CTCF functions as a critical regulator of cell-cycle arrest and death after ligation of the B cell receptor on immature B cells

Chen-Feng Qi*, Annica Martensson†, Michela Mattioli‡, Riccardo Dalla-Favera‡, Victor V. Lobanenkov*, and Herbert C. Morse III*§

*Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; †The Scripps Research Institute, La Jolla, CA 92037; and ‡Institute of Cancer Genetics and Departments of Pathology and Genetics and Development, Columbia University, New York, NY 10032

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The WEHI 231 B cell lymphoma is used as a model of self-tolerance by clonal deletion because B cell receptor (BCR) ligation results in apoptosis. Two critical events precede cell death: an early rise and fall in expression of MYC and cell-cycle arrest associated with enhanced expression of p21, p27, and p53. CTCF is a transcription factor identified as a repressor of MYC recently shown to cause cell growth inhibition. The present studies demonstrate that BCR ligation of WEHI 231 as well as of normal immature B cells greatly increased expression of CTCF in association with down-regulation of MYC followed by growth arrest and cell death. Conditional expression of CTCF in WEHI 231 mimicked BCR ligation with activated cells showing repressed expression of MYC, enhanced expression of p27, p21, p53, and p19^{ARF}, and inhibition of cell growth and induction of apoptosis. In keeping with a central role for CTCF in control of B cell death, conditional expression of a CTCF antisense construct in WEHI 231 resulted in inhibition of p27, p21, p53, and p19^{ARF} in association with enhanced expression of MYC. Activation of the endogenous CTCF locus by BCR ligation was also mimicked by three other routes to apoptotic death in WEHI 231: inhibition of the phosphoinositide 3-kinase or mTOR/FRAP signaling cascades and treatment with transforming growth factor (TGF)-β. Rapid activation of CTCF by BCR ligation or treatment with TGF-β was suppressed by ligation of CD40. These results demonstrate that CTCF is a common determinant to different pathways of death signaling in immature B cells.

Transcription factor CTCF is an evolutionarily conserved 11-zinc finger, DNA-binding nuclear phosphoprotein involved in multiple aspects of normal gene regulation including transcriptional repression and activation, gene silencing, chromatin insulation, and regulation of imprinted sites (reviewed in ref. 1). This functional flexibility is mediated by combinatorial use of the 11-zinc fingers to bind ≈50-bp target sites with dissimilar sequences yielding distinct CTCF–DNA complexes, some of which are methylation sensitive (2–4). CTCF was initially identified, cloned, and characterized as a factor that binds to multiple different sequences in avian, mouse, and human *MYC* promoters, where it uniformly functions to repress transcription (5, 6).

Conditional expression of CTCF in a variety of tumor cell lines induces profound growth arrest without apoptosis (7). Unexpectedly, inhibition of cell growth could not be explained solely by repression of MYC, suggesting the existence of other important target genes that regulate proliferation. An attractive answer to this puzzle came from the recent identification of p19^{ARF} (ARF) as another CTCF target with binding to the ARF promoter resulting in transcriptional activation (C.-F.Q., V.V.L., and H.C.M., unpublished observations). ARF is a critical component of the p53-ARF-MDM2 tumor suppressor axis that controls cell growth and death (reviewed in ref. 8). Within this axis, ARF binds to the p53 inhibitor, MDM2, facilitating activation of p53 resulting in downstream activation of its transcriptional targets, including p21 and BAX (reviewed in ref. 9). Direct evidence for *in vivo* regulation of ARF by CTCF came from conditional expression of *CTCF* in MCF-7 breast cancer cells that resulted in induction of *ARF* from the endogenous promoter and cell growth arrest, again in the absence of apoptosis (C.-F.Q., V.V.L., and H.C.M., unpublished observations).

