pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 34(1) : 27-33 (2017)
http://dx.doi.org/10.17555/jvc.2017.02.34.1.27

Journal of Veterinary Clinics
www.ksvc.or.kr

Vorinostat Induces Cellular Senescence in Fibroblasts Derived from Young and Aged Dogs

Min-Jung Kim, Hyun-Ju Oh, Erif Maha Nugraha Setyawan, Yoo-Bin Choi, Seok-Hee Lee and Byeong-Chun Lee[1]

College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea

(Received: October 18, 2016 / Accepted: January 19, 2017)

Abstract : Although HDACIs affect ubiquitously expressed histone deacetylase and increase cellular senescence, there has been little study on the effect of age on treatment with HDACIs. Accordingly, the purpose of this study was to compare cellular senescence status and vorinostat-induced senescence in fibroblasts derived from aged dogs compared to young dogs. Skin tissues were taken from young (1-year-old) and aged (7-year-old) male dogs, and fibroblasts were cultured without (control) or with 10 uM of vorinostat for 24 hr. Beta-galactosidase activity was assessed, and real-time polymerase chain reaction and western blotting were performed to analyze the expression levels of transcripts and proteins related to cellular senescence. Beta-galactosidase activity was higher in aged dogs compared to young dogs in the control group, and was increased by vorinostat treatment. Expression of p21, p53 and p16 transcripts was higher in the aged than in the young group, and all transcripts were affected by vorinostat in both young and aged groups. Western blot results showed lower H3K9 acetylation in the aged dogs compared to the young dogs, and the acetylation was increased by vorinostat treatment in both groups. However, there was no significant difference between the transcript or protein alterations induced by vorinostat.

Key words **:** vorinostat, age, cellular senescence, fibroblast, dog.

Introduction

The term “cellular senescence” was first used by Leonard Hayflick and Paul Moorhead approximately five decades ago to refer to the phenomenon that normal human fibroblasts had a finite proliferative capacity in culture (14). Recently, it is defined as a permanent and irreversible arrest of cell proliferation caused by potential oncogenic stressors, including dysfunctional telomeres, non-telomeric DNA damage, excessive mitogenic signals, non-genotoxic stress, etc (5). Although cellular senescence could not explain all the phenotypes of organismal aging, it has been speculated that a series of changes in cellular phenotypes during senescence could be an underlying cause of aging because many aging pathologies have been linked with the senescence response (4). For example, chronic DNA damage response signaling in senescent cells shows imperfect cell cycle checkpoints, which interfere with coordination of the S-phase and mitosis, thereby finally leading to cell-cycle withdrawal and chromosomal instability *in vitro* (16). Similarly, chromosome complement variations such as aneuploidy also occur in numerous tissues of aged humans *in vivo* (2). In addition to growth arrest, chromatin changes such as hypoacetylation and methylation in senescent cells can occur in punctate domains of heterochromatin called senescence-associated heterochromatin foci, which in turn cause alterations in gene expression (1). Indeed, the number of cells that express senescence markers *in vivo* increases with age, and is generally believed to contribute to age-related pathologies such as memory impairments and cancers (4).

Histone deacetylase inhibitors (HDACIs) have been identified as valuable drugs for modifying gene transcription by inducing acetylation of histones, affecting proteins that regulate gene expression including cell differentiation or growth arrest, and apoptosis (20,35). Based on their critical role in modulating cell physiology, there have been attempts to discover the pharmacokinetic properties of HDACIs, and apply these agents to the treatment of cancer, neurological diseases and immune disorders (11). Several HDACIs have already been approved by the US Food and Drug Administration (FDA) for the clinical treatment of cancers; for example, vorinostat and belinostat are approved for the treatment of cutaneous T cell lymphoma (10) and peripheral T cell lymphoma (39), respectively. HDACIs induced cell death in tumor cells by upregulation of pro-apoptotic genes and down-regulation of pro-survival genes (3). Furthermore, recent studies have suggested that HDACIs can be applied as treatments for age-dependent cognitive impairments such as learning and memory losses (28,37). A significant decrease of neurogenesis in the dentate gyrus (33) and hypoacetylation in the hippocampus after contextual fear conditioning (28) was found in aged mice that showed cognitive dysfunction. Remodeling of chromatin structure by post-translational modification of HDACIs could facilitate cell proliferation and neuroblast differentiation in the dentate gyrus (33) as well as the formation of long-term memories (28).

