

SIRT1 Is Significantly Elevated in Mouse and Human Prostate Cancer

Derek M. Huffman,¹ William E. Grizzle,² Marcas M. Bamman,^{3,5} Jeong-su Kim,^{3,5}
Isam A. Eltoum,² Ada Elgavish,⁴ and Tim R. Nagy¹

Departments of ¹Nutrition Sciences, ²Pathology, ³Physiology and Biophysics, and ⁴Genetics, University of Alabama at Birmingham; ⁵Geriatric Research, Education, and Clinical Center, Veterans Affairs Medical Center, Birmingham, Alabama

Abstract

Evidence suggests that the histone deacetylase, SIRT1, is a mediator of life span extension by calorie restriction; however, SIRT1 may paradoxically increase the risk of cancer. To better understand the relationship among SIRT1, energy balance, and cancer, two experiments were done. First, a transgenic mouse model of prostate cancer (transgenic adenocarcinoma of mouse prostate; TRAMP) was used to determine the role of energy balance on SIRT1 expression and the effect of cancer stage on SIRT1 and hypermethylated in cancer-1 (HIC-1). Second, immunohistochemistry was done on human prostate tumors to determine if SIRT1 was differentially expressed in tumor cells versus uninvolved cells. Results show that SIRT1 is not increased in the dorsolateral prostate (DLP) of calorie-restricted mice during carcinogenesis. In contrast, when examined in the DLP as a function of pathologic score, SIRT1 was significantly elevated in mice with poorly differentiated adenocarcinomas compared with those with less-advanced disease. HIC-1, which has been shown to regulate SIRT1 levels, was markedly reduced in the same tumors, suggesting that a reduction in HIC-1 may be in part responsible for the increased expression of SIRT1 in prostatic adenocarcinomas. Furthermore, immunostaining of human prostate tumors showed that cancer cells had greater SIRT1 expression than uninvolved cells. In conclusion, DLP SIRT1 expression from calorie-restricted mice was not altered during carcinogenesis. However, SIRT1 expression was increased in mice with poorly differentiated adenocarcinomas and in human prostate cancer cells. Because SIRT1 may function as a tumor promoter, these results suggest that SIRT1 should be considered as a potential therapeutic target for prostate cancer. [Cancer Res 2007;67(14):6612–8]

Introduction

Calorie restriction is the only known behavioral intervention proven to extend life span and inhibit or delay carcinogenesis (1). Evidence suggests that the class III histone deacetylase, SIRT1, may be an important mediator of life span extension by calorie restriction (2). Specifically, SIRT1 may function to promote cell survival (3–5) via histone modifications that increase genome stability and silence gene expression and through direct interactions with several apoptotic proteins, including p53 (5), Bax (4),

and members of the FOXO family of transcription factors (3). However, recent evidence suggests that the antiapoptotic affects of SIRT1 may paradoxically increase the risk of cancer (5–8).

Several studies have shown that targeted inhibition of SIRT1 *in vitro* and *in vivo* can effectively limit cancer progression and induce apoptosis (9–11). In addition, it has been shown that the tumor suppressor hypermethylated in cancer-1 (HIC-1), which is epigenetically silenced in many tumors, directly suppresses gene transcription of SIRT1 and that mice heterozygous for the *HIC-1* gene have more frequent tumor formation than wild-type animals (8). Moreover, high expression of SIRT1 has been reported in cancer cell lines, human tumors (breast, colon, and skin; refs. 12, 13) and in genetically modified mice (prostate and lung; refs. 8, 12).

The fact that calorie restriction can inhibit cancer incidence and progression (1), despite increasing SIRT1 protein content (4), suggests that a delicate balance of SIRT1 expression exists in the cell. Therefore, we tested the effect of changes in energy metabolism on SIRT1 protein expression during carcinogenesis in a mouse model of prostate cancer [transgenic adenocarcinoma of mouse prostate (TRAMP)]. Our hypothesis was that calorie-restricted mice would have greater SIRT1 expression in the dorsolateral prostate (DLP), despite having less advanced cancer (14). However, our results show that SIRT1 levels in the DLP were numerically, although not significantly, lower in calorie-restricted TRAMP mice during the early stages of carcinogenesis compared with other experimental groups.

Because we have shown previously that this dietary intervention inhibits cancer incidence and progression in the TRAMP model (14) and that high expression of SIRT1 in tumors and cancer cells has been reported, we sought to determine if SIRT1 expression was related to cancer stage in TRAMP mice. The results of this experiment show that SIRT1 expression is significantly increased in mouse prostatic adenocarcinomas. The increase in SIRT1 associated with poorly differentiated tumors was confirmed by immunostaining, such that it was not just a function of more cells per unit weight per volume.

We then measured HIC-1 levels from the same tumors and found a marked reduction in HIC-1 expression in poorly differentiated adenocarcinomas, suggesting that a reduction in HIC-1 may be in part responsible for the increased expression of SIRT1 in poorly differentiated tumors. Furthermore, Bax expression and the Bax/Bcl-2 ratio were significantly greater in poorly differentiated tumors from TRAMP mice, confirming that changes normally associated with cancer cells (15) were present in the tissues under investigation.

To determine if our results from the TRAMP model were applicable to human prostate cancer, we did immunohistochemical staining for SIRT1 in human prostate tumor biopsies. It was found that SIRT1 was more highly expressed in cancer cells than uninvolved cells, suggesting that our findings from the TRAMP

Requests for reprints: Derek M. Huffman, Division of Physiology and Metabolism, Department of Nutrition Sciences, University of Alabama at Birmingham, G005 Volker Hall, 1670 University Boulevard, Birmingham, AL 35294-3360. Phone: 205-934-4008; Fax: 205-934-7050; E-mail: dhuffman@uab.edu.

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model have relevance to the human disease. Because SIRT1 may function as a tumor promoter, these results strongly suggest that SIRT1 be considered as a potential therapeutic target for prostate cancer.

