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The Influence of Shc Proteins on Life Span in Mice

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The signaling molecule p66Shc is often described as a longevity protein. This conclusion is based on a single life span study that used a small number of mice. The purpose of the present studies was to measure life span in a sufficient number of mice to determine if longevity is altered in mice with decreased Shc levels (ShcKO). Studies were completed at UC Davis and the European Institute of Oncology (EIO). At UC Davis, male ***C57BL/6J*** WT and ShcKO mice were fed 5% or 40% calorie-restricted (CR) diets. In the 5% CR group, there was no difference in survival curves between genotypes. There was also no difference between genotypes in prevalence of neoplasms or other measures of end-of-life pathology. At 40% calorie restriction group, 70th percentile survival was increased in ShcKO, while there were no differences between genotypes in median or subsequent life span measures. At EIO, there was no increase in life span in ShcKO male or female mice on ***C57BL/6J***, 129Sv, or hybrid ***C57BL/6J***-129Sv backgrounds. These studies indicate that p66Shc is not a longevity protein. However, additional studies are needed to determine the extent to which Shc proteins may influence the onset and severity of specific age-related diseases.

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THE signaling molecule p66Shc is frequently described as a longevity protein. The Shc locus encodes three adaptor proteins ($p46^{Shc}$, $p52^{Shc}$, and $p66^{Shc}$) that are involved in transmitting signals from growth factor receptors (1). There is evidence that $p66^{Shc}$ participates in pathways that influence oxidative stress and apoptosis (2–5), and that cells lacking $p66^{Shc}$ are resistant to oxidative stress (3). However, the primary reason for linking p66Shc with longevity is a study in 1999, which found that life span was increased in $p66^{Shc}$ knockout compared with wild-type (WT) mice (3). It has recently been shown that the levels of $p46^{Shc}$ and $p52^{Shc}$ are also decreased in liver, muscle, and other tissues in these $p66^{Shc}$ knockout mice (6). Thus, these mice (we refer to as ShcKO) provide a model of overall decreases in Shc protein levels in multiple tissues. Although the initial longevity study is widely cited to support a role for $p66^{Shc}$ as a life span determinant, there are clear limitations to this study that need to be considered. First, the study was completed with a small number of animals (n = 15 ShcKO and n = 14 WT) and the WT mice used in this study did not have particularly long median (761 days) or maximum (~850 days) life spans (3). Second, the published manuscript did not provide details about animal husbandry, diet, housing conditions, health surveillance, or criteria for euthanasia, which can to varying extent influence life span. Third, no end-of-life pathology data were presented. Therefore, additional studies are needed to determine more thoroughly the influence of Shc proteins on life span.

In addition to the role Shc plays in oxidative stress (reviewed in (7)), there is accumulating evidence that Shc proteins also play an important role in metabolism. The activities of enzymes involved in β-oxidation and ketone body metabolism are increased in ShcKO compared with WT mice (8). Also, it has been shown that glucose tolerance and insulin sensitivity are increased in ShcKO versus WT mice (6). These metabolic changes are similar to those observed in calorie-restricted (CR) animals, and this has led to speculation that Shc proteins may play a role in the adaptation to CR. However, little is known about the interaction between CR and Shc proteins and no long-term studies have been completed to investigate life span in CR ShcKO mice.

The purpose of the present study was to complete life span studies in a sufficient number of animals to determine thoroughly the extent to which longevity and end-of-life pathology are altered in ShcKO mice. A further goal of the

study was to determine the influence of calorie restriction on life span in these animals.

Materials and Methods

Animal Husbandry, University of California – Davis (UCD)

ShcKO mice were provided by Dr. Pier Giuseppe Pelicci (European Institute of Oncology, Milan, Italy) and used to establish a breeding colony at UC Davis. The breeding stocks were backcrossed onto ***C57BL/6J*** mice to full congenic status. Heterozygous ShcKO mice were mated to produce the homozygous ShcKO, and WT mice used for the study. At 1–2 months of age, the mice were singly housed in polycarbonate cages placed within positive pressure, HEPA filtered units. All mice were provided continuous access to water and LM-485 diet (7012 Teklad, Harlan Laboratories, Madison, WI). The mice were housed in a vivarium maintained at 22°C–24°C and 40%–60% relative humidity with a 12-hour light/12-hour dark cycle. All experimental procedures were approved by the University of California Institutional Animal Care and Use Committee.