MYC and components of the p53-MDM2-ARF axis have been shown to be important determinants of life-and-death decisions that occur when immature B cells encounter antigen during their residence in the bone marrow. By using B cell lymphomas with properties of immature B cells, such as WEHI 231, it was found that crosslinking the Ig B cell receptor (BCR) resulted in cell-cycle arrest and apoptosis, thereby providing a model for self-tolerance by clonal deletion (10–12). At the molecular level, BCR ligation induced a striking up-regulation of MYC within 1 h followed by a rapid fall to below baseline levels (13, 14). The fall in MYC was critical to activation of the death pathway because stabilization of MYC prevented apoptosis (14). In addition, the fall in MYC levels was associated with increased expression of p53 (15), p21 (15), and p27 (16). The demonstration that MYC is a transcriptional repressor of p27 (17) provided a partial tie between these phenomena. The understanding of MYC regulation by CTCF noted above suggested that CTCF induction, repressing *MYC* transcription, might be pivotal to growth arrest and apoptosis in immature B cells. Here, we report that BCR ligation of WEHI 231 or normal immature B cells results in rapid induction of CTCF and that conditional expression of CTCF in WEHI 231 mimics the established range of effects of BCR ligation in this cell line. These observations define CTCF as a determinant in selection of the mature B cell repertoire through its actions as a central control element for clonal elimination of immature B cells.

Methods

Cell Culture and Reagents. WEHI 231 and CH33 cells were obtained from David Scott (Holland Laboratory, American Red Cross, Rockville, MD) and were cultured under standard conditions. WEHI 231 cells stably transfected with the p3/SS vector carrying the *lacI* gene expressing lactose repressor (15) were obtained from Gail Sonenshein (Boston University School of Medicine, Boston) and were cultured in the presence of hygromycin B (Hygro, 350 μg/ml, Roche Molecular Biochemicals). Cells containing p3/SS were electroporated with CTCF sense and antisense constructs.

The lymphoblastoid cell line EREB.MycER was as described (18). MycER was activated by addition of 4-hydroxytamoxifen

Abbreviations: ARF, p19^{ARF}; BCR, B cell receptor; TGF, transforming growth factor; TM, 4-hydroxytamoxifen; CX, cycloheximide; IPTG, isopropyl β-D-thiogalactosylpyranoside; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling; PI3K, phosphoinositide 3-kinase.

§To whom correspondence should be addressed. E-mail: hmorse@niaid.nih.gov.

(TM, 500 nM), of TM in the presence of cycloheximide (CX, 10 μg/ml), or of CX alone. Northern blots generated with 7–10 μg of poly(A)$^+$ mRNA were hybridized sequentially to a CTCF probe, a probe that recognizes MycER RNA, a c-MYC exon 1 probe for endogenous MYC RNA, and the β-actin probe as a control for RNA loading.

Normal WEHI 231 cells were treated with goat anti-mouse IgM antibody (1 μg/ml, Southern Biotechnology Associates), LY294002 (20 μM, LC Services, Woburn, MA), rapamycin (100 nM, Sigma), or recombinant human transforming growth factor (TGF)-β1 (2 ng/ml, R & D Systems). Cells were harvested and frozen for later preparation of nuclear proteins as described (19). Cell viability was assessed with standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assays. In some experiments, cells treated with anti-Ig or TGF-β were incubated with 1 μg/ml anti-CD40 antibody (HM40–3, BD Biosciences, San Diego).

To generate IL-7-driven bone marrow cultures, bone marrow cells from B10.D2 3-83 transgenic mice (20) 6–8 weeks of age were depleted of red blood cells and filtered to remove debris. Cells were cultured at 2.5×10^5 cells per ml in four 6-well plates in rIL-7 for 4–5 days. The IL-7 was then washed out, and the cells were replated at $1–1.5 \times 10^6$ per ml per well in new 6-well plates with or without 10 μg/ml anti-BCR antibody for an additional 24 h. The cells were >95% B220$^+$. Cells harvested at the indicated times after stimulation were frozen as pellets for later extraction of RNA and nuclear proteins. Procedures used in preparation of nuclear protein extracts and immunoblotting were as described (19). For immunohistochemistry, isopropyl β-D-thiogalactosylpyranoside (IPTG)-stimulated WEHI 231 cells were cultured on chamber slides (Lab-Tek), fixed with methanol, reacted with rabbit antibody to CTCF, counterstained with FITC-labeled goat anti-rabbit Ig, and assayed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) using the *in situ* cell-death detection kit (Roche). Pictures from immunofluorescence microscopy were taken with a Zeiss Axiophot.

