JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008, 59, Suppl 9, 201-212
www.jpp.krakow.pl

Original article

O. VAKHRUSHEVA[1], D. BRAEUER[2, 3], Z. LIU[1], T. BRAUN[1], E. BOBER[1]

SIRT7-DEPENDENT INHIBITION OF CELL GROWTH AND PROLIFERATION MIGHT BE INSTRUMENTAL TO MEDIATE TISSUE INTEGRITY DURING AGING

[1] Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany,
[2] Institute of Physiological Chemistry, Martin-Luther University, Halle, Germany,
[3] Landeszentrum fur Zell- und Gentherapie, Martin-Luther-Universitat, Halle, Germany

Mammalian sirtuins, Sirt1 – Sirt7, are recently discovered regulatory proteins, which play decisive roles in cellular metabolism, stress resistance, and proliferation. Sirtuins are homologs of the founder member of the sirtuin family, the yeast Sir2. Sir2 encodes a NAD^+-dependent histone deacetylase and its overexpression extends the lifespan through silencing of specific chromatin regions. Lifespan extension by Sir2 homologs was also demonstrated in more complex species such as C. elegans and D. melanogaster. A longevity function has been also postulated for mammalian sirtuins, however definitive proof is still lacking. Here, we have investigated the role of the mouse Sirt7 in the control of cellular growth and proliferation. Using Sirt7 knockout and overexpressing cells we demonstrate an anti-proliferative role of Sirt7. We also show that Sirt7 expression inversely correlates with the tumorigenic potential of several murine cell lines. Considering the known role of Sirt7 as an activator of rDNA transcription we propose that Sirt7 may enable cells to sustain critical metabolic functions by inhibiting cell growth even under severe stress conditions. We conclude, that these Sirt7 functions may improve tissue integrity in aged animals.

Key words: *sirtuins, Sirt1, Sirt7, proliferation, aging, tumorigenesis*

INTRODUCTION

Sirtuins in mammals constitute a family of seven genes (Sirt1 – Sirt7) and are often referred in literature as so called “longevity molecules”. Indeed, an increased activity of the founder member of this family, the yeast Sir2, increases

the lifespan. Overexpression of Sir2 homologous genes in C. elegans (Sir2.1) and in Drosophila (D. mel. 1) also extends the lifespan of these organisms (1). Yeast Sir2 stands for silent information regulation 2 and encodes a NAD+-dependent histone deacetylase, which silences specific chromatin regions within the yeast genome. Although a longevity promoting function was not clearly demonstrated for the mammalian sirtuins, accumulating data indicate critical roles of these molecules in controlling cellular proliferation, cell cycle, cellular metabolism as well as their important influence on tumor development (1, 2). There is growing interest in resolving the function of mammalian sirtuins and their potential role in preventing age-dependent physiological deterioration. However, the mechanisms how sirtuins may promote longevity and thus also protect against cancer, one of the most common age-dependent diseases, remain unresolved. Moreover, controversial data suggest that sirtuins may not only prevent but also promote cancer. Sirt1 can slow-down proliferation and activate stress defense mechanisms and DNA-repair, thus allowing time and means to the cell for preservation of the genomic integrity. On the other side, it was observed that certain cancer types become “addicted” to Sirt1 overexpression. In such cancers Sirt1 seems to improve tumor growth and prevent drug resistance (2-5).

Sirt1 deacetylates not only lysine residues of histones H1, H3 and H4, but also those of non-histone proteins, such as p53, FoxO proteins, Ku70, and others. Sirt1 targets are regulators of cell survival, anti-oxidative stress, DNA-repair, inflammation, and metabolism (1). Examples of Sirt1 molecular function include inhibition of the transcritptional regulatory potential of the pivotal cellular regulator p53 by its deacetylation. A diminished p53 activity increases cellular resistance against apoptosis and thus prevents cell loss under stress (6, 7). However, at the same time, cell division could proceed without the proper repair of DNA, and accumulating mutations could lead to genomic instability and tumor development.

We have concentrated on the functional analysis of the least investigated member of the mammalian sirtuin family, Sirt7. Sirt7 knockout mice display a premature aging-like phenotype and have a reduced mean and maximal life span of approximately 50% (8). Here we present an analysis of Sirt7 influence on cell survival and cell cycle control using Sirt7 overexpression in cell culture and mouse embryonic fibroblasts (MEFs) derived from Sirt7 knockout animals. Our data suggest that Sirt7 inhibits proliferation and promotes cell cycle arrest.

MATERIAL AND METHODS

Cells and culture conditions

Mouse embryonic fibroblasts (MEFs) were isolated from Sirt7-/- and wild type embryos at embryonic day 14.5 according to standard protocols. Cell lines were purchased from the American Type Culture Collection (ATCC). The study was approved by Local Ethical Committee of Max-Planck-Institute in Germany.

