TGF-β Induces Growth Arrest in Burkitt Lymphoma Cells via Transcriptional Repression of E2F-1*

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Transforming growth factor-β (TGF-β) is a potent regulator of tissue homeostasis and can act as both a tumor suppressor and a tumor promoter. The ability to induce cell cycle arrest is a major component of the tumor suppressor function of TGF-β. Lung, mammary, and skin epithelial cells exhibit a common minimal cytostatic program in response to TGF-β signaling involving the repression of the growth-promoting factors c-MYC, Id1, Id2, and Id3. Loss of c-MYC expression is a pivotal event in this process, resulting in derepression of the cyclin-dependent kinase inhibitors CDKN1A (p21) and CDKN2B (p15) and ultimately leading to growth arrest. It is not clear, however, which responses are necessary for TGF-β-mediated growth arrest in other cell types. Here, in human Burkitt lymphoma cells transformed by deregulated c-MYC expression, we demonstrate that efficient TGF-β-induced cytostasis can occur despite both maintenance of c-MYC levels and a lack of p21 and p15 induction. TGF-β treatment also results in induction, rather than repression, of Id1 and Id2 expression. In this context, growth arrest correlates with transcriptional repression of E2F-1, and overexpression of E2F-1 in Burkitt lymphoma cells largely overcomes the TGF-β-mediated G1 arrest phenotype. These data indicate that deregulation of c-MYC in lymphoma cells does not overcome the tumor suppressor function of TGF-β and that repression of E2F-1 transcription is sufficient for the efficient induction of cytostasis.

TGF-β1 (hereafter TGF-β)2 is the prototypical member of a large superfamily of extracellular cytokines that signal through serine/threonine kinase receptors to intracellular Smad effectors. This family of cytokines affects a multitude of cellular processes including homeostasis, angiogenesis, migration, and differentiation. TGF-β has dual roles in cancer, acting as both a tumor suppressor and a tumor promoter in a context-dependent manner. The tumor promotion function of TGF-β only occurs following subversion of its normal tumor suppressor activity, which, in many cell types, involves regulating cell proliferation through the potent induction of cytostasis (1).

TGF-β-mediated cytostasis is induced, at least in part, by Smad-dependent regulation of TGF-β target genes involved in cell cycle control. Progression through the cell cycle from G1 to S phase is dependent on cyclin-dependent kinase (CDK) activity. CDK4 and CDK6 bind cyclin D, whereas CDK2 associates with cyclin E. These kinases phosphorylate members of the pRB family of proteins (pocket proteins) to regulate their ability to bind E2F transcription factors, and ultimately, to control the function of E2F proteins in gene activation and repression. Loss of pRB function confers resistance to TGF-β (2). The TGF-β-induced cytostatic transcriptional program shared by lung, mammary, and skin epithelial cells involves the induction of the CDK inhibitor genes p21Cip1/Waf1 and p15Ink4b. Previous studies have indicated that a lack of p15 will not, by itself, result in a defective cytostatic response to TGF-β, suggesting that p15 induction alone is not sufficient for growth arrest (3–5). However, TGF-β signaling also results in the transcriptional repression of the growth-promoting transcription factor genes c-myc, Id1, Id2, and Id3 (6), the key event in this cytostatic response being the transcriptional repression of c-myc. Artificially avoiding c-MYC down-regulation prevents both TGF-β-mediated p15 induction and TGF-β-mediated cell cycle arrest (7). In addition, keratinocytes can be rendered insensitive to the anti-proliferative effects of TGF-β by activating an estrogen-inducible form of c-MYC (mycER) (8), whereas the ability to repress c-MYC is selectively lost in transformed breast cancer cells (5). The mechanism of transcriptional repression of c-myc by TGF-β has been well characterized. Following TGF-β signaling, a preformed cytoplasmic complex comprising activated receptor-regulated Smad3, the pRB family co-repressor p107, and E2F-4 accumulates in the cell nucleus with Smad4. The Smad3, p107–E2F-4 complex is recruited to a transcriptional inhibitory element adjacent to E2F binding sites within the c-myc promoter (5, 9–11) and inhibits transcription.

