Functional identification of LRF as an oncogene that bypasses RAS\(^{V12}\)-induced senescence via upregulation of CYCLIN E

Liesbeth C.W. Vredeveld\(^1\), Benjamin D. Rowland\(^1,3\), Sirithe Douma\(^1\), René Berhards\(^2\) and Daniel S. Peep\(^{1,*}\)

\(^1\)Division of Molecular Genetics and \(^2\)Division of Molecular Carcinogenesis, Center for Biomedical Genetics and Cancer Genomics Center, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

\(^3\)Present address: Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

"To whom correspondence should be addressed. Tel: +31 20 512 2002; Fax: +31 20 512 2011; Email: d.peep@uki.nl

Mutant RAS (RAS\(^{V12}\)) is known to transform most immortal cells but to induce premature senescence in primary cells. RAS\(^{V12}\)-induced senescence in murine cells depends on the induction of the ARF/p53 and the retinoblastoma (Rb) family tumor suppressor pathways. We and others have shown previously that oncogene-induced senescence in vitro can be used as a tool to identify new cancer-related genes. In addition, we have shown that oncogene-induced senescence corresponds to an in vivo tumor suppressive mechanism. Therefore, we extended our search for novel genes that bypass of RAS\(^{V12}\)-induced senescence, with the help of a previously designed unbiased functional screen with cDNA expression libraries. In this screen, we expected to find new mediators feeding into the p53 or Rb pathways or novel signaling factors. We report here the identification of leukemia/lymphoma related factor (Lrf) encoding a transcription and that it synergizes with RAS\(^{V12}\) in activating E2F. Indeed, LRF-mediated bypass of RAS\(^{V12}\)-induced senescence is accomplished by the induction of several E2F-target genes, including Cyclin E, Cyclin A and p107. Unexpectedly, LRF exerted this activity independent of several critical senescence inducers, such as p19\(^{ARF}\), p21\(^{CIP}\) and p16\(^{INK4A}\). We show that CYCLIN E is necessary for LRF-mediated bypass, suggesting that it corresponds to a critical mediator of LRF-driven oncogenic transformation. Thus, LRF bypasses RAS\(^{V12}\), induced senescence in a CYCLIN E-dependent manner, which conceivably contributes to its role in cancer.

Introduction

It is almost half a century ago that Hayflick showed that primary cells have a limited in vitro lifetime (1). It took several decades for researchers to uncover the underlying mechanism. Prolonged culturing of primary cells results in the emergence of critically short telomeres, thereby activating tumor suppressor networks (reviewed in ref. 2). This ultimately leads to replicative senescence, a state in which cells remain metabolically active yet are growth arrested. Similarly, unfavorable tissue culture conditions can induce the p53 and retinoblastoma (Rb) tumor suppressor pathways, leading to cell cycle arrest with several hallmarks of replicative senescence (reviewed in ref. 3). In addition, senescence can be induced prematurely in primary cells by overexpression of activated oncoproteins like HRAS\(^{V12}\) or BRAF\(^{E600}\). Also in this setting, the p53 and Rb pathways are involved in the induction of the proliferative arrest, now called oncogene-induced senescence (4).

Abbreviations: cDNA, complementary DNA; LRF, leukemia/lymphoma related factor; Rb, retinoblastoma; wt, wild-type; wt MEFS, wild-type mouse embryonic fibroblasts.

It is conceivable that the tumor suppressor p53 and Rb owe, at least in part, their important role in cancer suppression by acting as critical mediators of (oncogene induced) senescence.

We and others have recently found evidence indicating that oncogene-induced senescence is not only an in vitro phenomenon but acts as a genuine in vivo tumor suppressive mechanism ([5–9]; reviewed in ref. 10]). It is for this reason that in vitro senescence bypass screens have been designed to identify new oncoproteins and tumor suppressor genes (11,12). Here, we performed an unbiased screen with high-complexity retroviral complementary DNA (cDNA) expression libraries and report on the identification of Lf as a gene that abrogates RAS\(^{V12}\)-induced senescence.