Increased histone acetylation *in vivo* has been induced by administration of HDACIs *via* either localized (subcutane-

[1]Corresponding author.
E-mail : bclee@snu.ac.kr

ous (40), intrahippocampal (28), etc.) or general (oral (10), intravenous (39), etc.) routes. Considering the ubiquitous expression of histone deacetylase and resulting altered global gene expression in normal cells, the effects of HDACIs on normal tissue cells need to be evaluated. In human primary skin fibroblasts, treatment with vorinostat, MS275 or sodium butyrate increased DNA double strand breaks (32). Human mesenchymal stem cells treated with MS-275 or vorinostat induced cellular senescence and apoptosis, respectively, which are associated with a decrease in stem cell properties (8). Although those responses, including DNA double strand breaks, cellular senescence and apoptosis, are all related to the aging process (6), there has been little attempt to evaluate the effect of age on treatment with HDACIs. The present study was undertaken with cells from dogs because these animals are excellent models for human disease and aging processes due to their physiological similarities. We hypothesized that fibroblasts derived from aged dogs showed increased cellular senescence compared to those from young dogs, and that cellular senescence induced by vorinostat would be accelerated in fibroblasts derived from aged dogs compared those from young dogs.

Materials and Methods

Animal and chemical use

Animal experiments were done following standard procedures established by the Committee for Accreditation of Laboratory Animal Care and the Guidelines for the Care and Use of Laboratory Animals of Seoul National University (approval number is SNU-141201-4). All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise specified.

Fibroblasts derived from 1- and 7-year-old dogs

Primary cultures of dog skin fibroblasts were established following the procedures from previous studies with minor modification (23). Ear skin tissues from young (one-year-old) and aged (seven-year-old) male Gyeongju Donggyeong dogs were collected and transported aseptically to the laboratory. Each piece of tissue was washed with phosphate buffered saline (PBS; Invitrogen, Carlsbad, CA, USA), minced into small pieces (< 1 mm × 1 mm) and placed onto the bottom of a tissue culture dish. The cells were cultured with Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen) at 39°C in air containing 5% CO_2. Primary cells were cultured until confluence in this culture medium which was replaced every 3 to 4 days, and subcultured after trypsinization. Cells at passage 0 were cryopreserved using FBS supplemented with 10% dimethyl sulfoxide, and stored in liquid nitrogen until the following experiments were performed.

Cell culture and vorinostat treatment

Dog fibroblast cell cultures and treatment with vorinostat were done as described in a previous study (20). Cryopreserved cells were thawed and cultured with DMEM supplemented with 10% FBS and 1% non-essential amino acids (NEAA; Invitrogen) until confluency. At passage 1, cells were harvested by trypsinization and subcultured to reach about 40-50% of confluence on the day after subculture. The culture medium was replaced with fresh DMEM-FBS-NEAA without (control group) or with 10 uM vorinostat (vorinostat group) on one day after subculture. Twenty-four hours after the change of culture medium, all cells were retrieved by incubation for 3 min at 39°C with 0.25% trypsin-0.01% EDTA and collected by centrifugation. After discarding the supernatant, cell pellets were stored at −80°C until total RNA or protein extraction.

Assay of senescence-associated β-galactosidase activity

Dog fibroblasts were cultured in 35 mm dishes and senescence-associated beta-galactosidase activity was evaluated using a senescence beta-galactosidase staining kit (Cell Signaling Technology, Boston, MA, USA) according to the manufacturer's instructions. Twenty-four hours after vorinostat treatment, the culture media in both the control and vorinostat groups were removed. After rinsing with PBS, cells were fixed using Fixative Solution for 10-15 min at room temperature, and stained with Beta-Galactosidase Staining Solution at 37°C overnight in a dry incubator. At least 100 cells in each dish were examined for beta-galactosidase expression by microscopy (200X magnification).

