Molecular Cell
Matters Arising

p53-Dependent Transcription and Tumor Suppression Are Not Affected in Set7/9-Deficient Mice

Bernhard Lehnertz,[1,2] Jason C. Rogalski,[1] Felix M. Schulze,[1] Lin Yi,[1] Shujun Lin,[1] Jürgen Kast,[1] and Fabio M.V. Rossi[1,*]
[1]University of British Columbia, Biomedical Research Centre, 2222 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada
[2]Present address: IRIC, Université de Montréal, 2950, chemin de Polytechnique, Montréal, QC H3T 1J4, Canada
*Correspondence: fabio@brc.ubc.ca
DOI 10.1016/j.molcel.2011.08.006

SUMMARY

Methylation of specific lysine residues in the C terminus of p53 is thought to govern p53-dependent transcription following genotoxic and oncogenic stress. In particular, Set7/9 (KMT7)-mediated monomethylation of human p53 at lysine 372 (p53K372me1) was suggested to be essential for p53 activation in human cell lines. This finding was confirmed in a *Set7/9* knockout mouse model (Kurash et al., 2008). In an independent knockout mouse strain deficient in Set7/9, we have investigated its involvement in p53 regulation and find that cells from these mice are normal in their ability to induce p53-dependent transcription following genotoxic and oncogenic insults. Most importantly, we detect no impairment in canonical p53 functions in these mice, indicating that Set7/9-mediated methylation of p53 does not seem to represent a major regulatory event and does not appreciably control p53 activity in vivo.

INTRODUCTION

The tumor suppressor p53 is situated at a central signaling node mediating cellular responses to stress and is mutated in a majority of human cancers (Vousden and Lu, 2002). In response to cellular stress such as DNA damage or aberrant oncogene expression, p53 becomes stabilized and recruits a multitude of transcriptional coregulators to coordinate a complex gene expression network resulting in cell-cycle arrest or apoptosis (Brooks and Gu, 2003).

Reminiscent of signaling events on histone tails, site-specific methylation and acetylation within the regulatory C terminus of p53 have been proposed to either cooperate or counteract each other, thereby controlling opposing p53 activation states (Huang and Berger, 2008). Specifically, monomethylation of lysine 372 in human p53 (p53K372me1) by Set7/9 has been suggested to be a prerequisite for efficient p53-dependent gene induction, cell-cycle arrest, and apoptosis (Chuikov et al., 2004). p53K372me1 was found to inhibit the repressive monomethylation of K370 by Smyd2 (Huang et al., 2006) and has been proposed to facilitate acetylation of K373 and K382 (Ivanov et al., 2007). Thus, Set7/9-dependent methylation of p53 is currently considered a central regulatory event in the modulation of its transactivation potential. Importantly, however, most of the data in support of this hypothesis were obtained from studies in human cancer cell lines with compromised p53 activity. Therefore, the physiological significance of p53 methylation in primary cells largely remains to be investigated.

Kurash et al. (2008) reported that mice lacking Set7/9 display severe impairments in a number of canonical p53 activities. They showed that in response to genotoxic stress, p53 becomes methylated on K369 (p53K369), and that this methylation is absent in *Set7/9*$^{-/-}$ cells. It was also reported that p53 acetylation on multiple other lysines is abrogated and that the induction of two p53 target genes, *p21*$^{WAF1/CIP}$ and *Puma*, in response to cellular stress is greatly reduced in *Set7/9*$^{-/-}$ mice. As a result, expression of the oncogenes E1A and H-RasV12 was reported to easily transform MEFs lacking Set7/9. Based on this data, the authors concluded that Set7/9 is critically required for the tumor-suppressive function of p53 in primary mouse cells.

Here, we present data that not only cast doubt on the role of Set7/9 in regulating p53, but also question the overall impact of p53 C-terminal methylation on p53 activity in nontransformed cells.

RESULTS

Generation of a Conditional *Set7/9*$^{-/-}$ Strain

To disrupt *Set7/9* expression in the mouse, we chose a conditional targeting approach in which the majority of the gene, including the entire C terminus encoding the catalytically SET domain, was flanked with *loxP* sites (Figure S1). The knockout allele was then generated by crossing through a ubiquitous Cre-expressing transgenic strain. All mice used for this study were backcrossed into C57BL/6J for five generations. Their genetic background was therefore presumably comparable with that of the mice used by Kurash et al. (2008). Viable *Set7/9*$^{-/-}$ offspring were obtained at normal Mendelian ratios from *Set7/9*$^{+/-}$ intercrosses and did not display any gross abnormalities. Western analysis with antibodies raised against the full-length protein or an N-terminal peptide confirmed that we completely eliminated expression of the Set7/9 protein from the excised locus (Figure 1A). Further, although Set7/9 is classified as an H3K4-specific methyltransferase, we detected no change in global H3K4 methylation levels in *Set7/9*$^{-/-}$ MEFs (Figure 1A).