Cells were grown in presence of 20% O_2 and 5% CO_2 at 37°C in humidified chambers. Adriamycin, pifithrin and hydrogen peroxide were added to the culture medium for the time period of 24 h and at final concentrations of 1.5 µg/ml, 5µM and 1mM respectively.

Cell viability assay

For all experiments, cells were cultured in 24-well dishes. After reaching the 70% confluency cells were treated with adriamycin (1.5 µg/ml) or hydrogen peroxide (1mM) for 24 h. Subsequently, the cells were incubated in 100 µl of MTT solution (1mg/ml, Sigma M-2128) for 4h at 37°C. Then media were changed to DMSO/EtOH (1:1) solution. Plates were incubated for further 20 min at RT with constant agitation. 200 µl of each sample were measured at 550 nm as test wavelength and at 630 nm as the reference wavelength using Spectra Image Photometer (Tecan).

Sirt7 expression construct

1.7 kb DNA fragment containing the full-length Sirt7 cDNA was cloned into the plasmid vector (gene bank number: u71441) between 6 myc-tag sequences and the IRES-EGFP sequences.

Apoptosis

Apoptotic rate in cultured MEFs was measured using Cell Death Detection ELISA kit (Roche).

FACS analysis

Trypsinized cells were fixed with 70% EtOH, stained with propidium iodide and analyzed using ModFit LT software.

Colony forming efficiency assay (CFE)

10T1/2 fibroblasts were transfected with pIRES-EGFP/neo or pSirt7-IRES-EGFP/neo constructs, using Fugene6 reagent (Roche). Two days after transfection, cells were placed in selection medium containing geneticin (1.2 mg/ml) for 14 days. The cells were then fixed with 4% PFA, stained with hematoxylin, and counted. Formed colonies were separated in 2 groups according to the size (1-3 mm and 4-6 mm). CFE reduction was calculated using the following formula:

$$\% \text{ CFE-reduction} = 100 - [(n_p / n_c) \times 100]$$

where n_p is a number of pSirt7-IRES-EGFP formed colonies and n_c corresponds to pIRES-EGFP (control) colonies.

Quantitative real time PCR

Total RNA was isolated using the Trizol method and Q-RT-PCR was performed as described (9). Following primer sequences were used: Sirt7: 5'-CCCCGGACCGCCATCTCA-3' and 5'-ATCTCCAGGCCCAGTTCATTCAT-3'; Sirt1: 5'-TCCTTGGAGACTGCGATGTT-3' and 5'-ATTGTTGTTTGTTGCTTGGTCTAC-3'; GAPDH-1: 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3'

Gene reporter assays

Constructs containing p53, c-myc, E2F and pRb responsive elements upstream of a luciferase reporter gene were obtained from Clontech (Pathway Profiling System) and transfected into

HEK293 cells. 24h after transfection luciferase activity was estimated according to manufacture's protocol. All measurements were normalized to the internal ß-galactosidase levels.

RESULTS

Sirt7 knockout MEFs show higher viability than control wild type MEFs

In order to investigate whether Sirt7 affects cell survival we established several MEF cells from Sirt7 deficient, Sirt7 heterozygous and wild type mice and analyzed cell viability. Since we did not observed any differences between the heterozygous and wild type cells they are jointly referred to as control cells in the further course of this manuscript. Interestingly, under standard culture conditions Sirt7 knockout MEFs showed a slight but significant increase in viability (*Fig. 1A*, control). Even when cells were exposed against oxidative stress, the viability rates were still higher for the Sirt7 knockout than for the