Beginning at 3 months of age, daily food intake was measured in a subset of mice. Food intake was determined by collecting and weighing all food remaining in the food hopper and cage at the same time each day. At 4 months of age, the mice were randomly divided into four groups (n = 50 per group): 5% CR WT, 5% CR ShcKO, 40% CR WT, and 40% CR KO. The control groups were maintained on 5% CR to prevent excessive weight gain and obesity in these animals. Food intake measures from 3 to 4 months of age were used to determine the initial amount of LM-485 diet given to the mice. Food intake measures were continued through 18 months of age in a group of mice (n = 5 per genotype) allowed free choice consumption of food. Food intake in the CR groups was adjusted if changes in food intake were observed in the *ad libitum* fed mice. The daily food allotment given at 18 months of age was maintained until the end of the study. There were no differences in *ad libitum* food intake between the WT and ShcKO mice (Supplementary Figure 1), and thus both genotypes were given equal amounts of food for the duration of the study. One 5% CR WT mouse was dropped from the study and its data excluded from analysis because an animal husbandry error resulted in this mouse being fed a 40% CR diet for several weeks.

The mice were given fresh food daily and all animals were weighed weekly. Health checks were completed on the mice at least once per day. No other procedures were conducted on the mice in this study. The animals were allowed to live out their natural life span, and mice were only euthanized if they were moribund. Mice were considered moribund if they were unable to eat or drink, exhibited nonresponsiveness to an external stimulus (failure to move when gently prodded), or developed an ulcerated or bleeding tumor. Any animals that were euthanized or found dead in their cage were collected, their body cavities were opened, and the carcasses were placed in 10% neutral-buffered formalin solution and transported to the UC Davis Comparative Pathology Laboratory for necropsy and determination of cause of death. Date of death was recorded and used to calculate life span.

Sentinel mice were housed on the same racks as the study animals and were exposed to bedding from the study animals on a weekly basis. Sentinels were euthanized every 3 months for health screens, including aerobic cultures and serology (MHV, MPV, MVM, *M. pul.*, TMEV (GDVII), Ectro, EDIM, MAD1, MAD2, LCM, Reo-3). All tests were negative throughout the study.

Animal Husbandry, European Institute of Oncology

In addition to the study at UCD, life span studies were also completed on male and female ShcKO homozygous, heterozygous and WT mice [129Sv, ***C57BL/6J*** and hybrid F1 ***C57BL/6J***-129Sv strains] derived from the originally described ShcKO mice (3) at the European Institute of Oncology (EIO). The mice were housed in a SPF (FELASA) vivarium at the EIO. The mice were maintained in rooms with a 12-hour light/12-hour dark cycle at 22°C ± 2°C and 55% ± 10% relative humidity. The mice were allowed continuous access to water and Teklad 2018S diet (Harlan Teklad, Bresso-Milan, Italy). The mice were singly housed starting at 8 weeks of age. Health checks were completed on the mice at least once per day. No procedures were completed on the mice, and the mice were only euthanized if they were moribund. The same criteria for euthanasia at UCD (described above) were followed at EIO. Life span was determined from the recorded date of death for each animal. All experiments were performed according to the European guidelines for the use of animals in research and the requirements of Italian laws and regulations.

Sentinel mice were housed in the same rooms as the study animals and were exposed to bedding from the study animals. Sentinels were euthanized every 2 months and underwent the same health screens as the mice at UCD. The facility was included in a health-monitoring program developed in accordance with the Federation of European Laboratory Animal Science Associations (FELASA) guidelines (9). All tests were negative during the study.

Pathology

For all the UCD animals, a gross necropsy was performed, and a standardized set of tissues and lesioned organs were processed for histologic examination. These tissues included liver, kidneys, spleen, pancreas, heart, lungs, thymus, gastrointestinal tract (esophagus, stomach, small intestine, cecum, and large intestine), brain, reproductive

tract (testes, epididymis, prostate glands, and seminal vesicles), haired skin, and skeletal muscle. Other tissues were submitted for examination on the basis of the presence of gross lesions at the time of necropsy. Formalin-fixed tissues were processed and embedded in paraffin by routine methods. Tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Histologic examination was performed by STL, AK, and SMG.