Interestingly, wild-type (WT) and *Set7/9*$^{-/-}$ breeder pairs consistently yield comparable litter sizes (7.14 ± 1.96, n = 14 and 6.83 ± 2.71, n = 6), an observation that is at odds with a report suggesting that Set7/9 is an essential regulator of estrogen

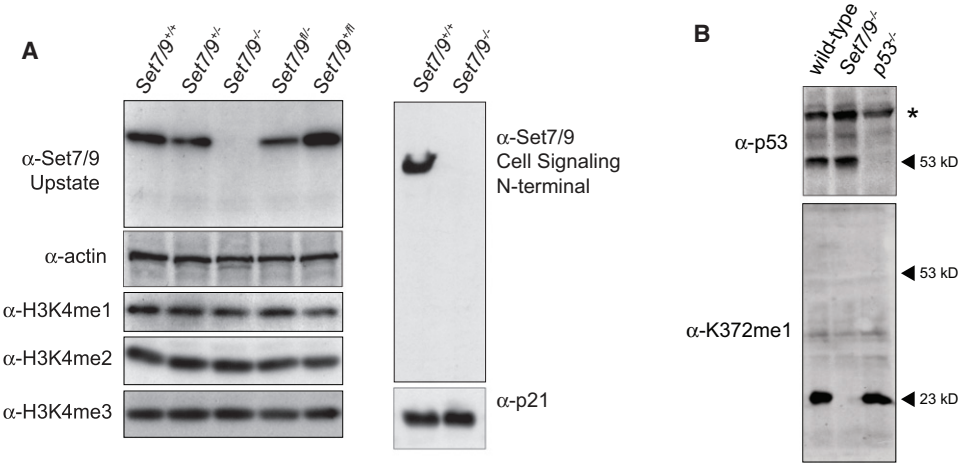

Figure 1. Characterization of a Set7/9 Conditional Knockout Strain

(A) Confirmation of the complete absence of Set7/9 in knockout MEFs. Global H3K4 methylation levels are unaffected in *Set7/9*$^{-/-}$ cells.

(B) The α-p53K372me1 antiserum does not bind activated p53 in Adriamycin-treated MEFs. A strong Set7/9-dependent cross-reacting band is observed at 23 kDa. The asterisk indicates an unspecific band used as a loading control. (See also corresponding Figures S1 and S2.)

receptor α (ERα) activity (Subramanian et al., 2008). Since ERα is required for postnatal sexual maturation (Lubahn et al., 1993), its impaired activity in *Set7/9*$^{-/-}$ mice should result in noticeably compromised male and female fertility. However, our data suggest that Set7/9 does not play a discernible role in the execution of ERα-dependent gene expression during sexual maturation.

The reported role of Set7/9 in regulating p53 by methylating p53K369 prompted us to investigate this modification in WT versus *Set7/9*$^{-/-}$ MEFs. We attempted to detect p53K369me1 in Adriamycin-treated cells using an antiserum raised against human p53K372me1 (Abcam cat. #16033) (Chuikov et al., 2004). Despite a divergent amino acid sequence (Figure S2A), this antiserum was also reported to react with mouse p53K369me1 (Kurash et al., 2008). In our hands, however, this antibody did not recognize murine p53 in lysates from Adriamycin-treated WT or *Set7/9*$^{-/-}$ cells (Figure 1B). Importantly, while showing strong reactivity toward a synthetic methylated human peptide spanning the sequence flanking p53K372me1 (Abcam #16203) in dot blot analyses, this antiserum entirely failed to recognize the corresponding mouse peptide including p53K369 in its methylated or unmethylated form (Figure S2B). At the same time, we consistently detected a cross-reacting protein of 23 kDa in whole-cell lysates of WT and *p53*$^{-/-}$ but not *Set7/9*$^{-/-}$ MEFs (Figure 1B). This indicates that whereas the antihuman p53K372me1 antiserum is not suitable to determine the methylation status of mouse p53K369, as claimed by Kurash et al. (2008), it likely recognizes a monomethylated lysine on an as-yet-unidentified Set7/9 substrate. Indeed, reintroduction of Set7/9 in *Set7/9*$^{-/-}$ MEFs by retroviral transduction led to the reappearance of the 23 kDa band, and the use of the same antiserum in immunoprecipitation experiments revealed the presence of a second protein that was recognized in WT but not in *Set7/9*$^{-/-}$ cells, about 43 kDa in size (Figures S2C and S2D). These data demonstrate that we have generated a functional knockout for Set7/9, but do not allow a definitive conclusion about whether p53K369 is indeed a substrate for Set7/9 in vivo.

Set7/9 Is Dispensable for p53-Dependent Senescence, Transcription, Cell-Cycle Arrest, and Apoptosis

Since p53 plays a key role in the response to culture-associated stress, cells deficient in p53 function escape culture-induced cell-cycle arrest and are easily immortalized. To investigate whether cells lacking Set7/9 display a similar impairment in culture-induced senescence, we investigated the growth kinetics of cultured MEFs from WT, *Set7/9*$^{-/-}$, and *p53*$^{-/-}$ mice following the 3T3 protocol (Todaro and Green, 1963). *p53*$^{-/-}$ MEFs grew exponentially throughout the culture period, as expected. In contrast, both *Set7/9*$^{-/-}$ and WT MEFs underwent a replicative crisis after passage 9 (Figure 2A), indicating that Set7/9 is not required for culture-induced senescence mediated by p53.

Genotoxic stress initiates the N-terminal serine phosphorylation of p53, blocking the interaction with Mdm2 and leading to the accumulation of the protein (Prives, 1998). Overexpression and RNAi experiments suggest that p53, once activated, depends on SET7/9 to induce expression of its target genes *p21* and *MDM2* (Chuikov et al., 2004) in human cells. We thus compared low-passage MEFs from WT and *Set7/9*$^{-/-}$ littermates, along with *p53*$^{-/-}$ controls, in their ability to stabilize p53 and induce p53-dependent transcription and cell-cycle arrest. We exposed MEFs to gamma radiation, Adriamycin, or the Mdm2 antagonist Nutlin 3 and compared protein levels of p53, p21, Mdm2, and Puma 18 hr after these treatments (Figure 2B). Under all conditions tested, we detected no expression of *p21* and *Mdm2* and only weak expression of *Puma* in *p53*$^{-/-}$ cells, as expected. By contrast, expression of these p53 target genes was readily detectable in untreated WT and *Set7/9*$^{-/-}$ MEFs, and their expression was upregulated in response to all treatments in both WT and *Set7/9*$^{-/-}$ MEFs. Importantly, the extent of p53 stabilization, its acetylation at K379, as well as

the expression of *p21*, *Mdm2*, and *Puma* was identical in *Set7/9*$^{-/-}$ MEFs and WT controls. This suggests that, in contrast to what was reported before (Ivanov et al., 2007; Kurash et al., 2008), Set7/9 is not required to control acetylation of K379 on p53 and the expression of p53 target genes in MEFs.