Transcript level analysis with real-time polymerase chain reaction

Total RNAs were extracted from the pellets of each group using an easy-spin Total RNA Extraction Kit (iNtRON Biotechnology Inc., Gyeonggi-do, Korea) according to the manufacturer's instructions. One microgram of the extracted RNAs was used to synthesize complementary DNA using an amfiRivert cDNA Synthesis Platinum Master Mix (GenDEPOT, Barker, Texas, USA) following the manufacturer's instructions. Real-time polymerase chain reaction was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using a Power SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions with slight modifications. Each reaction mix contained 200 ng complementary DNA, 10 ul SYBR, 7.2 ul distilled water and 0.4 ul (10 pM/ul) of each forward and reverse primer of p16, p19, p21, Rb1 and CDK4 (Table 1).

Western blotting

Western blot analysis was performed according to our previous study with a slight modification (20). Pro-PREP Protein Extraction Solution (iNtRON) was added to each cell pellet, and cells were incubated to lyse on ice for 20 min. After centrifugation, protein quantification of the supernatant using the Bradford reagent was performed, and 30 ug of each sample was loaded for separation by 15% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred onto polyvinylidene fluoride membranes, and the membranes were blocked with 5% skim milk in Tris-buffered saline and 0.1% Tween 20 (TBST) for 1 h at room temperature. Then, the membranes were incubated with diluted primary antibodies comprising mouse anti-beta actin antibody (Abcam, Cambridge, MA, USA),

Table 1. Primer sequences used for quantitative polymerase chain reaction

Gene	Primer sequences (5' to 3')	Product size (bp)	References
β-actin	F-ggcatcctgaccctgaagta	81	NM_001195845.1
	R-ggtgtggtgccagatcttct		
P16	F-ccggacttcaagaattgagc	112	JN086563.1
	R-tgataaaggcaagcatgcag		
P19	F-aggacccagcgaggttctat	112	XM_848853.3
	R-atcaacaccaacaagccaca		
P21	F-aatctgtcaggggcgtattg	116	XM_532125.4
	R-atgaaatgggggaagggtag		
P53	F-cagtgtggtggtgccttatg	139	NM_001003210.1
	R-ggagtcttccagggtgatga		
Rb	F-acgccaacaaaaatgactcc	138	XM_534118.4
	R-gctgccttcagcacttcttt		
CDK4	F-ccccgtccagtacagacagt	100	XM_844780.3
	R-aggcagagattcgcttgtgt		
HDAC1	F-cggtcatgtccaaagtgatg	116	XM_544435.4
	R-gtgccctttgatcgtgagat		

rabbit anti-acetyl-H3K9 antibody (Millipore, Billerica, MA, USA) and rabbit anti-p21 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in TBST at 4°C overnight. After washing in TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Pierce, Thermo Scientific, Rockford, IL, USA; Abcam) in TBST for 1 h at room temperature. After thorough washing in TBST, the membranes were developed by enhanced chemiluminescence.

Statistical analysis

Two-way analyses of variance (ANOVAs) with Bonferroni post-tests were performed to compare relative transcript levels and western band intensities between control and vorinostat treated cells by age. Paired t-tests were used to compare increased or decreased expression of transcripts and protein by vorinostat treatment. The significance level was $p < 0.05$. The data were analyzed using Graph Prism software (GraphPad, San Diego, CA, USA). All data presented in this study are the result of three or more independent experiments.

Results

Senescence-associated β-galactosidase activity

Cells derived from both young and aged dogs were cultured in a monolayer and showed flat and spindle-shaped fibroblasts, which is their typical morphology. However, as shown in Fig 1, the number of cells stained by senescence-associated β-galactosidase markers was higher in the aged group (6.0 ± 0.9/100 cells) compared to the young group (1.1 ± 0.7/100 cells). In both groups, the perinuclear staining for β-galactosidase activity was significantly increased after 10 uM vorinostat treatment. Similar to the pretreatment status, the number of β-galactosidase stained cells was higher in the aged group (9.5 ± 1.1/100 cells) than the young group (5.0 ± 0.5/100 cells).