Statistical Analysis

Maximum and mean body weights of WT and ShcKO mice in each diet group were compared using two-sample *t*-tests. Two-sample proportion tests were used to compare the incidence of cancer or organ pathology between genotypes and diet groups.

To compare overall survival, survival curves for each genotype and diet group were estimated with Kaplan-Meier estimators. A log rank test was used to test for differences in survival among the four genotype and diet groups and to conduct all pairwise comparisons of the groups. A Bonferroni adjustment was used to maintain the family-wise error rate at 0.05 for the pairwise comparisons. Because no survival times were censored, survival times were also directly compared between the groups. For this analysis, Sprent's nonparametric method for testing whether two distributions have the same median was generalized to test whether specified percentiles (10th through 90th) of survival times differed between groups (10). Finally, a Cox proportional hazard model was used to evaluate the effects of diet and genotype on survival, while adjusting for body weight. First, body weight was incorporated as a time-invariant predictor by using the mean and maximum body weight in two separate analyses. Second, mean weekly body weight was included as a time-varying predictor. For this analysis, because age at death was recorded in days, while body weight was recorded weekly, weekly body weights were converted to daily values as follows. The last recorded weight was used as the weight at the time of death (i.e., the last value was carried forward). While most mice had weights recorded at the beginning of the week of their death, for some mice the last reported weights were 1 or 2 weeks prior. Daily weights between weekly weights were imputed using linear interpolation.

Results

Body Weight

Weekly body weights for the UCD mice are shown in Figure 1. As expected, body weight was decreased ($p < .001$) in the 40% CR compared with 5% CR groups. At 40% CR, there was no difference between genotypes in mean lifetime body weight or maximum body weight (Table 1). In contrast, mean lifetime body weight and maximum body weight were increased ($p < .01$) in the WT compared with ShcKO mice at 5% CR (Table 1).

Longevity

The survival curves for the UCD mice are shown in Figure 2. There were no differences in the survival curves between the 5% CR WT and the ShcKO groups. Median and 10th percent survival for both groups were approximately 955 days and 1100 days, respectively (Table 2). As expected, median and 10th percent survival were increased ($p < .05$) in the 40% CR groups compared with either the 5% CR WT or ShcKO groups. With 40% CR, there was an increase ($p < .05$) in 70th percentile survival in the ShcKO compared with WT mice (Table 2). However, this difference between ShcKO and WT groups had disappeared by the 50th percent survival point, and no further differences between genotypes were observed for the remaining survival statistics.

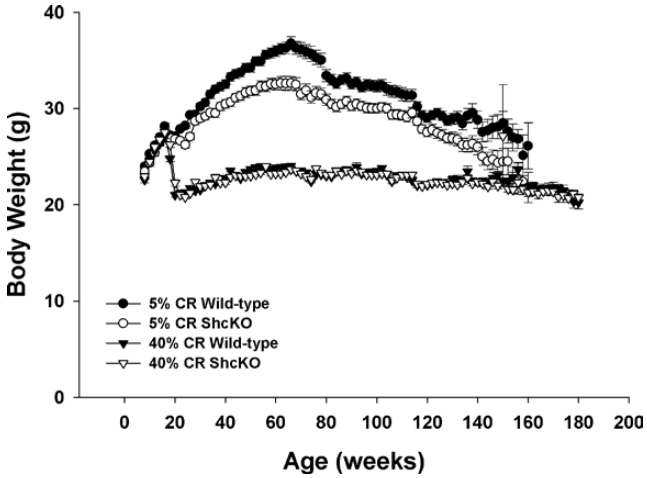

Figure 1. Mean body weights in wild-type (WT) and ShcKO ***C57BL/6J*** mice at UC Davis maintained on 5% or 40% calorie-restricted (CR) diets. All mice were housed at UC Davis ($n = 50$ for all groups, except $n = 49$ for 5% CR WT).

Table 1. Effect of Calorie Restriction and ShcKO on Maximum Body Weight and Body Weight Averaged Across the Life Span*

	5% CR WT	5% CR ShcKO	40% CR WT	40% CR ShcKO
Maximum body weight (g)	38.37 ± 4.45†	34.17 ± 4.38‡	27.90 ± 4.32§	27.46 ± 2.88§
Average body weight (g)	32.20 ± 2.91†	29.65 ± 3.12‡	22.71 ± 1.23§	22.60 ± 1.24§

Notes: *All body weight calculations used values collected after 20 weeks of age (this excludes the period of major weight loss following initiation of calorie restriction). Values within a row that contain different superscripts differ ($p < .05$).

