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The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene

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***KLF4* (*GKLF*/*EZF*) encodes a transcription factor that is associated with both tumour suppression and oncogenesis. We describe the identification of *KLF4* in a functional genomic screen for genes that bypass RAS^{V12}-induced senescence. However, in untransformed cells, KLF4 acts as a potent inhibitor of proliferation. KLF4-induced arrest is bypassed by oncogenic RAS^{V12} or by the RAS target cyclin-D1. Remarkably, inactivation of the cyclin-D1 target and the cell-cycle inhibitor p21^{CIP1} not only neutralizes the cytostatic action of KLF4, but also collaborates with KLF4 in oncogenic transformation. Conversely, KLF4 suppresses the expression of p53 by directly acting on its promoter, thereby allowing for RAS^{V12}-mediated transformation and causing resistance to DNA-damage-induced apoptosis. Consistently, KLF4 depletion from breast cancer cells restores p53 levels and causes p53-dependent apoptosis. These results unmask KLF4 as a regulator of p53 that oncogenically transforms cells as a function of p21^{CIP1} status. Furthermore, they provide a mechanistic explanation for the context-dependent oncogenic or tumour-suppressor functions of KLF4.**

Krüppel-like factor 4 (KLF4/GKLF/EZF)[1,2] is a transcription factor that can both activate and repress genes that are involved in cell-cycle regulation and differentiation. Among the KLF4-regulated cell-cycle genes, many upregulated genes are inhibitors of proliferation, whereas genes that promote proliferation are repressed[3]. This indicates that KLF4 regulates the expression of a set of cell-cycle genes to coordinately inhibit cellular proliferation. In keeping with this is the notion that ectopic expression of KLF4 acts cytostatically[1,4,5]. Moreover, KLF4 levels rise following DNA damage, cell-cycle arrest in response to serum withdrawal and contact inhibition[1,6]. Similarly, KLF4 levels are increased in the post-mitotic compartment of the gut and skin[1,2]. In mice, ectopic expression of Klf4 accelerates terminal differentiation, leading to premature skin-barrier acquisition[7], whereas *Klf4* deficiency prevents the terminal differentiation of colonic goblet cells[8] and the skin epithelium[9], which leads to neonatal death[9]. These observations establish KLF4 as a stress- and differentiation-associated inhibitor of proliferation[10], raising the possibility that KLF4 may have tumour-suppressive functions[8,11,12].

Indeed, *KLF4* expression is frequently lost in various human cancer types[11,13–19]. Recently, *KLF4* has been shown to undergo promoter methylation and loss of heterozygosity in gastrointestinal cancer[16,17]. Consistent with a tumour-suppressor function for KLF4, its overexpression reduces the tumorigenicity of colonic and gastric cancer cells *in vivo*[17,20]. These observations, taken together, indicate that KLF4 acts as a tumour suppressor. This notion is further supported by recent data showing that specific ablation of *Klf4* in the gastric epithelium of mice results in premalignant changes, including polypoid lesions[18].

Conversely, elevated KLF4 levels have also been linked to cancer: its mRNA and protein are overexpressed in up to 70% of mammary carcinomas[21]. Moreover, KLF4 has been found to be frequently overexpressed in squamous-cell carcinomas of the oropharynx[22]. Recently, ectopic Klf4 expression in mice has been shown to induce squamous epithelial dysplasia[23]. Together with the results described above, these observations create a paradox and indicate that not only loss, but also overexpression of KLF4 may contribute to tumorigenesis. This phenomenon is not understood, and is attributed to 'cell-type specificity'[22]. It raises the interesting possibility that, subject to the presence or absence of unidentified cellular factors, KLF4 can switch from a growth-inhibiting tumour suppressor to a growth-promoting oncogene.

Here, we report the isolation of *KLF4* in a functional, genome-wide screen for genes that bypass a senescence-like cell-cycle arrest that is elicited by oncogenic RAS^{V12}. As this was surprising in view of the cytostatic action of KLF4 in most cellular settings, we have used this finding as a starting point to elucidate the molecular principles by which KLF4 suppresses, and stimulates, cellular proliferation.

RESULTS

KLF4 allows bypass of RAS^{V12}-induced senescence

Although the *RAS* gene is frequently mutated in cancer[24], ectopic expression of an oncogenic mutant *RAS^{V12}* allele does not lead to transformation of primary fibroblasts but, instead, induces a senescence-like cell-cycle arrest. This requires the action of various tumour-suppressor genes that are frequently inactivated in human cancer, including *ARF*,

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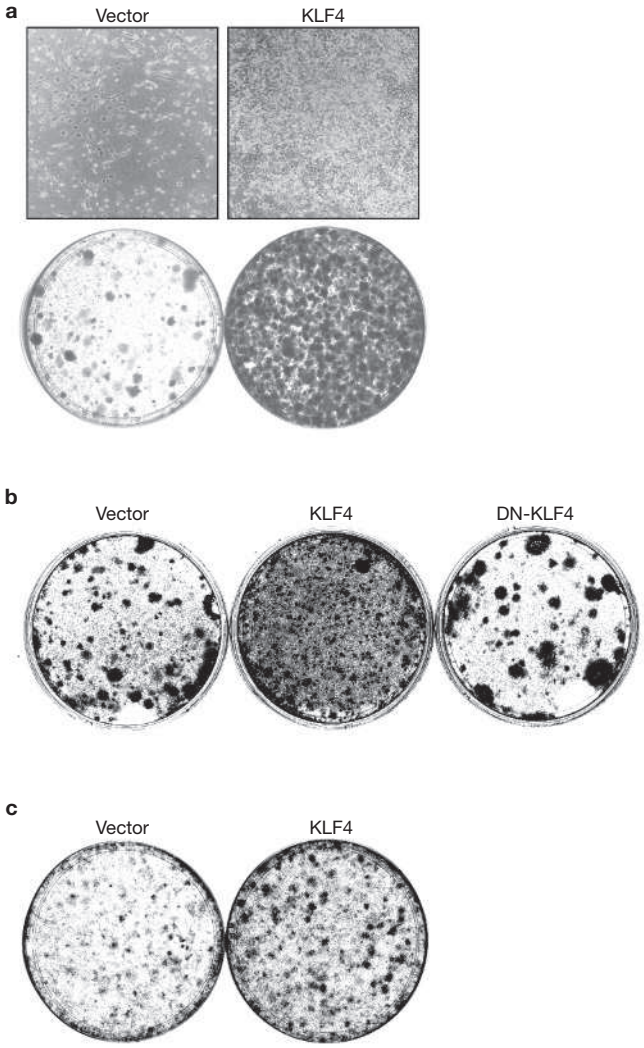

Figure 1 KLF4 allows the bypass of RAS^{V12}-induced senescence. (**a**) Colony-formation assay of BTR cells infected with control or KLF4-encoding retrovirus, maintained at the non-permissive temperature for 2 weeks (bottom). Detail at ×50 magnification (top). (**b**) Colony-formation assay of BTR cells infected with control, wild-type KLF4 or dominant-negative KLF4 (DN-KLF4)-encoding retrovirus, maintained at the non-permissive temperature for 2 weeks. (**c**) Colony-formation assay of wild-type mouse embryonic fibroblasts infected with RAS^{V12}- and KLF4-encoding retroviruses, 2 weeks after infection.

p53 and the retinoblastoma gene (*RB*) family[25,28]. RAS^{V12}-induced premature senescence can therefore be used as a model system to identify novel factors that interfere with these (or yet unknown) tumour-suppressor pathways. We have previously designed an unbiased, functional genome-wide screen for genes that bypass RAS^{V12}-induced senescence[29]. For this purpose, we used mouse embryonic fibroblasts (MEFs), which were conditionally immortalized with a temperature-sensitive (*ts*) mutant of SV40 large T antigen and co-expressing RAS^{V12}. This cell line (referred to as BTR) is transformed at 32°C, but undergoes RAS^{V12}-induced senescence at 39°C, when *ts* large T antigen is inactive. By using BTR cells in a functional screen with retroviral cDNA expression libraries, we have previously identified *DRIL1* as an immortalizing oncogene that collaborates with *RAS^{V12}* in oncogenic transformation[29].

Polymerase chain reaction (PCR) isolation of integrated cDNAs, followed by sequence analysis of four additional, independent cell lines that had escaped RAS^{V12}-induced senescence, revealed a wild-type full-length cDNA encoding KLF4. Indeed, following retroviral delivery to BTR cells, KLF4 efficiently bypassed RAS^{V12}-induced senescence (Fig. 1a). To address whether this function of KLF4 was dependent on its transcriptional-regulatory domain, we constructed a mutant of KLF4, which lacks its activation and repression domains. As this mutant functions in a dominant-negative manner over endogenous KLF4, we designated it DN-KLF4. This mutant failed to collaborate with RAS^{V12} (Fig. 1b), indicating that the transcriptional-regulatory domain is required for this function of KLF4. This observation was not restricted to this particular cell type, as primary MEFs also showed an increase in the number of transformed foci following retroviral co-expression of KLF4 and RAS^{V12}, as opposed to expression of the latter alone (Fig. 1c).