Conditional Expression Vectors. Construction of the IPTG-inducible episomal vector pEpiLac3 was described (21). The ≈2.7-kb *Spe*I–*Spe*I DNA fragment of the mouse *CTCF* cDNA clone p5.1 (5) was blunt-end ligated in the unique *Not*I site of the pEpiLac3. Before ligation, both vector and the insert were treated with T4 polymerase in the presence of dNTP to recess the ends. Two resulting constructs, called "pEpiLacCTCF*sense*" and "pEpiLacCTCF*anti*" containing CTCF cDNA in either orientation relative to the IPTG-inducible promoter, were identified by restriction enzyme mapping and verified by DNA sequencing (see the schematic outline of the sense construct shown in Fig. 2*A*). The sense and antisense CTCF constructs and the empty pEpiLac3 vector were transfected into the WEHI 231 cells by electroporation and then selected in Hygro by using the technique of Li and Lau (21). The Hygro-resistant cells were single-cell cloned and tested for expression of inducible CTCF by Western blotting and antisense CTCF by Northern blotting after the addition of 8 μM IPTG. Selected clones were then maintained in medium containing Hygro and G418.

Western Blots and Immunocytochemistry. Nuclear extracts were prepared from cells as detailed (19). Proteins (30 μg) were separated by SDS/PAGE, transferred to nitrocellulose membranes, and detected with the antibodies listed above. Bound antibodies were detected by an enhanced chemiluminescence system (Amersham Pharmacia).

Results

BCR Ligation Induces Sequential Activation of MYC, CTCF, and ARF. In keeping with previous studies, treatment of WEHI 231 cells with anti-IgM antibody resulted in a marked increase in MYC expression at 1 h followed by a dramatic fall to below control levels by 8 h (Fig. 1*A*). Analyses of the same samples for CTCF showed that levels were increased within 2 h after stimulation and increased progressively through 24 h. Levels of ARF, a transcriptional target for CTCF in the MCF-7 breast cancer cell line (C.-F.Q., V.V.L., and H.C.M., unpublished observations), were slightly increased at 2 h after induction and also increased through 24 h. Levels of β-actin, used as a loading control, were constant during this time course. These changes are consistent with a model in which enhanced expression of CTCF induced by BCR ligation contributes to down-regulation of MYC and induction of ARF.

To determine whether similar molecular events occur in normal B cells destined to respond to self-antigen by apoptosis, we studied cultures derived from IL-7-induced bone marrow B cells of 3-83 Ig transgenic mice, which have a BCR of known specificity for particular alleles of the mouse class I MHC (20). Bone marrow cells were cultured in IL-7 in the absence of antigen to permit generation of sIg$^+$ B cells. IL-7 was washed out, and the cells were treated with anti-Ig. Nuclear extracts prepared from cells at various times after BCR ligation were