Next, we analyzed the cell-cycle profiles of the same MEFs by BrdU incorporation and PI staining (Figures 2C and 2D). Whereas *p53*$^{-/-}$ MEFs were mostly unaffected by the treatments, as indicated by their continued BrdU incorporation, WT and *Set7/9*$^{-/-}$ cells responded to all p53 activating agents by efficiently stalling DNA synthesis. Moreover, upon extended exposure to Adriamycin over 24, 48, and 72 hr, WT and *Set7/9*$^{-/-}$ MEFs induced their respective growth arrests with equivalent efficiencies (Figure S3). These findings were in stark contrast to those of Kurash et al. (2008), who reported that *Set7/9*$^{-/-}$ but not WT MEFs continued to replicate in the presence of Adriamycin. Furthermore, Kurash et al. (2008) also reported that Adriamycin-treated *Set7/9*$^{-/-}$ cells displayed a normal cell-cycle profile with the majority of cells residing in G1, a finding that was interpreted as a result of diminished p53 activity. In contrast, we observed a characteristic accumulation of *p53*$^{-/-}$ cells in late S and G2/M phases after Adriamycin treatment, a result that has previously been attributed to pleiotropic activities of Adriamycin, triggering both p53-dependent G1 arrest and p53-independent G2 arrest (Attardi et al., 2004). Indeed, *p53*$^{-/-}$ MEFs also responded to sustained exposure to Adriamycin beyond 24 hr by markedly reducing their growth rate, likely due to extensive DNA damage caused by the intercalating action of Adriamycin resulting in the inability of these cells to continue replication (Figure S3). Taken together, our results indicate that Set7/9 is dispensable for p53-dependent transcription and for the G1/S phase arrest induced by DNA damage or culture-associated stress in MEFs.

To investigate whether cell-type-specific defects in the p53 pathway may be present in *Set7/9*$^{-/-}$ animals, we expanded our analysis to thymic progenitors. These cells are characterized by high proliferation rates and are very sensitive to apoptotic stimuli, making them an ideal model to study p53-mediated cell death (Lowe et al., 1993). We harvested thymocytes from WT and *Set7/9*$^{-/-}$ mice, subjected them to increasing dosages of ionizing radiation and quantified apoptosis within these samples by AnnexinV staining (Figure 2E). Regardless of the radiation dosage, we detected no significant difference in the rate with which *Set7/9*$^{-/-}$ and WT thymocytes entered apoptosis. Additionally, we observed no difference in the expression of p53 target genes *p21*, *Mdm2*, and *Bax* by quantitative real-time PCR in these cells (Figure 2F). Thus, Set7/9 is not required for p53-dependent apoptosis in thymic progenitors.

Set7/9$^{-/-}$ MEFs Are Fully Competent to Initiate Oncogene-Induced Senescence and Apoptosis and Are Not Predisposed to Transformation

Failsafe mechanisms that sense and counteract uncontrolled growth signals caused by viral or cellular oncogenes often act through the p19^{ARF}-mediated inhibition of Mdm2 and concurrent stabilization of p53. This eventually results in cell-cycle arrest, also known as premature senescence (Serrano et al., 1997), or in apoptosis (de Stanchina et al., 1998). To assess the integrity of tumor-suppressive responses in cells lacking Set7/9, early-passage WT, *Set7/9*$^{-/-}$, and *p53*$^{-/-}$ MEFs were infected with H-RasV12- or E1A-expressing retroviruses and subsequently assessed for the formation of colonies initiated by immortalized clones. Unlike *p53*$^{-/-}$ MEFs, which yielded a dramatically increased number of colonies under all conditions, *Set7/9*$^{-/-}$ MEFs were indistinguishable from WT controls in both colony numbers and size (Figures 3A and 3B). To determine if H-RasV12- or E1A-expressing MEFs grow in an anchorage-independent fashion and if they lost contact inhibition, two typical hallmarks of cellular transformation, we grew infected MEFs in soft agar-based culture medium or in high-density cultures. As expected, *p53*$^{-/-}$ MEFs gave rise to readily detectable multicellular colonies in soft agar medium when expressing H-RasV12 or E1A. In contrast, H-RasV12- or E1A-expressing WT controls and *Set7/9*$^{-/-}$ MEFs yielded no detectable colonies after 14 days of culture (Figure 3C). Similarly, and unlike what was reported in Kurash et al. (2008), when grown in high-density cultures for 21 days, H-RasV12-infected *p53*$^{-/-}$ MEFs formed foci indicative of cellular transformation, while WT and *Set7/9*$^{-/-}$ cells remained quiescent and in monolayers (Figure S4).