Materials and Methods

Transgenic animals. TRAMP mice on a pure C57BL/6 background were obtained from our breeding colony located at the University of Alabama at Birmingham (UAB). Transgenic females were bred with nontransgenic males, and all breeders were maintained on a standard 12L:12D photoperiod and fed standard rodent chow (Harlan Teklad, Inc.) and water *ad libitum*. After weaning, male pups were separated from females, and a tail biopsy was collected for determination of transgene incorporation by PCR as described previously (14, 16). All work was approved by the Institutional Animal Care and Use Committee of the UAB.

Experimental design—6-week feeding experiment. At 6 weeks of age, TRAMP mice were singly housed at 22°C and provided *ad libitum* access to a phytoestrogen-free diet (AIN-76A, Harlan Teklad) for 1 week. At 7 weeks of age, mice were randomly assigned to one of five groups: control ($n = 6$), *ad libitum* fed at 27°C (AL27; $n = 6$), *ad libitum* fed at 22°C (AL22; $n = 5$), pair fed at 27°C (PF27; $n = 6$), or pair fed at 22°C (PF22, $n = 6$).

Mice assigned to the control group were sacrificed at baseline, and all remaining mice were randomly distributed to one of four experimental treatments (AL27, AL22, PF27, and PF22) for 6 weeks (7–13 weeks old) as described previously (14). AL22 mice were housed at a “normal” ambient temperature (22°C) and given *ad libitum* access to food. Conversely, AL27 mice were fed *ad libitum* but were housed in a humidity-controlled incubator (model IS79SD, Powers Scientific, Inc.) at 27°C, a temperature that is closer to the thermoneutral zone for mice, thereby reducing the thermoregulatory demands needed to defend body temperature and hence energy expenditure (17). We have shown previously that TRAMP mice housed at 27°C reduce their food intake by 25% to 30%, with little to no effect on body weight and composition, compared with their *ad libitum*-fed counterparts at 22°C (14).

PF27 mice were housed at 27°C but were fed 2.2 g/d, an amount that we have shown previously to be 95% of *ad libitum* food intake at 27°C for TRAMP mice (14). PF22 mice were housed at 22°C and pair fed to PF27 mice (2.2 g/d), an amount that is ~30% less than what TRAMP normally consume *ad libitum* at 22°C. Overall, this experimental design allowed for examining the effects of changes in food intake without changing body composition (AL27 versus AL22), changes in body composition without changing food intake (PF27 versus PF22), 5% calorie restriction (AL27 versus PF27), or ~30% calorie restriction (AL22 versus PF22) on SIRT1 protein content in the DLP.

Body composition. Animals in the 6-week feeding experiment were anesthetized with isoflurane (2%) and placed in a prostrate position on the imaging plate, and body composition was assessed *in vivo* by dual-energy X-ray absorptiometry (GE-Lunar PIXImus, software version 1.45, GE-Lunar) 24 h before sacrifice. Fat mass, soft lean tissue mass, and percentage fat were determined as described previously (18).

Experimental design—cancer stage and SIRT1. To assess the effect of cancer stage on SIRT1 protein content, DLP samples were used from a group in a previous study examining the role of energy balance on cancer progression (14), in which mice were housed at 22°C and fed 2.2 g daily for 21 weeks. Tissues from these animals (PF22) were chosen because a few animals from this group had a pathologic score of 1 (normal prostate), thus providing a unique look at protein changes spanning from normal prostate to poorly differentiated adenocarcinomas.

Mouse tissue collection and Western blotting. At the conclusion of each experiment (the 6-week feeding experiment and the cancer stage and SIRT1 experiment), the DLP was rapidly collected, snap frozen in liquid nitrogen, and stored at -70°C until analysis. Frozen tissue samples were powdered by using a liquid nitrogen-cooled mortar and pestle and homogenized in an ice-cold lysis buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 0.5% NP40, 5 mmol/L EDTA, 100 μmol/L phenylmethylsulfonyl fluoride, 0.1 μmol/L

Okadaic acid, 1 mmol/L orthovanadate, 0.5× Protease inhibitor cocktail containing bestatin, leupeptin, and aprotinin (P2714, Sigma-Aldrich)]. Lysates were then assayed for total protein content by using the bicinchoninic acid technique with bovine serum albumin (BSA) as a standard.

For immunoblotting, 30 μg of total protein from the DLP were loaded onto 4% to 15% gradient mini gels (Bio-Rad Laboratories) and separated by SDS-PAGE. Proteins were then semidry transferred (Trans-Blot SD, Bio-Rad Laboratories) to polyvinylidene difluoride membranes and blocked for 1 h with 2% nonfat dry milk/2% BSA at room temperature. Membranes were incubated overnight at 4°C with either a rabbit polyclonal antibody to SIRT1 (1:2,000; Upstate Biotechnology), Bcl-2 (1:1,000; BD Biosciences), Bax (1:500; Abcam), a goat polyclonal antibody to HIC-1 (1:500; Santa Cruz Biotechnology), or a mouse monoclonal antibody (mAb) to actin (1:2,000), which was obtained from the Developmental Studies Hybridoma Bank (Department of Biological Sciences, University of Iowa).

After incubation with an appropriate horseradish peroxidase-conjugated secondary antibody, bands were visualized by chemiluminescence (Super West Dura kit, Pierce Biotechnologies) in a Bio-Rad ChemiDoc imaging system, and band densitometry was done using Bio-Rad Quantity One software.

Histopathology of TRAMP mouse prostate. For the cancer stage and SIRT1 experiment, a portion of the DLP from TRAMP mice was collected for histologic analysis. Briefly, the DLP was placed in an acid-alcohol fixative containing 96% ethanol, 1% glacial acetic acid, and 3% distilled water at 4°C overnight as described by Folkvord et al. (19). The fixed tissue was then embedded in paraffin, and 5-μm sections were mounted onto treated slides before Gomori trichrome staining.