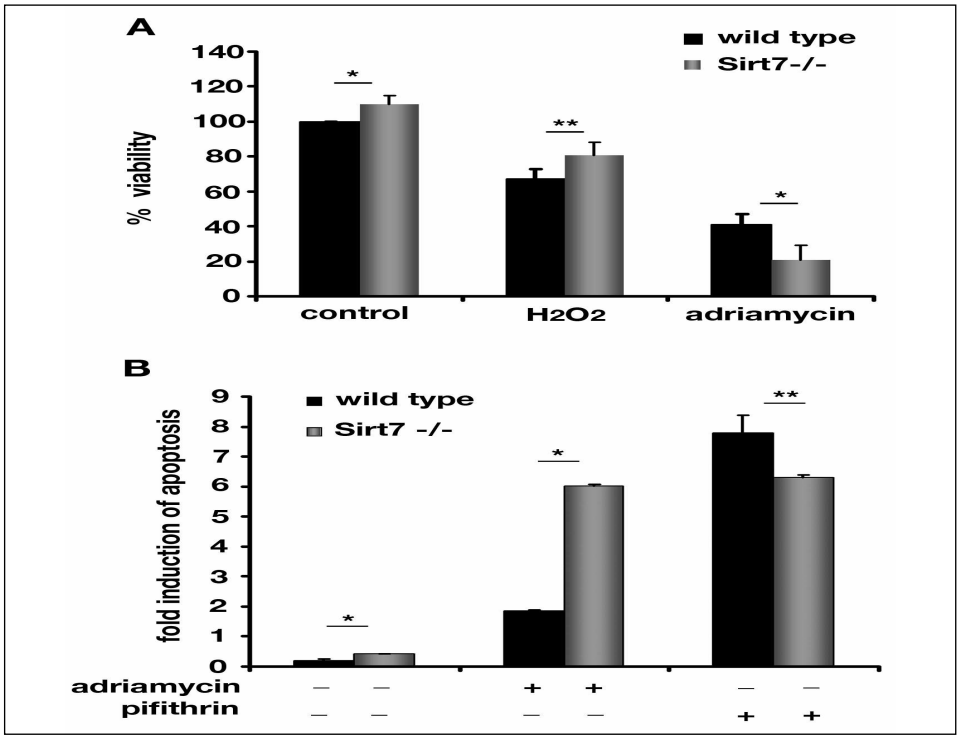

Fig. 1. Viability and induction of apoptosis in Sirt7 deficient mouse embryonic fibroblasts (MEFs). A. Sirt7 -/- and control MEFs were cultured under standard conditions and their viability estimated by MTT assay. B. Sirt7 -/- and wild type MEFs were treated with adriamycin and adriamycin together with pifithrin and scored for apoptosis. Mean values of at least three individual experiments are presented; * $P < 0.001$; ** $P < 0.05$.

control cells (*Fig. 1A*, H_2O_2). In contrast, treatment with genotoxic drug adriamycin diminished survival of Sirt7 -/- cells more strongly than control cells (*Fig. 1A*, adriamycin). The lower viability of Sirt7 -/- cells under genotoxic stress could be explained by a presence of a hyperactive, acetylated p53 protein, which induces apoptotic response after adriamycin exposure (10, 11). In fact, we observed higher levels of p53 acetylation in several tissues and cells derived from Sirt7 lacking mice (8; our unpublished data). Adriamycin treatment induced an approximately 3 fold higher level of apoptosis in Sirt7 -/- MEFs than in the control (*Fig. 1B*). Such massive apoptosis induction masked the higher viability potential of Sirt7 -/- cells so that less Sirt7 -/- cells survived under adriamycin exposure (*Fig. 1A*, adriamycin). In contrast, H_2O_2 treatment induced generally lower levels of apoptosis under our experimental conditions and thus the survival advantage was still preserved in Sirt7 deficient MEFs (*Fig. 1A*, H_2O_2, and results not shown). To definitely prove, whether the higher incidence of apoptosis was p53 dependent, the cells were exposed to adriamycin with a simultaneous addition of p53 inhibitor, pifithrin. As shown in *Fig. 1B*, inhibition of p53 caused a dramatic increase in apoptosis of control MEFs but did not further affected the Sirt7 -/- cells. This finding supports the presence of a hyperactive p53 protein in Sirt7 deficient cells.

Higher contribution of Sirt7 knockout MEFs to S and G2/M cell cycle phase

To get more insight into the role of Sirt7 in cell viability we investigated a possible involvement of Sirt7 in regulation of the cell cycle. We analyzed the contribution of Sirt7 -/- and control cells into G1, S, and G2/M phases of the cell cycle using FACS analysis. However, under standard culture conditions no statistically significant differences in the distribution of Sirt7 -/- and control cells to different cell cycle phases were observed (*Fig. 2*). Yet, after treatment with adriamycin significantly more Sirt7 -/- MEFs were found to be confined to the S and G2/M phases of the cell cycle than the control cells (*Fig. 2*). This effect could be reinforced by an addition of p53 inhibitor, pifithrin, along with adriamycin (*Fig. 2*). Thus, at least at certain conditions, Sirt7 deficiency promotes entering into the S and G2/M phases of the cell cycle.