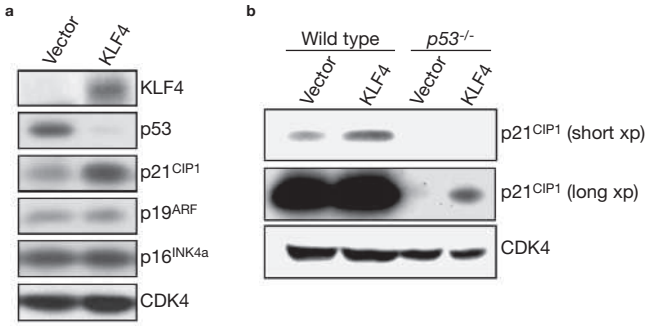

Figure 2 KLF4 suppresses p53, but induces p21^{CIP1}. (**a**) Western blot analysis of BTR cells, infected with retroviruses as indicated, and maintained at the non-permissive temperature for 2 weeks (similar results were obtained after 1 and 3 weeks). (**b**) Western blot analysis of wild-type and *p53$^{-/-}$* mouse embryonic fibroblasts infected with control or KLF4-encoding retrovirus. The p21^{CIP1} panels are shown as two different exposures (short and long xp) of the same blot. CDK4 serves as the loading control.

KLF4 suppresses p53, but induces p21^{CIP1}

This finding prompted us to address the molecular mechanisms that underlie the proliferative collaboration between KLF4 and RAS^{V12}. We therefore analysed the expression levels of a number of cell-cycle-related proteins that are known to have a key role in senescence signalling. Remarkably, KLF4 had opposing effects on the cyclin-dependent kinase inhibitor p21^{CIP1} and on p53. First, KLF4 expression led to an increase in p21^{CIP1} levels (a finding that corresponds with previously reported data[3,6]; Fig. 2a), which occurred independently of p53 (Fig. 2b). Second, KLF4 repressed p53 levels (Fig. 2a). This occurred independently of p19^{ARF}, as the levels of this p53 regulator remained unaffected in the presence of KLF4. Also, p16^{INK4a} — which, like p19^{ARF}, is expressed by the *INK4a/ARF* locus — was not deregulated by KLF4.

Biological effects of p53 suppression

As p53 is a critical mediator of RAS^{V12}-induced proliferative arrest, we hypothesized that KLF4, through the suppression of p53 levels, rescues RAS^{V12}-induced senescence. As expected, the reduction of p53 protein levels was accompanied by a decline in p53-dependent transcriptional activation (Fig. 3a). We then examined whether the suppression of p53 levels by KLF4 was sufficient for the observed rescue of RAS^{V12}-induced senescence. To test this, we used a short hairpin RNA (shRNA) retroviral vector (pRetroSUPER)[30] to reduce endogenous p53 levels and monitored the effect of this on the ability of RAS^{V12} to induce cell-cycle arrest. Although KLF4 and p53 shRNA suppressed p53 levels to a similar extent

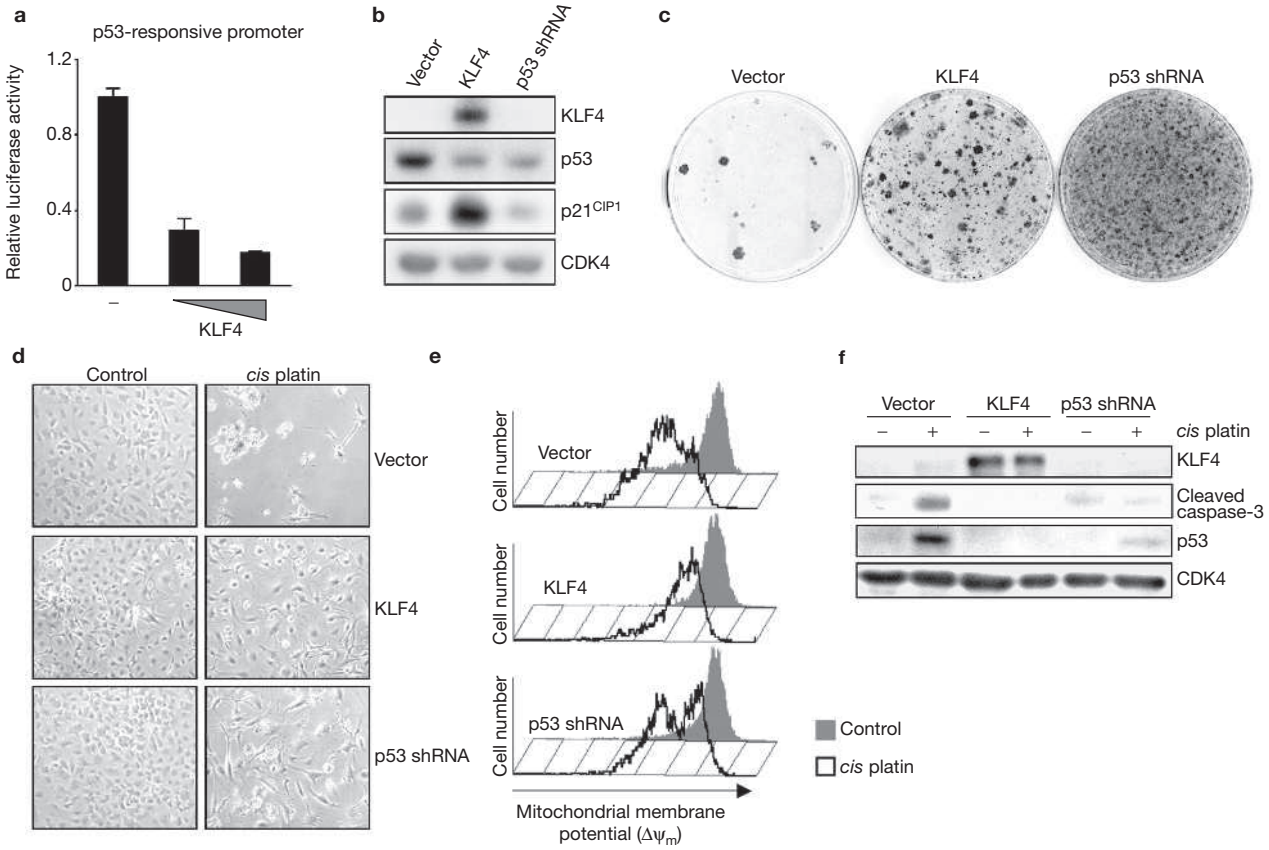

Figure 3 By suppressing p53 expression, KLF4 bypasses RAS^{V12}-induced senescence and causes resistance to DNA-damage-induced apoptosis. (**a**) Luciferase assay on cells transfected with a p53-responsive reporter and control or KLF4-encoding plasmids. Averages and standard deviations for three different experiments are shown, normalized to an internal cytomegalovirus *Renilla* luciferase control. (**b**, **c**) BTR cells were infected with KLF4- or p53-shRNA-encoding retrovirus, maintained at the non-permissive temperature for 2 weeks, and analysed by western blotting (**b**; CDK4 serves as the loading control) and in a colony-formation assay (**c**). (**d**–**f**) $p21^{CIP1-/-}$ mouse embryonic fibroblasts were infected with KLF4- or p53 shRNA-encoding retrovirus, treated with 50 μM cisplatin for 16 h and photographed at ×100 magnification (**d**), assayed for apoptosis by staining with DiOC6(3) and subsequent FACS analysis 48 h later (**e**), or analysed by western blotting (**f**). (**e**) The right-hand peaks reflect living cells, whereas the left-hand peaks (most prominent in the vector sample) are derived from apoptotic cells. Results from a representative experiment are shown (the vector and KLF4 data are representative of four independent experiments and the p53 shRNA data for two independent experiments), in which cisplatin treatment resulted in a 33.3% increase of apoptotic cells in the control cells, whereas in the KLF4- and p53-shRNA-expressing cells the increase of apoptotic cells was 11.3% and 21.2%, respectively.