Materials and methods

Cell culture experiments

BTR cells, temperature-sensitive Large T expressing MEFS (tsT MEFS) and wild-type mouse embryonic fibroblasts (wt MEFS) were maintained in Dulbecco’s modified Eagle’s medium (Gibco, Zwillndrecht, the Netherlands) supplemented with 10% fetal bovine serum (PAA Laboratories, Cölbe, Germany), 2 mM glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin (all Gibco) and 0.1 mM ß-mercapto-ethanol. NIH 3T3 cells were maintained in Dulbecco’s modified Eagle’s medium with 10% neonatal bovine serum (Invitrogen, Breda, the Netherlands), 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin (all Gibco). Phoenix packaging cells (http://www.stanford.edu/group/nolan/retroviral_systems/phx.html) were used to generate ectropic retroviruses to transduce cells, which were subsequently used to perform colony formations assays and growth curves [all as described previously in (11)].

Retroviral cDNA screen

The retroviral cDNA screen was performed as described previously (11) but with cDNA libraries in the pEYK3.1 vector (13). The libraries were prepared from human LMJ216 primary fibroblasts, human MCF-7 breast cancer cells and mouse embryo (day 14) cells. To discriminate between spontaneous colonies and colonies rescued by retroviral encoded by cDNA, we used the pEYK3.1 vector ([13]; Figure 1A) to shuttle the primary clones to E.coli. Subsequently, pEYK3.1 proviral DNA was isolated from multiple bacterial colonies (representing proviral sequences from single primary BTR cell clones) and transfected into Phoenix packaging cells to generate retrovirus. Fresh BTR cells were then transduced with these pools of retroviruses to perform the second round of screening. The polyclonal pool of DNA from the shuffled proviral inserts that appeared positive in the second round was analyzed by digestion and sequenced. Finally, single inserts were individually expressed by retroviral transduction of BTR cells in the third round of screening.

Assays

CYCLIN E-dependent kinase activity assay was performed as in (14), using the M20 (Santa CruzBiotechnology, Heidelberg, Germany) antibody for CYCLIN E immunoprecipitation and Histone H1 (Roche, Almere, the Netherlands) as a substrate. For the luciferase reporter assays, NIH 3T3 cells were electroporated with 2 µg of luciferase reporter driven by an artificial promoter containing six consensus E2F sites in front of the TATA box [6xE2F; (15)] or by the human CYCLIN E promoter [2.2 kb DNA fragment 5’ of the CYCLIN E gene with or without mutant E2F sites; (16)], as well as increasing amounts (0.5–2 µg) of leukemia/lymphoma related factor (LRF)-expressing plasmid, 0.1 µg of RAS\(^{V12}\)-expressing plasmid and 0.025 µg of E2F1 and DP1-expressing plasmid. TK-Renilla luciferase was co-introduced as an internal control. After 48 h, the luciferase was measured with the Dual Luciferase Reporter Assay System (Promega, Leiden, the Netherlands).

Plasmids

The full-length cDNA of mouse Lf/Zbtb7a was recloned from pEYK3.1 (pE) in pBABE (pB) and pC DNA 3.1 expression vectors via EcoRI digestion sites. Sense short hairpin RNA sequences for interference constructs for mouse Cyclin E1:
sh-CE#1 5'-TTGATGATGATGATGAAGGCCC-3', sh-CE#2 5'-GAGGTTTGGAGGATCATGT-3', and mouse p19ARF:5'-GTTCGTGCGATCCCGGAGA-3' were cloned into pRS as has previously been described for pRS-mp53 (17).

Antibodies
For western blotting, the following antibodies were used from Santa Cruz Biotechnology: M-156 for p16 INK4A, C-19 for p21 CIP1, C-22 for CDK4, H-295 for CYCLIN D1, M20 for CYCLIN D2, M-20 and C-19 (human specific) for CYCLIN E1, C-18 for p107 and C-19 for CYCLIN A; Calbiochem (Beeston Nottingham, UK): Ab-7 for p53; Abcam (Cambridge, UK): R-562 for p19ARF and Sigma-Aldrich Chemie (Zwijndrecht, the Netherlands): AC-74 for β-actin.

Quantitative reverse transcription–polymerase chain reaction
Reverse transcription was performed on total RNA with Superscript II first strand kit (Invitrogen). Quantitative reverse transcription-polymerase chain reaction was performed with the SYBR Green PCR Master Mix (Applied Biosystems, Nieuwekerk a/d IJssel, the Netherlands) on an ABI PRISM 7700 Sequence Detection System. All primer pairs span exon–exon barriers. Control primers were HPRT. Primer sequences: Rb: 5'-ATCTACCTCCCTTGCCCTGT-3' and 5'-GAAGGCGTGCACAGAGTGTA-3' and HPRT: 5'-CTGGTGAAAAGGACTCTCG-3' and 5'-TGAAGTACTCATTATAGTCAAGGGCA-3'.

