling, forming DNA hypersensitive sites (DHSs) in enhancer/promoters, functioning to disrupt nucleosomes to increase DNA accessibility to other transcription factors and cofactors while synergizing transcription factor/cofactor binding to targeted promoters/enhancers. We have previously detected specific interactions between NFAT and nuclear factor-κB (NF-κB), particularly in the DLBCL CD40L (CD40 ligand, also known as CD154) and BLYS (also known as BAFF) gene promoters.5,19 We have hypothesized that types of chromatin remodeling and other enhancer activation mechanisms similar to those reported in the T-cell GM-CSF gene, are also active in DLBCL NFAT-targeted growth and survival genes.
Changes in chromatin structure are catalyzed by ATP-dependent chromatin remodeling enzymes through 1 of 2 mutually exclusive subunits, brahma (Brm) and brahma-related gene-1 (Brg-1). While both Brg-1 and Brm can function as the central ATPase in the SWI/Syr/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex, each defines a discrete complex with unique biochemical activity. Because of its central function in epigenetic chromatin-remodeling mechanisms, dysregulation of SWI/SNF and its ATPase can lead to tumor development and growth. However, the impact of the chromatin remodeling mechanism in the biology of B-cell lymphomas is still unclear and unproven.

In this study, we demonstrate that transcription factor NFATc1 regulates growth and survival genes in DLBCL cells through a chromatin remodeling mechanism that involves recruitment of the SWI/SNF chromatin remodeling complex. In representative aggressive B-cell lymphoma cell lines, we found that, besides the critical TNF ligands CD40L and BLYS, the gene encoding the c-myc oncoprotein is also a target of NFATc1. Using the transcriptional regulation of c-myc by NFATc1 as a biologic model system, we discovered that the SWI/SNF ATPase Brg-1 was in fact involved in the NFATc1-mediated chromatin-remodeling mechanism by inducing DHS in DLBCL cells. Our data indicate a novel epigenetic chromatin remodeling mechanism for the transcription factor NFATc1 in the pathophysiology of aggressive lymphoma B cells and suggest that targeting NFATc1 could have therapeutic value.

Methods

Cells and reagents

Human DLBCL cell lines (MS, DS, DB, JM [McA], FN, EJ, HF, HB, MZ, LR, CJ, LP, and PL) were established from tissue biopsy or effusion specimens from patients as described elsewhere. The SUDHL-4 and OCI-LY10 DLBCL cell lines were obtained from Dr Michael Rosenblum (The University of Texas M. D. Anderson Cancer Center, Houston, TX). The Ramos and BJAB cell lines were obtained from ATCC. All cell lines, except for Ramos, EJ, CJ, and HB, were negative for c-myc t(8;14) translocation. This study was conducted in accordance with the Helsinki protocol and approved by the Institutional Review Board of The University of Texas M. D. Anderson Cancer Center. Informed consent was obtained from all patients whose tumor samples were used. The cells were cultured in RPMI medium (Invitrogen) containing 15% fetal calf serum (Hyclone). The SureSilencing small hairpin (sh) RNA vector (System Biosciences, Mountain View, CA) contains the human NFATc1 cDNA (nucleotide 243 to 2751) tagged at the N-terminus with a FLAG epitope and a site for CREB. The NFATc1 eukaryotic expression vector construct pSh1103 (kindly provided by Dr Gerald R. Crabtree, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY) contains the NFATc1 cDNA (nucleotide 243 to 2751) tagged at the N-terminus with a FLAG epitope and the CREB site.

Antibodies and plasmids

The following primary antibodies were used: polyclonal Brg-1 and Oct-1 and monoclonal NFATc1, NFATc2, c-myc, brm (Santa Cruz Technology), and polyclonal p65 and c-rel and monoclonal STAT3 (Millipore). The following primary antibodies were used: polyclonal Brg-1 and Oct-1 and monoclonal STAT3 (Millipore). The SureSilencing small hairpin (sh) RNA vector (System Biosciences, Mountain View, CA) contains the human NFATc1 cDNA (nucleotide 243 to 2751) tagged at the N-terminus with a FLAG epitope and a site for CREB. The NFATc1 eukaryotic expression vector construct pSh1103 (kindly provided by Dr Gerald R. Crabtree, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY) contains the NFATc1 cDNA (nucleotide 243 to 2751) tagged at the N-terminus with a FLAG epitope and the CREB site.