(Fig. 3b), the latter rescued RAS^{V12}-induced senescence more efficiently (Fig. 3c). This result shows that, indeed, the extent of p53 downregulation mediated by KLF4 is sufficient to rescue RAS^{V12}-induced arrest. Furthermore, it indicates that KLF4 also has an anti-proliferative effect that partially counteracts the effects of p53 downregulation. This apparently compromises the ability of KLF4 to collaborate with RAS^{V12} in stimulating cellular proliferation (this is addressed in more detail later).

p53 is vital not only for protection against RAS^{V12}-induced oncogenic transformation, but also represents a central player in the cellular DNA-damage response. Therefore, we hypothesized that KLF4, through the suppression of p53, prevents DNA-damage-induced apoptosis as well. As KLF4 induces $p21^{CIP1}$ (Fig. 2a, b; Fig. 3b), which may interfere with the DNA-damage response[31], we used $p21^{CIP1-/-}$ fibroblasts to address this. Treatment of control cells with the DNA-damaging agent cisplatin, a commonly used anti-cancer drug, resulted in the induction of p53 (Fig. 3f) and caused apoptosis (as judged by the appearance of floating cells, the cleavage of caspase-3 and decreased mitochondrial membrane potential; Fig. 3d–f). The induction of apoptosis was p53-dependent, as it was significantly reduced by a p53 shRNA. Importantly, in the presence of KLF4, cells became almost completely resistant to cisplatin-induced apoptosis (Fig. 3d–f). Thus, suppression of p53 by KLF4 has important functional consequences, in that it allows bypass of RAS^{V12}-induced senescence and causes resistance to DNA-damage-induced apoptosis.

KLF4 directly suppresses p53 transcription by binding to its promoter

In view of the significant biological consequences of KLF4-mediated regulation of p53, we explored its molecular basis. Many cellular signals target p53 for proteosomal degradation[28]. To examine whether KLF4, too, reduces p53 levels through enhanced proteosomal degradation, we treated KLF4-expressing cells with the proteosome inhibitor CBZ-LLL and analysed p53 levels. Whereas CBZ-LLL, as expected, induced an accumulation of p53, KLF4 suppressed the levels of p53 irrespective of CBZ-LLL treatment (Fig. 4a). This indicates that KLF4 does not regulate p53 through increased proteosomal degradation. Northern blotting revealed that, instead, KLF4 downregulated p53 at the RNA level (Fig. 4b).

As the KLF4 transcription factor repressed p53 expression at the RNA level, we assessed whether it actually binds to the p53 promoter *in vivo*, and whether KLF4 can suppress the activity of this promoter. First, to study if KLF4 binds to the p53 promoter, we performed

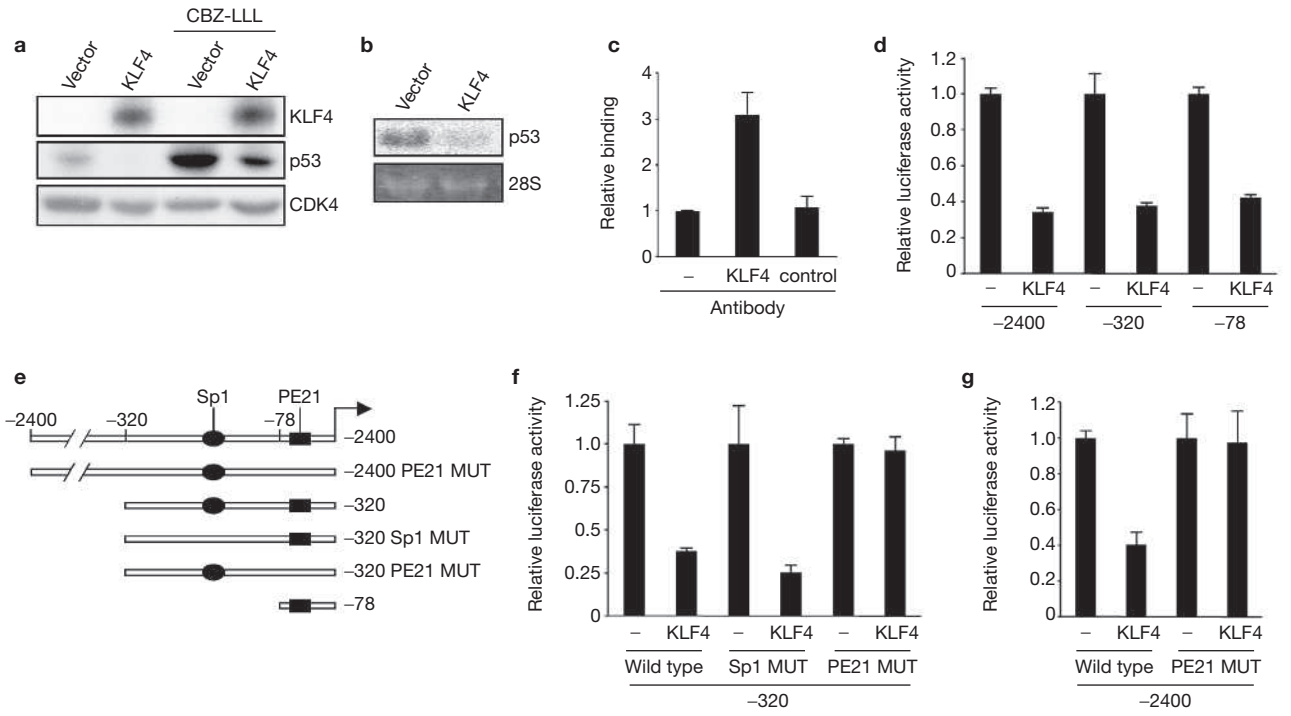

Figure 4 KLF4 directly suppresses p53 transcription by binding to its promoter. (**a**, **b**) *p21*$^{CIP1-/-}$ mouse embryonic fibroblasts were infected with KLF4-encoding or control retrovirus and analysed by western blotting (**a**) and northern blotting (**b**). Cells were treated with CBZ-LLL as indicated. **(c)** Chromatin immunoprecipitation (ChIP) analysis performed on MDA-MB-134 breast cancer cell lines, with KLF4-specific antibody, control or no antibody. Data are represented as the quantitative real-time polymerase chain reaction (PCR) signals from the proximal region of the p53 promoter (containing the PE21 element), relative to a glyceraldehyde dehydrogenase phosphate reaction performed on the same immunocomplexes. Averages and standard deviations are shown for three PCR reactions on a representative ChIP experiment. (**d, f, g**) Luciferase assay on cells transfected with the indicated p53 promoter reporter constructs and control or KLF4-encoding plasmids. MUT indicates reporter constructs with the core of the respective elements mutated. Averages and standard deviations for three different experiments are shown, normalized to an internal cytomegalovirus *Renilla* luciferase control. (**e**) Schematic depiction of the human p53 promoter and the mutants used in this study. The arrow represents the transcription start site at +1.

chromatin immune precipitation (ChIP) assays. For this purpose, we used human breast cancer cells (MDA-MB-134) that had wild-type p53 and that expressed high levels of endogenous KLF4 (data not shown), allowing analysis of the endogenous KLF4 protein in this setting. Quantitative real-time PCR performed on specific KLF4 immunocomplexes revealed the presence of the p53 promoter (Fig. 4c). This indicates that endogenous KLF4 binds to the p53 promoter.

To determine whether this was associated with the regulation of p53 transcription, we performed p53 promoter reporter assays. Indeed, KLF4 repressed the activity of the p53 promoter (Fig. 4d). To map the KLF4-responsive element, we generated two progressive deletion mutants of a 2.4-kb genomic DNA fragment encompassing the human p53 promoter (Fig. 4e). Deletion of most of this fragment, leaving intact only the most 3′ 78 bp, did not compromise the ability of KLF4 to repress the p53 promoter (Fig. 4d). This indicates that the KLF4-responsive element is located in the small, proximal fragment, spanning from –78 to –2. KLF4 is a close relative of Sp1 (ref. 10), and the p53 promoter contains a consensus Sp1-binding site[32]. However, this site is not located within the proximal 78 nucleotides (Fig. 4e) and its mutation did not affect the response to KLF4 (Fig. 4f). The proximal region contains a so-called PE21 element, which has previously been documented to mediate Oncostatin-M-induced repression of the p53 promoter[32]. Mutation of this PE21 element completely abolished KLF4-mediated repression of the p53 promoter (Fig. 4f, g), demonstrating that KLF4 requires the PE21 element for repression of the p53 promoter. Together, these results show that, in contrast to most regulators of p53 (ref. 28), KLF4 does not suppress p53 levels by increasing its proteosomal degradation, but rather by means of transcriptional repression through binding to a specific element within its promoter.

