

# Bach1-mediated suppression of p53 is inhibited by p19<sup>ARF</sup> independently of MDM2

Hironari Nishizawa,<sup>1,5</sup> Kazushige Ota,<sup>1,5</sup> Yoshihiro Dohi,<sup>1,3</sup> Tsuyoshi Ikura<sup>1,4</sup> and Kazuhiko Igarashi<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan

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Cellular senescence prevents the aberrant proliferation of damaged cells. The transcription factor Bach1 binds to p53 to repress cellular senescence, but it is still unclear how the Bach1–p53 interaction is regulated. We found that the Bach1–p53 interaction was inhibited by oncogenic Ras, bleomycin, and hydrogen peroxide. Proteomics analysis of Bach1 complex revealed its interaction with p19<sup>ARF</sup>, a tumor suppressor that competitively inhibited the Bach1–p53 interaction when overexpressed within cells. Reduction of MDM2 expression in wild-type murine embryonic fibroblasts (MEFs) did not result in slower proliferation, showing that Bach1 plays a role in keeping the proliferation of MEFs independent of MDM2. Consistent with this interpretation, expression of p21 was highly induced in MEFs when both Bach1 and MDM2 were abrogated. The level of Bach1 protein was reduced on knockdown of p53. These results suggest that p53 activation involves its dissociation from Bach1, achieved in part by the competitive binding of p19<sup>ARF</sup> to Bach1. The p19<sup>ARF</sup>–Bach1 interaction constitutes a regulatory pathway of p53 in parallel with the p19<sup>ARF</sup>–MDM2 pathway. (*Cancer Sci* 2012; 103: 897–903)

Cellular senescence is an irreversible cell cycle arrest that plays a role as a barrier against malignant transformation.<sup>(1)</sup> Cellular senescence is induced by the tumor suppressors p53 and pRb that integrate diverse signals under genotoxic and oncogenic stresses.<sup>(2,3)</sup> p53 is strictly regulated because of its potentially suicidal functions. For example, p53 possesses a very short half-life under normal conditions due to its polyubiquitination by the E3 ubiquitin ligase MDM2 and subsequent degradation.<sup>(4)</sup> p19<sup>ARF</sup> sequesters MDM2 from p53 by binding to MDM2, resulting in accumulation of p53.<sup>(5–7)</sup> Additional new mechanisms of p53 regulations have been reported, including Bach1,<sup>(8)</sup> CHD8,<sup>(9)</sup> and Cabin1<sup>(10)</sup> as negative regulators. Bach1 forms a complex with p53 on chromatin, recruiting histone deacetylase 1 and thus repressing the transcriptional activator function of p53. Bach1 inhibits cellular senescence of murine embryonic fibroblasts (MEFs) under oxidative stress and oncogenic stress imposed by activated Ras.<sup>(8)</sup> Thus Bach1 may potentially modulate the tumor suppressor function of p53. Bach1 represses the expression of a set of p53-target genes that collaborate to induce senescence.<sup>(11)</sup> However, the mechanism for the regulation of the Bach1–p53 interaction remains to be elucidated.

In addition to the cellular senescence, Bach1 represses the oxidative stress response.<sup>(12)</sup> Bach1 binds to a cis-regulatory Maf-recognition element (MARE) by forming a heterodimer with the small Maf oncoproteins to repress genes for oxidative stress including heme oxygenase-1 (HO-1) and ferritin.<sup>(12–14)</sup> As HO-1 protects cells and tissues from oxidative stress by reducing reactive oxygen species and producing carbon monoxide (CO),<sup>(15)</sup> Bach1-deficient mice, in which HO-1 is de-repressed in diverse tissues and cells, are more resistant to various insults including oxidative stress.<sup>(16–20)</sup> HO-1 also induces arrest of cell proliferation through CO-mediated

activation of the p21–Rb pathway.<sup>(21)</sup> Collectively, Bach1 connects two critical pathways of cellular senescence and the oxidative stress response. In this study, we asked how the Bach1–p53 interaction was regulated and found that the tumor suppressor p19<sup>ARF</sup> interacted with Bach1 and inhibited its interaction with p53. By examining genetic interactions among Bach1, MDM2, and p19<sup>ARF</sup>, we found that Bach1 and MDM2 were independently required for p53 suppression. These results suggest that p19<sup>ARF</sup> may activate p53 to induce senescence by relieving the Bach1-mediated inhibition in addition to the MDM2-mediated degradation of p53.

## Materials and Methods

**Isolation and culture of MEFs.** Mouse embryonic fibroblasts were isolated from 14.5-day-old embryos of various genotypes, as previously described.<sup>(8)</sup> The MEFs from single embryos were plated into a 100-mm diameter culture dish and incubated at 37°C. Cells were cultured in 20% or 3% oxygen by subculturing in a 60- or 100-mm diameter culture dish every 2–3 days, and cell number was determined at each passage.

**Plasmids.** The expression plasmids for FLAG-tagged Bach1 and its derivatives have been described previously.<sup>(22,23)</sup> Plasmids pCMXp53 and pCMV-SPORT6p19<sup>ARF</sup> were obtained from Life Technologies (Carlsbad, CA, USA). Biotin-tagged Bach1 expression plasmid was described previously.<sup>(24)</sup>

**Pull-down assays.** The 293T cells were transfected in 10-cm dishes with 2 or 3 µg each of indicated plasmids by Genejuice (EMD Chemicals, Darmstadt, Germany). After 24 h, the total protein extracts were prepared and precipitated with streptavidin beads or anti-FLAG M2 agarose beads as described previously.<sup>(8)</sup> Bleomycin and hydrogen peroxide were added to the culture medium at 25 or 50 µM and at 50 or 100 µM respectively for the last 8 h.