Results
A functional genetic screen for genes that bypass RASV12-induced senescence
A functional RASV12-induced senescence bypass screen was performed, as described previously (11), in the BTR cell system. Briefly, these cells, BALBc MEFs expressing a temperature-sensitive SV40 Large T (tsT) mutant and RASV12, allow for a rapid and tight induction of premature senescence at the non-permissive temperature (39.5°C), which is associated with a low background of spontaneous ‘escapees’ (11,18). In this cell system, disruption of either the p53 pathway (Figure 1C) or the Rb pathway (11) results in abrogation of oncogene-induced senescence. This is consistent with previous observations demonstrating that p53−/−, p19ARF−/− or Rb−/−/p107−/− MEFs fail to undergo RASV12-induced senescence (4,19,20). For the library, we used the pEYK3.1 vector system which allows for second round screening that requires neither wild-type (wt) murine Moloney leukemia virus to mobilize the proviral insert (11), nor subsequent PCR steps [see (13); and also Materials and Methods and Figure 1A for details].

We introduced by retroviral transduction three high-complexity retroviral cDNA expression libraries into BTR cells. After shifting the cells to the non-permissive temperature, most cells senesced. Indeed, in agreement with our previous observations (11,18), control-transduced BTR cells gave rise to only very few colonies. Amidst these senescent cells, ~60 colonies emerged 2 weeks after the temperature shift. The library-transduced colonies were picked, expanded and the colonies that appeared first were used to perform a second round of screening to discriminate true from false positives. For this, we used a system [pEYK3.1; Figure 1A; (13)], which allows for efficient shuttling of the proviral DNA. Thus, we digested the genomic DNA of the BTR cell clones, performed a religation step and transformed the recombinant shuttle vectors to E.coli. The genomic DNA was digested with either the NotI or Ascl ‘eight-cutters’ to reduce the chance that biologically active inserts would be lost during shuttling. After shuttling, bacterial colonies were pooled and plasmid DNA isolated. Subsequently, this DNA was transfected into Phoenix packaging cells to generate virus for a round of transduction of fresh BTR cells (Figure 1B).
Identification of LRF

Upon shuttling of the provirus from several primary clones, only DNA isolated from clone 23A(AscI) appeared positive in the second round of screening (Figure 2A). Importantly, this result indicated that the biological activity observed in the primary clone was transferable, rather than being caused by an endogenous mutation in the BTR cells. The 23A(AscI) polyclonal bacterial DNA pool contained three different cellular cDNAs, that is, Keratin, Stearoyl coA desaturase and Lrf. In the third screening round, we tested the individual inserts by retroviral transduction into fresh BTR cells, which revealed Lrf, but as expected not either of the remaining cDNAs, as the cDNA that was responsible for efficiently bypassing RAS V12-induced senescence (Figure 2C and D). The sequence of Lrf lacks an AscI site but contains a NotI site, which explains the lack of biological activity of the 23A(NotI) DNA pool (Figure 2A).

The pEYK3.1–LRF construct contained full-length mouse Lrf (NM_010731.3) encoding a 569 amino acid protein. It contains several modules, including a BTB/POZ domain, three Krüppel-like and one unusual Zinc fingers and a nuclear localization signal (Figure 2B). In an independent RAS V12-induced senescence bypass screen human ZBTB7B/hcKROX-α, the closest homologue of LRF was also identified as gene that can bypass RAS V12-induced senescence, although with lower biological activity than LRF (C.Martínez-Muñoz and D.S.Peeper, unpublished data). Upon expression of LRF at the non-permissive temperature, cells continued proliferating despite the presence of RAS V12 and showed a transformed phenotype (Figure 2C). Proliferation curves confirmed that LRF-expressing BTR cells expanded rapidly, whereas the control cells senesced (Figure 2D).