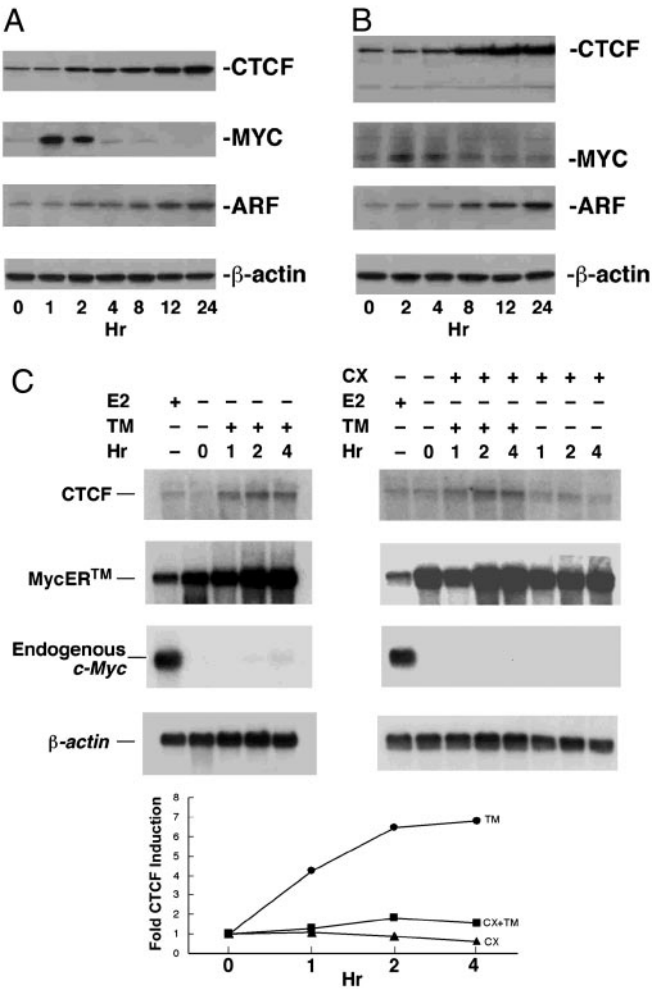

Fig. 1. Relations of BCR ligation to induction of CTCF. Immunoblot analyses of nuclear extracts of WEHI 231 (*A*) and 3-83 transgenic bone marrow-derived B cells (*B*) stimulated with anti-Ig and probed with antibody to MYC, CTCF, ARF, and β-actin. (*C*) Northern blot analyses of EREB.MycER cells after estrogen (E2) withdrawal and activation of MYCER by tamoxifin in the presence or absence of CX. (*Bottom*) Quantitation of CTCF expression in the presence of CX, CX plus TM, or TM alone is shown.

examined by immunoblotting for expression of CTCF, MYC, ARF, and β-actin as a loading control (Fig. 1*B*). As with WEHI 231, BCR ligation markedly but transiently stimulated high-level MYC expression with down-regulation coincident with heightened expression of CTCF followed by ARF (Fig. 1*B*).

CTCF Is a Transcriptional Target of MYC. Signaling pathways governing cell proliferation and differentiation are frequently characterized by negative feedback, with the ARF-MDM2-p53 axis being a prime example (8). Analyses of this circuitry have shown that genes such as MYC and E1A can induce ARF and p53, through unknown mechanisms, to trigger growth arrest and/or cell death (8). The observations that (*i*) the burst of MYC expression after BCR ligation is followed by expression of CTCF and (*ii*) CTCF activates ARF (C.-F.Q., V.V.L., and H.C.M., unpublished data) could be incorporated in this model by postulating that MYC activates CTCF.

To examine this possibility, we used the EREB.MycER B cell line (18) that permits independent control of cell proliferation and MYC expression. In these cells, proliferation is controlled by the EBV EBNA2 gene expressed as a chimeric fusion with the hormone-binding domain of the estrogen (E2) receptor. Estrogen removal leads to growth arrest associated with total down-regulation of endogenous c-MYC. In addition, these cells express an inactive c-MYC fused to a mutant estrogen receptor domain that can be rapidly activated by TM but not by estradiol. Northern blot analyses of mRNA from these cells after removal of estradiol and treatment with TM showed the expected marked increase in MycER transcripts associated with a rapid increase in CTCF transcripts detectable after 1 h (Fig. 1*C Left*). To determine whether c-MYC up-regulation of CTCF expression was direct, the same approach was used with cells treated simultaneously with the protein synthesis inhibitor CX. Induction of CTCF was significantly affected by cotreatment with CX (Fig. 1*C Right*, with quantitation at the bottom), consistent with an indirect effect of MYC activation on CTCF expression.

Conditional Expression of CTCF in WEHI 231 Induces Growth Arrest and Apoptosis. We considered it possible that CTCF might be the common switch for both growth arrest and death in immature B cells. If this were the case, induced expression of CTCF would mimic the effects of BCR ligation. To test this hypothesis, we generated IPTG-inducible vectors to express full-length CTCF in sense and antisense orientations (Fig. 2*A*) and introduced them to WEHI 231 cells transfected with the p3/SS vector carrying the *lacI* gene (15). Stable transfectants were cloned and selected for high expression of CTCF sense or antisense. A WEHI 231 clone containing the sense construct was cultured in the presence or absence of IPTG and compared with wild-type cells over 3 days for growth by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (Fig. 2*B*). Wild-type cells and transfected cells in the absence of IPTG showed comparable growth kinetics, whereas transfected cells cultured in the presence of IPTG showed a decrease in viable cells with time.