**Immunoblotting analysis.** The immunoblotting analysis was carried out with the following antibodies: anti-Bach1;<sup>(8)</sup> anti-MafK;<sup>(25)</sup> anti-tubulin (B7; Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-p19 (ab80; Abcam, Cambridge, UK); anti-p53 (CM5p; Leica Microsystems, Wetzlar, Germany); anti-HA (Roche Applied Science, Penzberg, Germany); and anti-Flag (Sigma-Aldrich, St. Louis, MO, USA). Whole-cell extracts were prepared from wild-type and Bach1-deficient MEFs and the extracts were separated by SDS-PAGE. Following SDS-PAGE, the proteins were electrotransferred to PVDF membrane (Millipore, Billerica, MA, USA) and reacted with antibodies as

<sup>2</sup>To whom correspondence should be addressed.

E-mail: igarashi@med.tohoku.ac.jp

<sup>3</sup>Present address: Department of Cardiovascular Physiology and Medicine Hiroshima University Graduate School of Biomedical Science Hiroshima 734-8551 Japan.

<sup>4</sup>Present address: Radiation Biology Center Kyoto University Sakyo-ku Kyoto 606-8501 Japan.

<sup>5</sup>These authors contributed equally to this work.

previously described.<sup>(8)</sup> The densities of signals were quantified with NIH Image 1.63 (<http://rsb.info.nih.gov/ni-image/>).

**RNA extract and quantitative RT-PCR.** Total RNAs was prepared from MEFs using the Total RNA Isolation minikit (Agilent Technologies). Quantitative PCR experiments were carried out with a LightCycler (Roche) using the following primers:

p19, 5'-GCTCTGGCTTTCGTGAACA-3', 5'-TCGAATCTG-CACCGTAGTTG-3';

MDM2, 5'-AGACAGGAGAAAGCGATAACA-3', 5'-TTTCC-CCTTATCGTCTGG-3';

Bach1, 5'-GCCCCGTATGCTTGTGTGATT-3', 5'-CGTGAG-AGCGAAATTATCCG-3';

p21, 5'-CCTGGTGATGTCGACCTGTT-3', 5'-GGGGAATCTTCAGGCCGCTC-3'; and  $\beta$ -actin, 5'-CG-TTGACATCCGTAAGACCTC-3', 5'-AGCCACCGATCCA-CACAGA-3'.

**RNA interference.** Stealth RNAi duplexes were designed for the target genes using the BLOCK-iT RNAi Designer (Invitrogen). For the knockdown of the target genes,  $2.0 \times 10^6$  cells were transfected with 6  $\mu$ L stock Stealth RNAi duplexes (20  $\mu$ M) using the basic nucleofection solution for MEFs (Lonza, Koeln, Germany). Stealth RNAi Negative Control duplexes (Invitrogen) were used as negative control RNAi. The transfected cells were divided into three parts and cultured in 60-mm diameter culture dishes. The cell number was determined at the indicated days. Sequences of the Stealth RNAi used in this study were: p19<sup>ARF</sup> RNAi, 5'-ACAACAUGUUC-ACGAAAGCCAGAGC-3'; and MDM2 RNAi, 5'-UCUUCU-GUCACUAAUGGAUCUCC-3'.

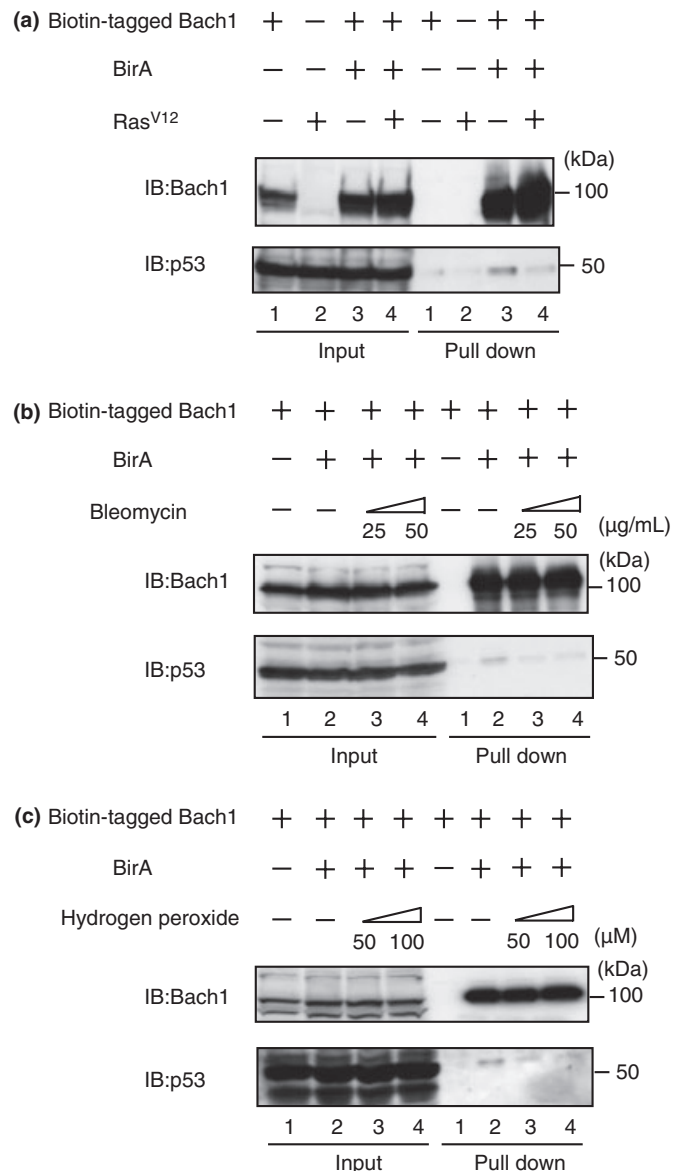
**Bach1 complex purification and analysis.** The Bach1 complex was purified from nuclear extracts prepared from MEL cells stably expressing eBach1 and they were analyzed as described previously.<sup>(8)</sup>