KLF4 depletion from breast cancer cells restores p53 levels and causes p53-dependent apoptosis

Above, we show that ectopically expressed KLF4 transcriptionally down-regulates p53 and that, in breast cancer cells, endogenous KLF4 binds to the p53 promoter. These observations suggest that at least one role of endogenous KLF4 in breast cancer cells is to actively repress p53 levels. Given the dominant role of p53 in apoptosis, it might be possible that the communication between KLF4 and p53 affects cell survival. To test these possibilities directly, we suppressed KLF4 expression with specific shRNAs and monitored the effect on p53 levels as well as on the survival of MDA-MB-134 cells. To exclude potential off-target effects, we designed three independent KLF4 shRNAs. All three shRNAs reduced KLF4 expression (Fig. 5a). More importantly, in all cases this resulted in an elevation of endogenous p53 levels. This indicates that, similar to ectopically expressed KLF4, endogenous KLF4 also suppresses p53. Furthermore, the shRNA that most effectively suppressed KLF4 expression (KLF4 shRNA #2) caused the strongest p53 induction, indicating that p53 suppression by KLF4 is a dose-dependent effect. Expression of a dominant-negative KLF4 protein also restored p53 levels (Fig. 5b), further excluding an off-target effect of the shRNAs.

We next investigated whether the restoration of p53 levels resulting from KLF4 depletion had any biological effect. Strikingly, following reduction of

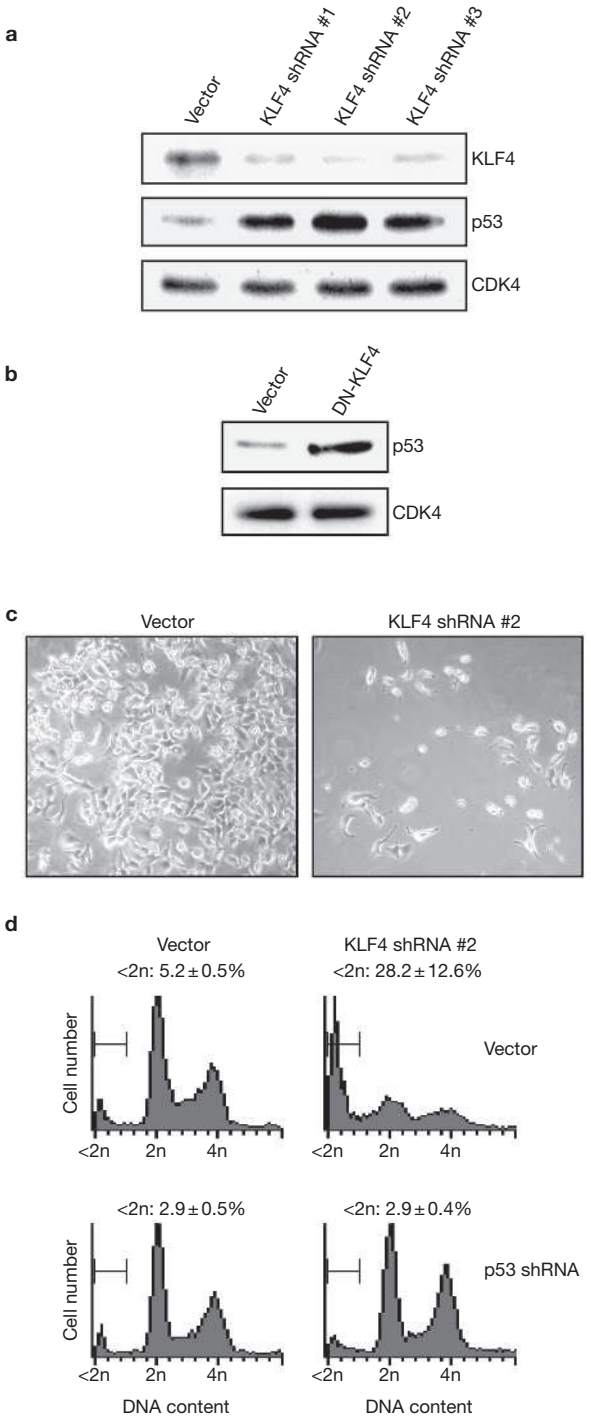

Figure 5 KLF4 depletion from breast cancer cells restores p53 levels and causes p53-dependent apoptosis. MDA-MB-134 cells were infected with retroviruses encoding shRNAs against KLF4 (**a**), or encoding a dominant-negative KLF4 (DN-KLF4) expression construct (**b**). After puromycin selection, cells were analysed by western blotting. CDK4 serves as the loading control. (**c**) MDA-MB-134 cells were transfected with KLF4 shRNA #2 or control plasmids that were selected for puromycin resistance, and photographed at ×50 magnification. (**d**) As in (**c**), but with cotransfected p53 shRNA or control plasmid. Cells were stained with propidium iodide and analysed by FACS 3 days post-transfection. The percentage ± standard deviations of apoptotic (sub-2n) cells for three independent experiments is shown; FACS profiles of a representative experiment are displayed.

KLF4 expression by shRNA, cells abruptly underwent apoptosis, leaving few viable cells after 3 days (Fig. 5c, d). Similar results were obtained in MCF7 cells (see Supplementary Information, Fig. S1). This result shows that endogenous KLF4 not only suppresses p53, but is also essential for the viability of these cells. It raises the possibility that suppression of p53 represents a crucial function of KLF4, at least in breast cancer cells that abundantly express KLF4. To determine the contribution of endogenous p53 in apoptosis that is instigated by KLF4 depletion, we co-transfected KLF4 shRNA and either control or p53 shRNA constructs. Whereas KLF4 reduction resulted in a 23% increase in the proportion of apoptotic cells, this effect was rescued by depletion of p53 (Fig. 5d). We confirmed that p53 shRNA did not compromise the efficacy of the KLF4 shRNA construct (data not shown). Thus, suppression of p53 is a crucial function of endogenous KLF4 and is essential for the survival of cultured breast cancer cells.

A central role for cyclin-D1

The results shown in Figs 1–5 shed light on the mechanisms by which KLF4 exerts its pro-mitogenic function — namely, at least in part, through the downregulation of p53. However, the pro-mitogenic effect of KLF4 that was revealed in the RAS^{V12} senescence bypass screen was surprising in view of the many reports highlighting KLF4 as a suppressor of proliferation that induces cell-cycle arrest in a variety of cell types[1,4]. Indeed, when we used KLF4-encoding retrovirus to infect primary wild-type MEFs, they also underwent a tight proliferative arrest, within 2 days (Fig. 6a). These observations, as well as the KLF4 literature, raise the possibility that KLF4, although acting as a inhibitor of proliferation in many settings, possesses a pro-mitogenic activity that is uncovered only within a specific genetic context — such as in the presence of RAS^{V12}.

To address the mechanism by which RAS^{V12}-dependent signals neutralize KLF4-induced cell-cycle arrest, we focused first on cyclin-D1, as it represents an established mitogenic target of RAS[33,34]. To examine its possible contribution, we reduced cyclin-D1 levels with an shRNA and determined whether this affected the mitogenic collaboration between KLF4 and RAS^{V12}. To ensure an identical extent of cyclin-D1 downregulation in all samples, we first created a cell population that was stably expressing cyclin-D1 shRNA, and these cells were subjected to subsequent infection with retroviruses encoding either KLF4 or, as controls, DRIL1 or p53 shRNA. Whereas, together, RAS^{V12} and KLF4 induced proliferation, this was impaired following cyclin-D1 depletion (Fig. 6b). This was not due to a general block of proliferation associated with cyclin-D1 shRNA, as the rescue of RAS^{V12}-induced arrest by DRIL1, which acts downstream of cyclin-D1 (ref. 29), remained unaffected. Similarly, p53 shRNA collaborated with RAS^{V12} in a cyclin-D1-independent manner. From these observations, we concluded that cyclin-D1 is essential for proliferative collaboration between KLF4 and RAS^{V12}.

To assess whether cyclin-D1 expression is sufficient to rescue KLF4-induced cell-cycle arrest, we co-transfected expression plasmids for KLF4 and cyclin-D1 and subsequently analysed the cell-cycle profiles. Whereas KLF4 induced G1 cell-cycle arrest in the control cell population, this was efficiently rescued by cyclin-D1 co-expression (Fig. 6d). Therefore, deregulated expression of cyclin-D1, a *bona fide* RAS target, neutralizes KLF4-induced cell-cycle arrest.