Sirt7 overexpression inhibits cell growth and proliferation

As our analysis of Sirt7 deficient MEFs suggested that Sirt7 has a potential to inhibit proliferation, we wanted to prove more directly, whether overexpression of Sirt7 can restrain cell growth. 10T1/2 fibroblasts were transfected with Sirt7-expressing construct, and stable cell lines overexpressing Sirt7 were established. Ectopic Sirt7 expression was confirmed by EGFP immunofluorescence, since EGFP protein was co-expressed with Sirt7 (see material and methods). Stable Sirt7 overexpressing cells were screened for colony forming efficiency (CFE). As expected, Sirt7 overexpression resulted in slowing-down of the growth of cell

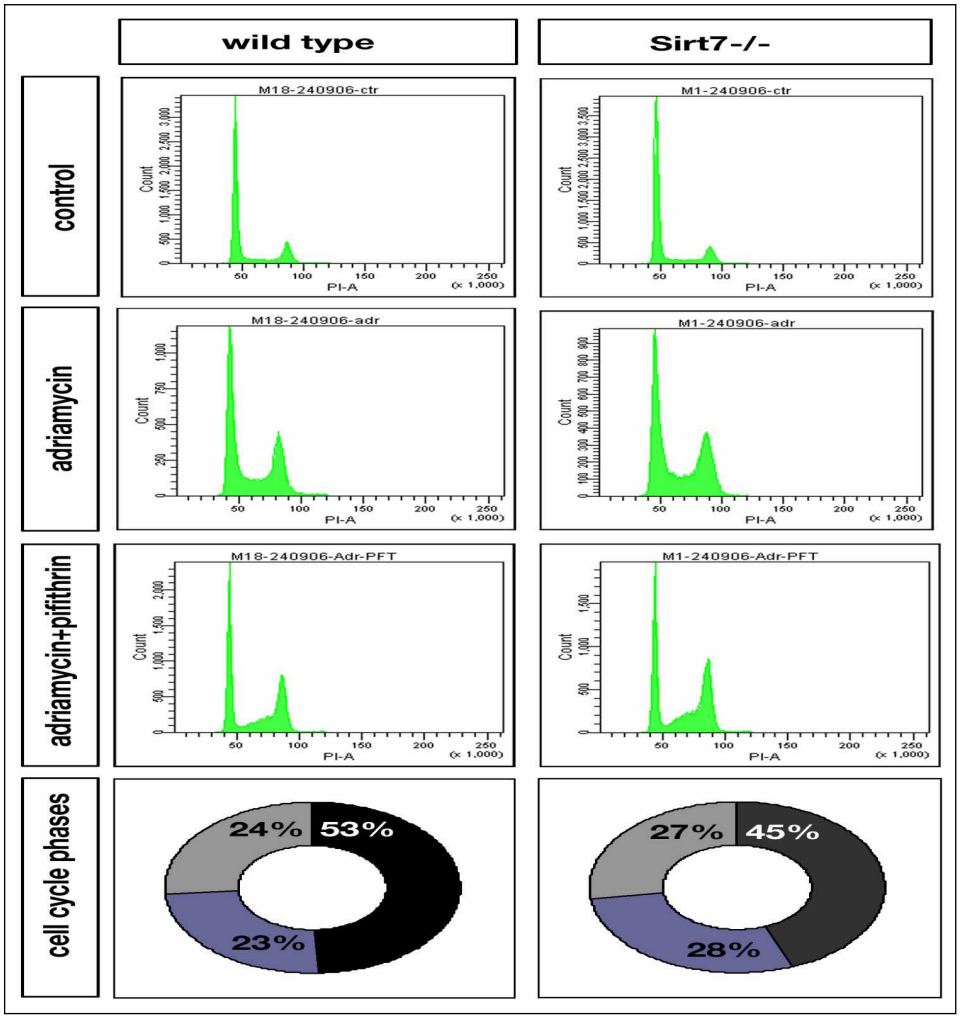

Fig. 2. Different confinement of Sirt7 -/- and wild type MEFs to cell cycle phases. Examples of FACS analyses for both cell types are presented. The cells were cultured under standard conditions without further treatment (control) or treated as indicated on the left. Below mean values derived from three independent experiments are shown in a diagram. Blue indicates S, grey G2/M, and black G1 phase of the cell cycle.

colonies. Cells overexpressing Sirt7 formed significantly less colonies (*Fig. 3*). In addition, the size of colonies expressing Sirt7 was smaller than that of colonies formed by control cells (*Fig. 3*). Overexpression of Sirt7 resulted in a 27% reduction of the number of forming colonies, while reduction of the colony size was of 17%.