MEFs transformed by combined E1A and H-RasV12 expression acquire the ability to form tumors when injected subcutaneously in immunocompromised mice (Jimenez et al., 2000). The growth of these tumors is strongly limited by p53, thus offering an additional readout for p53 activity. We double-infected WT, *Set7/9*$^{-/-}$, and *p53*$^{-/-}$ MEFs with E1A and H-RasV12 and injected them subcutaneously into NOD/SCID mice. After 14 days, the resulting tumors were harvested and weighed. As expected, transformed *p53*$^{-/-}$ MEFs gave rise to very large tumors while those derived from WT and *Set7/9*$^{-/-}$ MEFs were at least one order of magnitude smaller in size. In addition, we found that WT and *Set7/9*$^{-/-}$ tumors displayed no significant weight difference (Figure 3D). Together, these results indicate that loss of Set7/9 is not sufficient to bypass oncogene-induced senescence and does not attenuate apoptosis in response to E1A expression in MEFs.

Set7/9 Loss Does Not Accelerate Lymphomagenesis in *Eμ-Myc* Mice

Like E1A, ectopic expression of Myc induces apoptosis through activation of p19^{ARF} and p53 (Zindy et al., 1998). The translocation of the c-*myc* gene under the control of an immunoglobulin heavy chain (IgH) enhancer is a recurring lesion in the etiology of Burkitt's lymphoma. Transgenic mice expressing Myc under the control of the IgH enhancer (*Eμ-Myc*) develop B cell lymphomas at a mean age of 6 months and thus represent a sensitized model to study synergistic genetic events underlying lymphomagenesis (Adams et al., 1985; Hanahan et al., 2007). It is well established that spontaneous mutations in the p19^{ARF}-Mdm2-p53 axis occur in lymphomas that arise in these mice (Eischen et al., 1999), emphasizing a crucial role for p53 in safeguarding against hyperproliferative stimuli in this model. Importantly, targeted mutations in the proapoptotic p53-dependent effector genes *Bax* (Eischen et al., 2001), *Puma* (Garrison et al., 2008; Hemann et al., 2004), and *Bim* (Egle et al., 2004) also cooperate with the *Eμ-Myc* transgene and result in markedly accelerated malignancies. Given the proposed role for Set7/9 in

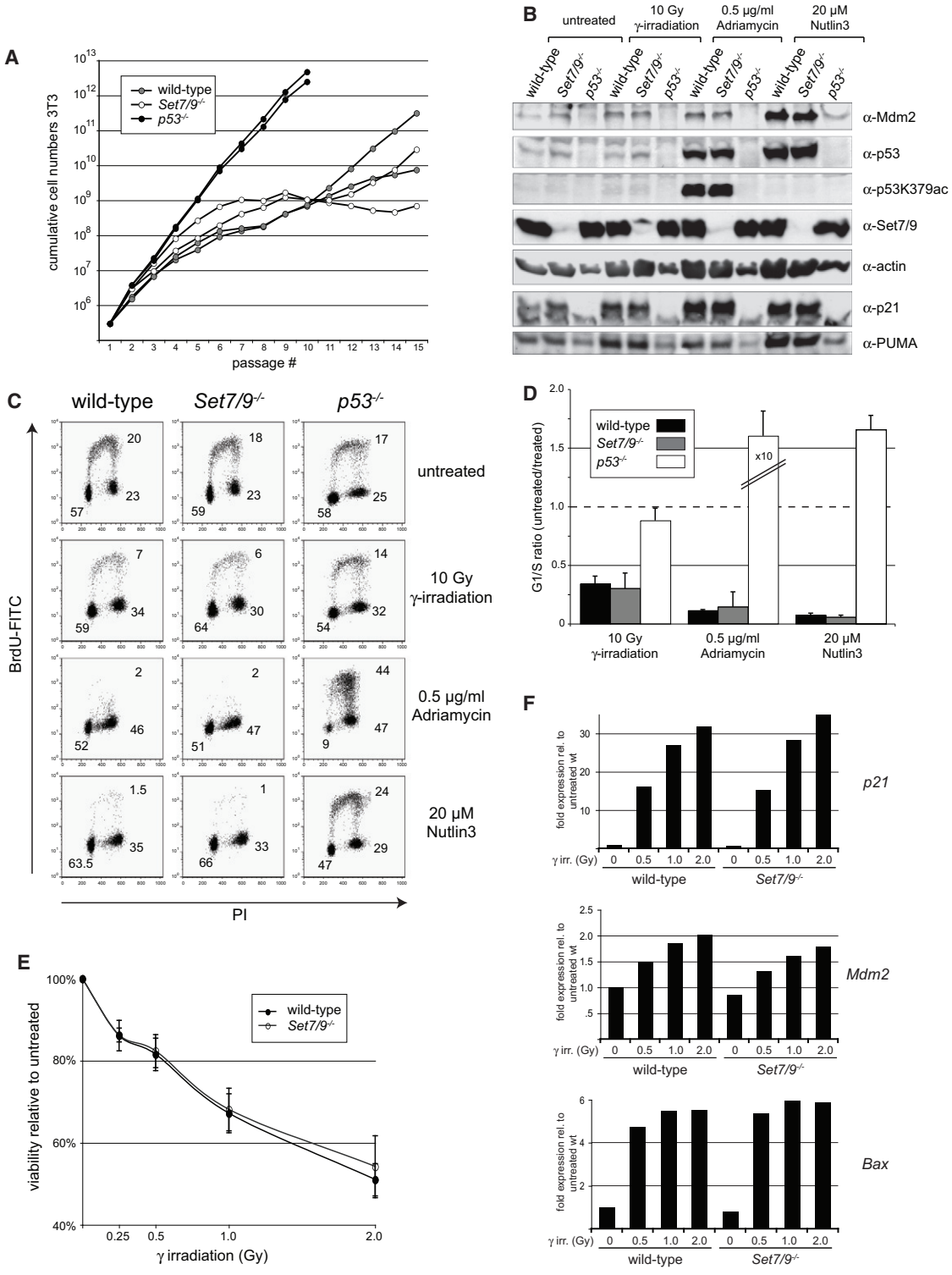

Figure 2. Culture-Induced Senescence, DNA Damage-Induced Cell-Cycle Arrest, and Apoptosis Are Unaffected in *Set7/9*$^{-/-}$ MEFs and Thymocytes

(A) Two independent MEF preparations for WT, *Set7/9*$^{-/-}$, and *p53*$^{-/-}$ genotypes were cultured according to the 3T3 protocol. Cells from duplicate plates were counted at each passage, and mean cumulative cell numbers were plotted. *Set7/9*$^{-/-}$ MEFs efficiently enter replicative crisis.