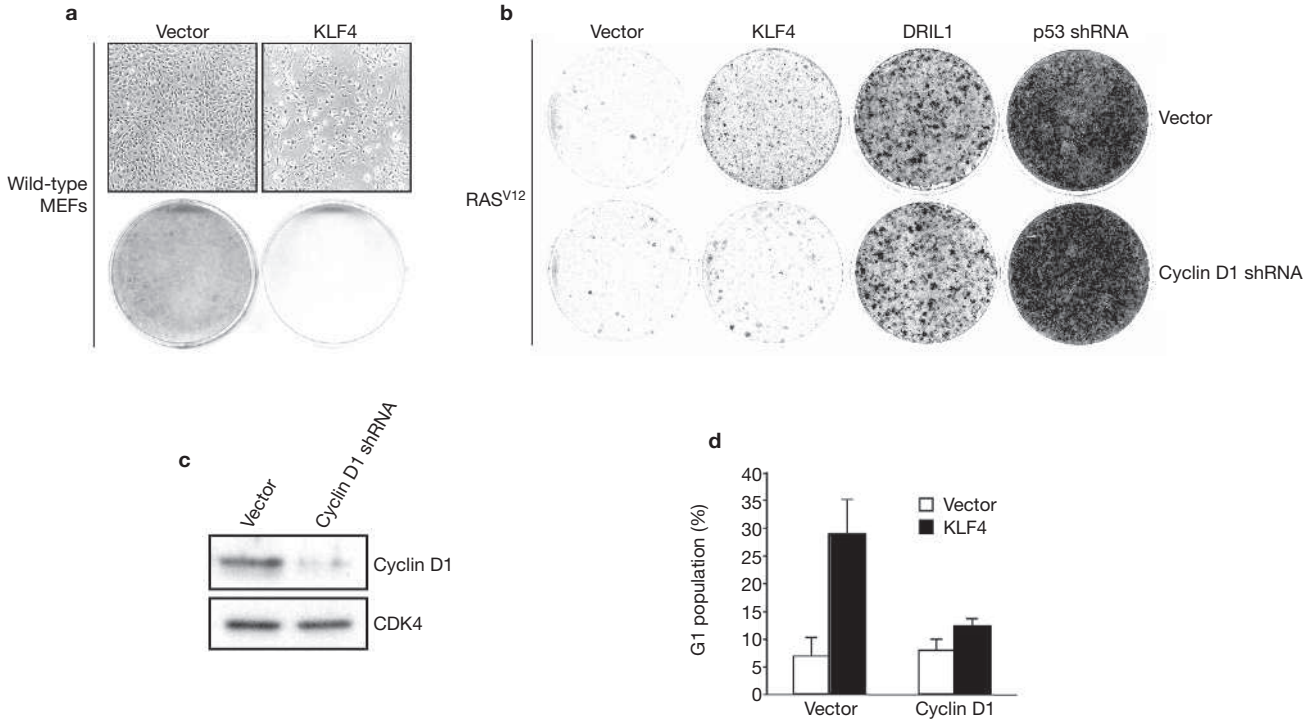

Figure 6 Cyclin-D1 is required for proliferative collaboration between KLF4 and RAS^{V12}. (**a**) Wild-type mouse embryonic fibroblasts were infected with KLF4-encoding or control retrovirus and used in a colony-formation assay (bottom). Details at ×50 magnification (top). (**b**) BTR cells were infected first with either control or cyclin-D1 shRNA retrovirus and subsequently with KLF4 expression, DRIL1-expression, p53 shRNA or control retrovirus at the permissive temperature. Dishes were stained after 7 days at the non-permissive temperature. (**c**) BTR cells were infected with control or cyclin-D1-shRNA-encoding retroviruses and analysed by western blotting. CDK4 serves as the loading control. (**d**) U2OS cells were transfected with expression plasmids as indicated and subjected to propidium iodide staining and FACS analysis 64 h later. The absolute percentage of G1 population is depicted. The average and standard deviations are shown for three independent experiments.

$p21^{CIP1}$ loss converts KLF4 from cell-cycle inhibitor into oncoprotein

The central role of cyclin-D1 in the proliferative collaboration between KLF4 and RAS led us to investigate the mechanism by which cyclin-D1 alleviates the proliferation-suppressing action of KLF4. As shown above, KLF4 induces the levels of $p21^{CIP1}$ (Fig. 2a, b; Fig. 3b). As neutralization of $p21^{CIP1}$ represents an important mitogenic activity of cyclin D1 (ref. 35), we hypothesized that it is specifically $p21^{CIP1}$ that is responsible for inhibiting the proliferation of KLF4-expressing cells. To test this, we inactivated $p21^{CIP1}$ in either of two ways. First, we infected two independent cultures of both primary wild-type and $p21^{CIP1-/-}$ MEFs with KLF4-encoding virus and subsequently measured their proliferative capacities. Whereas wild-type MEFs rapidly underwent cell-cycle arrest in response to KLF4 expression (Fig. 7a,b, top panels), $p21^{CIP1/-}$ MEFs were refractory to the cytostatic action of KLF4 (Fig. 7a,b, bottom panels). Second, in an independent assay, $p21^{CIP1}$ shRNA efficiently prevented cell-cycle inhibition by KLF4, to the same extent as ectopic expression of cyclin-D1 (Fig. 7c). These results demonstrate that KLF4 induces cell-cycle arrest in a $p21^{CIP1}$-dependent manner.

Together, the results discussed above show that KLF4 simultaneously suppresses p53 (which promotes cell proliferation) and induces $p21^{CIP1}$ (which inhibits cell proliferation). These opposing effects probably explain the observed difference in the abilities of KLF4 and p53 shRNA to bypass RAS^{V12}-induced cell-cycle arrest (Fig. 3c). Moreover, our results suggest that in the absence of $p21^{CIP1}$, KLF4 has the potential to contribute to cellular transformation. Indeed, whereas control-infected $p21^{CIP1-/-}$ MEFs, when grown to confluency, formed a monolayer, KLF4-expressing $p21^{CIP1-/-}$ MEFs grew to a much higher density (Fig. 7b, bottom panel) and produced many colonies that apparently had failed to undergo contact inhibition (Fig. 7d). These are both characteristics of oncogenically transformed cells. Additionally, in contrast to control $p21^{CIP1-/-}$MEFs, KLF4-infected $p21^{CIP1-/-}$ MEFs formed many colonies in a semi-solid medium (another hallmark of cellular transformation), similar to $p53^{-/-}$ MEFs expressing RAS^{V12} (Fig. 7e). Together, these results show that $p21^{CIP1}$ has a vital role in the establishment of cell-cycle arrest in response to KLF4 signalling. Moreover, following $p21^{CIP1}$ loss, the mitogenic properties of KLF4 apparently become exposed, which allows for cellular transformation to occur.

DISCUSSION

The isolation of the KLF4 transcription factor in a functional genomic screen for genes that bypass RAS^{V12}-induced senescence prompted us to unravel the molecular mechanism by which KLF4 stimulates or inhibits cell proliferation. We show here that it is the genetic context that has a decisive role in switching KLF4 between its opposing functions. Specifically, co-expression of an oncogenic RAS^{V12} allele or one of its mitogenic targets (cyclin-D1) is sufficient to neutralize the cytostatic effects of KLF4. Unexpectedly, loss of $p21^{CIP1}$ not only bypasses KLF4-induced cell-cycle arrest, but it also causes KLF4 to undergo a marked phenotypic change, in that it uncovers its oncogenic activity. This results in continued, KLF4-driven cellular proliferation, loss of contact inhibition, the ability to grow in an anchorage-independent manner and resistance to chemotherapeutic-induced apoptosis.