**Statistical analysis.** Data was presented as mean  $\pm$  SD. All the statistical analyses were done by Student's test or Welch's correction. A value of  $P < 0.05$  was considered of significance in all tests.

## Results

**Inhibition of the interaction between Bach1 and p53 by genotoxic stresses.** We applied biotin-tagging pull-down of Bach1<sup>(24)</sup> to examine changes in the interaction between Bach1 and p53 in response to p53 activating signals. When biotin-tagged Bach1 was expressed in 293T cells and pulled down with streptavidin beads, endogenous p53 was also recovered (Fig. 1a). When the cells were transfected with Ras<sup>V12</sup> plasmid, the Bach1-p53 interaction was clearly inhibited (Fig. 1a). Similarly, when the cells were treated with bleomycin or hydrogen peroxide, their interaction was also inhibited (Fig. 1b,c). These results suggest that dissociation of p53 from Bach1 is induced in response to several types of genotoxic stresses, presumably leading to p53 activation.

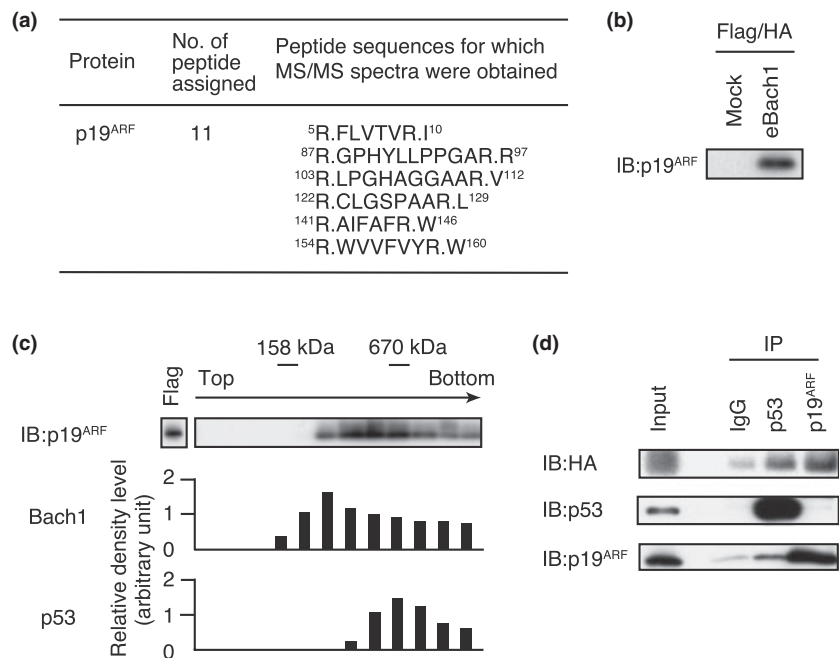
**Analysis of the Bach1 complex.** We speculated that genotoxic stresses activated a factor that inhibited the interaction between Bach1 and p53. A previous report described the purification of FLAG and HA-tagged Bach1 (eBach1) from MEL cells and characterized its associated proteins.<sup>(8)</sup> Further mass spectrometry analysis of Bach1-associated proteins identified p19<sup>ARF</sup> in the Bach1 complex (Fig. 2a), which was confirmed by Western blot analyses (Fig. 2b). When Bach1-enriched material obtained by single anti-FLAG affinity purification of eBach1 was size-fractionated by a 10–35% glycerol gradient sedimentation, p19<sup>ARF</sup> was found in the relatively faster sedimenting fractions where Bach1 and p53 were also present (Fig. 2c). To investigate the relationships among p53, p19<sup>ARF</sup>, and Bach1, p53 or p19<sup>ARF</sup> was immunoprecipitated from the Bach1-enriched material. p53 and p19<sup>ARF</sup> showed counter-enrichment