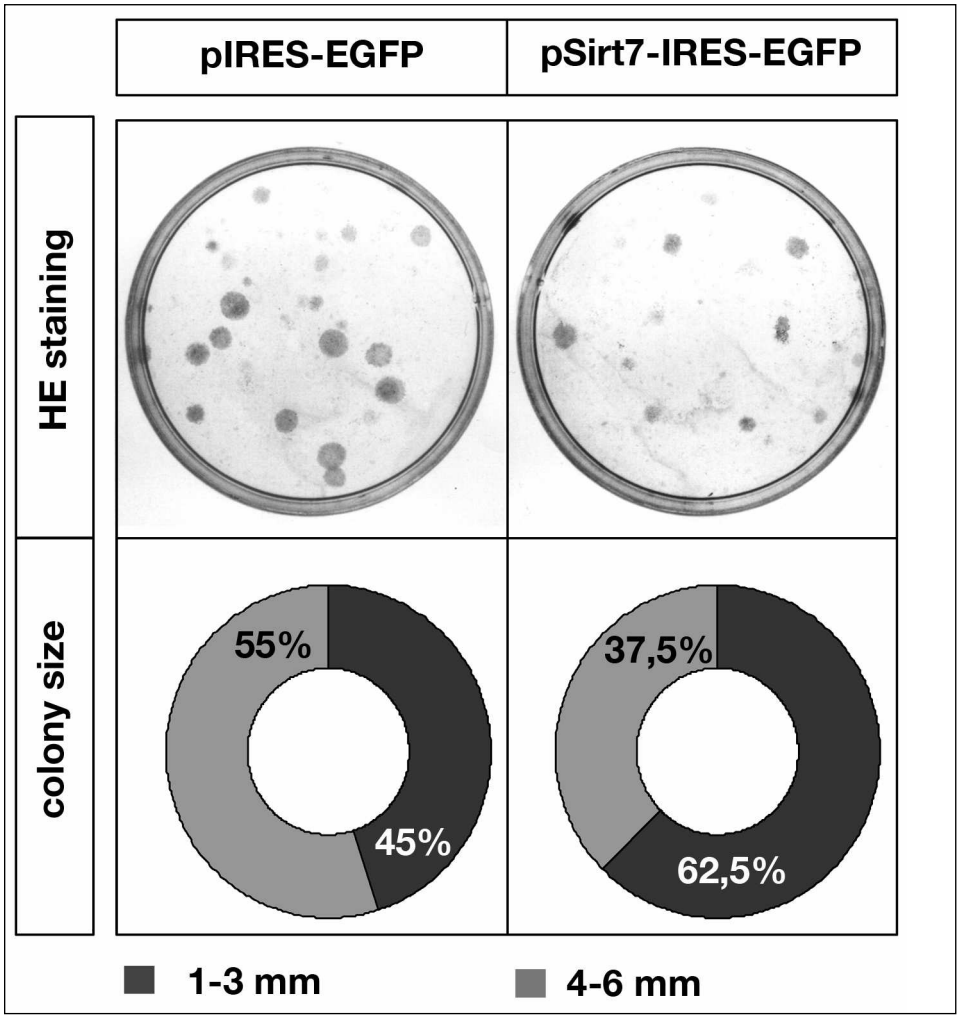

Fig. 3. Lower colony forming efficiency of Sirt7 overexpressing cells. 10T1/2 fibroblasts were stably transfected with Sirt7 expression construct (pSirt7-IRES-EGFP) or with vector alone (pIRES-EGFP). Upper pictures show examples of HE-stained colonies build by control and Sirt7 overexpressing cells. Note the lower number and size of Sirt7 overexpressing cell colonies. Lower pictures summarize distribution of small (1-3 mm diameter, marked black) and bigger-size colonies (4-6 mm diameter, marked grey) formed by both types of cells (expressed as the percentage of all colonies).

Sirt7 expression is reduced in several cancerogenic cell lines

The finding that Sirt7 delays cell growth suggested that Sirt7 expression might decrease during tumorigenesis. However, against this expectation, an increased Sirt7 expression was reported in thyroid and breast carcinomas (see discussion).

To investigate whether Sirt7 may be down-regulated in cells which are prone to develop cancer, we performed Sirt7 expression analysis in several murine cell lines of the known tumorigenic potential in comparison with non-tumorigenic cells. The expression data are summarized in *Fig. 4*. It is apparent that all of the tested murine tumorigenic cell lines: P19 (teratocarcinoma), NB41A3 (neuroblastoma), C3H/MCA (transformed fibroblast-derived cell line) showed a decreased Sirt7 expression as compared to the control non-tumorigenic cell line C3H/10T1/2 (*Fig. 4A*). Most significantly, the MCA cell line represents a derivative of the non-tumorigenic 10T1/2 fibroblasts, established after treatment of 10T1/2 cells with a carcinogen 3- methylcholanthrene. Sirt7 expression in this particular MCA cell is clearly decreased as compared to the expression level in the parental 10T1/2 cell (*Fig. 4A*). Similarly to Sirt7, another member of the sirtuin family, Sirt1, did not show a higher expression in the NB41A3 and MCA cells. However, Sirt1 expression was strongly increased in the teratocarcinoma cell line P19 (*Fig. 4A*). Interestingly, a higher Sirt1 expression was only observed in the undifferentiated P19 cells. Retinoic acid induced differentiation reverted Sirt1 expression in P19 cells to low levels. In contrast, Sirt7 was expressed constitutively at the low level in P19 cells independent of their differentiation status (*Fig. 4B*).