Decreased c-MYC expression then directly impacts on transcription of the p21 and p15 CDK inhibitor genes because the promoters of both genes are repressed by c-MYC. Repression of p15 occurs by direct interaction of c-MYC with MIZ-1, thereby inhibiting MIZ-1-mediated activation of the p15 promoter (12, 13). In addition, c-MYC directly binds to Smad2 and Smad3, preventing their functional cooperation with Sp1 and further interfering with p15 transcription (14, 15). Thus, when c-MYC expression levels decrease following exposure of the cell to TGF-β, repression is relieved, and at the same time, MIZ-1 is free to interact with activated Smad-Sp1 complexes to fully activate p15 transcription. Similarly, TGF-β signaling activates p21 transcription via a diverse range of factors acting at the
**TGF-β-mediated Growth Arrest via Repression of E2F-1**

![Graph](image)

**FIGURE 1.** TGF-β induces efficient G₁ cell cycle arrest in cells transformed by deregulated c-MYC expression. A, CA46 Burkitt lymphoma cells were seeded at 3 × 10⁵/ml overnight. Cells were then either left untreated or treated with 1 ng/ml TGF-β for the times indicated, stained with propidium iodide, and analyzed by flow cytometry. The linear gates M1, M2, M3, and M4 measure the proportion of apoptotic (sub-G₁) cells and cells in the G₁, S, and G₂M phases of the cell cycle, respectively. The percentages of CA46 cells in each phase of the cell cycle of a representative experiment are shown, counted at the times indicated. B, cells were treated as in A, and cell counts were determined at the indicated time points. Results are expressed as the mean (± S.D.) of two independent experiments each performed in triplicate. C, proliferation assay measured by [³H]thymidine incorporation during a 48-h time course of TGF-β treatment of CA46 cells. Data represent the mean (± S.D.) cpm (n = 3).

Cytostatic program induced by TGF-β. G₁ arrest occurs in the absence of c-MYC, Id1, and Id2 repression, and interestingly (because loss of the p21 (18) or p15 response is often associated with resistance to TGF-β-induced growth arrest), without induction of either p21 or p15. Inhibition of progression through G₁-S phase by TGF-β correlated instead with transcriptional repression of the E2F-1 gene, whereas overexpression of E2F-1 largely overcame the ability of TGF-β to induce G₁ arrest in BL cells. Our data support the conclusion that inhibition of E2F-1 is minimally required for induction of cytostasis and suggests that TGF-β can still effectively exert tumor suppressor function in cells lacking c-MYC, p15, and p21 responses.

**EXPERIMENTAL PROCEDURES**

**Cell Lines, Reagents, and Treatments**—CA46 Burkitt lymphoma cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM glutamine, penicillin, and streptomycin (100 units/ml) at 37 °C in 10% CO₂. For TGF-β treatment time courses, cells were seeded at 3 × 10⁵/ml and cultured overnight prior to treatment with 1 ng/ml TGF-β1 (Peprotech). HaCaT epithelial cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin, and streptomycin (100 units/ml) at 37 °C in 10% CO₂. For TGF-β treatment, cells were seeded at 3 × 10⁵/ml overnight prior to treatment with 1 ng/ml TGF-β1 (Peprotech). HaCaT epithelial cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin, and streptomycin (100 units/ml) at 37 °C in 10% CO₂. For TGF-β treatment, cells were seeded at 3 × 10⁵/ml overnight prior to treatment with 1 ng/ml TGF-β1 (Peprotech). HaCaT epithelial cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin, and streptomycin (100 units/ml) at 37 °C in 10% CO₂. For TGF-β treatment, cells were seeded at 3 × 10⁵/ml overnight prior to treatment with 1 ng/ml TGF-β1 (Peprotech). HaCaT epithelial cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, penicillin, and streptomycin (100 units/ml) at 37 °C in 10% CO₂. For TGF-β treatment, cells were seeded at 3 × 10⁵/ml overnight prior to treatment with 1 ng/ml TGF-β1 (Peprotech). HaCaT epithelial cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10%