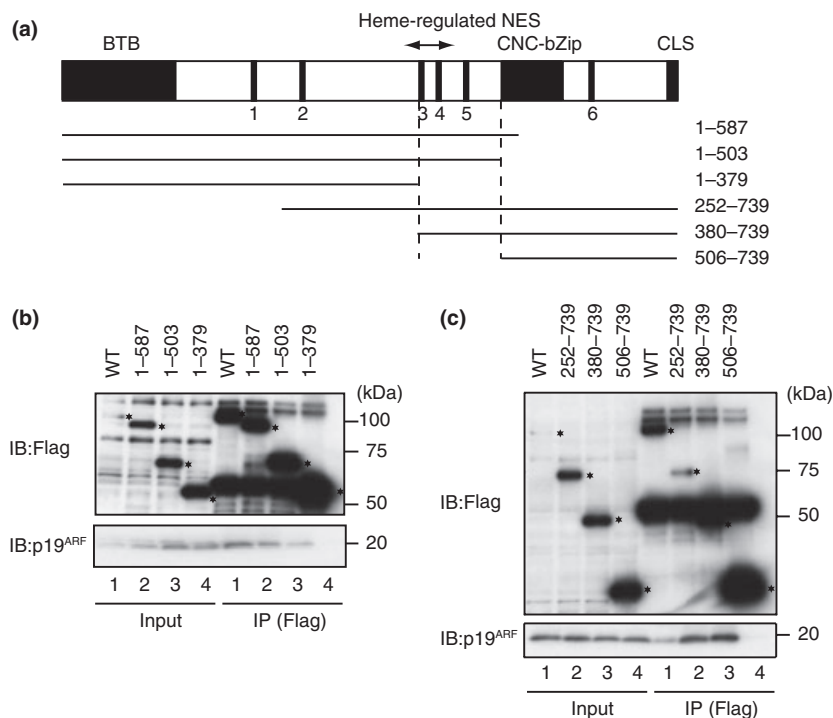
**Fig. 1.** Dissociation of Bach1 and p53 in response to Ras<sup>V12</sup>, DNA damage, and oxidative stress. Pull-down assays of FLBio-Bach1 and endogenous p53 in 293T cells in the presence of Ras<sup>V12</sup> (a), bleomycin (b), and hydrogen peroxide (c). Biotinylated Bach1 was pulled down with streptavidin beads, followed by immunoblotting (IB) analysis with the indicated antibodies.

of each other while Bach1 was precipitated with each antibody. These results show that p19<sup>ARF</sup> was a component of the Bach1 complex and raised the possibility that it may be involved in the regulation of the p53-Bach1 interaction.

**Mapping of the p19<sup>ARF</sup>-binding region on Bach1.** Bach1 possesses several functional domains including the N-terminal BTB domain mediating homodimer formation<sup>(26,27)</sup> and the C-terminal basic region-leucine zipper (bZip) domain forming heterodimers with Maf proteins to bind to DNA. To examine the specificity of the Bach1-p19<sup>ARF</sup> binding, the regions of Bach1 involved in the interaction with p19<sup>ARF</sup> were mapped using various deletion FLAG-Bach1 proteins (Fig. 3a). When overexpressed in 293T cells, the full-length FLAG-Bach1 was precipitated with anti-FLAG antibody together with exogenous p19<sup>ARF</sup> (Fig. 3b). Both the N-terminal and C-terminal deletion fragments failed to interact with p19<sup>ARF</sup> when a region preceding the bZip domain was deleted (Fig. 3b,c), indicating that



**Fig. 2.** Analysis of the purified Bach1 complex. (a) The identification of p19<sup>ARF</sup> by mass spectrometry. Amino acid sequences of peptides were determined by mass spectrometry/mass spectrometry (MS/MS) analysis. (b) Flag- and HA-purified Bach1 complex sample was analyzed by immunoblotting (IB) analysis using p19<sup>ARF</sup> antibody. (c) Glycerol gradient analysis of the Bach1 complex. The fractions were further analyzed by IB using p19<sup>ARF</sup> antibody. Bach1 and p53 in each fraction analyzed by IB using Bach1 or p53 antibodies were quantified by densitometry. (d) Flag-purified Bach1 complex sample was immunoprecipitated (IP) and analyzed by IB with the indicated combinations of antibodies.