Sirt7 can activate transcriptional function of c-myc and p53 proteins

In the light of previous reports on increased Sirt7 expression in thyroid and breast cancer the here observed Sirt7-dependent inhibition of cell proliferation

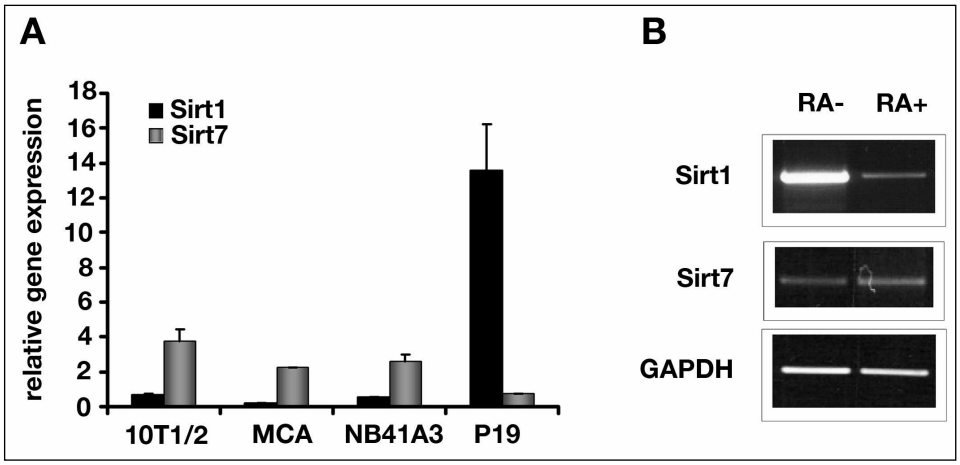

Fig. 4. Sirt7 expression is decreased in tumorigenic cell lines. A. Quantitative RT-PCR estimation of Sirt7 (grey) and Sirt1 (black) expression levels in tumorigenic (MCA, NB41A3, P19) cell lines as compared to control cell line (10T1/2). B. Semiquantitative RT-PCR analysis of Sirt1 and Sirt7 expression in differentiated (after retinoic acid treatment, RA+) and undifferentiated (RA-) P19 teratocarcinoma cell line. GAPDH was used as loading control.

was a rather unanticipated function. The recently demonstrated ability of Sirt7 to activate rDNA transcription also argues for a proliferation promoting rather than inhibiting function of Sirt7 (12). Since Sirt7 was postulated to deacetylate and inactivate p53 (8), we asked whether Sirt7 could also influence transcriptional function of p53. P53 is known to induce transcription of cell cycle inhibitors, such as p21 and thus cause cell cycle arrest. In addition, we also investigated the influence of Sirt7 on c-myc protein, a known activator of rDNA transcription (13-15). Constructs containing p53- or myc-responsive elements and basic promoter sequences in front of a luciferase reporter gene were transfected together with Sirt7 expression constructs into the HEK293 cells. As shown in *Fig. 5*, overexpression of Sirt7 led to 10-fold and 5-fold activation of p53- and c-myc reporter plasmids respectively. No activation was observed, when reporter plasmids containing E2F- or Rb-response elements were used (*Fig. 5*). Thus, it can be concluded, that Sirt7 is able to activate p53- and c-myc-dependent transcription. Transfection of constructs expressing another sirtuin involved in cell cycle control, Sirt2 (16, 17), had no effect on reporter-genes activities. Because Sirt2 is known to fluctuate between cytoplasm and nucleus (17), we ensured the nuclear localization by adding the nls-sequence into the Sirt2