5% CR WT = 5% calorie-restricted wild-type; 5% CR ShcKO = 5% calorie-restricted ShcKO; 40% CR WT = 40% calorie-restricted wild-type; 40% CR ShcKO = 40% calorie-restricted ShcKO.

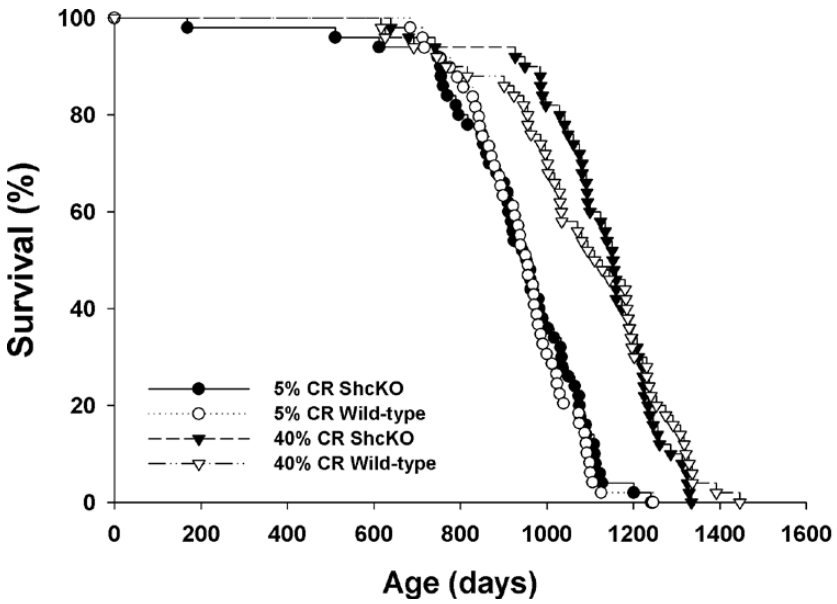

Figure 2. Survival curves for wild-type (WT) and ShcKO ***C57BL/6J*** mice maintained on 5% or 40% calorie-restricted (CR) diets. All mice were housed at UC Davis ($n = 50$ for all groups, except $n = 49$ for 5% CR WT).

To investigate whether differences in body weight between the 5% CR WT and ShcKO mice influenced life span, body weight (average weekly weight, maximum weight or average body weight across the life span) was included as a covariate in a Cox proportional hazard model (11). Survival did not differ significantly ($p > .10$) between genotypes on the 5% CR diet with inclusion of any measure of body weight as a covariate.

In the studies at the EIO, there were no differences in combined male and female survival curves for ShcKO and WT mice on ***C57BL/6J*** (Figure 3), 129Sv (Figure 4) and hybrid F1 ***C57BL/6J*** x 129Sv (Supplemental Figure 2) backgrounds. When the survival curves were separated by sex, there was an increase ($p < .05$) in life span in WT versus ShcKO males on a 129Sv background (Figure 4B) and there was an increase ($p < .05$) in life span in Shc heterozygous versus WT females on a hybrid F1 ***C57BL/6J*** x 129Sv background (Supplemental Figure 2C). However, it should be noted that there were only a small number of Shc heterozygous ($n = 16$) and WT ($n = 18$) females on the hybrid F1 background, and thus, these results should be viewed with caution. There were no other differences between either male or female ShcKO and WT mice.

Pathology

The necropsy results for the four groups of mice are summarized in Tables 3 and 4. The most frequent cause of death in this study was hepatic histiocytic sarcoma, which occurred in 75 of the 199 animals examined (Table 3). Typically neoplastic histiocytes expanded the sinusoids resulting in attenuation and necrosis of extensive regions of hepatic parenchyma. Metastasis was noted most frequently to the spleen ($n = 29$), lung ($n = 16$), kidney ($n = 8$), and local lymph nodes ($n = 6$). An additional 33 animals had evidence of marked Kupffer cell hyperplasia. Kupffer cells are presumed to be the cell of origin of hepatic histiocytic sarcomas, and Kupffer cell hyperplasia was interpreted as a preneoplastic lesion. Other neoplasms were much less frequent and included lymphoma ($n = 16$), most commonly a multicentric process, hemangiosarcoma ($n = 6$), which typically arose from the spleen, and pulmonary adenomas ($n = 17$). Lymphoid depletion ($n = 28$) and expansion of extramedullary hematopoiesis ($n = 29$) in the spleen were frequently seen as reactive/secondary changes to other pathology. Other common, age-related and incidental findings were degeneration and atrophy of the seminiferous tubules of the testicles ($n = 82$), ectasia