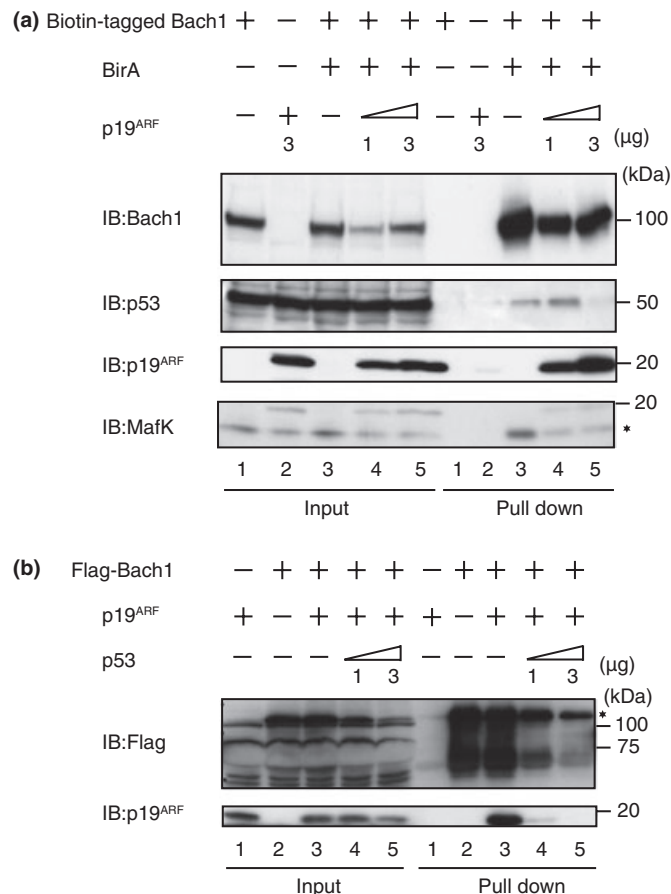


**Fig. 3.** Mapping of the p19<sup>ARF</sup>-interacting region on Bach1. (a) A schematic representation of Bach1 and its deletion derivatives. CP motifs are indicated with filled boxes and numbers. BTB domain/POZ domain, CNC homology-basic region-leucine zipper (bZip), and cytoplasmic localization signal (CLS) are also shown with filled boxes. Heme-regulated nuclear export signal (NES) is shown with an arrow. The boundaries of the p19<sup>ARF</sup>-binding region are shown by the vertical dashed lines. (b,c) Co-immunoprecipitation assays of Bach1 and p19<sup>ARF</sup>. The 293T cells were transfected with indicated combinations of expression plasmids for FLAG-tagged Bach1 derivatives and p19<sup>ARF</sup>, then subjected to immunoprecipitation (IP) with anti-FLAG antibodies followed by immunoblot (IB) analysis with the p19<sup>ARF</sup> antibodies. The asterisks indicate bands of FLAG-tagged Bach1 derivatives.

this region between amino acid residues 380 and 503 was involved in the interaction with p19<sup>ARF</sup>.

**Competitive binding of p53 and p19<sup>ARF</sup> to Bach1.** The above results, and the fact that Bach1 inhibits p53 function, suggested that Bach1–p19<sup>ARF</sup> interaction would regulate p53 function. To clarify the interaction among Bach1, p53, and p19<sup>ARF</sup>, pull-down assays with biotin-tagged Bach1 were carried out (Fig. 4a). When biotin-tagged Bach1 was overexpressed in 293T cells, pull-down of Bach1 with streptavidin beads brought down endogenous p53 (lane 3). Simultaneous overexpression of p19<sup>ARF</sup> clearly inhibited coprecipitation of Bach1 with p53 while p19<sup>ARF</sup> bound to Bach1 in a dose-dependent manner (lanes 4 and 5). Furthermore, overexpressed p19<sup>ARF</sup> inhibited the coprecipitation of Bach1 with MafK.

To confirm the competitive binding of p19<sup>ARF</sup> and p53 to Bach1, p53 was overexpressed and its effect on the Bach1–p19<sup>ARF</sup> interaction was examined in immunoprecipitation assays (Fig. 4b). When FLAG-Bach1 and p19<sup>ARF</sup> were expressed in 293T cells, immunoprecipitation of FLAG-Bach1 brought down p19<sup>ARF</sup> (lane 3). An additional overexpression of p53 clearly inhibited coprecipitation of p19<sup>ARF</sup> with FLAG-Bach1 (lanes 4 and 5). These observations suggest that p53 and p19<sup>ARF</sup> mutually inhibit their interaction with Bach1 and that p19<sup>ARF</sup> also affects the Bach1–MafK interaction.



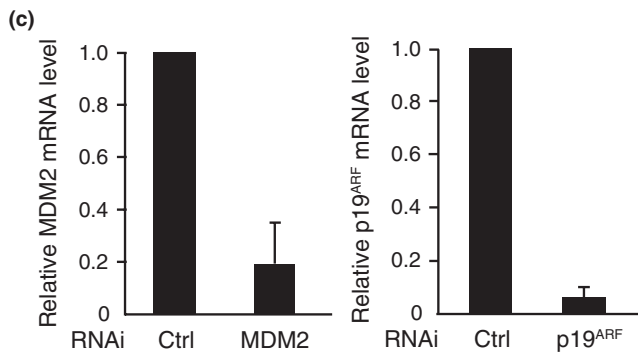
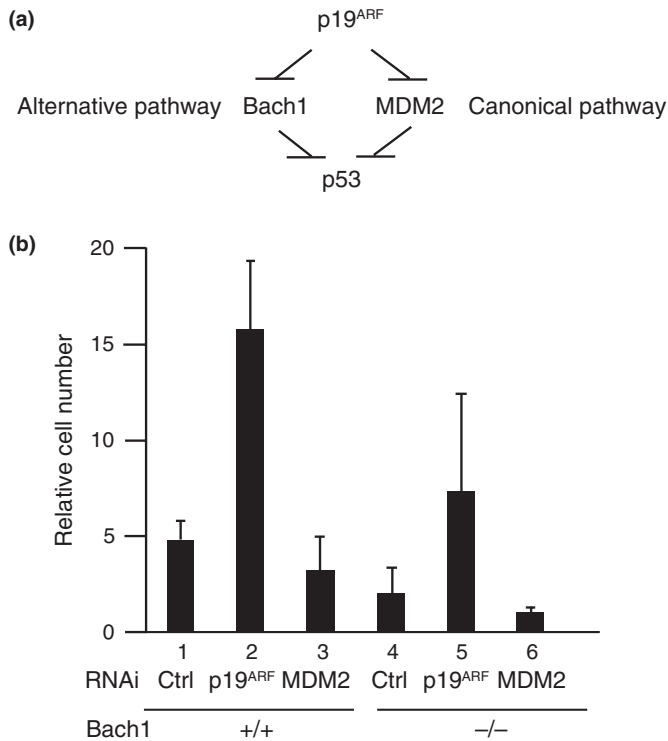
**Fig. 4.** Interactions among Bach1, p53, and p19<sup>ARF</sup>. (a) 293T cells were transfected with expression plasmids for FLBio-Bach1 and p19<sup>ARF</sup>. Biotinylated Bach1 was pulled down with streptavidin beads, followed by an immunoblotting (IB) analysis with the indicated antibodies. (b) 293T cells were transfected with FLAG-Bach1, p19<sup>ARF</sup>, and p53. Extracts were subjected to immunoprecipitation (IP) with anti-FLAG antibodies and were analyzed by IB. The asterisks indicate endogenous MafK or FLAG-Bach1 bands.