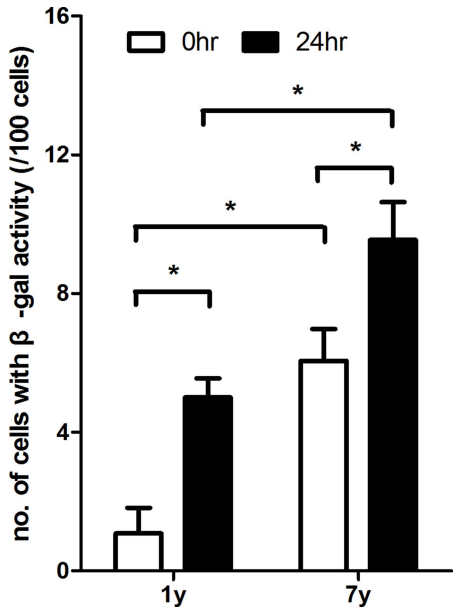

Fig 1. β-galactosidase activity of 1 year old and 7 years old dog fibroblasts without (0 h) or with 24 h of vorinostat treatment (24 h). β-galactosidase activity was significantly increased after 24 h of vorinostat treatment in both 1 year old and 7 years old dog fibroblasts. Asterisk indicates statistical significance, $p < 0.05$.

Analysis of relative expression of senescence-related transcripts

Even in the control group, some of the relative transcript expressions related to cellular senescence by both telomere dependent and independent pathways showed significantly different expression levels (Fig 2, Fig 3). Transcripts of p16,

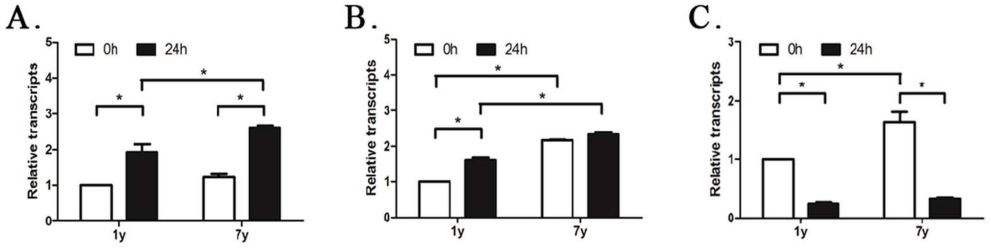

Fig 2. Relative transcript expression levels of genes related to cellular senescence by the telomere dependent pathway and to apoptosis of dog fibroblasts derived from 1 year old and 7 years old donors without or with 24 h of vorinostat treatment. Expression profiles of (a) p19, (b) p21 and (c) p53 ($p < 0.05$). Each experiment was performed at least three times.

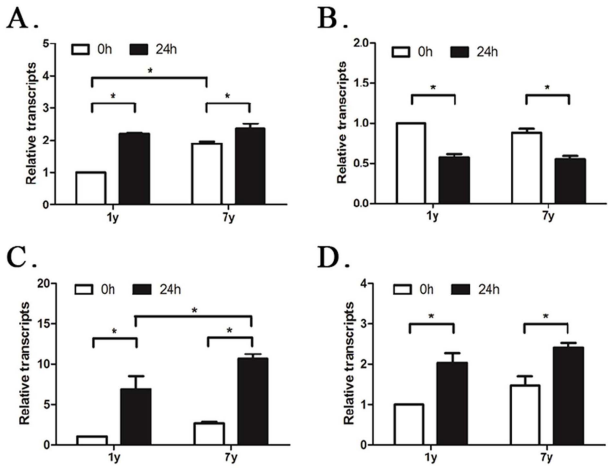

Fig 3. Relative transcript expression levels of genes related to cellular senescence by the telomere independent pathway of dog fibroblasts derived from 1 year old and 7 years old donors without or with 24 h of vorinostat treatment. Expression profiles of (a) p16, (b) CDK4, (c) Rb and (d) HDAC1 ($p < 0.05$). Each experiment was performed at least three times. Rb, retinoblastoma; HDAC, histone deacetylase.