Table 2. Life Span Statistics for Wild-type and ShcKO Mice*

	5% CR WT	5% CR ShcKO	40% CR WT	40% CR ShcKO
70th percentile	879† (828, 930)	867† (805, 929)	1002‡ (931, 1073)	1082§ (1035, 1129)
50th percentile	956† (915, 997)	953† (904, 1002)	1111‡ (984, 1238)	1153‡ (1128, 1178)
30th percentile	1012† (967, 1057)	1034† (967, 1101)	1202‡ (1153, 1251)	1212‡ (1178, 1246)
10th percentile	1099† (1080, 1118)	1111† (1085, 1137)	1322‡ (1291, 1353)	1287‡ (1232, 1342)
Longest lived	1246	1241	1447	1335

Notes: *Survival times (days) with approximate 95% confidence interval indicated in parenthesis. Values in a row that contain different superscripts differ at $p < .05$ determined by Sprent's nonparametric method.

5% CR WT = 5% calorie-restricted wild-type; 5% CR ShcKO = 5% calorie-restricted ShcKO; 40% CR WT = 40% calorie-restricted wild-type; 40% CR ShcKO = 40% calorie-restricted ShcKO.

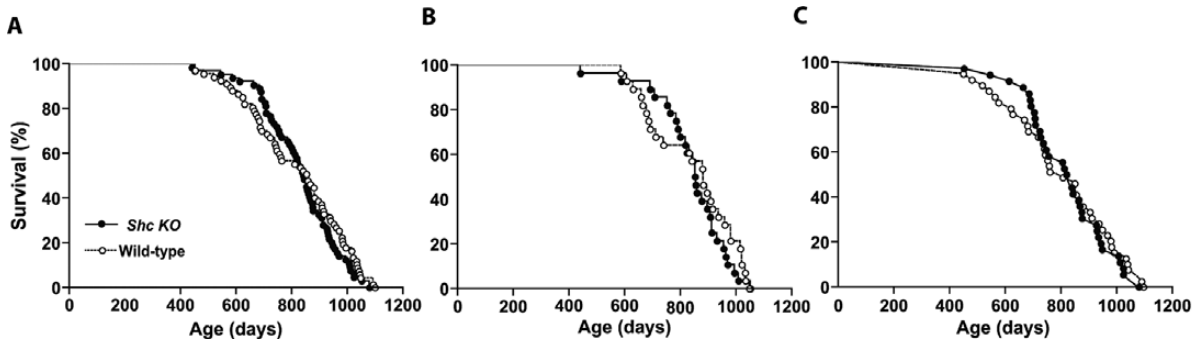

Figure 3. Survival curves for wild-type (WT) and ShcKO ***C57BL/6J*** mice fed *ad libitum*. All mice were housed at the European Institute of Oncology. **(A)** Male and female mice, **(B)** male mice only, **(C)** female mice only (n = 28 WT males, n = 28 ShcKO males, n = 39 WT females, n = 36 ShcKO females).