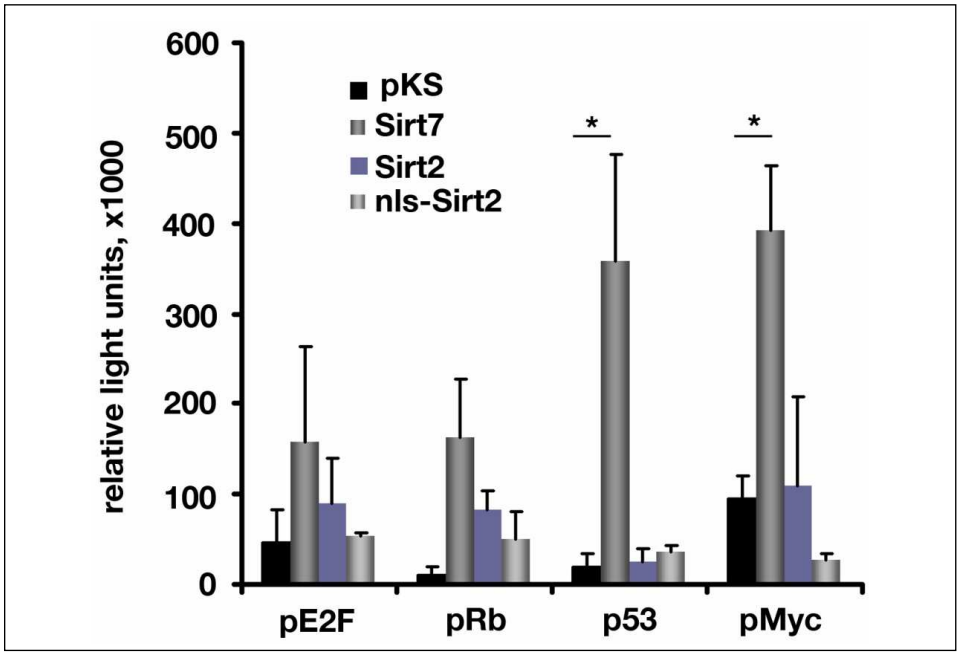

Fig. 5. Sirt7 activates transcriptional targets of c-myc and p53. Luciferase activity driven by E2F, Rb, p53, and c-myc responsive elements after transfection with indicated expression constructs (see text). * $P < 0.001$.

expression plasmid (nls-Sirt2, *Fig. 5*). This plasmid was constitutively expressed in the nucleus (data not shown). However, even the nls-Sirt2 expression plasmid was not able to induce transcription of p53 or c-myc targets.

DISSCUSSION

Sirtuins constitute a vigorously investigated group of proteins implicated in control of tumor development and aging (2, 18). The most experimental data accumulated so far concentrates on the function of the founding member of mammalian sirtuins, Sirt1 (18, see below). Still relatively little is known about the role of the remaining sirtuins in these processes. In this work we analyzed influence of Sirt7, a less investigated member of the mouse sirtuin family, on cell growth and proliferation. Interestingly, our results demonstrate clear anti-proliferative effects of Sirt7. The removal of Sirt7 in Sirt7 knockout mice-derived MEFs led to an increased viability under standard culture conditions and under oxidative stress. Moreover, a higher proportion of Sirt7 deficient MEFs was found to be confined to the S and G2/M phases of cell cycle than control wild type cells. In agreement with the proliferation inhibiting potential, the overexpression of Sirt7 led to smaller number and size of Sirt7 overexpressing cell colonies as demonstrated in colony forming efficiency assay. Furthermore, cells of tumorigenic potential revealed lower Sirt7 expression as compared to parental, non-tumorigenic cells.

This anti-proliferative capacity of Sirt7 is a rather unexpected finding. Sirt7 was previously demonstrated to be an activator of the rDNA transcription and thus to promote proliferation (12). In addition, an increased Sirt7 expression was reported for thyroid and breast cancer in human patients (19-21). How do these finding agree with the slowing down of proliferation by Sirt7? Similar discrepancies were already described concerning the role of Sirt1 in cancerogenesis. Inhibition of Sirt1 by siRNA enhances proliferation of primary human fibroblasts thus suggesting that Sirt1 might limit tumor development (22). In fact, ectopic Sirt1 expression in mouse model of colon cancer reduced tumor formation, proliferation, and animal morbidity (23). On the other hand, Sirt1 has been demonstrated in multiple assays to increase cell survival. In addition, an increased Sirt1 expression was detected in a variety of tumors (discussed in 1, 2 and 18).

It is conceivable that Sirt7 is able to promote cellular metabolism through direct activation of rDNA transcription (12; our unpublished observations) and also by activating the transcriptional function of c-myc, as demonstrated in this paper. At the same time Sirt7 may slow-down cell proliferation through activation of cell cycle inhibitors, such as a p53 target p21 and through further as yet not known mechanisms. Thus, in cancer cells, which lost p53 activity, increased Sirt7 expression might be important to sustain higher metabolic demands of such cells

without affecting their proliferation potential. Inhibition of Sirt7 function in such cells could help to eliminate them by means of “metabolic starvation”. Such therapies could be of special interest for targeting of quiescent cancer stem cells, which are resistant to the most anti-tumor therapies (24, 25). However, cancer cells develop various heterogenic strategies, which render them resistant to induction of different types of programmed cell death by anti-cancer drugs (26). Moreover, in tumors, which still posses an active p53 protein, Sirt7 may also support survival by inhibition of p53 function through deacetylation and thus prevent apoptosis. Therefore, further investigations of exact roles of Sirt7 in different types of tumors are necessary to develop well-tailored anti-tumor therapies by modulating Sirt7 function.