p21 and p53 in the fibroblasts derived from the 7-year-old group were 1.9 folds, 2.2 folds and 1.6 folds higher than those from the 1-year-old group, but transcripts of Rb and p19 were not different between the two groups. However, after vorinostat treatment, while there were no significant differences in transcript expressions for p16 and p53 between both groups, expression of Rb, p19 and p21 transcripts were significantly increased in the aged group after the vorinostat treatment by 1.5 folds, 1.4 folds and 1.3 folds, respectively. In addition, although transcripts of CDK4 were decreased and HDAC1 were increased by vorinostat treatment in both the 1- and 7-year-old groups, they were not affected by age. Vorinostat treatment also increased transcript expression of p16, Rb, p19 and p53 in both the 1- and 7-year-old groups.

Changes in protein expression of p16, p21, and histone acetylation

Western blot results showed age-dependent protein expression of p16 (Fig 4). The protein expression of p16 was 1.7 folds higher in the 7-year-old control group compared to the 1-year-old control group. The vorinostat treatment doubled p16 protein expression levels in both young and aged groups, resulting in 1.5 folds higher expression in the 7-year-old group than the 1-year-old group. Similarly, although there was no significant difference in p21 protein expression between the young and aged groups, vorinostat increased expression two-fold in each group. The p21 protein expression in the 7-year-old group was 1.4 folds increased compared to that of the 1-year-old group. Furthermore, acetylation of H3K9 expression was 0.3 folds lower in the control aged group compared to the young group. After vorinostat treatment, H3K9 acetylation was 4.9 folds and 13.2 folds increased in the 1- and 7-year-old groups, respectively, but still 0.8 folds lower in the aged group than the young group.

Discussion

Dogs are used as animal models for human aging research not only because of their similarities in anatomy and physiol-

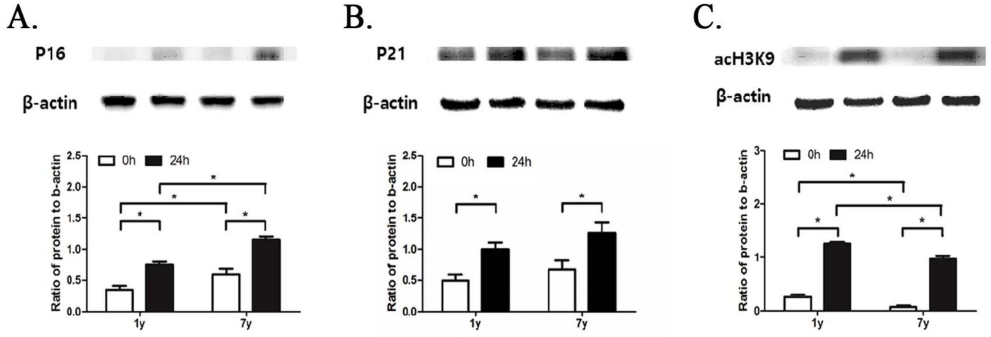

Fig 4. Western blot results showing (A) p16, (B) p21 and (C) H3K9 acetylation levels in dog fibroblasts derived from 1 year old and 7 years old donors without or with 24 h of vorinostat treatment.

ogy to humans but also because of their increased longevity and more similarity of DNA and protein sequences with humans compared to other laboratory animals including mice and rats (27). Domesticated dogs are a spontaneous animal model of human cancer because dogs have a shared environment with humans and naturally develop the same cancers as humans (29). HDACIs also showed similar responses between human and dogs for the treatment of osteosarcoma (38), and consequently there have been trials to evaluate the effects of vorinostat on various canine cancer cell lines *in vitro* (21) and to treat canine hemangiosarcoma with vorinostat *in vivo* (7). In addition, there is growing interest in dogs as a useful translational geroscience model because they share human environments (18). Therefore, primary cell cultures derived from different aged dogs were used to compare the effect of aging on HDACI treatment in this study.