**Plasmids**—Promoter luciferase reporter constructs were generated by PCR of a region of human genomic DNA spanning the E2F-1 transcription start site from −1808 to +9 using high fidelity Platinum Pfx DNA polymerase (Invitrogen) and the following primers: forward, 5′-tgctgcatggatatgctcaatgg-3′, and reverse, 5′-aagctccgactccatttacct-3′. The product was cloned into pCR-Blunt (Invitrogen) and subcloned into pGL3-basic (Promega) using MluI/XhoI restriction enzyme digests. Constructs were sequenced prior to use. pcDNA3.1 and pcDNA3.1-E2F-1 were kindly provided by Professor Kevin Ryan, The Beatson Institute.
Transfection of BL Cells and Generation of Stable Cell Lines—

5 × 10⁵ CA46 cells were transfected in triplicate with 2 μg of reporter plasmid and 1 μg of pCMV-β-galactosidase using an Amaxa nucleofector as recommended by the manufacturer. Cells were incubated overnight before being re-fed with medium with or without TGF-β. Luciferase and β-galactosidase activity was assessed after 8 h as described previously (19). Stable cell pools were generated by transfection with 5 μg of DNA followed by selection with 500 μg/ml geneticin (Invitrogen).

[^3H]Thymidine Incorporation—Cells were seeded overnight at 3 × 10⁵/ml in 96-well plates and then cultured for up to 48 h with and without TGF-β. Cells were pulsed with[^3H]thymidine, and the amount incorporated into cell DNA was measured as described previously (20).

Immunoblotting and Antibodies—Radioimmune precipitation buffer lysates were analyzed by SDS-PAGE and Western blotting using rabbit polyclonal antibodies recognizing p15 (sc-612), p21 (sc-397), p57 (sc-1040), c-MYC (9E10) (sc-40), ID1 (sc-488), ID2 (sc-489), ID3 (sc-490), ATF-3 (sc-188), CDK2 (sc-163), CDK4 (sc-260), and pRB (sc-50) (Santa Cruz Biotechnology), rabbit polyclonal antibodies raised against phosphorylated-Smad2 (Ser-465/476), phosphorylated-Smad3 (Ser-433/435), and phosphorylated-S6 kinase (Cell Signaling), and mouse monoclonal antibodies raised against E2F-1 (Upstate Biotechnology), Smad2/3 (BD Biosciences), and actin (Sigma). Secondary antibodies were horseradish peroxidase-conjugated goat anti-rabbit Ig (Dako) and horseradish peroxidase-conjugated sheep anti-mouse Ig (Dako). Bound immunocomplexes were detected by enhanced chemiluminescence (ECL; Amersham Biosciences).

Cell Cycle Analysis by Flow Cytometry—Cells were fixed in 80% ethanol and labeled with propidium iodide. At least 10⁵ events were analyzed for DNA content using a flow cytometer (BD FACScan or FacsCalibur) and CellQuest software.

RNase Protection Assay (RPA)—Total cellular RNA was extracted using TRIzol. RPAs were carried out exactly as described previously (19) using the appropriate multiprobe template sets (BD Biosciences).

Real-time qRT-PCR—Total cell RNA was isolated using TRIzol as recommended by the manufacturer. cDNA and qRT-PCR reactions were prepared using SYBR green 2-step qRT-PCR kits (Finnzymes) and specific primers (Qiagen). Amplified products were analyzed by Chromo4 continuous fluorescence detector and Opticon Monitor3 software. Samples were amplified in triplicate, and the mean relative amount of specific RNA expressed after normalization to the amount of 18 S rRNA is shown unless stated otherwise.