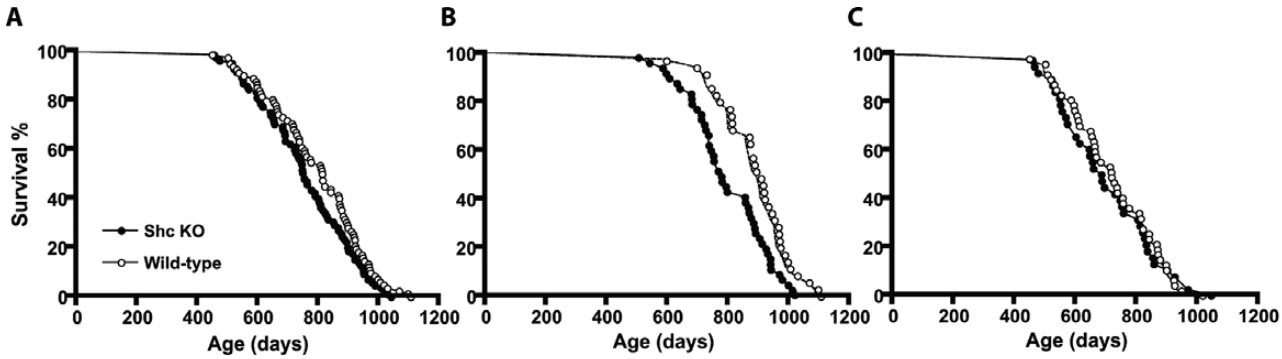

Figure 4. Survival curves for wild-type (WT) and ShcKO 129Sv mice fed *ad libitum*. All mice were housed at the European Institute of Oncology. **(A)** Male and female mice, **(B)** male mice only, **(C)** female mice only (n = 35 WT males, n = 47 ShcKO males, n = 47 WT females, n = 38 ShcKO females).

and inspissation of the seminal vesicles (n = 21), and mineralization of the vessels of the thalamus (n = 23). There was no evidence of infectious pathogenic agents in any of the mice.

Statistical comparisons between the four groups were limited to broad categories (cancer incidence or overall presence of pathology within a specific organ), since the low frequency of specific pathology findings prevented more refined comparisons. There were no significant differences in cancer incidence between any of the groups of mice. The only significant difference between ShcKO and WT mice was a decrease in gastrointestinal pathology in the 5% CR ShcKO compared with WT mice. This primarily reflected the fact that there was a greater incidence of small intestine amyloidosis in the WT (14%) compared with the ShcKO (2%) mice.

The end-of-life pathology was similar between the 5% and 40% CR groups (Table 4), except there were clear decreases in dilation of seminal vesicles and ectasia and inspissations in the 40% versus 5% CR mice. In contrast, the incidence of testicular degeneration was increased in the 40% CR compared with 5% CR groups.

Table 3. Prevalence of Neoplasms in Wild-type and ShcKO Mice

Neoplasm	5% CR WT (n = 49)	5% CR ShcKO (n = 49)	40% CR WT (n = 50)	40% CR ShcKO (n = 49)
Histiocytic sarcoma	17	16	19	23
Lymphoma	5	6	4	1
Other	6	8	4	5

Notes: 5% CR WT = 5% calorie-restricted wild-type; 5% CR ShcKO = 5% calorie-restricted ShcKO; 40% CR WT = 40% calorie-restricted wild-type; 40% CR ShcKO = 40% calorie-restricted ShcKO.

Table 4. Pathology at Time of Death in Control and Calorie-restricted Mice

	5% CR WT (n = 49)	5% CR ShcKO (n = 49)	40% CR WT (n = 50)	40% CR ShcKO (n = 49)
Liver				
Histiocytic sarcoma	17	16	20	19
Kuppfercell hyperplasia	7	4	7	15
Extramedullary hematopoiesis	11	5	5	4
Hepatocellular degeneration	4	3	2	2
Lymphohistiocytic hepatitis	4	2	3	2
Lymphoma	3	3	0	1
Hemangiosarcoma	0	1	3	0
Reproductive				
Testes				
Seminiferous tubule atrophy and degeneration	14	14	24	30
Seminal vesicles				
Presence of eosinophilic secretory material	17	23	1	2
Dilation of	15	21	1	2
Ectasia and inspissation	4	11	3	3
Prostate				
Prostatitis	0	2	0	2
Spleen				
Extramedullary hematopoiesis	4	7	12	6
Histiocytic sarcoma	8	7	5	9
Lymphoid depletion	11	4	8	5
Hemangiosarcoma	2	1	1	0
Hemosiderosis	0	0	3	0
Lymphoma	1	1	1	0
Kidney				
Lymphoplasmacytic interstitial nephritis	13	10	14	12
Tubular mineralization	9	3	7	7
Hyaline droplet nephropathy	6	6	6	2
Hydronephrosis	3	4	3	1
Histiocytic sarcoma	3	2	0	3
Glomerular sclerosis	3	1	2	1
Lymphoma	1	1	2	1
Tubular cyst	3	0	0	2
Tubular degeneration	0	1	0	3
Lymphocytic infiltrates	0	0	0	1
Lung				
Histiocytic sarcoma	5	6	5	5
Pulmonary adenoma	4	5	3	5
Pneumonia	1	6	4	5
Lymphoma	2	0	3	0
Pulmonary carcinoma	0	2	1	0
Lymphocytic leukemia	1	1	0	0
Pulmonary carcinoma	0	0	1	0
Heart				
Ventricular dilation	1	3	6	8
Aortic mineralization	0	1	2	2
Ventricular thrombosis	0	1	1	3
Atrial thrombosis	0	2	0	2
Atrial dilation	1	0	0	2