Pathologic changes in the DLP were scored as described previously (14, 16, 20) on a 1 to 6 scale by a pathologist who was blinded to the experiment (I.A.E.). For the purposes of this study, only tissues with a pathologic score of 1 ($n = 5$), 3 ($n = 6$), or 6 ($n = 6$), indicating normal tissue, high-grade prostatic intraepithelial neoplasia (PIN), or poorly differentiated prostatic adenocarcinomas, respectively, were used.

Immunostaining of TRAMP mouse prostate. Immunostaining for the SIRT1 and cancer stage experiment was done on either normal prostates (pathologic score, 1), high-grade PIN lesions (score, 3), or adenocarcinomas (score, 6) from TRAMP mice ($n = 4$ per group). Initially, a 5-μm section of each specimen was mounted onto treated slides. Following rehydration, sections were treated with 3.0% H₂O₂ for 5 min to quench endogenous peroxidase activity, blocked with preimmune goat serum (1%) for 20 min, and incubated with a rabbit polyclonal antibody to SIRT1 (1:200) for 1 h. A delete was included in the same run for each slide by omitting primary antibody from the staining procedure. All slides were then incubated with antirabbit link (Jackson ImmunoResearch) for 20 min followed by a biotin-streptavidin containing label (Signet) for 20 min. Finally, slides were incubated for 3 min with the substrate 3,3'-diaminobenzidine (DAB) for visualization of the antigen-antibody complex and counterstained lightly with hematoxylin.

Human prostate tumor biopsies. Anonymous human prostate cancer cases (Gleason score, 6–10) were obtained from Tissue Procurement at UAB, and all procedures were approved by the UAB Institutional Review Board. All tumor samples ($n = 30$) were incorporated on one tissue array paraffin block in triplicate, which included cell lines (DU145, LNCaP, and MCF7), liver, tonsil, and kidney as controls.

Immunostaining of human prostate cancer cases. For immunostaining of human prostate cancer cases, 5-μm sections from the tissue array block containing all cancer cases ($n = 30$) in triplicate were mounted onto treated slides. Antigen retrieval was done for the detection of SIRT1 in human prostate tumors on both the slide and matched delete in 1 mmol/L EDTA (pH 8) using a pressure cooker on high steam for 10 min.

Following rehydration (Bax and Bcl-2) or antigen retrieval (SIRT1), slides were treated with 3.0% H₂O₂ for 5 min to quench endogenous peroxidase activity and blocked with preimmune goat serum (1%) for 20 min. Sections were then incubated with either a mouse mAb to Bax (1:150; Santa Cruz Biotechnology), Bcl-2 (1:100; Roche), or a rabbit mAb to SIRT1 (1:100; Epitomics) for 1 h. A delete was included in the same run for each slide in

which primary antibody was omitted from the staining procedure. Sections were then incubated with a biotin-streptavidin detection system (Signet) followed by DAB reagent for 2 min (Bax) or 7 min (SIRT1 and Bcl-2) and counterstained lightly with hematoxylin.

Evaluation of immunostaining. Immunostaining intensity of various target proteins in mouse and human prostate specimens were scored on a scale of 0 (no staining) to 4 (intense staining) by a pathologist (W.E.G.). To quantify the staining intensity of each cell type, a weighted average was obtained by multiplying the percentage of cells at each staining intensity by the intensity score. The percentage of cells positively stained was determined by calculating the percentage of cells not scored "0" (no staining). Because human prostate cancer cases were included in triplicate on the tissue array, the final intensity score and percentage staining for these tissues are reported as the average of three values.

Statistical analyses. Body weight, body composition, and protein content were analyzed using ANOVA and followed up with planned contrasts when appropriate. Nonparametric data were analyzed using the Kruskal-Wallis test and planned contrasts were done with the Mann-Whitney *U* test when appropriate. Spearman correlations were done on immunohistochemical markers to determine associations between variables. Outcomes were considered statistically significant when $P < 0.05$. All analyses were done using SPSS (version 10.0).

Results

6-Week Feeding Experiment in TRAMP Mice

Calorie-restricted mice (PF22) had less total, fat, and lean mass. No significant differences were observed for body weight or composition among groups at the beginning of the study (data not shown). *Ad libitum*-fed mice at 27°C (AL27) and 22°C (AL22) had similar body weight and composition after 6 weeks of treatment, and both groups had more total, fat, and lean mass than PF27 and PF22 mice (Fig. 1A–D). The PF27 group, who were fed 95% of *ad libitum* food intake at 27°C, weighed less and had less fat and lean mass than both groups of *ad libitum*-fed mice but more than PF22 mice ($P < 0.001$). Furthermore, compared with their

ad libitum-fed controls at 22°C (AL22), PF22 mice had ~30% less body mass, 53% less fat mass, 28% less lean mass, and a resultant 5% lower percentage fat.

SIRT1 expression in mouse prostate was not increased by calorie restriction. SIRT1 protein expression was assessed by Western blot in TRAMP mice killed at baseline, or following one of four interventions for 6 weeks designed to manipulate energy expenditure and/or intake. There was no significant difference in SIRT1 expression among groups ($P = 0.44$; Fig. 2A). However, SIRT1 expression was numerically, albeit not significantly, less in mice killed at baseline and PF22 mice, which consumed the least calories and were leaner and lighter than other experimental groups. In contrast, expression was notably high in some animals from the AL27, AL22, and PF27 groups.

SIRT1 and Cancer Stage in TRAMP Mice

SIRT1 was significantly increased in mouse prostatic adenocarcinomas. When the DLP from mice treated up to 21 weeks were examined by Western blot as a function of pathologic score, there was no difference in protein content between normal prostates and PIN lesions, but SIRT1 protein was 2.5-fold greater in poorly differentiated adenocarcinomas compared with those with less-advanced disease ($P < 0.05$; Fig. 2B). The increase in phenotypic expression of SIRT1 in poorly differentiated tumors was confirmed by immunostaining; staining intensity was ~2-fold greater in cancer cells versus luminal and basal cells from normal glands, or PIN lesions ($P < 0.05$; Fig. 3A–D). There was no difference in staining intensity between luminal cells from normal prostates and PIN lesions ($P = 0.99$; Fig. 3A, B, and D). However, in PIN lesions, there was a significantly greater staining intensity in luminal cells than basal cells ($P < 0.05$; Fig. 3B and D), but no difference in staining intensity was observed between luminal and basal cells in normal prostates ($P = 0.20$; Fig. 3A and D).