Several important morphological changes occur in senescent cells and have been used to evaluate the degree of cellular senescence. These include cell hypertrophy, cell flattening, increased senescence-associated beta-galactosidase activity, formation of senescence associated heterochromatic foci, etc (31). Our results showed that, even without HDACI treatment, fibroblasts derived from the aged dogs group showed significantly increased beta-galactosidase activity compared to those from the young group. In human skin samples, the frequency and intensity of beta-galactosidase positive cells in the dermis were increased with age, and the beta-galactosidase activity also varied in skin fibroblasts with replicative age (9). However, contrary to our results, it has been reported that beta-galactosidase activity was not affected by the skin donor's age (9). This might be the results of early passage (passage 2) and different confluency status (about 50%) in our study. In addition, vorinostat accelerated beta-galactosidase activity with flattened morphology in both young and aged groups, which is consistent with the effects of other HDACIs such as butyrate and TSA (30).

Cellular senescence is regulated by at least two different pathways acting together or sometimes independently: a telomere-dependent pathway *via* p53/p21 and a telomere-independent pathway controlled by p16 retinoblastoma (Rb) (1). The DNA damage response induced by dysfunctional telomeres often leads to either apoptosis or replicative senescence by activation of p53 (36). The p53 activation promotes transactivation of its downstream target genes, including the cyclin-dependent kinase (cdk) inhibitor, p21. Expression of cyclin/cdk2 is inhibited in response to stimuli of p21, which initiate the phosphorylation and inactivation of Rb (12). Hypophosphorylated Rb induces cell arrests in the G1 phase of the cell cycle. To avoid cell cycle arrest through contact inhibition, cells at subconfluency were used in our study. The p53/21 pathway has been generally regarded as an important regulator of replicative senescence, and alteration of transcript and proteins expression or proteasome activity *via* this pathway also has been reported in senescent fibroblasts (15). Even the primary cells from old rats showed higher nuclear p21 and p53 proteins than young, growing rats (24), but in our study the p21 protein expression levels were not different between young and aged dog groups. The vorinostat treatment used in our study also induced cellular senescence by upregulation of p21 transcripts, which is one of the crucial changes during induction of replicative senescence in cell culture (17), and downregulation of p53. These changes are also seen in cultures of peripheral blood lymphocytes treated with sodium butyrate (19). Furthermore, it increased the expression of p19 which is a tumor suppressor linked to degradation of p53 in the telomere-dependent pathway. However, these changes induced by vorinostat were not significantly different between the young and aged groups.

In the telomere-independent pathway, a cyclin-dependent kinase (CDK) inhibitor p16 inhibits CDK4 and CDK6 which initiate the phosphorylation and inactivation of Rb. Hypophosphorylated Rb represses the transcriptional activation of E2F by recruiting chromatin remodeling proteins such as HDAC1s (13). The present study showed that in the control groups, both transcript and protein expression levels of p16 showed increases in the aged group compared to the young group. Although up-regulation of p16 is a reliable biomarker of cellular and organismal aging in rodents and humans (34), little is known about gene expression involved in cellular senescence in dogs. It seems that telomere length, which is one of the widely used organismal aging markers in humans, is not shortened by aging and is affected by breed in dogs (25). Thus, the upregulation of p16 transcript and protein levels in the aged group suggests that p16 could be a valuable marker of dog aging. The vorinostat treatment successfully increased acetylation of H3K9 in both young and aged dog fibroblasts, which is similar to other studies that found increased acetylation of H4 by sodium dibutyrate (26) or of H3K9 by valproic acid (22). The vorinostat used in our study accelerates cellular senescence by increasing transcript expression of p16 and decreasing CDK4, which results in an increase of Rb1 and HDAC1 transcripts. These results are similar to those found with other HDACI such as sodium dibutyrate on human fibroblasts (26) and valproic acid on dog adipose tissue-derived stem cells (22). However, in our study there were no significant differences in p16 protein and transcript (CDK4, Rb1, and HDAC1) expression levels between the young and aged groups.