RESULTS

TGF-β Induces Growth Arrest in CA46 BL Cells—BL cell lines can undergo G₁ growth arrest in response to TGF-β (16). BLs arise following translocation of the c-myc locus to the immunoglobulin heavy-chain locus (t(8;14)), resulting in deregulated c-MYC expression (17). Because BL cells overexpress c-MYC and the repression of c-MYC is a pivotal event in TGF-β-mediated cytostasis (directly affecting transcription of p15, p21, and Id2 in epithelial cells), we investigated how cells harboring a deregulated c-myc locus might respond to TGF-β treatment. CA46 BL cells were seeded overnight at 3 × 10⁵/ml, during which time nearly 80% of cells were synchronized in the G₁ phase of the cell cycle as shown by propidium iodide staining (Fig. 1A). Cells were then either left untreated or treated with TGF-β for 48 h. Control cells progressed from G₁ through the cell cycle, whereas TGF-β-treated cells exhibited an efficient G₁ cell cycle arrest (Fig. 1A). This was confirmed by a reduction in
Cell Cycle Arrest of BL Cells Is Independent of c-MYC and CDK Inhibitor Regulation—To establish whether TGF-β-mediated growth arrest in BL cells occurs via induction of the minimal epithelial cytostatic program, we compared expression of c-MYC, p15, and p21 following TGF-β treatment of CA46 BL and HaCaT epithelial cells (Fig. 2A). As expected, rapid TGF-β-dependent repression of c-MYC and induction of p15 and p21 was observed in HaCaT cells. However, these changes were not observed in CA46 BL cells. High c-MYC expression levels were maintained throughout, and p15 and p21 were undetectable despite TGF-β treatment readily stimulating phosphorylation of Smad2 in these cells (Fig. 2A). In addition to the well characterized effects on p15 and p21, TGF-β can induce other CDK inhibitors (for example, p27 in CH31 and WEHI231 murine B-cells (21) and p57 in hematopoietic stem cells (22)). We therefore analyzed expression of the different CDK inhibitors in both CA46 and HaCaT cells using a multiprobe RPA (Fig. 2B). We readily detected the TGF-β-mediated induction of p21 and p15 RNA in HaCaT cells, but none of the CDK inhibitors assayed were induced in CA46 cells. TGF-β-induced growth arrest in CA46 BL cells therefore is independent of induction of the previously described TGF-β-responsive CDK inhibitors p27, p57, p21, and p15.

TGF-β Induces Id1 and Id2 in CA46 Cells—TGF-β-mediated cytostasis in epithelial cells is also associated with repression of the growth-promoting transcription factors Id1, Id2, and Id3. Sustained repression of Id2 occurs following reduced c-MYC expression and an increase in Mad4 levels, resulting in the replacement of activating MYC-MAX heterodimers with repressive MAD-MAX complexes on the Id2 promoter (23). Id1 repression meanwhile is mediated by Smad3-dependent activation of ATF3, which is then recruited by Smad3 to the Id1 promoter (24). However, during TGF-β-induced growth arrest of CA46 BL cells, the expression of both Id1 and Id2 proteins increased, peaking at 4 h (Fig. 2C), whereas Id3 was not expressed in CA46 cells (data not shown). The differences in Id protein regulation between the two cell types may be explained not only by the difference in c-MYC response but also by the lack of induction of the Smad co-repressor ATF3 in CA46 cells (Fig. 2D). Taken together, our findings so far indicate that the induction of cell cycle arrest in CA46 cells is entirely independent of the minimal cytostatic program associated with TGF-β-induced arrest of epithelial cells.

TGF-β-mediated Cell Cycle Arrest of BL Cells Correlates with Loss of E2F-1 Expression—To determine the likely mechanism of cell cycle arrest in CA46 cells, we analyzed the effect of TGF-β on cell cycle-related proteins (Fig. 3). At 0 h, most cells are synchronized in G1 (as described in Fig. 1A). Progression through the cell cycle during the subsequent 48-h incubation was associated with the cyclin/cdk-dependent phosphorylation of the E2F transcriptional co-repressor pRb (Fig. 3). As expected, in the presence of TGF-β, pRb, was maintained in its active, hypo-phosphorylated form (Fig. 3). We observed very little effect on basal expression of the cyclin-dependent kinases 2 and 4 and no effect on the phosphorylation state of p70 S6 kinase (a substrate of the phosphatase protein phosphatase 2A and a component of a post-transcriptional mediated G1 arrest pathway activated by TGF-β in mammary epithelial cells (26)). Expression of the transcription factor E2F-1, however, was dramatically decreased within 4–8 h of TGF-β treatment.