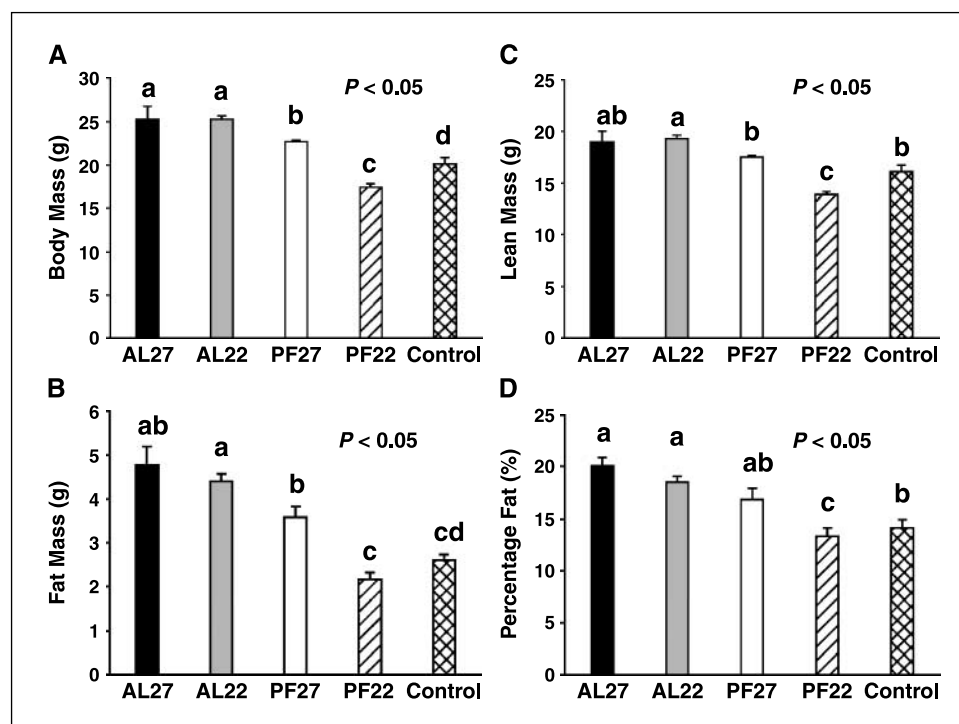


Figure 1. Phenotypic characteristics of mice in the 6-wk feeding experiment assigned to one of five groups: *ad libitum* fed at 27°C (AL27; $n = 6$), *ad libitum* fed at 22°C (AL22; $n = 5$), pair fed at 27°C (PF27; $n = 6$), pair fed at 22°C (PF22; $n = 6$), or control ($n = 6$). Measurements were taken at baseline (data not shown) and after 6 wks of treatment for body mass (A), fat mass (B), lean mass (C), and percentage fat (D). Columns, mean; bars, SE. Different letters (a–d) denote a significant difference between groups, such that groups not sharing a similar letter are significantly different from each other ($P < 0.05$).

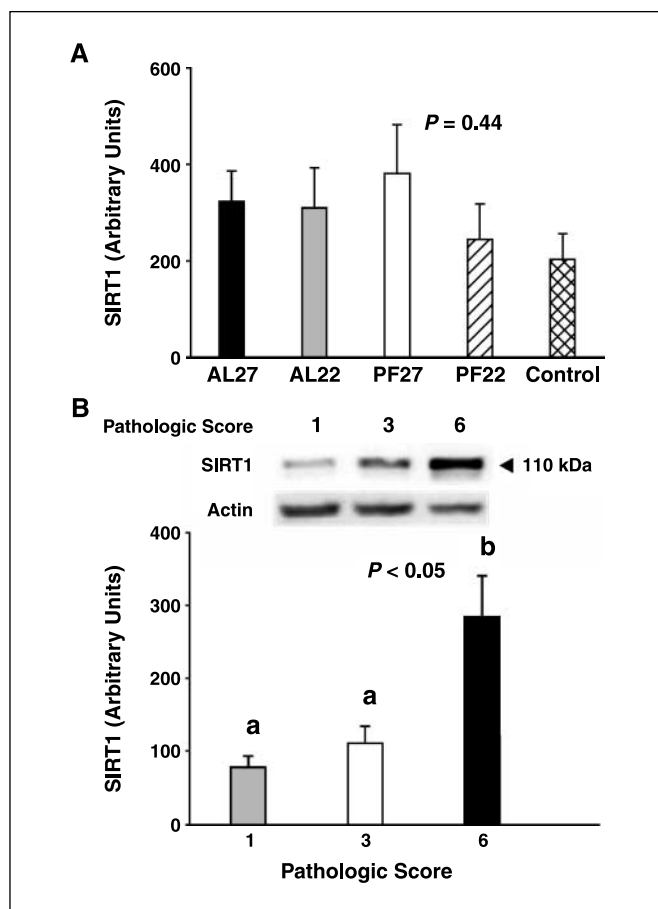


Figure 2. SIRT1 protein content in the DLP from TRAMP mice. **A**, SIRT1 protein expression in the DLP of TRAMP mice in the 6-week feeding experiment. Mean protein expression for SIRT1 in the DLP from experimental groups. There were no significant differences among groups ($P = 0.44$). **B**, SIRT1 protein expression in the DLP of TRAMP mice in the SIRT1 and cancer stage experiment with pathologic score of 1 ($n = 5$), 3 ($n = 6$), or 6 ($n = 6$), indicating normal tissue, high-grade PIN, or poorly differentiated prostatic adenocarcinoma, respectively. Representative Western blot (top). Mean protein expression for SIRT1 in the DLP at each pathologic score (bottom). Columns, mean; bars, SE. Different letters denote (*a* and *b*) a significant difference between groups, such that groups not sharing a similar letter are significantly different from each other ($P < 0.05$).