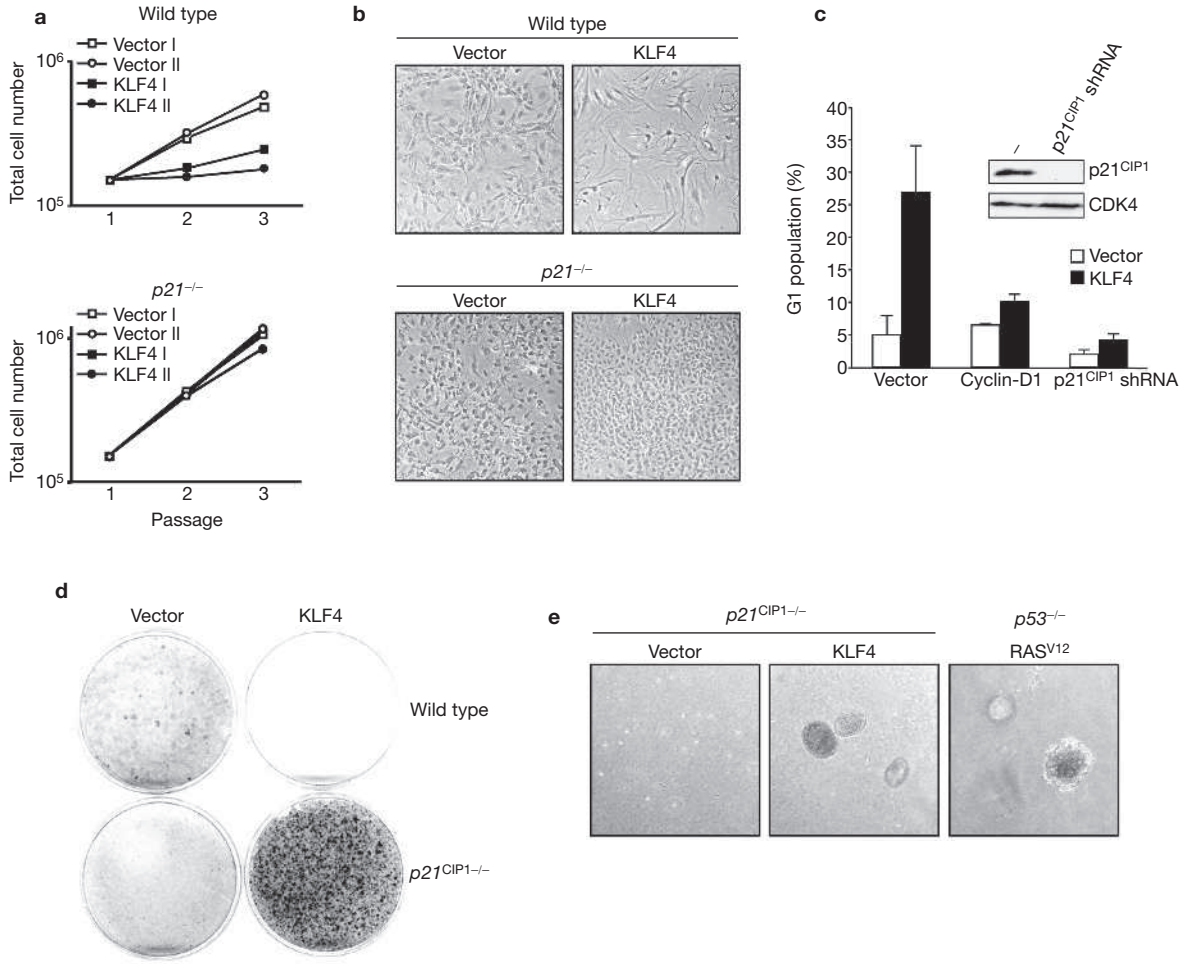

Figure 7 p21CIP1 loss converts KLF4 from cell-cycle inhibitor into oncoprotein. (**a**) Proliferation curves of two independent experiments of wild-type and *p21*$^{CIP1-/-}$ mouse embryonic fibroblasts (MEFs) infected with KLF4-encoding or control retrovirus. (**b**) Wild-type and *p21*$^{CIP1-/-}$ MEFs infected with KLF4-encoding or control retrovirus were photographed at ×100 magnification, 7 days after seeding. (**c**) U2OS cells were transfected with control, cyclin-D1 expression or p21^{CIP1} short hairpin RNA (shRNA) plasmids and analysed by FACS, as described for Fig. 6d, or by western blotting (insert). (**d**) Cells were treated as in (**b**), and used in a colony-formation assay. A representative of four independent experiments is shown. (**e**) Cells grown on plates as shown in (**d**) were trypsinized and used in a soft-agar assay and photographed at ×100 magnification after 2 weeks. In four different experiments, per 2.5×10^4 cells seeded, control *p21*$^{CIP1-/-}$ MEFs never formed any colonies visible to the naked eye, KLF4-expressing *p21*$^{CIP1-/-}$ MEFs consistently formed between 300 and 400 colonies, and RAS^{V12} expressing *p53*$^{-/-}$ MEFs formed 500 colonies.

Numerous 'classical' examples exist of oncogenic collaboration between mutant *RAS* and immortalizing genes, including *MYC*, *E1A*, *TBX2* and *DRIL1* (refs 29,36–38). Similarly, inactivation of tumour-suppressor genes, such as *p19ARF* or *p53*, each of which leads to immortalization, cooperates with RAS^{V12} in stimulating proliferation[28]. KLF4 is unique amongst these genes in that it acts as a potent cell-cycle inhibitor rather than an immortalizing gene in most settings, including in primary cells. However, within an appropriate genetic milieu, KLF4 apparently switches to become a stimulator of cell proliferation.

the duality of KLF4 function is reminiscent of the transforming growth factor-β (TGF-β) pathway. The TGF-β cytokine acts cytostatically in early-stage cancers, whereas it promotes tumorigenicity of more malignant lesions, which is conceivably related to changes in its genetic context during tumour progression[39]. It is worth noting that the signalling pathways that are used by KLF4 (refs 4,6; this paper) and TGF-β[40–42] both seem to converge on p21^{CIP1}. Furthermore, we show that KLF4 represses p53 transcription through a so-called PE21 element in the p53 promoter. This element has previously been reported to be responsible for repression of p53 expression by the cytokine Oncostatin-M[32]. Intriguingly, there are a number of additional phenotypic similarities between Oncostatin-M and KLF4. Both can, depending on the cellular context, function either as an inhibitor or a promoter of cell proliferation (ref. 43; this paper). In addition, besides suppressing p53 transcription, both can also induce p21^{CIP1} (ref. 44) levels, again supporting a prominent role for p21^{CIP1} in such settings.

Our data highlight that, in specific genetic settings, p21^{CIP1} acts as a gatekeeper to prevent oncogenic transformation. Surprisingly, it is not frequently deleted in human tumours[45], which may, at least in part, be related to its contribution to assembling the cyclin-D1–CDK4/6 kinases[35]. However, we demonstrate here that activation of the RAS^{V12}–cylin-D1 pathway can be functionally equivalent to genetic inactivation of p21^{CIP1}, in that both are sufficient to prevent KLF4 from inhibiting proliferation (Fig. 8a, b). In contrast to *p21*^{CIP1} loss, the former two events occur frequently in human cancer, with RAS being activated in 30% of human tumours[24] and cyclin-D1 protein being overexpressed in 50% of human mammary carcinomas[28]. Interestingly, KLF4 is overexpressed in the

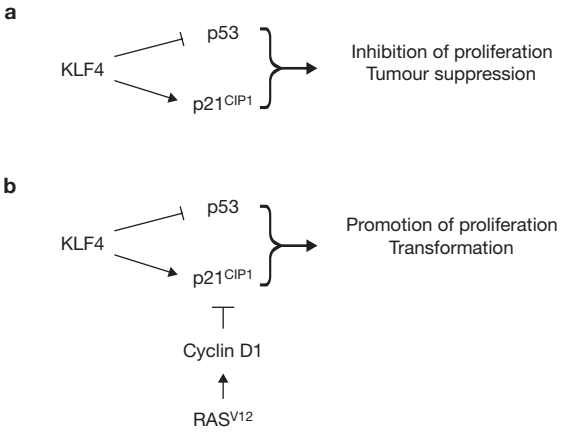

Figure 8 A model for KLF4 function in tumour suppression and oncogenic transformation. (**a**) KLF4 simultaneously represses p53 levels and induces $p21^{CIP1}$, the latter of which represents the dominant response in normal cells, resulting in cell-cycle arrest. This probably represents part of the tumour-suppression function of KLF4. (**b**) In the absence of $p21^{CIP1}$, or in the presence of RAS^{V12}–cyclin-D1 signalling, the anti-proliferative effect of KLF4 is neutralized. This results in cellular transformation and contributes to the oncogenic function of KLF4. Although KLF4 may have functions in addition to those identified here, our results indicate that the downregulation of p53 and the induction of $p21^{CIP1}$ represent key features of KLF4 signalling. Arrows represent activating signals and inhibitory symbols represent repressing or inactivating signals.

majority of mammary carcinomas[10,21,22], which could suggest that KLF4 and functional loss of $p21^{CIP1}$ collaborate in human cancer.

Together, these data strongly suggest that the physiological, tumour-suppressive role of KLF4 is related to its ability to act as a $p21^{CIP1}$-dependent suppressor of proliferation. This is supported by the observation that mice deficient for Klf4 in their gastric epithelium, as well as developing premalignant lesions, also display decreased $p21^{CIP1}$ levels[18]. By contrast, in tumours that overexpress KLF4 and in which $p21^{CIP1}$ function is neutralized, the inhibitory effect of KLF4 on p53 is likely to represent its dominant function, thereby promoting cell proliferation and/or cell survival. In keeping with this, p53 is not frequently mutated in sporadic breast cancer. This may be explained, at least in part, by the high levels of endogenous KLF4 in these tumours.