Antibodies were crosslinked to Dynabeads Protein A (Dynal Biotech) according to the manufacturer’s instructions. The DNA-binding activity of NFAT and other factors to the NFAT consensus site was analyzed by an enzyme-linked immunosorbent assay (ELISA)–based assay according to the manufacturer’s instructions. The TransAM NFAT Family Transcription Factor Assay Kit (Active Motif) was used to detect the DNA-binding activity of NFATc1 and other transcription factors, followed by horseradish peroxidase–conjugated secondary antibody, and were quantified by spectrophotometry at 450 nm with a reference wavelength of 650 nm.

Analysis of NFAT DNA-binding activity

Antibodies were crosslinked to Dynabeads Protein A (Dynabeads Technology) according to the manufacturer’s instructions. The DNA-binding activity of NFAT and other factors to the NFAT consensus site was analyzed by an enzyme-linked immunosorbent assay (ELISA)–based assay according to the manufacturer’s instructions. The DNA-binding activity of NFAT and other factors to the NFAT consensus site was analyzed by an enzyme-linked immunosorbent assay (ELISA)–based assay according to the manufacturer’s instructions. The TransAM NFAT Family Transcription Factor Assay Kit (Active Motif) was used to detect the DNA-binding activity of NFATc1 and other transcription factors, followed by horseradish peroxidase–conjugated secondary antibody, and were quantified by spectrophotometry at 450 nm with a reference wavelength of 650 nm.

ChIP assays

Chromatin immunoprecipitation (ChIP) assays were performed using the ChIP assay kit and protocol provided by Millipore. Cells were crosslinked with 1% formaldehyde in medium for 10 minutes at 37°C, washed with cold PBS, resuspended in cell sodium dodecyl sulfate lysis buffer (provided with kit) for 10 minutes on ice, and sonicated at 10-second intervals 3× with a sonicator. Samples were subjected to centrifugation for 10 minutes at 16,000g (13,000 rpm) at 4°C, and the supernatants were diluted with ChIP dilution buffer. To reduce nonspecific background, samples were precleared with protein A Dynabeads with normal immunoglobulin G (IgG) for 30 minutes at 4°C with agitation. Primary antibodies were added to the samples, which were incubated overnight at 4°C. The protein A-antibody–DNA complexes were washed...
purified DNA from immunoprecipitation studies and DNA inputs were used for quantitative real-time (rt) polymerase chain reaction (Q-PCR). Data were analyzed using the SuperArray ChIP-QPCR Data Analysis Template (SABiosciences).

**Quantification of DNase I sensitivity**

DNase I sensitivity analysis was performed according to the protocol of McArthur et al.27 DLBCL cells were harvested and resuspended in ice-cold lysis buffer (100mM KCL, 50mM Tris-CL [pH 7.9], 50% [vol/vol] glycerol, 200mM β-mercaptoethanol, and 5mM MgCl2) and incubated for 10 minutes. Nuclei were recovered from the lysed cells by subjecting the suspension to centrifugation at 13,000g for 15 minutes at 4°C and resuspending the cells in Buffer A (50mM Tris-Cl [pH 7.9], 3mM MgCl2, 0.2mM phenylmethylsulfonyl fluoride, 100mM NaCl, and 1mM dithiothreitol). Nuclei were digested with the indicated amount of DNase I for 3 minutes at room temperature in Buffer A. The samples were then treated with proteinase K and RNase A, and the DNA was recovered using the QIAGEN PCR purification kit. DNase-treated DNA was subjected to Q-PCR.

**RNA isolation and rt PCR**

Total RNA isolation was performed using Trizol LS Reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription of RNA was carried out with cDNA archive kit (Applied Biosystems). Synthesized cDNA was subjected to Q-PCR for the detection of related genes transcripts was carried out with cDNA archive kit (Applied Biosystems). Synthesized according to the manufacturer’s instructions. Reverse transcription of RNA isolation and rt PCR using the QIAGEN PCR purification kit. DNase-treated DNA was subjected to Q-PCR.