HIC-1 was reduced, but Bax and the Bax/Bcl-2 ratio were increased in mice with prostatic adenocarcinomas. Western blots indicated that HIC-1 protein content was significantly reduced in prostatic adenocarcinomas compared with normal prostates and high-grade PIN lesions ($P < 0.05$; Fig. 4A). In contrast, Bax was increased by ~7-fold in the same tumors (pathologic score, 6) compared with those with normal prostates (pathologic score, 1) or high-grade PIN lesions (pathologic score, 3; $P < 0.01$; Fig. 4B). Bcl-2 was numerically decreased 53% in poorly differentiated tumors (pathologic score, 6) compared with normal prostates, but the main effect for group did not reach statistical significance ($P = 0.48$; Fig. 4C). Consequently, the Bax/Bcl-2 ratio was greatest in poorly differentiated adenocarcinomas ($P < 0.01$; Fig. 4D).

SIRT1 and Human Prostate Cancer

Frequency of SIRT1 staining was greatest in cancer cells and luminal cells. The percentage of cells that stained positive for SIRT1 in human prostate cancer cases was greatest in cancer cells

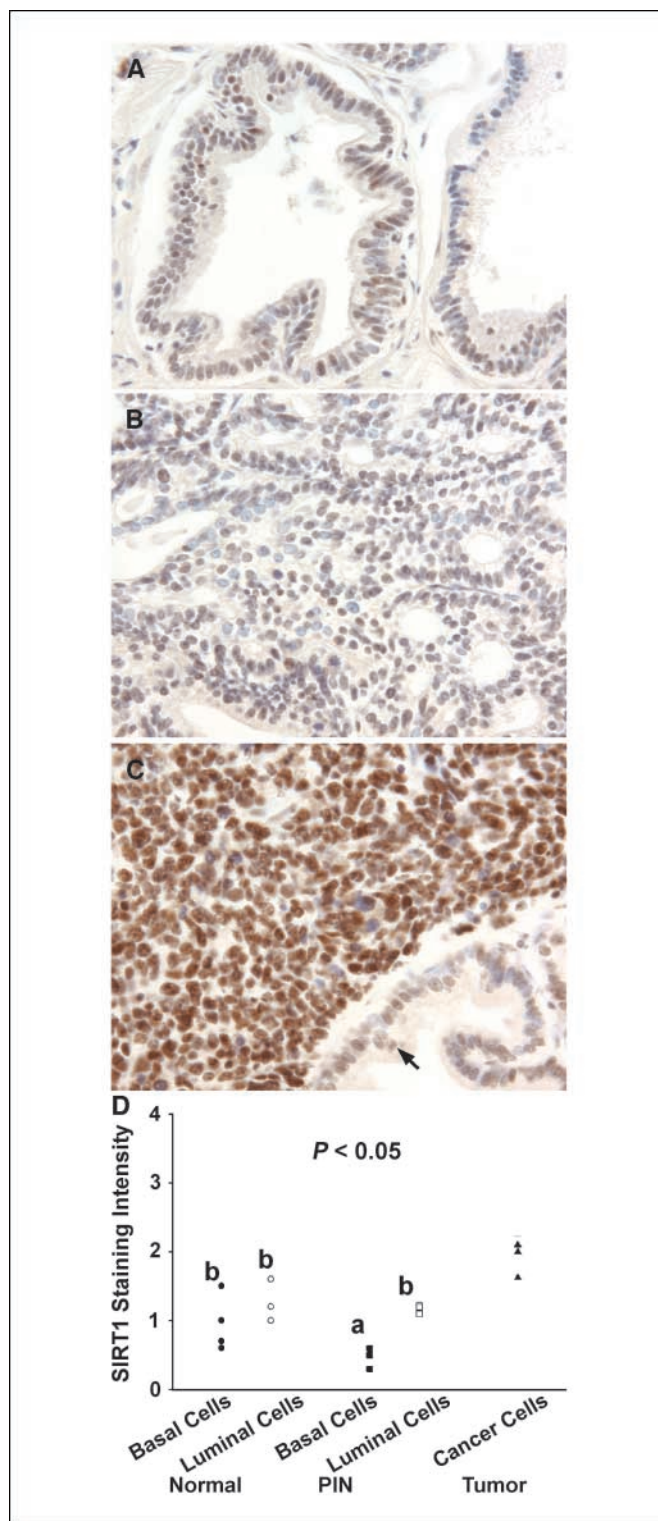


Figure 3. SIRT1 immunostaining taken at $\times 400$ magnification in the DLP from TRAMP mice with pathologic score of 1 ($n = 4$), 3 ($n = 4$), or 6 ($n = 4$), indicating normal tissue, high-grade PIN, or poorly differentiated prostatic adenocarcinoma, respectively. SIRT1 staining in a normal prostate (A), SIRT1 staining in a high-grade PIN lesion (B), and SIRT1 staining in poorly differentiated adenocarcinomas (C); note the contrast in staining between the cancer cells and the adjacent uninvolved gland (arrow). SIRT1 staining intensity in normal cells, PIN lesions, and tumors (D). Different symbols represent SIRT1 staining intensity for cell types present in each specimen. Different letters (*a*–*c*) denote a significant difference between groups, such that groups not sharing a similar letter are significantly different from each other ($P < 0.05$).

and uninvolved luminal cells. Basal cells from the uninvolved glands had less percentage positive staining than either luminal cells or cancer cells, but all had significantly more percentage positive staining than stromal cells ($P < 0.05$; Fig. 5A-C).