TGF-β Induces Transcriptional Repression of E2F-1 in BL Cells—We next investigated the mechanism of TGF-β-mediated inhibition of E2F-1 expression. Using a multiprobe RPA assay, we found that TGF-β treatment resulted in the down-regulation of E2F-1 RNA levels followed by a decrease in the RNA levels of the E2F DNA binding partners DP-1 and DP-2 and the family member E2F-4 (Fig. 4A). We confirmed this decrease in E2F-1 RNA levels by quantitative RT-PCR (Fig. 4B). Treatment of the cells with the protein synthesis inhibitors cycloheximide and anisomycin blocked the TGF-β-mediated inhibition of E2F-1 mRNA expression, suggesting either that the mechanism of E2F-1 regulation requires induction of a TGF-β-responsive transcriptional repressor or that continued synthesis of a labile factor is required for efficient repression of the E2F-1 gene (Fig. 4B). The effects of TGF-β and actinomycin D (an inhibitor of transcription) on E2F-1 RNA levels were comparable (Fig. 4C), suggesting that TGF-β inhibits transcription of the E2F-1 gene. To confirm this, we cloned a region of the E2F-1 promoter spanning −1808 to +9 relative to the gene transcription start site into a luciferase reporter construct (a schematic diagram is shown in Fig. 4D) and assayed its responsiveness to TGF-β signaling following transfection into CA46 cells. Transcription driven by this region of the E2F-1 promoter was repressed to background levels when cells were treated with TGF-β for 8 h (Fig. 4E).
Re-expression of E2F-1 Rescues BL Cells from TGF-β-induced G₁ Arrest—To determine whether transcriptional regulation of E2F-1 is sufficient for TGF-β-mediated growth arrest in cells with deregulated c-MYC expression, we constitutively re-expressed E2F-1 in CA46 cells. The TGF-β responsiveness of stable cell pools containing empty vector (CA46-pcDNA) or an E2F-1 expression vector (CA46-E2F-1) were compared. E2F-1 was overexpressed in cells transfected with the expression vector when compared with control cell pools and, importantly, both cell pools were equally responsive to TGF-β as evidenced by equivalent levels of repression of E2F-1 RNA (Fig. 5A) and of Smad2 and Smad3 phosphorylation (Fig. 5B). The high levels of E2F-1 expression were maintained even after treatment with 1 ng/ml TGF-β for 24 h (Fig. 5, A and B).

Elevated expression of cell-cycle-stimulating oncogenes (such as E2F-1) can be associated with increased apoptosis through an E2F-1-p14ARF-p53 pathway, by induction of the p53-related homologue p73, or by increasing transcription of BH3-only proapoptotic proteins such as PUMA and NOXA (reviewed in Ref. 27). We saw no increase in apoptosis in CA46 cells stably expressing E2F-1 (Fig. 5E). It is possible that the mutant p53 (28) expressed in CA46 cells and a lack of BAX/BAK (29) expression allows successful E2F-1 overexpression in this cell background. In fact, at low cell-seeding densities (≤1.2 × 10⁵/ml), E2F-1-overexpressing cells had a proliferative advantage over control cells (data not shown). Enforced expression of E2F-1 efficiently reduced TGF-β-mediated growth arrest measured in cell-counting experiments (Fig. 5, C and D), and this effect was apparent irrespective of cell-seeding density. The reduction in the cytostatic response correlated with a reduction in the ability of TGF-β to induce G₁ arrest shown in representative histograms of propidium iodide-stained cells analyzed by flow cytometry (Fig. 5E) and as the average increase in cells undergoing a G₁ arrest in response to TGF-β (Fig. 5F). Overall our data indicate that TGF-β-mediated growth arrest in human B lymphoma cells is independent of c-MYC regulation and is mediated predominantly by the repression of E2F-1 transcription.