(B) Early-passage MEFs were treated with p53-activating agents; whole-cell lysates were prepared 18 hr later and subjected to western analysis. Under all conditions tested, the extent of p53K379 acetylation and the expression of *Mdm2*, *p21*, or *Puma* were comparable in *Set7/9*$^{-/-}$ and WT MEFs.

enabling p53-dependent transcription, we asked whether Myc-driven lymphomagenesis was accelerated in *Set7/9*$^{-/-}$ mice. We bred the *p53* null, *Set7/9* null, and floxed alleles to *Eμ-Myc*$^{+}$ mice and transferred *Eμ-Myc*$^{+}$*; Set7/9*$^{fl/+}$, *Eμ-Myc*$^{+}$*; Set7/9*$^{-/-}$, or *Eμ-Myc*$^{+}$*; p53*$^{+/-}$ fetal liver cells into lethally irradiated C57/B6 *CD45.1*$^{+}$ recipients (Figure 3E). We monitored these mice for enlarged lymph nodes and/or other indications of B cell lymphoma onset over a time of 6 months. Consistent with a previous report (Schmitt et al., 2002), all mice in the *Eμ-Myc*$^{+}$*; p53*$^{+/-}$ cohort developed lymphomas at a greatly accelerated pace. In contrast, *Eμ-Myc*$^{+}$*; Set7/9*$^{-/-}$ recipients exhibited no shortened disease latency (Figure 3E) in comparison to *Eμ-Myc*$^{+}$*; Set7/9*$^{fl/+}$ controls, indicating that Set7/9 has no discernible role in regulating p53 activity during lymphomagenesis.

DISCUSSION

Overall, the results we obtained investigating p53 function in our *Set7/9*$^{-/-}$ mouse strain are not reconcilable with previous reports, despite the use of very similar assays.

In several crucial points, our results are at odds with those of Kurash et al. (2008), as well as aspects of the reports by Chuikov et al. (2004) and Ivanov et al. (2007), thus challenging the notion that p53 function depends on Set7/9-mediated lysine methylation in its regulatory C terminus. In summary, we find that (1) the methylation of murine p53K369 cannot be detected using an antiserum raised against human p53K372me, (2) the induction of p53 target genes in response to DNA damage and oncogenic stress is unaltered in *Set7/9*$^{-/-}$ cells, and accordingly, (3) no impairment in p53-dependent apoptosis and cell-cycle arrest is observed in the absence of Set7/9. Importantly, this is in agreement with the data obtained independently by Testa and coworkers (Campaner et al., 2011). Hence, while our data do not formally exclude that p53 may be a Set7/9 substrate in vivo, they strongly suggest that the proposed role of Set7/9 in p53 regulation requires considerable re-evaluation. Our data also highlight the requirement to confirm mechanisms of p53 regulation hypothesized based on in vitro studies of transformed cell lines in primary cells in vivo (Johnson and Attardi, 2006; Toledo and Wahl, 2006).

Further, our results are consistent with a number of studies that have addressed the roles of posttranslational lysine modifications on p53. For example, mutational conversion of all seven lysines in the C terminus of mouse p53, including K369, into arginines entails only minor changes in tumor-suppressive p53 activities in vivo (Feng et al., 2005; Krummel et al., 2005). Moreover, in human cells, only the combined mutation of eight lysines, including two lysines in the DNA binding domain, yields a transcriptionally inert form of p53 (Tang et al., 2008). This result, while not discounting the overall importance of lysine modifications, strongly suggests that they play highly redundant and/or exquisitely tissue-specific roles. This is in line with the notion that the activity of p53, because of its fundamental role in tumor suppression, is regulated by a multitude of functionally redundant mechanisms.

Since cancer formation is a multistep process in which neoplastic cells progressively acquire genetic or epigenetic alteration (Hanahan and Weinberg, 2000), Set7/9 might become a critical lesion in cells that have become deficient in other p53-regulating pathways. However, our results indicate that loss of Set7/9 by itself is inconsequential for canonical p53 functions in vivo.

EXPERIMENTAL PROCEDURES

Generation of *Set7/9* Conditional Knockout Mice

Conditional targeting of the Set7/9 gene was done in R1 ESCs and is described in more detail in Figure S1.

MEF Derivation and Culture

MEFs were prepared from 13.5 dpc embryos according to standard procedures and frozen within 2 days of culture in DMEM, 10% FBS, nonessential amino acids, l-glutamine, Pen/Strep, and 100 μM β-mercaptoethanol. Thawed MEF preps were designated as passage 1 and were used until passage 4 maximum.

3T3 Assay

MEFs were plated in duplicate at 3 × 10^{5} cells per 6 cm dish. Every third day, cells were trypsinized, counted, and replated at original densities over 15 passages.

Thymocyte Apoptosis

Thymocytes were γ-irradiated in a ^{64}Co source at 0.5, 1, and 2 Gy. After overnight culture, viability was determined by AnnexinV-APC and 7AAD staining on a FACScalibur. For RT-PCR, thymi from sex-matched WT and *Set7/9*$^{-/-}$ littermates were harvested 2 hr after whole-body irradiation.