Intensity of staining for SIRT1 and Bax were greatest in cancer cells. Staining intensity for SIRT1 was ~2-fold greater in cancer cells than luminal cells from uninvolved glands ($P < 0.05$; Fig. 5A, B, and D). However, luminal cells showed greater staining intensity than basal cells or stromal cells ($P < 0.05$; Fig. 5A, B, and D). Bax immunostaining was readily detectable in cancer cells (percentage positive cells, 98.7%; staining intensity, 2.4) but was relatively undetectable in cells outside the tumor (data not shown). There was very little Bcl-2 staining observed in cancer cells (data not shown), and there was no significant relationship in cancer cells between Bax staining intensity and SIRT1 staining intensity ($r = 0.21$; $P = 0.26$) or SIRT1 staining intensity and Gleason Score ($r = 0.30$; $P = 0.10$).

Discussion

Previous studies have reported high SIRT1 protein expression in human cancers (12, 13), tumors from genetically altered mice (8, 12), and in cancer cell lines (7, 21), including lung (H1299 and NSCLC) and breast (MCF7). Conversely, one study reported a reduction in SIRT1 mRNA from colorectal tumors versus normal mucosa (22). However, our results are the first to clearly show that greater SIRT1 protein expression is associated with cancer

cells from both mouse and human prostate tumors. This suggests that high expression of SIRT1 is a common and relevant pathologic event in prostate cancer and potentially other cancers as well (8, 12, 13).

Furthermore, the observation from the 6-week feeding experiment that SIRT1 in the DLP was not greater, and, in fact, was numerically lower in the lean-restricted mice (PF22), was intriguing because we have shown recently that cancer incidence and progression are delayed in PF22 TRAMP mice (14). Taken together, the present finding coupled with our previous data (14) do not support the hypothesis that the cancer-preventive effects of calorie restriction in the TRAMP model involve an increased expression of SIRT1.

Additionally, our results showed diminished expression of HIC-1 in poorly differentiated tumors from TRAMP mice. HIC-1 is a tumor suppressor that has been shown to act in concert with p53 (23) but is often found to be epigenetically silenced in cancer (24, 25) due to hypermethylation of the HIC-1 promoter (26). Indeed, it has been shown that TRAMP mice overexpress DNA methyltransferase-1 (DNMT1) in advanced tumors (27), and increased DNMT1 activity has been linked to hypermethylation of the *HIC-1* gene (26). Furthermore, Chen et al. (8) showed that when expressed, HIC-1 forms a transcriptional repression complex with SIRT1, which can directly bind the SIRT1 promoter and suppress SIRT1 transcription. However, *Hic1*^{+/-} mice, which have reduced levels of HIC-1, frequently develop tumors and have elevated levels of SIRT1 in both normal and cancer cells (8).

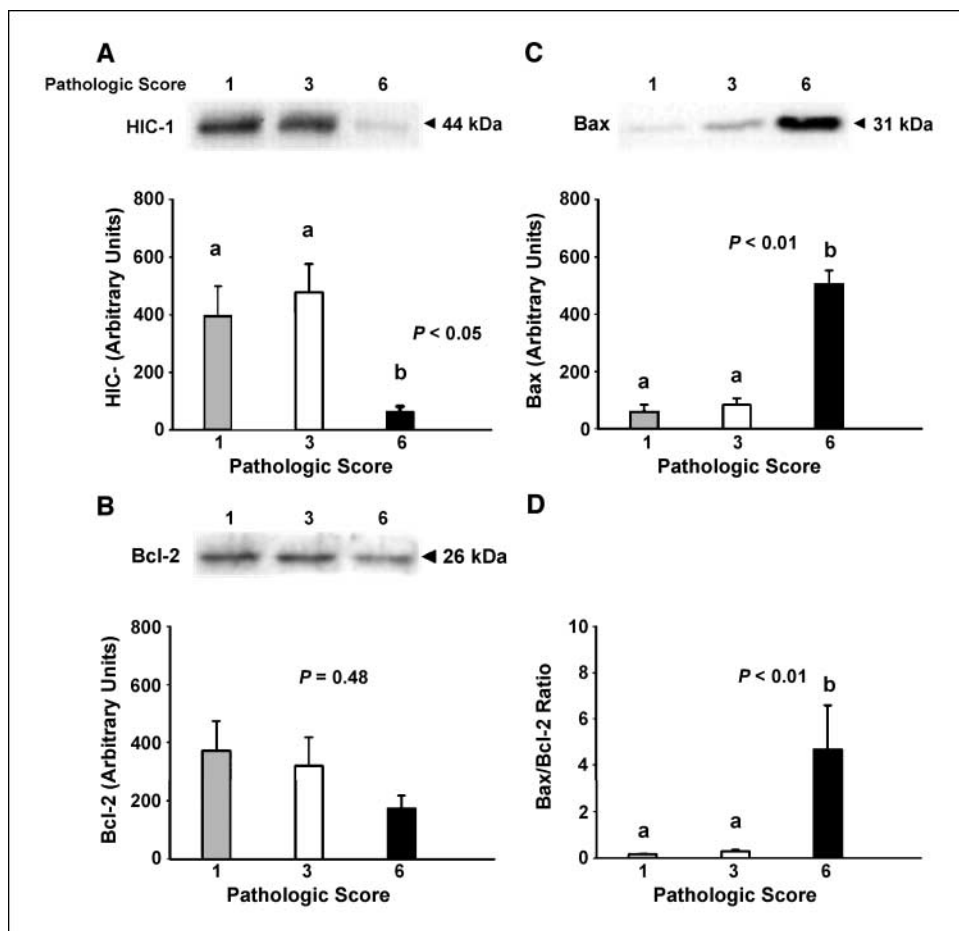
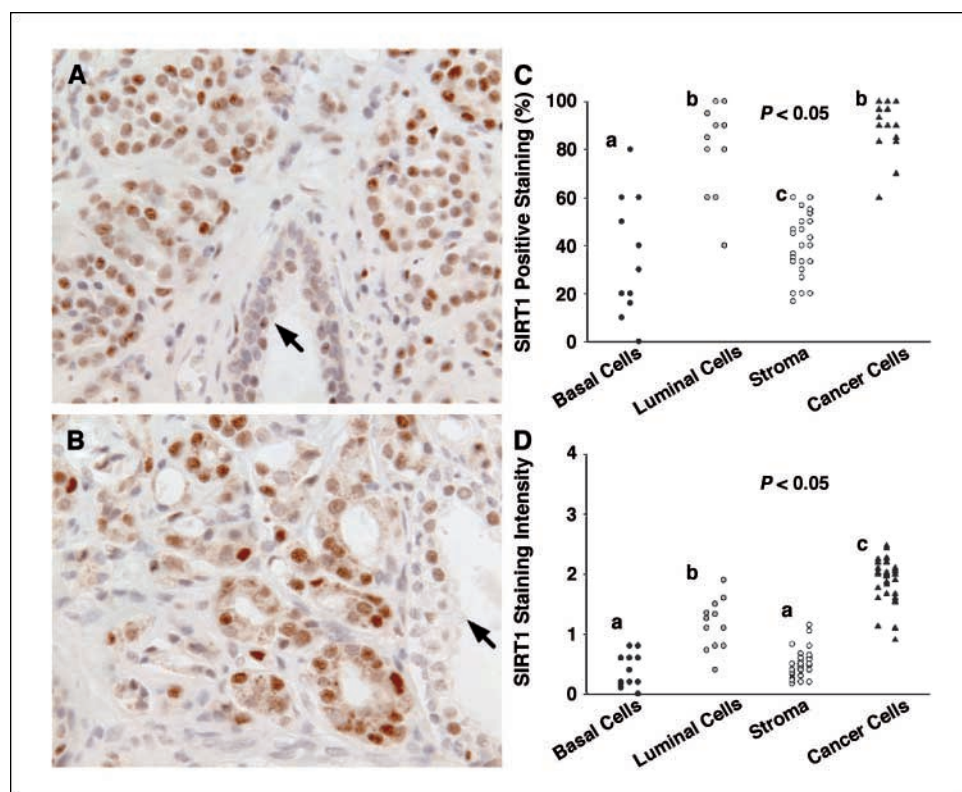