The importance and practical applications of HDACI have been enlarged especially in elderly people because they are considered to have the highest risk factors for a range of diseases, such as cancer or cognitive disorders, that can respond to HDACIs treatment. Our results showed that 10 uM vorinostat treatment for 24 hr induced cellular senescence in fibroblasts derived from young and aged dogs, but there was no significant difference in the induction of senescence between the two groups. This could imply that acceleration of senescence induced by HDACIs is not influenced by the initial acetylation status and safe application of HDACIs to aged animals or people is possible. Further studies are needed to evaluate the organismal aging effect induced by HDACIs treatment on aged compared to young dogs.

Acknowledgments

This study was supported by NRF (#2014R1A1A20599-28), RDA (#PJ010928032017), Korea IPET (#316002-05-2-SB010), Nature Cell (#550-20150030), Research Institute for

Veterinary Science, Natural Balance Korea and the BK21 plus program.

References

1. Adams PD. Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 2009; 36: 2-14.
2. Arendt T, Mosch B, Morawski M. Neuronal aneuploidy in health and disease: a cytomic approach to understand the molecular individuality of neurons. Int J Mol Sci 2009; 10: 1609-1627.
3. Bolden J, Shi W, Jankowski K, Kan C, Cluse L, Martin B, MacKenzie K, Smyth G, Johnstone R. HDAC inhibitors induce tumor-cell-selective pro-apoptotic transcriptional responses. Cell Death Dis 2013; 4: e519.
4. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol 2013; 75: 685-705.
5. Campisi J, di Fagagna FdA. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007; 8: 729-740.
6. Chen J-H, Hales CN, Ozanne SE. DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res 2007; 35: 7417-7428.
7. Cohen L, Powers B, Amin S, Desai D. Treatment of canine haemangiosarcoma with suberoylanilide hydroxamic acid, a histone deacetylase inhibitor. Vet Comp Oncol 2004; 2: 243-248.
8. Di Bernardo G, Squillaro T, Dell'Aversana C, Miceli M, Cipollaro M, Cascino A, Altucci L, Galderisi U. Histone deacetylase inhibitors promote apoptosis and senescence in human mesenchymal stem cells. Stem Cells Dev 2009; 18: 573-582.
9. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 1995; 92: 9363-9367.
10. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, Chiao JH, Reilly JF, Ricker JL, Richon VM. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 2007; 109: 31-39.
11. Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014;
12. Ferbeyre G, De Stanchina E, Lin AW, Querido E, McCurrach ME, Hannon GJ, Lowe SW. Oncogenic ras and p53 co-operate to induce cellular senescence. Mol Cell Biol 2002; 22: 3497-3508.
13. Ferreira R, Naguibneva I, Pritchard LL, Ait-Si-Ali S, Harel-Bellan A. The Rb/chromatin connection and epigenetic control: opinion. Oncogene 2001; 20:
14. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25: 585-621.
15. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. Science 2006; 311: 1257-1257.
16. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature 2009; 461: 1071-1078.
17. Ju Z, Choudhury AR, Rudolph KL. A dual role of p21 in stem cell aging. Ann N Y Acad Sci 2007; 1100: 333-344.
18. Kaeberlein M, Creevy KE, Promislow DE. The dog aging project: translational geroscience in companion animals. Mamm Genome 2016; 1-10.
19. Kalousek I, Brodska B, Otevrelova P, Röselova P. BimEL dependent apoptosis induced in peripheral blood lymphocytes with n butyric acid is moderated by variation in expression of c myc and p21 (WAF1). Cell Biochem Funct 2008; 26: 509-521.
20. Kim MJ, Oh HJ, Kim GA, Suh HN, Jo YK, Choi YB, Kim DH, Han HJ, Lee BC. Altering histone acetylation status in donor cells with suberoylanilide hydroxamic acid does not affect dog cloning efficiency. Theriogenology 2015;