**Functions of Bach1 and MDM2 downstream of p19<sup>ARF</sup>.** A key regulatory target of p19<sup>ARF</sup>, MDM2, was not present in the Bach1 complex judging from the results obtained by the mass spectrometry and Western blot analyses (data not shown). Therefore, we speculated that Bach1 might be independent of MDM2 and constitute an alternative pathway for the p19<sup>ARF</sup>-mediated p53 activation (Fig. 5a). To explore the relationship between Bach1, MDM2, and p19<sup>ARF</sup>, reduction of the expressions of MDM2 or p19<sup>ARF</sup> was carried out in wild-type and *Bach1*-deficient MEFs using RNAi (Fig. 5b,c). *Bach1*-deficient MEFs showed slower proliferation due to p53 activation, as reported previously<sup>(8)</sup> (Fig. 5b, lanes 1,5). Elimination of p19<sup>ARF</sup> by knockdown in wild-type MEFs tended to result in more vigorous proliferation than control MEFs with statistical significance (lanes 1,2). A similar effect of p19<sup>ARF</sup> elimination was also observed in *Bach1*-deficient MEFs, although it was not statistically significant (lanes 4,5). Together with our previous reports showing that Bach1 inhibits p53-driven senescence,<sup>(8,11)</sup> we suggest that p53 restricted proliferation of wild-type and *Bach1*-deficient cells under the experimental conditions and that p19<sup>ARF</sup> was involved at least in part in the p53 activation. Reducing MDM2 in wild-type cells resulted in a mild reduction of proliferation, if any (lane 3). Because *Bach1*-deficient MEFs poorly proliferated, the effect of MDM2 reduction was not clear but seemed to further inhibit proliferation (lanes 4,6). Therefore, these results are consistent with the idea that Bach1 inhibited p53 in a way independent of MDM2. This interpretation was further corroborated by changes in p21 expression (Fig. 6). mRNA levels of p21 were higher in *Bach1*-deficient MEFs than control wild-type cells as reported previously.<sup>(8)</sup> MDM2 knockdown resulted in higher p21 expression in both cells, but the levels appeared to be higher in the absence of Bach1 than its presence. The frequency of apoptosis after MDM2 knockdown was similar in *Bach1*-deficient MEFs and wild-type MEFs (data not shown), suggesting a specific function of Bach1 in proliferation. Taken together, our observations suggest that Bach1 and MDM2 constitute parallel pathways both required for inhibiting p53 and maintaining proliferation in MEFs.

**Regulation of Bach1 expression.** MDM2 is a transcriptional target of p53 and constitutes a negative feedback loop by promoting the degradation of p53. The inhibitory function of Bach1 on p53 suggested that the expression of Bach1 might also be regulated by p53. When p53 was reduced by knockdown, mRNA and protein levels of Bach1 decreased in MEFs under normal culture conditions (Fig. 7a,b). Next, we examined whether the expression of Bach1 was affected in response to DNA damage that activates p53. After treating cells with bleomycin, Bach1 mRNA and protein did not show appreciable change (Fig. 7c,d). Higher levels of p21 mRNA after bleomycin treatment showed the activation of p53 (Fig. 7e). Culturing cells under 20% oxygen causes oxidative stress.<sup>(28)</sup> The levels of Bach1 protein decreased when MEFs were cultured under 20% oxygen but not physiological 3% oxygen (Fig. 7f,g). The levels of p21 mRNA increased under 20% oxygen (Fig. 7h). These observations suggest that normal, lower levels of p53 participate in transcriptional regulation of Bach1 but higher levels of p53 induced by genotoxic and oxidative stresses do not further activate Bach1 expression.<sup>(29)</sup> We surmise that p53 maintains the expression of Bach1, thereby forming a negative feedback loop under normal culture conditions. However, this regulation may be superseded by another regulation under oxidative stress. Bach1 protein may be reduced in response to oxidative stress by a post-translational mechanism.

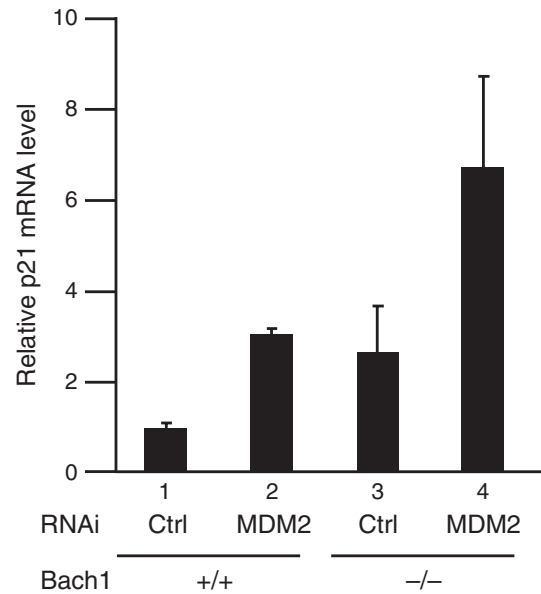
## Discussion

How Bach1 is regulated during the process of cellular senescence is poorly clarified. This issue is critical to understand