RT-PCR

RNA was prepared using RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. cDNA was generated using the Invitrogen Superscript III RT protocol. Real-time PCR was done using a Roche Lightcycler 480 and gene-specific Taqman reagents (Applied Biosystems, p21: Mm00432448_m1, Mdm2: Mm00487656_m1, Bax: Mm00432050_m1, GAPDH Hs02758991_g1).

Western Analysis, Immunoprecipitation, and Antibodies

Whole-cell lysates were done in IP buffer (50 mM Tris [pH 7.5], 150 NaCl, 1 mM EDTA, 1% NP40, Roche Complete protease inhibitors) and processed in standard western analysis. Antibodies were: α-Set9 (Upstate), α-SET7/9 (Cell Signaling clone C24B1), α-p53 (CM-5, Novocastra), α-p53 (PAb 240, BD), α-human p53K372me1 (Abcam #16033), α-acetyl-p53K379 (Cell Signaling #2570), α-H3K4me (Abcam), α-p21 (C-19, Santa Cruz), α-p21 (Ab-6,

(C) The cell-cycle status of the same MEF populations was analyzed by BrdU incorporation and PI staining. The percentages of cells in the G1, S, and G2/M stages are indicated. WT and *Set7/9*$^{-/-}$ MEFs respond to all treatments by withdrawing from the cell cycle. *p53*$^{-/-}$ MEFs are characterized by an increased arrest in late S and G2 phase in response to Adriamycin.

(D) Cell-cycle analysis summary. G1/S ratios were calculated from three replicate experiments as illustrated in (C). Error bars represent standard deviations.

(E) Quantification of apoptosis in irradiated thymocytes. Thymocytes were prepared from four individual mice of each genotype and exposed to increasing radiation dosages. Six hours later, the relative viability was determined by AnnexinV staining and 7AAD exclusion. Error bars represent standard deviations.

(F) Quantitative RT-PCR analysis of p53 target genes in irradiated thymocytes. WT and *Set7/9*$^{-/-}$ mice (n = 4) were exposed to whole-body irradiation at the indicated dosages. Two hours later, thymocytes were harvested for RT-PCR. We detected no impaired induction of *p21*, *Mdm2*, and *Bax* in *Set7/9*$^{-/-}$ cells. (See also corresponding Figures S3 and S4.)

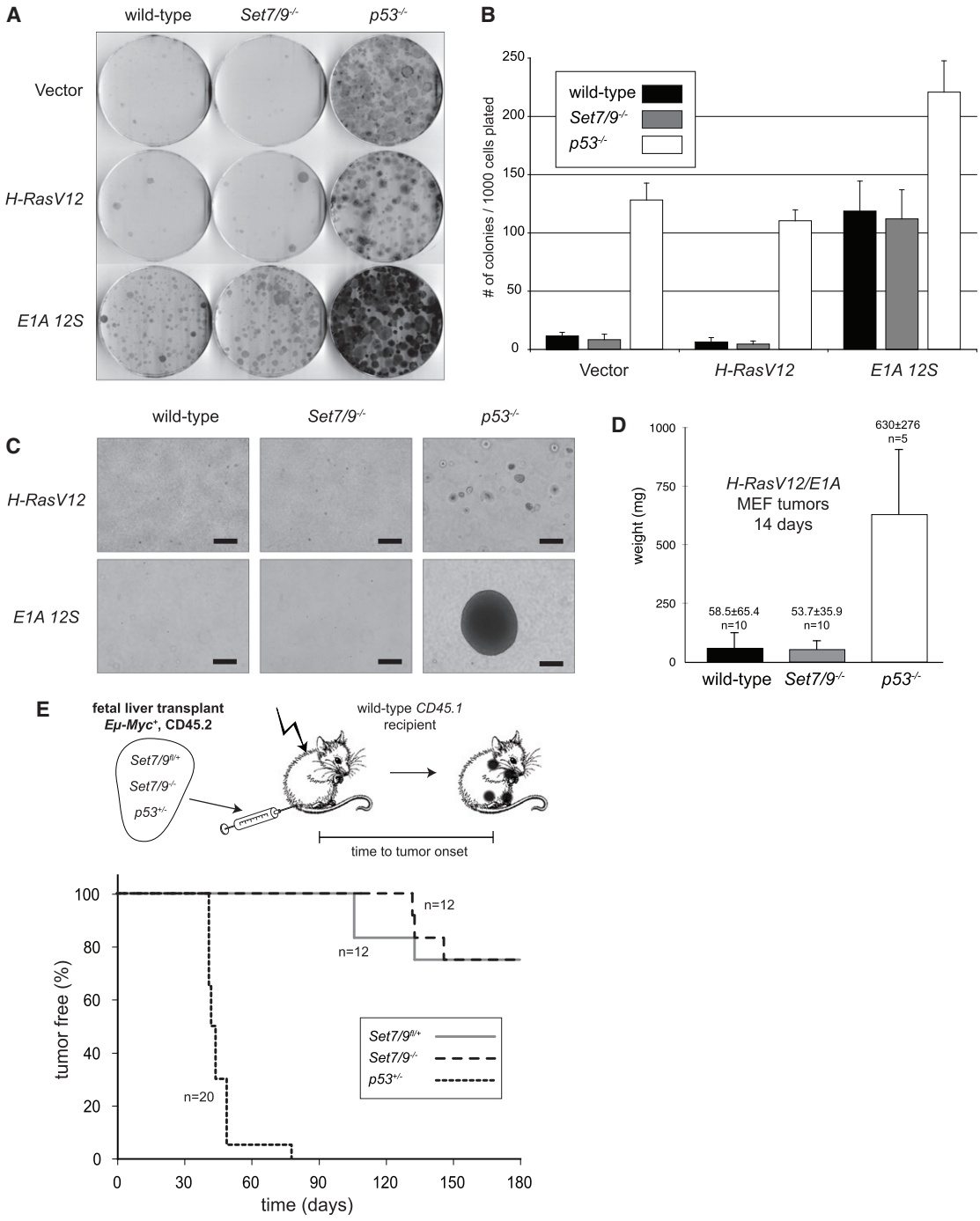

Figure 3. *Set7/9*$^{-/-}$ Cells Respond Normally to Hyperproliferative Stimuli and Are Not Predisposed to Transformation