**Q-PCR for ChIP and DNase1 assays**

DNA samples from ChIP assays or DNase I sensitivity assays and primers were added to the additional rt PCR reagents (RT2 SYBR Green/ROX qPCR Master Mix) in a 25-μL reaction volume and subjected to the following cycles: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. All samples were analyzed in triplicate. DNA contamination was evaluated by performing PCR on the nonreverse transcribed control of each sample. The relative expression levels of the genes of interest were normalized to endogenous reference 18S and relative to a control sample as a calibrator using the formula: 2^−ΔΔCT. The Threshold Cycle reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold.

**Production of recombinant retroviruses and infection of lymphoma cells**

Recombinant retroviruses were produced by cotransfecting either the pMSCV-GFP or pMSCV-canNFATc1 proviral vectors together with pVSV-G (Clontech), encoding the vesicular stomatitis virus-glycoprotein, into the GP293 pantrophic packaging cell line (Clontech) using FuGENE6 (Roche). Medium was replaced after 24 hours, and viral supernatants were harvested 2 days after transfection and stored at −80°C. For infections, lymphoma cells were incubated in viral supernatant containing 8 μg/mL polybrene (Sigma-Aldrich), and were centrifuged at 2000 rpm for 1.5 hours at room temperature. Cells were expanded in growth medium for subsequent analysis and typically used within 2-4 days of infection. Flow cytometric analysis of GFP routinely revealed that greater than 70% of cells were virally infected.

**Confocal microscopic analysis**

Cells were fixed with 100% cold methanol for 10 minutes. Nonspecific protein binding was prevented by blocking the cells with 5% fetal calf serum in PBS for 30 minutes. Cells were stained with the appropriate primary antibodies (1:200 dilution) for 2 hours at room temperature or overnight at 4°C. After 3 washes with PBS, cells were stained with the appropriate anti–donkey secondary antibodies (labeled with fluorescein isothiocyanate or Texas Red 1:200 dilution) for 45 minutes and washed with PBS. Coverslips were applied with Slow Fade Reagent (Molecular Probes). The cells were visualized by a FluoView 500 (FV500) laser scanning confocal microscope (Olympus America). Images were captured with a PlanApo 60×/1.4 oil objective using the appropriate filter sets. Digital images were obtained using the manufacturer’s FluoView v.5.0 software. Image resolutions were adjusted using Adobe Photoshop CS3.

**Results**

**Model system for studying the functional role of NFATc1 in DLBCL**

A recent study showed that NFATc1 regulates c-myc transcription in pancreatic cancers.14 To examine the possible correlation of NFATc1 and c-myc protein expression in aggressive NHL-B, 8 cell lines (a well-known Burkitt lymphoma cell line Ramos and 7 of our DLBCL cell lines) were examined for expression of NFATc1 and c-myc proteins by Western blot analysis. As shown in Figure 1A, expression of the c-myc protein correlated with nuclear NFATc1 protein expression in all cell lines examined. One DLBCL cell line (DS) was negative for both NFATc1 and c-myc protein expression. The mRNA level of c-myc was shown to correlate with the DNA binding activity of NFATc1 in representative DLBCL cell lines (P = .166; Figure 1B). Similar results were obtained from an NFAT and c-myc TMA in 100 representative DLBCL cases. NFATc1 and c-myc TMA slides containing 100 DLBCL cases, as well as examples for positive and negative NFATc1 and c-myc immunostaining are shown in Figure 1C. Seventy-five percent of DLBCL cases showed positive staining (> 30% positive cells) for both NFATc1 and c-myc expression (Figure 1D). Seven cases showed negative staining for c-myc that also showed negative for NFATc1 staining. Of the 72 cases that were positive staining for NFATc1 staining, 59 (82%) had nuclear NFATc1 staining as well as c-myc nuclear staining. NFATc1 is also highly expressed in reactive
in the nuclear compartment (Figure 2D). These results suggest that c-myc is a potential target gene for NFATc1 transcription in aggressive B-cell lymphomas.