**Fig. 5.** Functions of Bach1, MDM2, and p19<sup>ARF</sup> in the regulation of p53. (a) A proposed model of p19<sup>ARF</sup>-p53 pathways through MDM2 (canonical pathway) and Bach1 (alternative pathway). (b) Proliferation of pre-senescent wild-type or *Bach1*-deficient mouse embryonic fibroblasts expressing Stealth RNAi duplexes of control (Ctrl RNAi) or MDM2 or p19<sup>ARF</sup> is compared at day 5 as values relative to day 1. The experiments were carried out independently three times in triplicate and are expressed as means  $\pm$  SD. (c) p19<sup>ARF</sup> and MDM2 mRNA levels 2 days after RNAi were determined by quantitative RT-PCR as relative mean values  $\pm$  SD compared to day 1 control RNAi.

how upstream signals of p53 are integrated to activate or inhibit p53 in the process of cellular senescence. This study showed that p19<sup>ARF</sup> bound to Bach1 in a way mutually exclusive of p53 and suggest that p19<sup>ARF</sup> may alleviate Bach1-mediated inhibition of p53 by binding to Bach1 (Fig. 8). Diverse stimuli including Ras<sup>V12</sup> and oxidative stress induce p19<sup>ARF</sup>.<sup>(28,30)</sup> Therefore, the observed dissociation of Bach1 from p53 (Fig. 1) is likely to involve p19<sup>ARF</sup>. In our model (Fig. 8), p19<sup>ARF</sup> sends divergent signals through inhibiting both Bach1 and MDM2, converging upon p53 activation by different mechanisms (i.e., stabilization and de-repression on chromatin, respectively). Such a two-tiered regulation known as a bi-parallel network motif,<sup>(31)</sup> is expected to confer a sigmoidal response beneficial to integration of multiple inputs