Analyses of IPTG-induced cultures by flow cytometry indicated that both cell-cycle arrest and apoptosis contributed to the absence of growth (data not shown). Microscopic evaluations of the cells cultured in IPTG also suggested that the cell loss might be due to apoptosis (not shown). To evaluate this possibility, WEHI 231 cultures with added IPTG were followed by using immunohistochemical staining specific for CTCF and the TUNEL assay as a marker for apoptotic cells (Fig. 2*C*). TUNEL-positive cells first appeared in substantial numbers between 6 and 12 h after initiation of treatment with IPTG, and by 24 h, >70% of cells were TUNEL positive. Conditional expression of CTCF in WEHI 231 thus resulted in induction of both growth arrest and apoptosis.

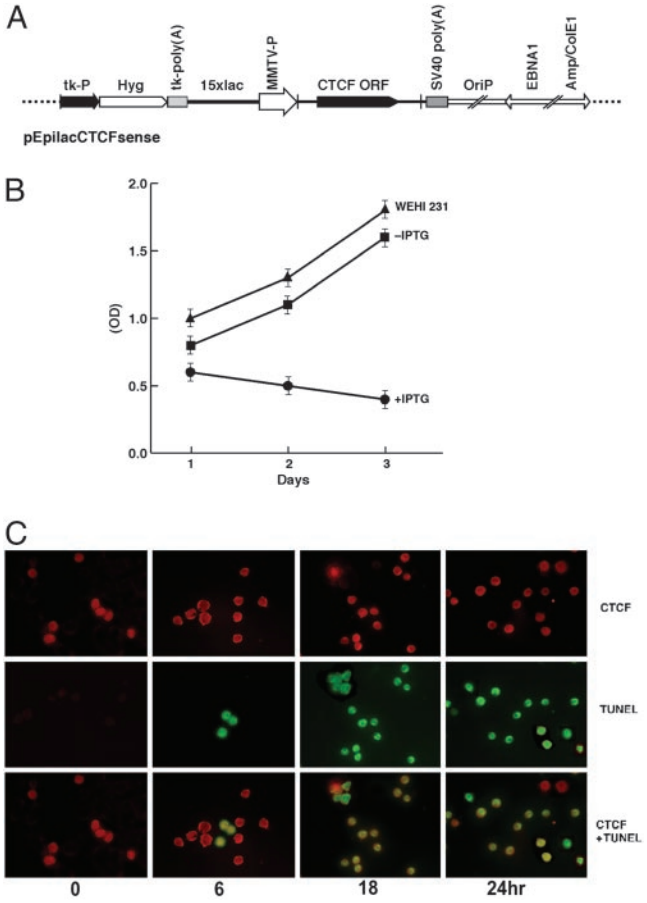

Fig. 2. Conditional expression of CTCF in WEHI 231 cells induces growth arrest and apoptosis. (*A*) Structure of IPTG-inducible CTCF sense constructs. The antisense construct contains the full CTCF cDNA in the opposite orientation. (*B*) 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide growth assays of normal WEHI 231 cells and WEHI 231 cells with IPTG-inducible CTCF sense in the presence (+IPTG) or absence (−IPTG) of the chemical. (*C*) Cells with the IPTG-inducible CTCF sense construct tested for expression of CTCF (green) and by TUNEL (red) and superimposed images (CTCF + TUNEL).

Regulation of MYC, p27, p21, p53, and ARF Proceeds in Inverse Directions in WEHI 231 Cells Expressing Conditional CTCF Sense and CTCF Antisense Constructs. The combined use of sense and antisense vectors in a single cell type provides a powerful means for evaluating signaling pathways determined by expression of a gene. To further understand the consequences of CTCF expression in immature cells of the B lymphocyte lineage, we therefore studied WEHI 231 cells containing IPTG-inducible constructs for expression of molecules deemed critical to life-and-death decisions in this cell line: MYC, p21, p27, p53, and ARF (Fig. 3).