Finally, even though we show that inhibition of KLF4 expression can drive cultured breast cancer cells into apoptosis, our results predict that (systemic) targeting of KLF4 in anti-cancer therapy will yield tissue-specific effects. Indeed, in cells that have functional $p21^{CIP1}$ (that is lack $p21^{CIP1}$-neutralizing lesions, such as a deregulated RAS–cyclin-D1 pathway), KLF4 probably acts as a tumour suppressor, rather than as an oncogene. Clearly, inactivation of KLF4 would not be desirable in such settings. Furthermore, our observation that high levels of KLF4 cause resistance to drug-induced apoptosis may be of relevance to therapeutic intervention of those mammary carcinomas that overexpress KLF4. It predicts that these tumours may have a selective survival advantage after treatment relative to untransformed cells, contrary to the objective of cancer therapy. Rather, inactivation of KLF4 may be desirable, specifically in tumours that both overexpress KLF4 and have a compromised $p21^{CIP1}$ function. □

METHODS

Cell culture experiments. A functional genome-wide screen for genes that rescue RAS^{V12}-induced senescence in BTR cells has been described previously[29]. KLF4 was isolated from a mouse embryo retroviral cDNA library (Clontech, Mountain View, CA). Retroviral infections of BTR cells were performed at 32°C and cultures were shifted to the non-permissive temperature of 39°C at 2 d after infection. MEFs were cultured and infected as described previously[46]. For colony-formation assays, cells were fixed with 4% formaldehyde and stained with 0.1% crystal violet. Soft-agar assays were performed as described previously[29]. To inhibit the proteosome, 10 μM CBZ-LLL was added to the cell culture medium 5 h prior to preparing cell extracts for western blot analysis.

Protein, RNA and FACS analyses. Western blotting was performed as described previously[46]. For northern blot analysis, RNA was isolated using Trizol, separated on a 1% agarose gel, transferred to nitrocellulose and hybridized with a radiolabelled murine p53 cDNA probe. Apoptosis assays were performed by treatment with DiOC6(3) (Invitrogen, Carlsbad, CA) in phosphate-buffered saline for 15 min at 37°C and subsequent FACS analysis. For cell-cycle experiments, U2OS cells were transfected with 2 μg of control or pcDNA3.1–KLF4 plasmids, 4 μg of pCMV–cyclin-D1 or pSUPER–$p21^{CIP1}$ and 200 ng pBABEpuro. Twenty-four hours after transfection, cells were selected with 4 μg ml^{-1} puromycin for 40 h. Nocodazole (50 ng ml^{-1}) was added 48 h after transfection and FACS analysis was performed 16 h later.

Promoter analyses. The reporter assays in Fig. 3a were performed by transfection of NIH-3T3 cells with 10 μg of pGL2–p100–luciferase, 50 ng of cytomegalovirus (CMV) *Renilla* luciferase and 0, 1 or 10 μg of pcDNA3.1–KLF4 or pcDNA3.1. Luciferase activity was measured 48 h after transfection. p53 promoter analyses were performed by transfection of Phoenix cells with 5 μg of pGL2–p53 promoter 2.4 kb or mutants, 2 ng of CMV *Renilla* luciferase and 2 μg of pcDNA3.1–KLF4 or pcDNA3.1. Luciferase activity was measured 72 h after transfection. ChIP experiments were performed, in essence as described previously[46]. MDA-MB-134 cells were cross-linked for 30 min and antibodies H-180 (for KLF4) and C-14 (for TrkB) (both Santa Cruz Biotechnology, Santa Cruz, CA) were used. Real-time PCR was performed using primers targeting the region of the p53 promoter that includes the PE21 element (5′-GCCCTTACTTGTCATGGCGA-3′ and 5′-CAATCCCATCAACCCCTGC-3′) and primers for exon 8 of GAPDH (5′-TGAACGGGAAGCTCACTGG-3′ and 5′-CACCACTGACACGTTGGCAG-3′).

Plasmids. From pcDNA3–KLF4 (ref. 47), KLF4 was subcloned into pLIPE and pBABEpuro retroviral vectors. Dominant-negative KLF4 was constructed by deleting the *Msc*I fragment, which contains the activation and repression domains, resulting in a KLF4 that retains its DNA-binding domain. For RNA interference experiments, we used pSUPER–$p21^{CIP1}$ (ref. 48); pRetroSUPER–murine p53 (ref. 49); pRetroSUPER–human p53 (ref. 30); pRetroSUPER–cyclin-D1 (encoding an shRNA with the sense sequence: GATGAAGGAGACCATTCCC); and pRetroSUPER–KLF4 #1 (GATCAAGCAGGAGGCGGTCTC), #2 (GGACGGCTGTGGATGGAAA) and #3 (GAGTTCCCATCTCAAGGCACACC). The p53 promoter deletion mutants '–320' and '–78' were constructed by cloning the *Hind*III and *Alu*I fragments from pGL2–basic–luciferase containing the 2.4 kb human p53 promoter[50] into the *Hind*III and *Sma*I sites of pGL2–basic, respectively. The Sp1 and PE21 mutants were generated by site-directed mutagenesis, using primers with the core elements mutated (GAATCCTGACTCTGCACCCTAAGAACCAACTCCATTTCCTTTG C at –160 and GCCTCGCAGGGGTTGATGAGCTCGGGGTTTTCCCCTCCC at –55, respectively; the mutations are underlined).

Antibodies. Primary antibodies used were: Ab-7 for murine p53 (Calbiochem, San Diego, CA); R562 for $p19^{ARF}$ (Abcam, Cambridge, UK); and H-180 for KLF4, Do-1 for human p53, C-19 for $p21^{CIP1}$, H-295 for cyclin-D1, M-156 for $p16^{INK4a}$ and C22 for CDK4 (all Santa Cruz Biotechnology).

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Note: Supplementary Information is available on the Nature Cell Biology website.

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COMPETING FINANCIAL INTERESTS
The authors declare that they have no competing financial interests.