DISCUSSION

The key event in TGF-β-mediated cytostatic responses of epithelial cells is the transcriptional regulation of c-myc and its target genes. In the context of deregulated c-MYC expression, however, for example, in BL cells transformed by t(8;14) translocation of the c-myc gene, it was unknown whether TGF-β cell cycle arrest could occur by a similar mechanism. Here we provide evidence that efficient TGF-β-mediated growth arrest can occur in human lymphoma cells despite a lack of the core cytostatic program events including loss of c-MYC and Id transcription factors and without induction of CDK inhibitors.

The absence of any substantive effect on c-MYC, p15, p21, and p27 expression following TGF-β stimulation is more usually associated with increased proliferation (30) rather than with the antiproliferative response shown here in BL cells. In the human breast cancer cell line MCF-10A (deficient in the p15INK4b locus), transformation with Ras-ErB2 induces resistance to TGF-β cytostasis by virtue of an inability to down-regulate c-MYC. In these cells, therefore, the combination of a defective c-MYC response and lack of p15 is enough to render the cells completely insensitive to TGF-β-induced cytostasis (5). However, we have demonstrated that these same defects in the cytostatic program are in no way detrimental to the ability...
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The observed differences may be cell type-specific, although not confined to epithelial versus hematopoietic cells because c-MYC down-regulation may not be a prerequisite for TGF-\(\beta\)-mediated growth arrest in canine thyroid epithelial cells (31).

TGF-\(\beta\) has been observed to repress expression of the Id transcription factors in epithelial cells (32). In contrast, we found that TGF-\(\beta\) treatment of CA46 BL cells readily induced expression of Id1 and Id2, indicating that this may be an example of cell type differences in the TGF-\(\beta\) response. Consistent with our findings, others have also observed Id proteins to be induced by TGF-\(\beta\) family members. During development, Id proteins are essential for lymphocyte development and function (33). TGF-\(\beta\) treatment of primary murine lymphocyte progenitors results in induction of Id3, causing growth arrest and apoptosis of B220+ve B cell progenitors (34, 35), whereas TGF-\(\beta\) induction of Id2 has a role in negatively regulating IgE class switching in mature B cells (36). Interestingly BMP-6 also induces Id1 Ramos BL cells (37) and induces both Id1 and Id3 in human bone marrow lymphocyte progenitors and the pre-B-ALL cell line Nalm-6 (38). It has yet to be determined whether induction of Id proteins shown here has a functional role in TGF-\(\beta\)-induced growth arrest in human BL cells.

In our study, TGF-\(\beta\)-mediated growth arrest in BL cells correlated with transcriptional repression of E2F-1. Loss of E2F-1 RNA expression has been reported in mink lung (2) and HaCaT epithelial cells (39) after 24 and 12 h of TGF-\(\beta\) signaling, respectively. We now show that in BL cells, TGF-\(\beta\) signaling affects E2F-1 transcription rapidly (within 4 h) and requires de novo protein synthesis. We also demonstrate for the first time the TGF-\(\beta\)-dependent repression of E2F-1 promoter activity using luciferase reporter constructs containing −2 kb of the wild type E2F-1 promoter. Transcrip-
tional autoregulation of E2F-1 occurs through two E2F binding sites near the transcription start (shown in Fig. 4D). The same sites also mediate E2F-1 repression in G0/G1 phase of the cell cycle through binding of repressor E2F complexes made up of E2F-4 and E2F-5 bound to pocket proteins p107, p130, and perhaps pRb (40). TGF-β can induce these repressor E2F complexes in HaCaT cells (39, 41), which, in concert with histone deacetylases, repress E2F target genes such as cdc25a (41). It is possible that a similar mechanism exists in BL cells to regulate E2F-1 transcription; however, analysis of Smad binding elements (5'-GTCT-3' or its reverse complement 5'-AGAC-3') (42), within the −1808 to +9 E2F-1 promoter region assayed, also reveals at least 14 potential Smad binding elements.3 It is therefore possible that activated Smads are recruited to the E2F-1 promoter along with E2F repressor complexes (as in the case of c-myc repression). We are currently investigating the mechanism of E2F-1 repression in BL cells.