Next, we determined whether LRF allowed cells to bypass also spontaneous senescence. To this end, we transduced wt and temperature-sensitive Large T expressing MEFs with LRF-encoding retrovirus and shifted the temperature-sensitive Large T expressing MEFs to the non-permissive temperature. Indeed, also this resulted in abrogation of the senescence response (Figure 2E and F). To determine whether LRF can collaborate with more oncogenes than just RAS, we co-expressed it together with cMYC in wt MEFs. LRF strongly enhanced the immortalizing activity of cMYC (Figure 2G). These cells have the ability to form colonies in soft agar with moderate efficiency compared with wt MEFs expressing RAS V12 and cMYC, whereas wt MEFs immortalized with LRF and RAS V12 are not oncogenically transformed as measured in this assay (data not shown). These findings confirm previous observations (21) and demonstrate that LRF overexpression can bypass both premature and cellular senescence.
LRF-mediated bypass of RASV12-induced senescence is accompanied by induction of CYCLINs E and A and occurs independently of critical senescence inducers

To address the mechanism by which LRF rescues RASV12-induced senescence, we analysed the levels of various proteins known to be involved in senescence induction as well as cell cycle proteins. Inactivation of p19ARF is known to collaborate with RASV12 in oncogenic transformation. However, in a time course experiment, protein levels of p19ARF and p16INK4A showed no difference in control BTR cells compared with their LRF-expressing counterparts, indicating that the LRF-mediated rescue abrogation of senescence is not mediated by suppression of p19ARF or p16INK4A (Figure 3A). This is in contrast with the findings of Maeda et al. (21), as they observed that LRF represses p19ARF transcription. We found that p53 was only slightly downregulated in LRF-expressing BTR cells, even though one of its transcriptional targets p21CIP in fact showed a minor upregulation. Another p53 target, MDM2, was not regulated by LRF. So p53 is downregulated but its targets have been found to be involved in senescence are not regulated in this experimental setting. Consistent with these observations, knockdown of p53 enhanced the ability of LRF to abrogate senescence-mediated bypass (supplementary Figure 1A and B is available at Carcinogenesis Online). This suggests that p53 is not involved in the LRF-mediated bypass of RASV12-induced senescence.

In contrast, the E2F-targets genes Cyclin E, Cyclin A and p107 showed a clear upregulation in the cells that were rescued from RASV12-induced senescence by LRF, whereas the D-type CYCLINs showed no change in protein levels (Figure 3A). To examine whether the increase in CYCLIN E protein levels is accompanied by an increase in CYCLIN E-associated activity, we performed a CYCLIN E kinase activity assay with Histone H1 as a substrate. As shown in Figure 3B, LRF-expressing BTR cells indeed display increased CYCLIN E kinase activity compared with control cells. In principle, the activation of E2F-dependent transcription could be indirect, as a result of the downregulation of Rb by LRF (22), yielding an increase in free E2F competent to activate transcription. However, Rb transcript levels are not regulated by LRF in the BTR cells (Figure 3C). Thus, LRF-mediated bypass of RASV12-induced senescence correlates with induction of several E2F-target genes including CYCLIN E.

CYCLIN E induction is essential for LRF-mediated bypass of RASV12-induced senescence

To investigate the role of CYCLIN E in the LRF-mediated bypass of RASV12-induced senescence, we depleted it using two independent non-overlapping short hairpin RNA (shRNA). Silencing of Cyclin E strongly suppressed the ability of LRF to bypass senescence (Figure 4A and C). Importantly, this was not due to a general cytostatic effect, as the proliferative capacity of both NIH 3T3 mouse fibroblasts (Figure 4A) and BTR cells expressing (wild-type) SV40 LT at the non-permissive temperature (Figure 4C) remained unaffected by silencing of Cyclin E. To determine if CYCLIN E is not only required for LRF to bypass RASV12-induced senescence but also sufficient to bypass it, we overexpressed it in the BTR cell system. However, overexpression of CYCLIN E was unable to exert this effect, whether alone or in combination with overexpression of CYCLIN A (Figure 5B and C). Thus, although CYCLIN E is not sufficient to bypass RASV12-induced senescence, it is necessary for the LRF-mediated bypass from RASV12-induced senescence.