Figure 4. DLP protein content from TRAMP mice with pathologic score of 1 ($n = 5$), 3 ($n = 6$), or 6 ($n = 6$), indicating normal tissue, high-grade PIN, or poorly differentiated prostatic adenocarcinoma, respectively. A representative Western blot and corresponding figure are shown. Mean protein expression in the DLP from TRAMP mice with different pathologic scores for HIC-1 (A), Bax (B), Bcl-2 (C), and the Bax/Bcl-2 ratio (D). Columns, mean; bars, SE. Different letters (a and b) denote a significant difference between groups, such that groups not sharing a similar letter are significantly different from each other ($P < 0.05$).

Figure 5. SIRT1 immunostaining in human prostate cancer cases. *A* and *B*, SIRT1 staining at $\times 400$ magnification from two different prostate cancer cases. Note the greater staining intensity in the surrounding cancer cells than adjacent stromal cells and the uninvolved gland (arrows). Also notice that greater staining is observed in the luminal cells than basal cells in the uninvolved gland. *C*, percentage of cells positively stained for SIRT1 in the cancer cases. *D*, SIRT1 staining intensity in human prostates for basal cells and luminal cells from uninvolved glands, stromal cells, and cancer cells. Symbols represent SIRT1 staining intensity for cell types present from each individual case. Different letters (*a–c*) denote a significant difference between groups, such that groups not sharing a similar letter are significantly different from each other ($P < 0.05$).



Therefore, the apparent loss of HIC-1 expression in poorly differentiated tumors from TRAMP mice provides one viable explanation for the concurrent increase in SIRT1 expression.

In addition to direct evidence, reports on epigenetic changes, and specifically histone acetylation status from tumors and cancer cell lines, may provide indirect clues for a SIRT1-cancer connection. Fraga et al. (28) reported a greater loss of monoacetylation at Lys¹⁶ and trimethylation at Lys²⁰ on histone 4 (H4Lys16 and H4Lys20) as a common hallmark of primary tumors and cancer cell lines compared with controls. Furthermore, Seligson et al. (29) found that among patients with low-grade prostate tumors (Gleason, <7), many patients had a substantial reduction in acetylation levels of Lys⁹ on histone 3 (H3Lys9), and these individuals had greater 10-year tumor recurrence rates than those with greater H3Lys9 acetylation levels. Despite the fact that no study has directly linked these epigenetic changes to a specific enzyme(s), it is intriguing that both H3Lys9 and H4Lys16 are known targets of SIRT1 deacetylase activity (30). Therefore, based on our data and others, it is tempting to speculate that an elevation in SIRT1 could be responsible for these observed epigenetic changes, although the potential role of other histone deacetylases and histone acetyltransferases in this context are not known.

Our results show that mouse and human prostate cancer cells are associated with elevated SIRT1 levels, and most studies suggest that SIRT1 may function as a tumor promoter via its inhibitory effects on p53, Bax-Ku70, and FOXO transcription factors (9, 11, 31). For example, one study found that the SIRT1 inhibitor, Sirtinol, induced senescence-like growth arrest in MCF7 and lung cancer H1299 cells (11). In addition, Chu et al. (9) reported that SIRT1 mRNA and protein levels were elevated in multidrug-resistant cell lines and tumors compared with controls. However, inhibiting

SIRT1 using RNA interference was effective in reversing this resistance phenotype in cells, suggesting that inhibiting SIRT1 may improve the effectiveness of chemotherapy agents for cancer treatment (9). Furthermore, Heltweg et al. (10) using a SIRT1 inhibitor, cambinol, induced apoptosis in BCL6-expressing Burkitt lymphoma cells and reduced tumor growth in mice with Burkitt lymphoma xenografts. Therefore, ample evidence suggests that SIRT1 may play an important role in tumor promotion.