We have shown here that TGF-β-mediated repression of E2F-1 in lymphoma cells transformed by c-MYC deregulation induces cell cycle arrest; however, it is also possible that the loss of E2F-1 expression may affect tumor cell survival. Blocking E2F-1 function in osteosarcoma cells overexpressing wild type c-MYC induces apoptosis, which is caspase-dependent but independent of the ability of deregulated c-MYC to activate the ARF/p53 response to oncogenic stress (43). In this situation, E2F-1-induced expression of the prosurvival factor BCL-2 may account for the requirement for E2F activity concomitant with Myc overexpression because BCL-2 can counteract the function of the proapoptotic BH3-only protein BIM induced by c-MYC. In some Burkitt lymphomas, however, the situation is complicated by selection of c-MYC mutants, which cannot induce BIM expression and consequently have diminished apoptotic potential (44). Whether BL cell lines rely on E2F-1 as a survival factor and whether TGF-β-induced apoptosis in this cell type involves E2F-1 repression therefore requires further investigation.

Because re-expression of E2F-1 in CA46 cells largely overcomes the effect of TGF-β in BL cells, we conclude that down-regulation of E2F-1 is the predominant mechanism by which TGF-β can induce cell cycle arrest in the absence of c-MYC regulation. It remains to be determined whether TGF-β can directly repress expression of E2F-1 in other cell types and whether this contributes to the cytostatic program. Our data suggest that in human B cells, deregulation of the c-MYC expression is not sufficient to evade the tumor suppressor function of TGF-β. It is possible that some lymphomas may escape from the potent tumor suppressor functions of TGF-β by deregulation of the E2F-1 pathway, and we are currently investigating this hypothesis.

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3 L. C. Spender, unpublished observations.