(A) Passage 2 MEFs of the indicated genotypes were infected with control, H-RasV12, or E1A retroviruses. After Puro selection, 1000 cells were plated on 10 cm dishes. Colonies scored by crystal violet staining after 18 days revealed a comparable propensity of WT and *Set7/9*$^{-/-}$ MEFs to be transformed by these oncogenes.

(B) Summary of transformation assays. Average number of colonies per 1000 cells calculated from ten repeats covering four WT, four *Set7/9*$^{-/-}$, and two *p53*$^{-/-}$ MEF preparations. Error bars represent standard deviations.

(C) Unlike *p53*$^{-/-}$ MEFs, *Set7/9*$^{-/-}$ MEFs do not acquire anchorage-independent growth when transduced with H-RasV12 or E1A retroviruses. Scale bar is 1 mm.

(D) Tumorigenicity of E1A/H-RasV12-infected MEFs. WT, *Set7/9*$^{-/-}$, and *p53*$^{-/-}$ MEFs were doubly infected with E1A and H-RasV12, selected by hygro and puro, and injected subcutaneously into NOD/SCID mice. The weight of tumors harvested after 14 days was comparable between WT and *Set7/9*$^{-/-}$ cells. n = 10 for WT and *Set7/9*$^{-/-}$, n = 5 for *p53*$^{-/-}$, error bars represent standard deviations.

(E) No acceleration in Myc-mediated lymphomagenesis in the absence of Set7/9. *Eμ-Myc*$^{+}$ fetal liver cells (day 14.5) of the indicated genotypes were transplanted into irradiated recipient mice by tail vein injection. The time until lymphoma onset, indicated by enlarged lymph nodes, is plotted.

Oncogene), α-Mdm2 (Calbiochem clone 2A10), α-Bax (Calbiochem Ab-1), and α-Puma (Cell Signaling #4976).

Retroviral Gene Transfer

The retroviral transfer vectors pWZL E1A hygro and pBabe RasV12 puro were obtained from Scott Lowe (CSHL). Virus production was performed in Phoenix E cells according to standard procedures. Infected MEFs were selected with hygromycin (200 μg/ml) or puromycin (2 μg/ml) for 48 hr.

Tumor Graft Assays

4×10^6 pWZL E1A hygro and pBabe RasV12 puro double-infected MEFs were injected subcutaneously into NOD/SCID mice. The resulting tumors were dissected and weighed after 14 days.

Cell-Cycle Analysis

Early-passage MEFs were treated with p53-activating agents overnight and pulsed with 10 μM BrdU for 60 min. BrdU/PI detection was performed using FITC-conjugated anti-BrdU (BD Cat. No. 556028) according to the manufacturer's instructions.

Eμ-Myc$^+$ Lymphoma Onset Assay

Eμ-Myc$^+$ (JAX #02728) × *Set7*$^{fl/+}$, *Set7*$^{-/-}$, or *p53*$^{+/-}$ fetal livers were transplanted into lethally irradiated recipients. The recipients were euthanized at lymphoma onset, characterized by enlarged lymph nodes and spleens.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and can be found with this article online at doi:10.1016/j.molcel.2011.08.006.

ACKNOWLEDGMENTS

We thank Scott Lowe for providing oncogene retroviruses; Michael Long, Jeff Northrop, Colby Zaph, and Matt Lorincz for critical reading of the manuscript; and all Rossi lab members for stimulating discussions. All experiments were performed according to standards approved by the UBC animal care committee. B.L. was supported by a PhD scholarship from the German Academic Exchange Service (DAAD).

Received: March 30, 2010
Revised: January 14, 2011
Accepted: August 3, 2011
Published: August 18, 2011

REFERENCES

Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D., and Brinster, R.L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature *318*, 533–538.

Attardi, L.D., de Vries, A., and Jacks, T. (2004). Activation of the p53-dependent G1 checkpoint response in mouse embryo fibroblasts depends on the specific DNA damage inducer. Oncogene *23*, 973–980.

Brooks, C.L., and Gu, W. (2003). Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. Curr. Opin. Cell Biol. *15*, 164–171.

Campaner, S., Spreafico, F., Burgold, T., Doni, M., Rosato, U., Amati, B., and Testa, G. (2011). The methyltransferase Setd7 is dispensable for the p53-mediated DNA damage response. Mol. Cell *43*, this issue, 681–688.

Chuikov, S., Kurash, J.K., Wilson, J.R., Xiao, B., Justin, N., Ivanov, G.S., McKinney, K., Tempst, P., Prives, C., Gamblin, S.J., et al. (2004). Regulation of p53 activity through lysine methylation. Nature *432*, 353–360.

de Stanchina, E., McCurrach, M.E., Zindy, F., Shieh, S.Y., Ferbeyre, G., Samuelson, A.V., Prives, C., Roussel, M.F., Sherr, C.J., and Lowe, S.W. (1998). E1A signaling to p53 involves the p19(ARF) tumor suppressor. Genes Dev. *12*, 2434–2442.