LRF and RASV12 synergize in activating E2F-dependent transcription

Since we found three established E2F-targets genes (CYCLIN E, CYCLIN A and p107) being upregulated in BTR cells expressing LRF and found that this was not mediated by changes in Rb mRNA levels, we examined whether LRF enhances E2F-dependent transcriptional activation. We transfected into NIH 3T3 cells a luciferase reporter plasmid, which is driven by six tandem E2F sites (15). This experiment was performed in the presence of E2F and DP1, which we titrated in such amounts that the reporter was only minimally activated (Figure 6A). LRF stimulated the promoter activity in a dose-dependent manner, to up to 60 times. Interestingly, this was even further enhanced in the presence of RASV12, to up to 190 times. Next,
we used the same experimental setting to determine the effect of LRF on the CYCLIN E promoter (16), which showed the same pattern: LRF increased E2F/DP1 activity and this was further enhanced by the presence of RASV12 (Figure 6B). The effect of LRF was E2F dependent in the context of RASV12, as mutation of the corresponding sites in the CYCLIN E promoter greatly impaired activation by LRF. In conclusion, LRF synergizes with RAS V12 to strongly activate E2F-dependent transcription, which conceivably underlies its ability to stimulate expression of several E2F targets, thereby contributing to abrogation of the premature senescence program.

Discussion
To identify new regulators of oncogene-induced cellular senescence, we performed a RASV12-induced senescence bypass screen and found LRF as a potent hit. We used the pEYK3.1 shuttling system to efficiently perform second round screens. One of the advantages of this system is the lack of requirement for PCR steps to isolate hits. Lrf is highly GC-rich and therefore very difficult to amplify by PCR; this might explain why, despite its strong biological effect, it has not been identified in previously performed similar screens that rely on PCR steps (11,18).

LRF was originally cloned as a family member of two well-known leukemia/lymphoma related oncogenes, BCL-6 and PLZF (23), and the corresponding gene is located on chromosome 19p13.3, a hot spot for chromosomal aberrations in human cancer. The closest homologues of LRF are cKrox-a and AMP-1 (cKrox-c) (24). All the above-mentioned proteins are members of the POK protein family, which have a BTB/POZ domain for homomeric and heterodimeric complex formation and other protein interactions, and Krüppel-like C2H2 zinc fingers, for binding to specific DNA sequences (reviewed in ref. 25). It was recently described that LRF overexpression induces oncogenic transformation in vitro, whereas mice with transgenic expression of LRF in lymphoid cells develop aggressive lymphomas. Furthermore, aberrant overexpression of LRF is found not only in certain types of lymphoma (diffuse large B-cell lymphoma and follicular lymphoma) (21) but also in several other human cancers, including non-small lung cancer (26,27).

LRF is a transcription factor that is involved in several differentiation processes, like osteoclastogenesis (28), chondrogenesis (29), adipogenesis (30) and early lymphoid cell-fate decision (31). Originally, LRF was identified as a factor involved in elongation blocking of HIV-1 (32,33), whereas in the presence of the HIV-1 transcription factor Tat full-length transcription is induced (34). This might involve recruiting other (transcription) factors and/or keeping the promoter in an open and active conformation. Most POK proteins are transcriptional repressors (reviewed in ref. 35) and LRF, too, has been shown to repress transcription of several genes (24,28). This occurs via recruitment of HDAC1 and Sin3A repressor complexes to the promoters (22,29,36) and/or by inhibiting the transcriptional activity of Sp1 and p53 (24,37,38). However, LRF can also enhance transcription, by increasing the nuclear localization and stabilization of transcription factors (39) and by activating Sp1-transcriptional activation (40). Whether LRF inhibits or activates transcription probably depends on the different promoter elements, on cofactors and in what ratio they bind to promoters. This is already known for SP1 (reviewed in Fig. 4. CYCLIN E induction is essential for LRF-mediated bypass of RASV12-induced senescence. (A and C) NIH 3T3, BTR-LRF and BTR-SV40LT cells at the non-permissive temperature were transduced with retrovirus expressing short hairpins for CYCLIN E1 (sh-CE). Colony formations was stained 2 weeks after transduction. (B and D) Cell extracts were prepared and analysed on western blot for CYCLIN E levels; CDK4 and β-actin serve as loading controls.