21. Kisseberth WC, Murahari S, London CA, Kulp SK, Chen C-S. Evaluation of the effects of histone deacetylase inhibitors on cells from canine cancer cell lines. Am J Vet Res 2008; 69: 938-945.
22. Kurihara Y, Suzuki T, Sakaue M, Murayama O, Miyazaki Y, Onuki A, Aoki T, Saito M, Fujii Y, Hisasue M. Valproic acid, a histone deacetylase inhibitor, decreases proliferation of and induces specific neurogenic differentiation of canine adipose tissue-derived stem cells. J Vet Med Sci 2014; 76: 15.
23. Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, Shamim MH, Kim JJ, Kang SK, Schatten G, Hwang WS. Dogs cloned from adult somatic cells. Nature 2005; 436: 641.
24. Machida S, Booth FW. Increased nuclear proteins in muscle satellite cells in aged animals as compared to young growing animals. Exp Gerontol 2004; 39: 1521-1525.
25. McKevitt TP, Nasir L, Devlin P, Argyle DJ. Telomere lengths in dogs decrease with increasing donor age. J Nutr 2002; 132: 1604S-1606S.
26. Munro J, Barr NI, Ireland H, Morrison V, Parkinson EK. Histone deacetylase inhibitors induce a senescence-like state in human cells by a p16-dependent mechanism that is independent of a mitotic clock. Exp Cell Res 2004; 295: 525-538.
27. Paoloni M, Khanna C. Translation of new cancer treatments from pet dogs to humans. Nat Rev Cancer 2008; 8: 147-156.
28. Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, Cota P, Wittnam JL, Gogol-Doering A, Opitz L. Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 2010; 328: 753-756.
29. Pinho SS, Carvalho S, Cabral J, Reis CA, Gärtner F. Canine tumors: a spontaneous animal model of human carcinogenesis. Transl Res 2012; 159: 165-172.
30. Place RF, Noonan EJ, Giardina C. HDACs and the senescent phenotype of WI-38 cells. BMC Cell Biol 2005; 6: 37.
31. Pospelova TV, Chitikova ZV, Pospelov VA. An integrated approach for monitoring cell senescence. Methods Mol Biol 2013; 383-408.
32. Purrucker JC, Fricke A, Ong MF, Rübe C, Rübe CE, Mahlknecht U. HDAC inhibition radiosensitizes human normal tissue cells and reduces DNA Double-Strand Break repair capacity. Oncol Rep 2010; 23: 263-269.
33. Rao MS, Hattiangady B, Shetty AK. The window and mechanisms of major age related decline in the production of new neurons within the dentate gyrus of the hippocampus. Aging cell 2006; 5: 545-558.
34. Ressler S, Bartkova J, Niederegger H, Bartek J, Scharffetter Kochanek K, Jansen Dürr P, Wlaschek M. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 2006; 5: 379-389.
35. Sardiu ME, Smith KT, Groppe BD, Gilmore JM, Saraf A, Egidy R, Peak A, Seidel CW, Florens L, Workman JL. Suberoylanilide Hydroxamic Acid (SAHA)-Induced Dynamics of a Human Histone Deacetylase Protein Interaction Network. Mol Cell Proteomics 2014; 13: 3114-3125.

36. Sikora E, Arendt T, Bennett M, Narita M. Impact of cellular senescence signature on ageing research. Ageing Res Rev 2011; 10: 146-152.
37. Stilling RM, Fischer A. The role of histone acetylation in age-associated memory impairment and Alzheimer's disease. Neurobiol Learn Mem 2011; 96: 19-26.
38. Wittenburg LA, Bisson L, Rose BJ, Korch C, Thamm DH. The histone deacetylase inhibitor valproic acid sensitizes human and canine osteosarcoma to doxorubicin. Cancer Chemother Pharmacol 2011; 67: 83-92.
39. Yeo W, Chung HC, Chan SL, Wang LZ, Lim R, Picus J, Boyer M, Mo FK, Koh J, Rha SY. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. J Clin Oncol 2012; 30: 3361-3367.
40. Yoo DY, Kim W, Kim IH, Nam SM, Chung JY, Choi JH, Yoon YS, Won M-H, Hwang IK. Combination effects of sodium butyrate and pyridoxine treatment on cell proliferation and neuroblast differentiation in the dentate gyrus of D-galactose-induced aging model mice. Neurochem Res 2012; 37: 223-231.