Acknowledgments. The excellent technical assistance of K. Kolditz and M. Wiesnet is greatly acknowledged. This study was approved by Institutional Review Committe at Martin-Luther University, Halle.

Conflict of interest statement: None declared.

REFERENCES

1. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J* 2007; 404: 1-13.
2. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. *Oncogene* 2007; 26: 5489-5504.
3. Chu F, Chou PM, Zheng X, Mirkin BL, Rebbaa A. Control of multidrug resistance gene mdr1 and cancer resistance to chemotherapy by the longevity gene sirt1. *Cancer Res* 2005; 65: 10183-10187.
4. Huffman DM, Grizzle WE, Bamman MM, *et al.* SIRT1 is significantly elevated in mouse and human prostate cancer. *Cancer Res* 2007; 67: 6612-6618.
5. Kojima K, Ohhashi R, Fujita Y, *et al.* A role for SIRT1 in cell growth and chemoresistance in prostate cancer PC3 and DU145 cells. *Biochem Biophys Res Com* 2008; 373: 423-428.
6. Vaziri H, Dessain SK, Ng Eaton E, *et al.* hSIR2 (SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* 2001; 107: 149-159.
7. Luo J, Nikolaev AY, Imai S, *et al.* Negative control of p53 by Sir2a promotes cell survival under stress. *Cell* 2001; 107: 137-148.
8. Vakhrusheva O, Smolka C, Gajawada P, *et al.* Sirt7 increases stress resistance of cardiomyocytes and prevents inflammatory cardiomyopathy in mice. *Circ Res* 2008; 102: 703-710.
9. Neuhaus P, Oustanina S, Loch T, *et al.* Reduced mobility of fibroblast growth factor (FGF)-deficient myoblasts might contribute to dystrophic changes in the musculature of FGF2/FGF6/mdx triple-mutant mice. *Mol Cell Biol* 2003; 17: 6037-6048.
10. Ito A, Lai CH, Zhao X, *et al.* P300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. *EMBO J* 2001; 20: 1331-1340.
11. Tang Y, Zhao W, Chen Y, Zhao Y, Gu W. Acetylation is indispensable for p53 activation. *Cell* 2008; 133: 612-626.
12. Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev* 2006; 20: 1075-1080.

13. Oskarsson T, Trumpp A. The Myc trilogy: lord of RNA polymerases. *Nat Cell Biol* 2005; 7: 215-217.
14. Grandori C, Gomez-Roman N, Felton-Edkins ZA, *et al.* c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nat Cell Biol* 2005; 7: 311-318.
15. Arabi A, Wu S, Ridderstrake K, Bierhoff H, *et al.* c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. *Nat Cell Biol* 2005; 7: 303-310.
16. Dryden SC, Nahhas FA, Nowak JE, Goustin AS, Tainsky MA. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* 2003; 23: 3173-3185.
17. North BJ, Verdin E. Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. *PLoS ONE* 2007; 29: e784.
18. Kim E-J, Um S-J. SIRT1: roles in aging and cancer. *BMB Rep* 2008; 41: 751-756.
19. De Nigris F, Cerutti J, Morelli C, *et al.* Isolation of a SIR-like gene, SIR-T8, that is overexpressed in thyroid carcinoma cell lines and tissues. *Br J Cancer* 2002; 87: 1479.
20. Frye R. "SIRT8" expressed in thyroid cancer is actually SIRT7. *Br J Cancer* 2002; 87: 1479.
21. Ashraf N, Zino S, Macintyre A, *et al.* Altered sirtuin expression is associated with node-positive breast cancer. *Br J Cancer* 2006; 95: 1056-1061.
22. Abdelmosen K, Pullmann Jr R, Lal A, *et al*. Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell* 2007; 25: 543-557.
23. Firestein R, Blander G, Michan S, *et al.* The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. *PLoS ONE* 3: e2020.
24. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003; 3: 895-902.
25. Kucia M, Ratajczak MZ. Stem cells as a two edged sword – from regeneration to tumor formation. *J Physiol Pharmacol* 2006; 57: Supp 7, 5-16.
26. Motyl T, Gajkowska B, Zarzynska J, Gajewska M, Lamparska-Przybysz M. Apoptosis and autophagy in mammary gland remodelling and breast cancer chemotherapy. *J Physiol Pharmacol* 2006; 57: Supp 7, 17-32.

Received: November 5, 2008
Accepted: December 20, 2008

Author's address: Dr. Eva Bober, Max-Planck-Institut fur Herz- und Lungenforschung. W.G. Kerckhoff Institut, Parkstrasse 1, D-61231 Bad Nauheim; Phone: +49(0)6032.705-271; Fax: +49(0)6032.705-419; e-mail: eva.bober@mpi-bn.mpg.de