To test whether NFATc1 does in fact, act as a transcriptional regulator of c-myc, DLBCL MS cells were cotransfected with a c-myc luciferase reporter plasmid containing either a full-length c-myc promoter comprising positions −2446 to +334 (Del-1) or a c-myc promoter fragment containing positions −109 to +334 (Del-6) relative to the P2 transcription start site, both of which contain an NFAT site (Figure 3A), along with an NFATc1 expression plasmid in combination with an NFATc1 shRNA expression plasmid or a dominant-negative NFAT expression construct. The results of these experiments showed that NFATc1 enhanced c-myc promoter activity in DLBCL cells and that this promoter activity can be repressed by cotransfecting the cells with a dominant-negative NFAT plasmid or reduced by cotransfection with NFATc1 shRNA (Figure 3B). The c-myc promoter fragment Del-6 had luciferase activity similar to that of the full-length promoter (Figure 3B), and NFATc1 enhanced Del-6 promoter

lymph node tissue, but mostly in the cytoplasmic compartment with very little nuclear staining (Figure 1E). These findings suggest that c-myc is a likely NFATc1 target gene in aggressive NHL-B cells, and that nuclear NFATc1 could play an important role in the biology of NHL-B.

Next, we suppressed NFAT activation in representative DLBCL cell lines (which express NFATc1 at high levels) with an NFAT inhibitor, FK-506, and then examined the levels of c-myc protein and mRNA. Treatment with FK-506 down-regulated both dephosphorylated NFATc1 (active form) and c-myc protein (Figure 2A) as well as c-myc mRNA (Figure 2B) expression in these cells. Moreover, specific down-regulation of NFATc1 by small hairpin RNA (shRNA) in MS cells also inhibited c-myc protein expression (Figure 2C). Knockdown of NFATc1 by shRNA also down-regulated c-myc mRNA expression in representative DLBCL cell lines (Figure 2D). Conversely, transduction of DLBCL DS cells (negative for both NFATc1 and c-myc) with a retroviral vector containing a constitutively active mutant of NFATc1 induced c-myc protein expression (Figure 2C). Constitutively active NFATc1 in DS cells appears as punctated dots in both the cytoplasm as well as
models for studying transcriptional regulation of c-myc in defining the functional role of NFATc1 in transcriptional regulation of the important growth and survival genes in B-cell lymphomas.

Involvement of the chromatin-remodeling complex protein Brg-1 with NFATc1 within the c-myc promoter

The transcription factor NFATc1 is known for its active role in forming enhanceosomes and was recently recognized as a nucleosome reorganization factor involved with the chromatin remodeling complex proteins. To examine whether NFATc1 is involved in the chromatin remodeling mechanism in lymphoma B cells, we tested the direct association of NFATc1 protein and the chromatin remodeling complex ATP enzymes Brm and Brg-1 using DNA-binding ELISAs. In assays using nuclear extracts purified from representative DLBCL cell lines, both Brm and Brg-1 proteins bound to the consensus NFAT-binding sequence (Figure 4A).

Because Brg-1 bound to the NFAT-binding site with higher intensities than Brm, our remaining studies focused primarily on Brg-1 and its activity in mediating NFATc1 function. Next, we used gel-shift assays to test whether Brg-1 bound to the NFAT-binding site within the c-myc promoter. Figure 4B shows that antibodies against Brg-1 competed out the NFAT-binding complex, suggesting that Brg-1 proteins are part of the NFATc1 complex in DLBCL cells. Further analysis by immunoprecipitation assays...
showed that Brg-1 interacts directly with NFATc1 (Figure 4C), and confocal microscopic analysis confirmed that Brg-1 and NFATc1 colocalized in the nuclear compartment in punctate nuclear complexes (Figure 4D). These results indicate a direct link between the transcription factor NFATc1 and the chromatin remodeling complex protein Brg-1 in DLBCL and suggest that NFATc1 can function actively in chromatin remodeling mechanism.

**NFATc1 recruits Brg-1 and other transcription factors to the c-myc promoter**

We then examined whether NFATc1 was involved in recruiting Brg-1 and other transcription factors to the NFATc1-targeted gene promoters. Ectopic expression of a constitutively active mutant of NFATc1 in DLBCL DS cells (negative for NFATc1) was sufficient to recruit Brg-1 as well as other transcription factors, such as NF-κB-p65, c-rel, and STAT3, to the NFAT DNA-binding site in the c-myc promoter (Figure 5A). Conversely, inhibiting NFATc1 in DLBCL MS and SUDHL-4 cells with shRNA diminished Brg-1 and other transcription factors binding to the c-myc gene promoters (Figure 5B).