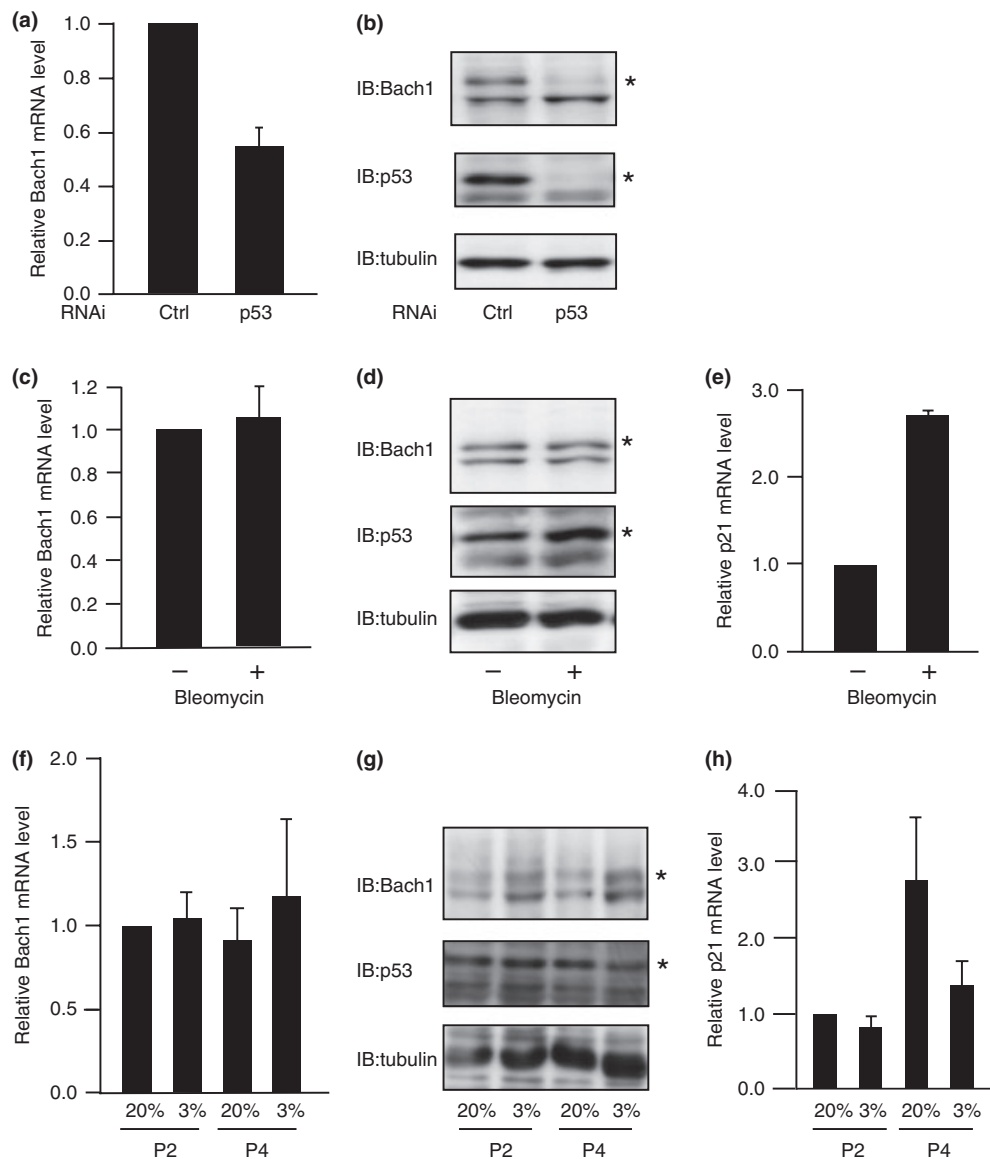


**Fig. 6.** Functions of Bach1 and MDM2 in the regulation of the *p21* gene. p21 mRNA levels at day 3 in pre-senescent wild-type or *Bach1*-deficient mouse embryonic fibroblasts expressing control or MDM2 siRNAs. The experiments were carried out independently three times and the results are expressed as the mean  $\pm$  SD.

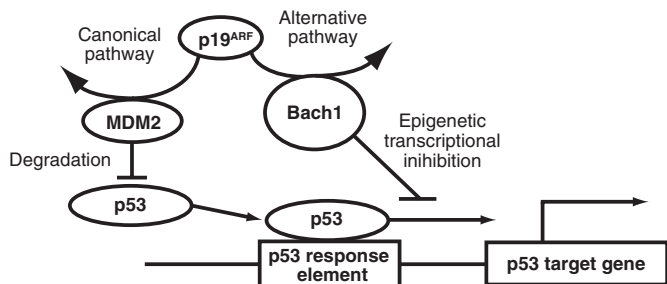
and to preclude spontaneous activation of the senescence program in response to milder stress. Our results in Figures 5(b) and 6 suggest that a partial inhibition of both Bach1 and MDM2 may synergistically activate p53, providing a mechanistic basis for signal integration. It is not clear how p19<sup>ARF</sup> regulates the Bach1-p53 interaction. We have found that the p19<sup>ARF</sup>-Bach1 interaction is indirect (data not shown) and that Bach1-p53 interaction is also indirect.<sup>(8)</sup> Therefore, identification of a protein that mediates their interaction is necessary. It will also be necessary to examine interactions of the endogenous proteins and their changes in response to genotoxic stresses.

Furthermore, p19<sup>ARF</sup> may regulate the p53-independent function of Bach1, because p19<sup>ARF</sup> inhibited the interaction between Bach1 and MafK as well (Fig 4a). It should be noted that the p19<sup>ARF</sup>-binding region of Bach1 contains the Crm1-dependent, heme-regulated nuclear export signal.<sup>(22,32)</sup> Therefore, p19<sup>ARF</sup> could affect the MARE-dependent repressor activity of Bach1 by interacting with this region. Knockdown of p19<sup>ARF</sup> did not affect HO-1 expression in wild-type MEFs (data not shown), suggesting that the effect of p19<sup>ARF</sup> on Bach1 may be specific to a subset of target genes and/or a definite condition.

Our results revealed that Bach1 and MDM2 are both required for the inhibition of p53 in MEFs. Both of them constitute negative feedback loops of p53 (reference 33 and this study). However, their regulatory roles seem to be distinct. Bach1-mediated inhibition operates under normal culture conditions but it becomes void under oxidative stress due to the reduction of Bach1 protein (Fig. 7f). In addition, induction of p53 by DNA damage did not result in further accumulation of Bach1 (Fig. 7d). Therefore, the Bach1-mediated inhibition of p53 might be restricted to normal, homeostatic conditions. This can explain the fact that, while the inhibitory role of Bach1 on p53 predicts the presence of human cancers in which BACH1 is overexpressed, there has been no report on BACH1 overexpression until now. The mechanism by which oxidative stress decreased Bach1 protein is not clear yet. Considering that a cysteine residue of Bach1 is a target of the oxidative



**Fig. 7.** Regulation of Bach1 expression by p53. (a,b) Effects of p53 knockdown on Bach1 mRNA (a) and protein (b) levels are compared. Effects of bleomycin on Bach1 mRNA (c) and protein (d) levels are compared; p21 mRNA levels are compared in (e). (f-h) Mouse embryonic fibroblasts were cultured under 20% or 3% oxygen. Levels of Bach1 mRNA (f) and protein (g) or p21 mRNA (h) are compared at indicated time points. The experiments were carried out independently three times and the mRNA levels are expressed as the mean  $\pm$  SD. Representative images of immunoblotting (IB) are shown. Ctrl, control.



**Fig. 8.** Model of the regulatory network involving p19<sup>ARF</sup>, p53, and Bach1. p19<sup>ARF</sup> regulates both Bach1, which inhibits p53-dependent senescence, and MDM2.

stressor diamide,<sup>(34)</sup> redox modification could be involved in the regulation. Because oxidative stress inhibits the Bach1-p53 interaction (Fig. 1) and decreases the Bach1 protein level

(Fig. 7e), we speculate that oxidative stress induces senescence in part by affecting Bach1-mediated inhibition of p53. Considering the functions of Bach1 and p19<sup>ARF</sup> in diverse stress responses, further study of Bach1 and p19<sup>ARF</sup> is beneficial for the elucidation of the signal networks observed in the oxidative stress response, cellular senescence, and tumor suppression.

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## Disclosure Statement

The authors have no conflicts of interest.