Western blot analyses of cells induced to express CTCF-sense message showed efficient induction of CTCF along with increased levels of ARF, a finding consistent with the demonstration of ARF as a direct transcription target for CTCF, and decreased levels of MYC, the first known target for transcriptional repression by CTCF (5, 6, 22). The data document the expected downstream consequences of ARF activation, namely enhanced expression of p53 (23–25) and p21, a transcriptional target of p53 (26). It is noteworthy that levels of p27 were also increased, because previous studies indicated that the signals leading to apoptosis in WEHI 231 cells are dependent on both p21 and p27 (15). In addition, it has been reported that MYC represses transcription of p27 such that down-regulation of MYC would promote p27 expression (17).

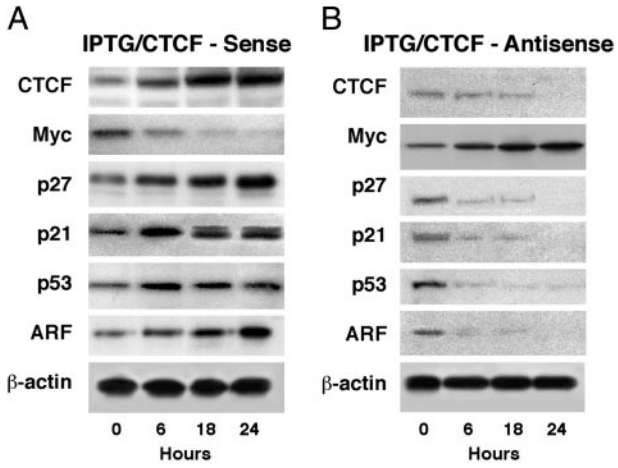

Fig. 3. Conditional expression of CTCFsense (*A*) and CTCFanti (*B*) in WEHI 231 induces reciprocal changes in expression of MYC, p27, p21, p53, and ARF. Cells were incubated with IPTG for the indicated times and tested for expression of the proteins by immunoblotting of nuclear extracts. The antisense vector contains the sense insert in the opposite orientation.

Studies of cells expressing IPTG-induced full-length CTCF antisense showed that CTCF protein levels were reduced by 6 h after the beginning of treatment and were barely detectable by 24 h. Predictably, levels of MYC increased over this time frame, whereas those of ARF, p53, and p21 decreased. The demonstration that p27 levels were decreased in cells expressing CTCF antisense, whereas levels were increased after induction of sense transcripts raised the possibility that p27 might be a transcriptional target for CTCF as well as for MYC.

Pathways Signaling Activation of CTCF. Recent studies demonstrated that p27 levels in WEHI 231 cells were increased after inhibition of phosphoinositide 3-kinase (PI3K) signaling by treatment with LY294002 or interference with mTOR/FRAP, a regulator of $p70^{S6K}$, by treatment with rapamycin (27). In light of our new data, these results could be interpreted to suggest that the function of these inhibitors was to induce activation of CTCF with secondary activation of p27. We tested this hypothesis by evaluating CTCF levels after treatment of normal WEHI 231 cells with these inhibitors (Fig. 4*A*). We also studied the effects of these agents on another cell line susceptible to cell death after ligation of the BCR, CH33 (28). Western blot analyses showed that treatment of both cell lines with either of these agents led to marked increases in CTCF levels (Fig. 4*A* and data not shown). This indicates that the PI3K and mTOR/FRAP pathways are involved in control of CTCF expression in immature B cells.

Others have shown that both WEHI 231 and CH33 die by apoptosis after treatment with TGF-β (29, 30). Studies of both cell lines treated with this agent (Fig. 4*A* and data not shown) revealed activation of CTCF at levels comparable to those seen with inhibition of PI3K or mTOR/FRAP signaling. Of interest, it has been shown that ligation of CD40 prevents apoptosis of WEHI 231 induced either by BCR ligation or treatment with TGF-β (30). To investigate the possibility that these antiapoptotic effects of CD40 ligation were mediated through control of CTCF expression, we examined cells treated with an anti-CD40 mAb during treatment with anti-Ig or TGF-β (Fig. 4*B*). CD40 signaling markedly suppressed the course of CTCF induced by either treatment. These data indicate that several signaling pathways may be involved in mediating clonal elimination of immature B cells through regulation of CTCF.