However, not all evidence implicates SIRT1 as a tumor promoter. Yeung et al. (21) showed that in response to tumor necrosis factor- α , SIRT1 physically interacts with and deacetylates the RelA/p65 subunit of nuclear factor- κ B, which can induce apoptosis rather than cell survival. In addition, Fu et al. (32) has reported recently that SIRT1 diminishes androgen receptor (AR) expression and activity in prostate cancer cell lines, and this was reversed by addition of a SIRT1 antagonist. Furthermore, in response to SIRT1 administration, they reported substantial growth arrest in cells expressing AR but no effect in prostate cancer cells not expressing AR. Because prostate cancer can be driven by androgen-dependent or androgen-independent mechanisms, the potential effect of SIRT1 on prostate cancer, as with other cancers, is likely quite complex.

In conclusion, these data show that DLP SIRT1 protein levels from TRAMP mice were not significantly altered by calorie restriction during the early stages of carcinogenesis. However, SIRT1 was significantly increased in prostatic adenocarcinomas, which was confirmed by immunostaining, such that it was not just a function of more cells per unit weight per volume. In contrast, HIC-1 levels were markedly reduced in the same tumors, suggesting

that a reduction in HIC-1 may be in part responsible for the increased expression of SIRT1. In addition, Bax expression and the Bax/Bcl-2 ratio were significantly greater in poorly differentiated tumors. Furthermore, immunostaining of human prostate tumors showed that cancer cells were associated with greater SIRT1 expression than uninvolved cells. Because SIRT1 may function as a tumor promoter, these results suggest that SIRT1 should be considered as a potential therapeutic target for prostate cancer.

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References

- Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. *Annu Rev Med* 2003;54:131–52.
- Kaerberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev* 1999;13:2570–80.
- Brunet A, Sweeney LB, Sturgill JF, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 2004;303:2011–5.
- Cohen HY, Miller C, Bitterman KJ, et al. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 2004;305:390–2.
- Luo J, Nikolaev AY, Imai S, et al. Negative control of p53 by Sir2 α promotes cell survival under stress. *Cell* 2001;107:137–48.
- Guarente L. Calorie restriction and SIR2 genes—towards a mechanism. *Mech Ageing Dev* 2005;126:923–8.
- Lim CS. SIRT1: tumor promoter or tumor suppressor? *Med Hypotheses* 2006;67:341–4.
- Chen WY, Wang DH, Yen RC, et al. Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell* 2005;123:437–48.
- 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;65:10183–7.
- Heltweg B, Gatbonton T, Schuler AD, et al. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res* 2006;66:4368–77.
- Ota H, Tokunaga E, Chang K, et al. Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells. *Oncogene* 2006;25:176–85.
- Kuzmichev A, Margueron R, Vaquero A, et al. Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc Natl Acad Sci U S A* 2005;102:1859–64.
- Hida Y, Kubo Y, Murao K, Arase S. Strong expression of a longevity-related protein, SIRT1, in Bowen's disease. *Arch Dermatol Res* 2007;299:103–6.
- Huffman DM, Johnson MS, Watts A, et al. Cancer progression in the transgenic adenocarcinoma of mouse prostate mouse is related to energy balance, body mass, and body composition, but not food intake. *Cancer Res* 2007;67:417–24.
- Huang YW, Wang LS, Chang HL, et al. Molecular mechanisms of (–)-gossypol-induced apoptosis in human prostate cancer cells. *Anticancer Res* 2006;26:1925–33.
- Mentor-Marcel R, Lamartiniere CA, Eltoum IE, Greenberg NM, Elgavish A. Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). *Cancer Res* 2001;61:6777–82.
- Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277–359.
- Nagy TR, Clair AL. Precision and accuracy of dual-energy X-ray absorptiometry for determining *in vivo* body composition of mice. *Obes Res* 2000;8:392–8.
- Folkvord JM, Viders D, Coleman-Smith A, Clark RA. Optimization of immunohistochemical techniques to detect extracellular matrix proteins in fixed skin specimens. *J Histochem Cytochem* 1989;37:105–13.
- Wechter WJ, Leipold DD, Murray ED, Jr., et al. E-7869 (R-flurbiprofen) inhibits progression of prostate cancer in the TRAMP mouse. *Cancer Res* 2000;60:2203–8.
- Yeung F, Hoberg JE, Ramsey CS, et al. Modulation of NF- κ B-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004;23:2369–80.
- Ozdag H, Teschendorff AE, Ahmed AA, et al. Differential expression of selected histone modifier genes in human solid cancers. *BMC Genomics* 2006; 7:90.
- Wales MM, Biel MA, el Deiry W, et al. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. *Nat Med* 1995;1:570–7.
- Waha A, Koch A, Meyer-Puttlitz B, et al. Epigenetic silencing of the HIC-1 gene in human medulloblastomas. *J Neuropathol Exp Neurol* 2003;62:1192–201.
- Hayashi M, Tokuchi Y, Hashimoto T, et al. Reduced HIC-1 gene expression in non-small cell lung cancer and its clinical significance. *Anticancer Res* 2001;21:535–40.
- Vertino PM, Yen RW, Gao J, Baylin SB. *De novo* methylation of CpG island sequences in human fibroblasts overexpressing DNA (cytosine-5)-methyltransferase. *Mol Cell Biol* 1996;16:4555–65.
- McCabe MT, Low JA, Daignault S, et al. Inhibition of DNA methyltransferase activity prevents tumorigenesis in a mouse model of prostate cancer. *Cancer Res* 2006; 66:385–92.
- Fraga MF, Ballestar E, Villar-Garea A, et al. Loss of acetylation at Lys¹⁶ and trimethylation at Lys²⁰ of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005;37:391–400.
- Seligson DB, Horvath S, Shi T, et al. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005;435:1262–6.
- Vaquero A, Scher M, Lee D, et al. Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* 2004;16:93–105.
- Ford J, Jiang M, Milner J. Cancer-specific functions of SIRT1 enable human epithelial cancer cell growth and survival. *Cancer Res* 2005;65:10457–63.
- Fu M, Liu M, Sauve AA, et al. Hormonal control of androgen receptor function through SIRT1. *Mol Cell Biol* 2006;26:8122–35.