**NFATc1 induces DNase I hypersensitive sites in NFAT-targeted gene promoters**

Because NFATc1 is able to recruit chromatin remodeling complex protein Brg-1 and other transcription factors to chromatin, we hypothesized that an NFATc1/Brg-1 complex could play a prominent role in modulating NFATc1 target genes by inducing DHS, which leads to an open chromatin structure accessible to transcription factors. The c-myc promoter has been well mapped for DHS and has a prominent DHS within the NFAT-binding site. To determine whether NFATc1 is in fact involved in forming DHS in DLBCL, we quantitated DNase I hypersensitivity in promoters of NFATc1-targeted genes by treating with DNase I, followed by Q-PCR. The results show that the DNA regions containing the NFAT-binding site within the c-myc promoter, as well as in other NFATc1-targeted gene promoters such as CD40L and BLyS, are hypersensitive to DNase I treatment (Figure 6). However, the same DNA regions were insensitive to DNase I treatment when NFATc1 was down-regulated, either with FK-506 treatment or NFATc1 shRNA transfection (Figure 6), suggesting that NFATc1 plays a prominent role in DHS formation in DLBCL.

**Discussion**

NFAT family proteins are multifunctional activators, and also important calcium-inducible transcription factors that activate expression of a wide range of immune response genes, especially in activated T lymphoid cells, but our previous studies have indicated that NFAT proteins are also important in B lymphocytes, and particularly in aggressive human NHL-B. Recent experimental evidence has revealed a key role for NFAT transcription factors in human tumor progression, particularly in hematologic malignancies. In this study, we report that NFATc1 regulates c-myc as well as other growth and survival genes in DLBCL through a chromatin remodeling mechanism involving the recruitment of the chromatin remodeling ATPase enzyme Brg-1. These findings suggest that nuclear NFATc1 is intrinsically involved in the pathophysiology of DLBCL and pharmacologic interdiction of this pathway could provide new therapeutic avenues for clinical intervention.

NFAT-dependent promoters and enhancers rapidly undergo extensive inducible chromatin remodeling to form DHS.

NFAT is likely to be a driving force behind this chromatin remodeling, which has recently been proposed as a major NFAT function, because it has been shown that NFAT sites alone are sufficient to activate DHS in a chromatin context. Chromatin remodeling may well be a primary function of NFAT elements, because even high-affinity NFAT sites are relatively weak transcriptional activators in the absence of the collaborating transcription factors with which they normally associate. Transcription factor–activating protein 1 (AP-1) is the most common partner directly recruited by NFAT, at the level of DHS, and composite NFAT–AP-1 DNA response elements are very efficient in evicting nucleosomes. NFAT–AP-1 complexes recruit both histone acetyltransferases (HATs) and the ATP-dependent SWI-SNF family of chromatin remodelers, that together provide the functional proteins needed to modify and rearrange nucleosomes.

Recent studies have shown that NFAT may also help to organize chromatin domains and enable enhancer-promoter communication. In activated T cells, inducible intrachromosomal looping occurs between the tumor necrosis factor (TNF) gene promoter and 2 NFAT-dependent enhancers located at –9 kb and +3 kb relative to the start site of transcription. This topology places the TNF gene and the adjacent lymphotoxin genes in separate loops, thereby allowing independent regulation of the TNF gene within a multigene locus. These new data support earlier studies indicating that NFAT functions through the disruption of nucleosomes within specific gene enhancers, mobilizing nucleosomes across extensive chromatin domains linking enhancers and promoters. Our studies identify NFAT as a factor in DLBCL cells, that creates a chromatin environment that is permissive for both recruitment and aggregation of factors that control transcriptional processes within important targeted promoters and enhancers. The specific role that...
NFAT plays in the complex process of gene locus activation is still unclear, but it is clearly an effective facilitator for initiating the first essential step of creating a critical accessible “open” chromatin environment,28 that we have shown to function in NFAT-targeted G/S survival genes in DLBCL.