Table 4. *(Continued)*

	5% CR WT (n = 49)	5% CR ShcKO (n = 49)	40% CR WT (n = 50)	40% CR ShcKO (n = 49)
Endocardial mineralization	1	2	0	0
Myocardial fibrosis	0	1	0	2
Myocardial degeneration	0	0	2	0
Aortic valvular thrombosis	1	0	0	0
Myocardial mineralization	0	0	1	0
Pericardial mineralization	0	0	1	0
Gastrointestinal/urinary tract				
Large Intestine				
Vessel congestion	1	1	0	0
Edema	0	0	1	0
Epithelial necrosis	1	0	0	0
Lymphoplasmacytic colitis	0	0	1	0
Mucosal necrosis	1	0	0	0
Small Intestine				
Amyloidosis	7	1	2	0
Chronic intraluminal hemorrhage	0	0	0	2
Lymphoplasmacytic enteritis	0	0	2	0
Chronic hemorrhage	0	0	0	1
Epithelial necrosis	1	0	0	0
Stomach				
Gastritis	2	1	2	1
Amyloidosis	1	1	0	0
Anaplastic sarcoma	1	0	0	1
Mesentery				
Lymphoma	0	0	1	0
Ureter				
Hydroureter	1	0	0	0
Urethra				
Urethritis	1	0	0	1
Subepithelial hemorrhage	0	0	0	1
Brain				
Mineralization (Thalamus)	3	6	6	8
Histiocytic sarcoma	0	0	1	0
Meninges lymphoma	1	0	0	0
Lymph nodes				
Histiocytic sarcoma	1	1	1	3
Lymphadenitis	2	1	1	1
Lymphoma	2	1	1	0

Notes: 5% CR WT = 5% calorie-restricted wild-type; 5% CR ShcKO = 5% calorie-restricted ShcKO; 40% CR WT = 40% calorie-restricted wild-type; 40% CR ShcKO = 40% calorie-restricted ShcKO.

Discussion

The results of the present study indicate that ShcKO does not increase median or maximum life span in mice, and therefore, Shc is not a longevity gene. These conclusions conflict with a previous study that found that life span was increased by 30% in ShcKO compared with WT mice (3). There are at least three differences between the studies, which could contribute to the disparate life span results.

Strain Differences

The strains of mice used in the life span studies could influence the results. As a first step toward determining whether the strain could play a role in the life span differences between studies, it is important to determine if the life span values of the WT animals are consistent with published values for their strain. The median life span of the mice used in the present study met or exceeded typical life span values for C57BL/6 mice (12–15). In contrast, the median survival (761 days) of the WT mice in the study by Migliaccio et al. was below median life span values for male and female 129 mice maintained in a specific-pathogen free facility (14). The increase in life span observed in the study by Migliaccio et al. may primarily be a result of rescue of whatever stress was causing the low life span of the WT mice used in this study, and there is considerable evidence that Shc deficiency promotes stress resistance (7,16–18). To further test the influence of mouse strain on longevity of ShcKO mice, life span measurements were completed on ***C57BL/6J*** and 129Sv WT and ShcKO mice at the EIO. Life span was not increased in the ShcKO mice regardless of the mouse strain. Thus, mouse strain is not responsible for the differences in life span between studies.

Differences in Mouse Numbers

A second possible reason for differences between the studies is that they used different numbers of mice, with 49–50 mice per group in the present UCD study and 14–15 mice per group in the 1999 study. A concern with the use of 15 (or fewer) mice per group is the fact that each animal has a large impact on the survival curve. It can be difficult to reproduce these studies which are so heavily influenced by a few animals, and thus, a larger sample size is needed to allow for random fluctuations about the true life span trend in finite-sample experiments. It has been recommended that life span studies include 40–50 mice per group to avoid excessive influence of individual animals on survival curves, provide adequate power to detect differences between groups of mice and allow more detailed statistical analysis of survival curves (19). It is possible that the increase in life span in the ShcKO group in the previous study was an anomaly resulting from the small number of animals used in the study.