Fig. 5. Overexpression of CYCLIN E and A is not sufficient to bypass RASV12-induced senescence. (A and C) BTR cells were transduced with retrovirus encoding human CYCLIN E1 and/or CYCLIN A and shifted to the non-permissive temperature 2 days after transduction. LRF-overexpressing cells were used as positive control. Colony formations was stained 2 weeks after transduction and (B) analysed on western blot with a human specific antibody for CYCLIN E and β-actin, serving as loading control.
nescence inducers, like p19 ARF, p21CIP and p16INK4A. Depletion of CYCLIN E tentatively downregulate p19ARF. Similarly, in contrast to previous reports cells and can collaborate with other oncogenes like RAS V12 and (21). We confirm here that LRF immortalizes primary murine et al. shown for LRF-mediated activation of the FASN promoter by Choi et al. for which LRF activates E2F-dependent transcription remains to be not involve the downregulation of Rb by LRF, the precise mechanism activated, E2F-dependent transcription. Although we show that it does notwithstanding, CYCLIN E may not be the sole CYCLIN to be CLIN E abolishes the ability of LRF to bypass senescence. This is not involved. However, CYCLIN E may not be the sole CYCLIN to be involved. However, CYCLIN E is not sufficient to bypass RASV12-induced senescence, not even in the presence of CYCLIN A. Lastly, we find that LRF can enhance, and in synergy with RASV12, supposedly activate, E2F-dependent transcription. Although we show that it does not involve the downregulation of Rb by LRF, the precise mechanism by which LRF activates E2F-dependent transcription remains to be determined. It may be a direct activation mechanism, as has been shown for LRF-mediated activation of the FASN promoter by Choi et al. (40).

Our results are partially in agreement with those obtained by Maeda et al. (21). We confirm here that LRF immortalizes primary murine cells and can collaborate with other oncogenes like RASV12 and cMYC. In contrast, in our experimental system LRF does not consistently downregulate p19ARF. Similarly, in contrast to previous reports on LRF suppressing the levels of p21CIP (38), CYCLIN A and p107

(30), we find these genes to be upregulated in our experiments. These differences might be explained by the use of cell lines from different species and/or origins and different experimental setups (e.g. transfection versus retroviral expression). Indeed, how a promoter is regulated by LRF might depend on the presence of different cofactors and in what ratios to LRF they bind to the promoter elements (40). Furthermore, we find, like for Rb−/−/p107−/− MEFs (20), that MEFs expressing LRF are immortalized but not oncogenically transformed by RASS12. This finding is in agreement with our data suggesting that LRF acts primarily on the Rb/E2F pathway.

Increasing evidence points to an important role of LRF/FBI-1 in cancer. The mouse model directing high levels of LRF to immature T and B lymphoid cells (icE/Ju-Lrf) develops aggressive tumors (21). Ablated LRF/FBI-1 expression has been found in several human tumors, however, its relation to expression of the human counterpart of p19ARF, 14ARF, varies. In a subset of B-cell lymphomas (diffuse large B-cell lymphoma), high LRF/FBI-1 levels correlated with low p14ARF 21, whereas an inverse relationship was found in non-small cell lung cancer (26). The relationship of LRF/FBI-1 expression to clinical outcome also differs; LRF/FBI-1 expression predicts good clinical outcome in diffuse large B-cell lymphoma yet bad clinical outcome in non-small cell lung cancer (ref. 21,27). These differences might depend on the cells of origin of the tumors, the collaborating oncogenes, and which other targets are regulated by LRF/FBI-1. In aggregate, the available evidence indicates that LRF/FBI-1 deregulates several important genes associated with cancer, including p14/p19ARF, Rb, p21CIP and, as we show here, Cyclin E and other E2F targets, which conceivably explains its oncogenic activity in different settings.

Supplementary material

Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

Funding

Dutch Cancer Society (KWF Kankerbestrijding); Vidi and Vici grants from the Netherlands Organisation for Scientific Research (NWO) to L.C.W.V., B.D.R., D.S.P.

Acknowledgements

We acknowledge E.Koh and G.Daley for sharing the pEYK3.1 libraries, A.Dluhy for the pRS-m53, pRS-p19ARF and pRS-nCyclin E1 constructs, K.Helin for the 6xE2F reporter construct, Y.Geng for human CYCLIN E reporter constructs, members of Peper laboratory for helpful discussions, and T.Kuilen and C.Höming-Hözel for critical reading of the manuscript.

Conflict of Interest Statement: None declared.

References

34. Pendergast, P.S. et al. (2002) FBI-1 can stimulate HIV-1 Tat activity and is targeted to a novel subnuclear domain that includes the Tat-P-TEFb-containing nucleolar speckles. Mol. Biol. Cell, 13, 915–929.

Received June 12, 2009; revised November 6, 2009; accepted November 21, 2009