Egle, A., Harris, A.W., Bouillet, P., and Cory, S. (2004). Bim is a suppressor of Myc-induced mouse B cell leukemia. Proc. Natl. Acad. Sci. USA *101*, 6164–6169.

Eischen, C.M., Weber, J.D., Roussel, M.F., Sherr, C.J., and Cleveland, J.L. (1999). Disruption of the ARF-Mdm2-p53 tumor suppressor pathway in Myc-induced lymphomagenesis. Genes Dev. *13*, 2658–2669.

Eischen, C.M., Roussel, M.F., Korsmeyer, S.J., and Cleveland, J.L. (2001). Bax loss impairs Myc-induced apoptosis and circumvents the selection of p53 mutations during Myc-mediated lymphomagenesis. Mol. Cell. Biol. *21*, 7653–7662.

Feng, L., Lin, T., Uranishi, H., Gu, W., and Xu, Y. (2005). Functional analysis of the roles of posttranslational modifications at the p53 C terminus in regulating p53 stability and activity. Mol. Cell. Biol. *25*, 5389–5395.

Garrison, S.P., Jeffers, J.R., Yang, C., Nilsson, J.A., Hall, M.A., Rehg, J.E., Yue, W., Yu, J., Zhang, L., Onciu, M., et al. (2008). Selection against PUMA gene expression in Myc-driven B-cell lymphomagenesis. Mol. Cell. Biol. *28*, 5391–5402.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Hanahan, D., Wagner, E.F., and Palmiter, R.D. (2007). The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. Genes Dev. *21*, 2258–2270.

Hemann, M.T., Zilfou, J.T., Zhao, Z., Burgess, D.J., Hannon, G.J., and Lowe, S.W. (2004). Suppression of tumorigenesis by the p53 target PUMA. Proc. Natl. Acad. Sci. USA *101*, 9333–9338.

Huang, J., and Berger, S.L. (2008). The emerging field of dynamic lysine methylation of non-histone proteins. Curr. Opin. Genet. Dev. *18*, 152–158.

Huang, J., Perez-Burgos, L., Placek, B.J., Sengupta, R., Richter, M., Dorsey, J.A., Kubicek, S., Opravil, S., Jenuwein, T., and Berger, S.L. (2006). Repression of p53 activity by Smyd2-mediated methylation. Nature *444*, 629–632.

Ivanov, G.S., Ivanova, T., Kurash, J., Ivanov, A., Chuikov, S., Gizatullin, F., Herrera-Medina, E.M., Rauscher, F., 3rd, Reinberg, D., and Barlev, N.A. (2007). Methylation-acetylation interplay activates p53 in response to DNA damage. Mol. Cell. Biol. *27*, 6756–6769.

Jimenez, G.S., Nister, M., Stommel, J.M., Beeche, M., Barcarse, E.A., Zhang, X.Q., O'Gorman, S., and Wahl, G.M. (2000). A transactivation-deficient mouse model provides insights into Trp53 regulation and function. Nat. Genet. *26*, 37–43.

Johnson, T.M., and Attardi, L.D. (2006). Dissecting p53 tumor suppressor function in vivo through the analysis of genetically modified mice. Cell Death Differ. *13*, 902–908.

Krummel, K.A., Lee, C.J., Toledo, F., and Wahl, G.M. (2005). The C-terminal lysines fine-tune P53 stress responses in a mouse model but are not required for stability control or transactivation. Proc. Natl. Acad. Sci. USA *102*, 10188–10193.

Kurash, J.K., Lei, H., Shen, Q., Marston, W.L., Granda, B.W., Fan, H., Wall, D., Li, E., and Gaudet, F. (2008). Methylation of p53 by Set7/9 mediates p53 acetylation and activity in vivo. Mol. Cell *29*, 392–400.

Lowe, S.W., Schmitt, E.M., Smith, S.W., Osborne, B.A., and Jacks, T. (1993). p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature *362*, 847–849.

Lubahn, D.B., Moyer, J.S., Golding, T.S., Couse, J.F., Korach, K.S., and Smithies, O. (1993). Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc. Natl. Acad. Sci. USA *90*, 11162–11166.

Prives, C. (1998). Signaling to p53: breaking the MDM2-p53 circuit. Cell *95*, 5–8.

Schmitt, C.A., Fridman, J.S., Yang, M., Baranov, E., Hoffman, R.M., and Lowe, S.W. (2002). Dissecting p53 tumor suppressor functions in vivo. Cancer Cell *1*, 289–298.

Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., and Lowe, S.W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell *88*, 593–602.

Subramanian, K., Jia, D., Kapoor-Vazirani, P., Powell, D.R., Collins, R.E., Sharma, D., Peng, J., Cheng, X., and Vertino, P.M. (2008). Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Mol. Cell *30*, 336–347.

Tang, Y., Zhao, W., Chen, Y., Zhao, Y., and Gu, W. (2008). Acetylation is indispensable for p53 activation. Cell *133*, 612–626.

Todaro, G.J., and Green, H. (1963). Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J. Cell Biol. *17*, 299–313.

Toledo, F., and Wahl, G.M. (2006). Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat. Rev. Cancer *6*, 909–923.

Vousden, K.H., and Lu, X. (2002). Live or let die: the cell's response to p53. Nat. Rev. Cancer *2*, 594–604.

Zindy, F., Eischen, C.M., Randle, D.H., Kamijo, T., Cleveland, J.L., Sherr, C.J., and Roussel, M.F. (1998). Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. Genes Dev. *12*, 2424–2433.