Differences in Animal Husbandry

The third potential reason for the difference between studies is differences in animal husbandry. One area of possible difference is level of food intake. The mice in the present UCD study were fed a mildly (5%) restricted diet. However, it seems unlikely that this had a major influence on the results, since the body weights of the mice in both studies were similar and there was no evidence of obesity being a factor in either study. Furthermore, there were no differences in longevity between WT and ShcKO in the studies at the EIO using *ad libitum* fed animals. The relatively low life span of the WT group in the previous study (3) strongly suggests that husbandry for these animals was not ideal, and the observed results represent a response to some level of environmental stress. It is possible that ShcKO will improve survival in response to some stressors. However, it is important to note that another study found that life span is decreased in ShcKO compared with WT mice when housed in an outdoor enclosure intended to mimic a natural environment (20). It has also been reported that p66Shc levels are higher in fibroblasts from centenarians compared to elderly (50–80 years) or young (17–38 years) people (21). These results suggest that chronic decreases in p66Shc levels, at least in one cell type, are not required for a long life. It remains to be determined which specific environmental conditions may alter survival in animals with low Shc levels. Nonetheless, the results of the present study indicate that life span is not increased in ShcKO mice when compared with WT animals, which meet expected life span values for their strains under the highest husbandry standards. Thus, these results indicate that Shc is not a life span gene.

Shc Deficiency, Calorie Restriction, and Health Span

A secondary goal of the present study was to determine whether ShcKO influences life span in 40% CR mice. The results of the study showed that 70th percentile survival was significantly increased in the 40% CR ShcKO compared with WT mice, while median life span and subsequent survival statistics were not different between the genotypes. These results indicate that ShcKO had an influence on disease processes that resulted in early death in the 40% CR mice but had no influence on rate of aging. Shc proteins have been shown to play a role in energy metabolism (6,8,22–25) and reactive oxygen species production (2,26,27), and it is likely that these actions of Shc proteins could influence some age-related diseases and possibly alter life span under certain conditions. In particular, ShcKO mice have increased insulin sensitivity (6) and are resistant to ischemic injury (28), atherosclerosis (26) and apoptosis induced by a variety of stressors (17). These effects of ShcKO would be expected to have a positive influence on health span even if life span is not lengthened. There is also evidence that ShcKO decreases adiposity (6,22,25) and increases capacity for fatty acid oxidation (8) and these changes may influence life span under certain conditions, such as diet-induced obesity. Consistent with this idea, it has been reported that life span is increased in obese, leptin deficient mice (ob/ob) on a ShcKO versus WT background (25). The influence of ShcKO on age-related disease and health span with various diets and models of obesity is an area that warrants further investigation.

Shc Deficiency and End-of-Life Pathology

Similar to previous studies in C57BL/6 mice (12,29), neoplastic disease was the primary cause of death with histiocytic sarcoma and lymphomas as the major neoplasms. There was no difference in prevalence of neoplasms or other measures of end-of-life pathology between the ShcKO and WT mice. Although there is considerable interest in the role Shc proteins play in tumorigenesis (18), the results of the present study indicate that the decreased level of Shc proteins in the ShcKO mice had no influence on the occurrence of neoplasia. A clear limitation of the present study, however, was the fact that only end-of-life pathology was measured, and it was not possible to determine whether ShcKO or level of restriction influenced the age of onset of any of the measures of pathology. Although knockdown of Shc proteins had little influence on end-of-life pathology in either 5% or 40% CR mice, additional studies are needed to determine if Shc proteins influence the onset or duration of major age-related diseases. Another limitation of the present study is the fact that C57BL/6 mice die primarily from neoplasms (in particular lymphomas and histiocytic sarcomas). Further studies are needed to determine if ShcKO influences pathology in animal models that die primarily from causes other than those commonly observed in C57BL/6 mice.

Conclusions

The results of the present study indicate that median and maximum life spans are not increased in ShcKO compared with WT mice. Thus, Shc is not a life span gene. However, ShcKO did increase survival time in the first portion (70th percentile) of the survival curve in 40% CR mice. This indicates that ShcKO does have an influence on some disease processes. Shc proteins play a role in insulin signaling, stress resistance, and energy metabolism, and additional studies are needed to determine the extent to which these actions of Shc proteins influence health span and age-related changes in physiological function and disease.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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