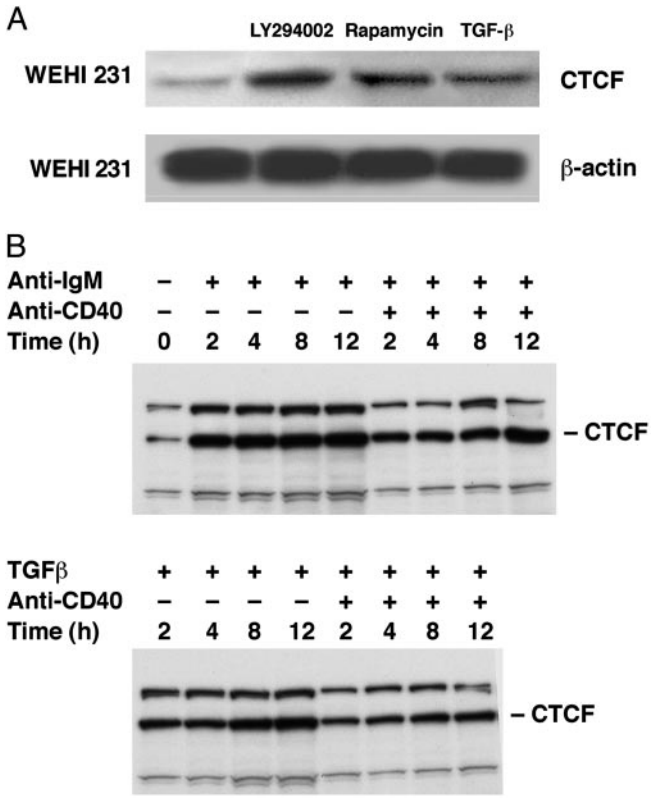

Fig. 4. Signaling pathways controlling CTCF expression. (*A*) WEHI 231 cells were treated with LY294002, rapamycin, or TGF-β for 24 h and assayed by immunoblotting for expression of CTCF in nuclear extracts. (*B*) WEHI 231 treated with anti-IgM or TGF-β with or without anti-CD40 for the indicated times were assayed by immunoblotting for expression of CTCF.

Discussion

The results of this study place CTCF at the center of a series of signaling pathways that orchestrate life-and-death decisions in immature B cells (Fig. 5). The critical finding is that expression of CTCF is markedly increased by any of a series of signals that induce apoptosis in WEHI 231-BCR ligation, inhibition of PI3K, inhibition of mTOR/FRAP, and treatment with TGF-β. In addition, CD40 ligation-induced rescue from apoptosis induced by BCR ligation or treatment with TGF-β greatly delays high-level expression of CTCF. The relevance of these findings for normative B cell biology is strongly suggested by the fact that BCR ligation of naïve, immature B cells from IL-7-stimulated bone marrow cultures also induced high levels of MYC followed by CTCF and ARF.

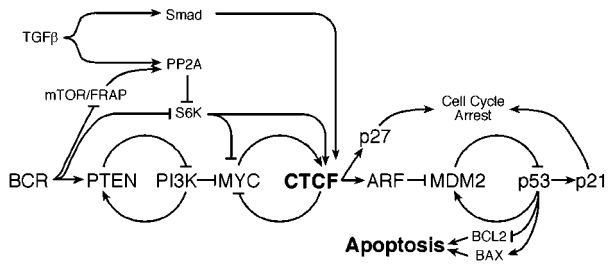

Fig. 5. Signaling pathways in immature B cells treated with anti-Ig to crosslink BCR or with TGF-β. Transcriptional activation of a gene is indicated by an underline of the name and an arrow to the right. MDM2 is transcriptionally activated by p53 but is inhibited at the protein level by interactions with ARF.

Previous studies demonstrated important contributions of the IκBα–NFκB signaling pathway in transcriptional up-regulation of MYC induced by BCR crosslinking or treatment of WEHI 231 with TGF-β (30, 31) (Fig. 5). Our work also revealed a critical link between BCR ligation, inhibition of the PI3K signaling pathways, and regulation of MYC and CTCF. This was revealed by the strong activation of CTCF seen after inhibition of PI3K with LY294002. Based on preliminary results from our laboratory and more definitive studies to be reported elsewhere, the mechanism responsible for inhibition of PI3K signaling after BCL ligation in WEHI 231 can be ascribed to activation of the inositol phosphatase, PTEN/MMC1 (reviewed in ref. 32; G. Cary, C.-F.Q., and D. Scott, unpublished results). The mechanisms involved in PTEN activation by BCR ligation are not known, and the manner in which reduced PI3K signaling contributes to alterations in CTCF and MYC expression remains to be determined.