The consequence of an active NFAT transcription factor playing a role in chromatin remodeling in regulating important growth and survival genes, such as c-myc, could be beneficial to many cancer cells, including B-cell lymphomas. Previous studies have shown that deregulated c-myc expression due to alternative myc translocation, amplification, or mutation is a negative prognostic indicator in DLBCL.38 Constitutive NFAT activation in DLBCL, on the other hand, could be one plausible molecular mechanism for maintaining continuous c-myc expression. In fact, c-myc can control the NFAT pathway in B lymphocytes by directly amplifying the calcium signal that leads to sustained intracellular calcium and maintaining continual NFAT nuclear translocation, thereby enabling concurrent expression of Myc- and calcium-regulated target genes.39 In this sense, deregulated myc or NFATc1 in DLBCL could provide a positive regulatory feedback loop in which NFATc1 and c-myc mutually reinforce each other’s expression, subsequently maintaining neoplastic cell growth and survival. Recent studies have already established that NFATc1 is a key transcription factor involved in cell growth and survival as well as cellular transformation in vitro in various cell types.5,11,13,16,19 However, the consequence of an active NFATc1 in an in vivo genetic-engineered mouse model setting has not been demonstrated and warrants further investigation.

We discovered that the chromatin remodeling ATPase dependent enzyme Brg-1 binding to growth and survival gene promoters is dependent on the direct interaction with NFATc1 in DLBCL. Brg-1 has been shown to unfold or displace nucleosomes to create “open” chromatin structures.40 Therefore, we hypothesized that once Brg-1 is recruited and interacts with NFATc1 at the chromatin loci, these factors are able to unfold these chromatin loci to create DNase I hypersensitive sites, resulting in transcriptionally active and accessible chromatin, allowing other transcription factors to bind to these sites. Although chromatin remodeling proteins have been implicated in the oncogenic transformation,41 the underlying mechanisms are still poorly understood. SNF5, a core subunit of the SWI/SNF complex, is a potent tumor suppressor that is specifically inactivated in several types of human cancer.42,43 A recent study showed that lymphomagenesis in the absence of SNF5 does not result from SWI/SNF inactivation but rather that oncogenesis is dependent on continued presence of Brg-1.44 These findings along with our current findings suggest that Brg-1 plays an important role in the biology of B-cell lymphomas and that targeted inhibition of Brg-1 ATPase activity could provide a novel and effective therapeutic approach for aggressive B-cell lymphomas.

In summary, this study has shown a key function for the transcription factor NFATc1 in regulating important growth and survival genes (ie, c-myc, CD40L, BlyS, and NF-M) in DLBCL through an epigenetic chromatin remodeling mechanism that requires recruitment of the ATPase enzyme Brg-1. Our finding that NFATc1 is a component of a chromatin remodeling complex not only extends these findings by implicating chromatin remodeling complexes in the pathogenesis of DLBCL, but also provides a mechanistic insight into NFATc1 function and the biochemical pathways regulated by NFATc1. These results also suggest that targeting the NFAT/Brg-1 pathway in DLBCL could have therapeutic potential, particularly for patients with relapsed or refractory DLBCL.

Acknowledgments

This work was supported in part by the Odyssey Program and the Kimberly-Clark Foundation Award for Scientific Achievement at The University of Texas M. D. Anderson Cancer Center (to L.V.P.). This work was also supported by National Cancer Institute grants CA-RO1-100836 (to R.J.F.) and CA-16672-26 (Cancer Center Support Grant), and a grant from the Leukemia & Lymphoma Society (to R.J.F.).

Authorship

Contribution: L.V.P. and R.J.F. contributed to all scientific aspects of the manuscript (designed and performed research and analyzed data) and wrote the paper; A.T.T. and C.L. contributed by performing experiments and analyzing data; and C.B.-R. contributed to all scientific aspects of the manuscript (designed and performed research and analyzed data) and wrote the paper; A.T.T. and C.L. contributed by performing experiments and analyzing data; and C.B.-R. contributed to all scientific aspects of the manuscript (designed and performed research and analyzed data) and wrote the paper; A.T.T. and C.L. contributed by performing experiments and analyzing data; and C.B.-R. contributed to all scientific aspects of the manuscript (designed and performed research and analyzed data) and wrote the paper; A.T.T. and C.L. contributed by performing experiments and analyzing data; and C.B.-R. contributed to the immunohistochemical analysis.

Conflict-of-interest disclosure: The authors declare no conflicting financial interests.

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