REFERENCES

1. Pardali, K., and Moustakas, A. (2007) Biochim. Biophys. Acta 1775, 21–62
2. Schwarz, J. K., Bassing, C. H., Kovesdi, I., Datto, M. B., Blazing, M., George, S., Wang, X. F., and Nevins, J. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 485–487
3. Javaran, A., and Massague, J. (1997) Nature 387, 417–422
4. Latres, E., Malumbres, M., Sotillo, R., Martin, J., Ortega, S., Martin-Caballero, J., Flores, J. M., Cordón-Cardo, C., and Barbacid, M. (2000) EMBO J. 19, 3496–3506
5. Chen, C. R., Kang, Y. B., and Massague, J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 992–999
6. Siegel, P. M., and Massague, J. (2003) Nat. Rev. Cancer 3, 807–821
7. Warner, B. J., Blain, S. W., Seoane, J., and Massague, J. (1999) Mol. Cell. Biol. 19, 5913–5922
8. Alexandrow, M. G., Kawabata, M., Aakre, M., and Moses, H. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3239–3243
9. Chen, C. R., Kang, Y., Siegel, P. M., and Massague, J. (2002) Cell 110, 19–32
10. Yagi, K., Fujishashi, M., Aoki, H., Goto, D., Kuwano, H., Sugamura, K., Miyazono, K., and Kato, M. (2002) J. Biol. Chem. 277, 854–861
11. Frederick, J. P., Liberati, N. T., Waddell, D. S., Shi, Y., and Wang, X. F. (2004) Mol. Cell. Biol. 24, 2546–2559
12. Seoane, J., Pouponnot, C., Staller, P., Schader, M., Eilers, M., and Massague, J. (2001) Nat. Cell Biol. 3, 400–408
13. Staller, P., Peukert, K., Kierra, M., Seoane, J., Lukas, J., Karsunky, H., Morony, T., Bartek, J., Massague, J., Hanel, F., and Eilers, M. (2001) Nat. Cell Biol. 3, 392–399
14. Feng, X. H., Lin, X., and Derynck, R. (2000) EMBO J. 19, 5178–5189
15. Feng, X. H., Liang, Y. Y., Liang, M., Zhai, W., and Lin, X. (2002) Mol. Cell. Biol. 9, 133–143
16. Inman, G. J., and Allday, M. J. (2000) J. Gen. Virol 81, 1567–1578
17. Madisen, L., and Groudine, M. (1994) Genes Dev. 8, 2212–2226
18. Nicolas, F. J., and Hill, C. S. (2005) Oncogene 22, 3698–3711
19. Spender, L. C., Whiteman, H. J., Karstegl, C. E., and Farrell, P. J. (2005) Oncogene 24, 1873–1881
20. Spender, L. C., Cannell, E. J., Hollyoake, M., Wensing, B., Gawn, J. M., Brimmell, M., Packham, G., and Farrell, P. J. (1999) J. Virol. 73, 4678–4688
21. Kamesaki, H., Nishizawa, K., Michaud, G. Y., Cossman, J., and Kiyono, T. (1998) J. Immunol. 160, 770–777
22. Scardura, J. M., Boccuni, P., Massague, J., and Nimer, S. D. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 15231–15236
23. Siegel, P. M., Shu, W., and Massague, J. (2003) J. Biol. Chem. 278, 35444–35450
24. Kang, Y., Chen, C. R., and Massague, J. (2003) Mol. Cell 11, 915–926
25. Laiho, M., DeCaprio, J. A., Ludlow, J. W., Livingston, D. M., and Massague, J. (1990) Cell 62, 175–185
26. Petritsch, C., Beug, H., Balmain, A., and Oft, M. (2000) Genes Dev. 14, 3093–3101
27. Qin, J., Rees, J. A. (2007) Curr. Opin. Cell Biol. 19, 649–657
28. Cherney, B. W., Bhatia, K. G., Sgadari, C., Gutierrez, M. I., Mostowski, H., Pike, S. E., Gupta, G., Magrath, I. T., and Tosato, G. (1997) Cancer Res. 57, 2508–2515
29. Doucet, J. P., Hussain, A., Al-Rasheed, M., Gaidano, G., Gutierrez, M. I., Magrath, I. T., and Bhatia, K. (2004) Leuk. Lymphoma 45, 357–362
30. Khalil, N. (1999) Microbes Infect. 1, 1255–1263
31. Depoortere, F., Pirson, I., Bartek, J., Dumont, J. E., and Roger, P. P. (2000) Mol. Biol. Cell 11, 1061–1076
32. Rotzer, D., Krampert, M., Sulyok, S., Braun, S., Stark, H. J., Boukamp, P., and Werner, S. (2006) Oncogene 25, 2070–2081
33. Engel, I., and Murre, C. (2001) Nat. Rev. Immunol. 1, 193–199
34. Kee, B. L. (2005) J. Immunol. 175, 4518–4527
35. Kee, B. L., Rivera, R. R., and Murre, C. (2001) Nat. Immunol. 2, 242–247
36. Sugai, M., Gonda, H., Kusunoki, T., Katakai, T., Yokota, Y., and Shimizu, A. (2003) Nat. Immunol. 4, 25–30
37. Kersten, C., Sivertsen, E. A., Hystad, M. E., Forfang, L., Smeland, E. B., and Myklebust, J. H. (2005) BMC Immunol. 6, 9
38. Kersten, C., Dosen, G., Myklebust, J. H., Sivertsen, E. A., Hystad, M. E., Smeland, E. B., and Rian, E. (2006) Exp. Hematol. 34, 72–81
39. Li, J. M., Hu, P. P., Shen, X., Yu, Y., and Wang, X. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4948–4953
40. Trimarchi, J. M., and Lees, J. A. (2002) Nat. Rev. Mol. Cell. Biol. 3, 11–20
41. Iavarone, A., and Massague, J. (1999) Mol. Cell. Biol. 19, 916–922
42. Yang, Y. C., Piek, E., Zavadil, J., Liang, D., Xie, D., Heyer, J., Pavlidis, P., Kucherlapati, R., Roberts, A. B., and Bottinger, E. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10269–10274
43. Santoni-Rugiu, E., Duro, D., Farkas, T., Mathiasen, I. S., Jaattela, M., Bartek, J., and Lukas, J. (2002) Oncogene 21, 6498–6509
44. Hemann, M. T., Bric, A., Teruya-Feldstein, J., Herbst, A., Nilsson, J. A., Cordon-Cardo, C., Cleveland, J. L., Tansey, W. P., and Lowe, S. W. (2005) Nature 436, 807–811

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