Our analyses of CTCF expression in WEHI 231 and normal B cells after BCR ligation and in EREB.MycER cells treated with TM in the presence of CX suggested that CTCF might be a direct transcriptional target for MYC. The reduced induction in the presence of CX and negative results from chromatin immunoprecipitation analyses of two potential MYC targets in the CTCF proximal promoter (not shown) weigh against this notion. Nonetheless, CTCF is clearly rapidly activated after specific induction of MYC.

The mechanisms responsible for continued high-level expression of CTCF after MYC is down-regulated after BCR ligation are not known. Attractive possibilities suggested by our studies include effects emanating from down-regulation of PI3K or S6K function and downstream consequences of Smad activation. Regardless of the mechanism(s) involved, it is clear that persistent, high-level expression of CTCF in WEHI 231 successfully mimicked the many molecular and biologic phenotypes that result from BCR ligation: enhanced expression of ARF, p53, p21, and p27 and down-regulation of MYC, together with cell-cycle arrest and apoptosis. The observation that conditional expression of CTCF antisense generates a mirror image of this condition validates the designation of CTCF as a critical control element in the life-and-death decisions made by immature B cells. Directly testing this model by expressing CTCF antisense in cells undergoing BCR ligation is, unfortunately, not possible because this construct induces apoptosis on its own after 24 h (data not shown).

It is quite striking that activation of TGF-β signaling pathways in WEHI 231 also resulted in high-level CTCF expression. It has been shown that TGF-β-mediated inhibition of proliferation in many cell types is due to down-regulation of MYC and that this repression results from binding of a Smad complex to a TGF-β-inhibitory element in the *MYC* promoter (33). In contrast, TGF-β-driven, Smad-independent growth suppression can occur in some cells through the mTOR/FRAP-PP2A-S6K pathway, with TGF-β activation of PP2A resulting in inhibition of S6K activity (34). It remains to be determined whether one or both of these alternative pathways to growth inhibition are used in immature B cells. The fact that S6K inhibition results from treatment of WEHI 231 with rapamycin or TGF-β suggests this as a component of a common pathway for at least some signals to induce growth arrest in this B cell subset. One common effect is, more than likely, translational repression of MYC because the mTOR/FRAP pathway and phosphorylation of 4E-BP1 are crucial determinants of MYC translational activity (35). It must be remembered, however, that not all Smad functions are dependent on DNA binding and that many proteins interact with Smads to modify their activity. This could result in TGF-β signaling pathways that are distinct between B cell and other lineages as well as between different stages of B cell differentiation.

Together with previous data, these results suggest the initial "immediate-early" MYC response of immature B cells to BCR ligation is substantially like that in more mature cells, but in a contextual manner, this is rapidly overcome by enhanced expression and activity of PTEN, CTCF, and PP2A that act in concert to repress MYC (Fig. 5). As noted above, reduced PI3K and S6K signaling may act to directly enhance CTCF expression. CTCF then acts through ARF, a direct transcriptional target, to activate p53 and its downstream target, p21. Direct interaction of CTCF with the promoter of p27 results in transcriptional activation (C.-F.Q., unpublished observations), a function that would synergize with down-regulation of MYC, a transcriptional repressor of p27 (36). Coexpression of p27 and p21 induces apoptosis in WEHI 231 (15), completing the death cycle initiated by BCR ligation.

Alternative fates to death induced by crosslinking the BCR on immature B cells are receptor editing and anergy (37–40) as well as apoptosis. It would be of considerable interest to examine whether MCR stimuli that result in these alternative fates for immature B cells elicit quantitatively different levels of CTCF expression that contribute to developmental arrest but not death.

Depending on the range of B cell differentiation in which high-level CTCF can induce apoptosis, our results may have implications for treatment of B cell lineage lymphomas. Selective pharmacologic activation of CTCF in sensitive cells could provide a therapeutic strategy for treating a range of B cell neoplasms.

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