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1. Shields, J. M., Christy, R. J. & Yang, V. W. Identification and characterization of a gene encoding a gut-enriched Kruppel-like factor expressed during growth arrest. *J. Biol. Chem.* **271,** 20009–20017 (1996).
2. Garrett-Sinha, L. A., Eberspaecher, H., Seldin, M. F. & de Crombrugghe, B. A gene for a novel zinc-finger protein expressed in differentiated epithelial cells and transiently in certain mesenchymal cells. *J. Biol. Chem.* **271,** 31384–31390 (1996).
3. Chen, X., Whitney, E. M., Gao, S. Y. & Yang, V. W. Transcriptional profiling of Kruppel-like factor 4 reveals a function in cell cycle regulation and epithelial differentiation. *J. Mol. Biol.* **326,** 665–677 (2003).
4. Chen, X. *et al.* Kruppel-like factor 4 (gut-enriched Kruppel-like factor) inhibits cell proliferation by blocking G1/S progression of the cell cycle. *J. Biol. Chem.* **276,** 30423–30428 (2001).
5. Geiman, D. E., Ton-That, H., Johnson, J. M. & Yang, V. W. Transactivation and growth suppression by the gut-enriched Kruppel-like factor (Kruppel-like factor 4) are dependent on acidic amino acid residues and protein-protein interaction. *Nucleic Acids Res.* **28,** 1106–1113 (2000).
6. Zhang, W. *et al.* The gut-enriched Kruppel-like factor (Kruppel-like factor 4) mediates the transactivating effect of p53 on the p21WAF1/Cip1 promoter. *J. Biol. Chem.* **275,** 18391–18398 (2000).
7. Jaubert, J., Cheng, J. & Segre, J. A. Ectopic expression of kruppel like factor 4 (Klf4) accelerates formation of the epidermal permeability barrier. *Development* **130,** 2767–2777 (2003).
8. Katz, J. P. *et al.* The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. *Development* **129,** 2619–2628 (2002).
9. Segre, J. A., Bauer, C. & Fuchs, E. Klf4 is a transcription factor required for establishing the barrier function of the skin. *Nature Genet.* **22,** 356–360 (1999).
10. Black, A. R., Black, J. D. & Azizkhan-Clifford, J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. *J. Cell Physiol.* **188,** 143–160 (2001).
11. Ohnishi, S. *et al.* Downregulation and growth inhibitory effect of epithelial-type Kruppel-like transcription factor KLF4, but not KLF5, in bladder cancer. *Biochem. Biophys. Res. Commun.* **308,** 251–256 (2003).
12. Dang, D. T., Mahatan, C. S., Dang, L. H., Agboola, I. A. & Yang, V. W. Expression of the gut-enriched Kruppel-like factor (Kruppel-like factor 4) gene in the human colon cancer cell line RKO is dependent on CDX2. *Oncogene* **20,** 4884–4890 (2001).
13. Dang, D. T. *et al.* Decreased expression of the gut-enriched Kruppel-like factor gene in intestinal adenomas of multiple intestinal neoplasia mice and in colonic adenomas of familial adenomatous polyposis patients. *FEBS Lett.* **476,** 203–207 (2000).
14. Ton-That, H., Kaestner, K. H., Shields, J. M., Mahatanankoon, C. S. & Yang, V. W. Expression of the gut-enriched Kruppel-like factor gene during development and intestinal tumorigenesis. *FEBS Lett.* **419,** 239–243 (1997).
15. Shie, J. L. *et al.* Role of gut-enriched Kruppel-like factor in colonic cell growth and differentiation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279,** G806–G814 (2000).
16. Zhao, W. *et al.* Identification of Kruppel-like factor 4 as a potential tumor suppressor gene in colorectal cancer. *Oncogene* **23,** 395–402 (2004).
17. Wei, D. *et al.* Drastic down-regulation of Kruppel-like factor 4 expression is critical in human gastric cancer development and progression. *Cancer Res.* **65,** 2746–2754 (2005).
18. Katz, J. P. *et al.* Loss of Klf4 in mice causes altered proliferation and differentiation and precancerous changes in the adult stomach. *Gastroenterology* **128,** 935–945 (2005).
19. Luo, A. *et al.* Discovery of Ca(2+)-relevant and differentiation-associated genes downregulated in esophageal squamous cell carcinoma using cDNA microarray. *Oncogene* **1,** 1 (2003).
20. Dang, D. T. *et al.* Overexpression of Kruppel-like factor 4 in the human colon cancer cell line RKO leads to reduced tumorigenecity. *Oncogene* **22,** 3424–3430 (2003).
21. Foster, K. W. *et al.* Increase of GKLF messenger RNA and protein expression during progression of breast cancer. *Cancer Res.* **60,** 6488–6495 (2000).
22. Foster, K. W. *et al.* Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLF. *Cell Growth Differ.* **10,** 423–434 (1999).
23. Foster, K. W. *et al.* Induction of KLF4 in basal keratinocytes blocks the proliferation-differentiation switch and initiates squamous epithelial dysplasia. *Oncogene* **24,** 1491–1500 (2005).
24. Bos, J. L. Ras oncogenes in human cancer: a review. *Cancer Res.* **49,** 4682–4689 (1989).
25. Palmero, I., Pantoja, C. & Serrano, M. p19ARF links the tumour suppressor p53 to Ras. *Nature* **395,** 125–126 (1998).
26. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88,** 593–602 (1997).
27. Peeper, D. S., Dannenberg, J. H., Douma, S., te Riele, H. & Bernards, R. Escape from premature senescence is not sufficient for oncogenic transformation by Ras. *Nature Cell Biol.* **3,** 198–203 (2001).
28. Sherr, C. J. & McCormick, F. The RB and p53 pathways in cancer. *Cancer Cell* **2,** 103–112 (2002).
29. Peeper, D. S. *et al.* A functional screen identifies hDRIL1 as an oncogene that rescues RAS-induced senescence. *Nature Cell Biol.* **4,** 148–153 (2002).
30. Brummelkamp, T. R., Bernards, R. & Agami, R. Stable suppression of tumorigenicity by virus-mediated RNA interference. *Cancer Cell* **2,** 243–247 (2002).
31. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J. & Leder, P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* **82,** 675–684 (1995).
32. Li, C. *et al.* The critical role of the PE21 element in oncostatin M-mediated transcriptional repression of the p53 tumor suppressor gene in breast cancer cells. *Oncogene* **20,** 8193–8202 (2001).
33. Filmus, J. *et al.* Induction of cyclin D1 overexpression by activated ras. *Oncogene* **9,** 3627–3633 (1994).
34. Peeper, D. S. *et al.* Ras signalling linked to the cell-cycle machinery by the retinoblastoma protein. *Nature* **386,** 177–181 (1997).
35. Sherr, C. J. & Roberts, J. M. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* **13,** 1501–1512 (1999).
36. Jacobs, J. J. *et al.* Senescence bypass screen identifies TBX2, which represses Cdkn2a (p19(ARF)) and is amplified in a subset of human breast cancers. *Nature Genet.* **26,** 291–299 (2000).
37. Land, H., Parada, L. F. & Weinberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304,** 596–602 (1983).
38. Ruley, H. E. Adenovirus region E1A enables viral and cellular transforming genes to transform primary cells in cultures. *Nature* **304,** 602–606 (1983).
39. Siegel, P. M. & Massague, J. Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. *Nature Rev. Cancer* **3,** 807–820 (2003).
40. Li, C. Y., Suardet, L. & Little, J. B. Potential role of WAF1/Cip1/p21 as a mediator of TGF-β cytoinhibitory effect. *J. Biol. Chem.* **270,** 4971–4974 (1995).
41. Elbendary, A. *et al.* Transforming growth factor β1 can induce CIP1/WAF1 expression independent of the p53 pathway in ovarian cancer cells. *Cell Growth Differ.* **5,** 1301–1307 (1994).
42. Datto, M. B. *et al.* Transforming growth factor β induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc. Natl Acad. Sci. USA* **92,** 5545–5549 (1995).
43. Zarling, J. M. *et al.* Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. *Proc. Natl Acad. Sci. USA* **83,** 9739–9743 (1986).
44. Bellido, T., O'Brien, C. A., Roberson, P. K. & Manolagas, S. C. Transcriptional activation of the p21(WAF1,CIP1,SDI1) gene by interleukin-6 type cytokines. A prerequisite for their pro-differentiating and anti-apoptotic effects on human osteoblastic cells. *J. Biol. Chem.* **273,** 21137–21144 (1998).
45. Shiohara, M. *et al.* Absence of WAF1 mutations in a variety of human malignancies. *Blood* **84,** 3781–3784 (1994).
46. Rowland, B. D. *et al.* E2F transcriptional repressor complexes are critical downstream targets of p19(ARF)/p53-induced proliferative arrest. *Cancer Cell* **2,** 55–65 (2002).
47. Jenkins, T. D., Opitz, O. G., Okano, J. & Rustgi, A. K. Transactivation of the human keratin 4 and Epstein-Barr virus ED-L2 promoters by gut-enriched Kruppel-like factor. *J. Biol. Chem.* **273,** 10747–10754 (1998).
48. Berns, K. *et al.* A large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* **428,** 431–437 (2004).
49. Dirac, A. M. & Bernards, R. Reversal of senescence in mouse fibroblasts through lentiviral suppression of p53. *J. Biol. Chem.* **278,** 11731–11734 (2003).
50. Raman, V. *et al.* Compromised HOXA5 function can limit p53 expression in human breast tumours. *Nature* **405,** 974–978 (2000).

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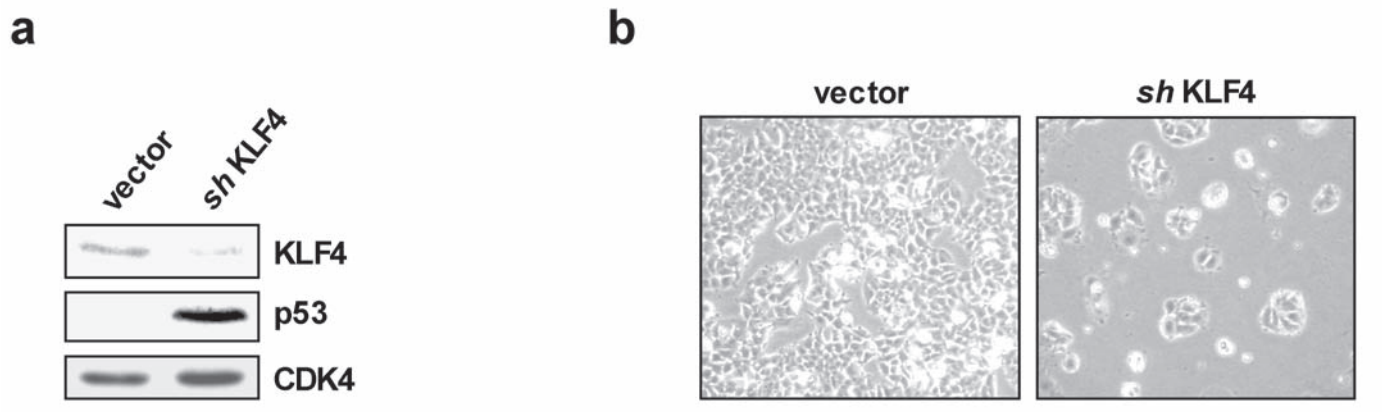

Figure S1 Endogenous KLF4 suppresses p53 expression and promotes survival of MCF7 cells. MCF7 cells were infected with control or sh KLF4 (#2) encoding retroviruses and analyzed by Western Blotting (a) and photography at 50 x (b) after five days. CDK4 serves as loading control.