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Thirdhand tobacco smoke exposure increases the genetic background-dependent risk of pan-tumor development in Collaborative Cross mice

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Increasing evidence has shown that thirdhand smoke (THS) exposure is likely to induce adverse health effects. An important knowledge gap remains in our understanding of THS exposure related to cancer risk in the human population. Population-based animal models are useful and powerful in investigating the interplay between host genetics and THS exposure on cancer risk. Here, we used the Collaborative Cross (CC) mouse population-based model system, which recapitulates the genetic and phenotypic diversity observed in the human population, to assess cancer risk after a short period of exposure, between 4 and 9 weeks of age. Eight CC strains (CC001, CC019, CC026, CC036, CC037, CC041, CC042 and CC051) were included in our study. We quantified pan-tumor incidence, tumor burden per mouse, organ tumor spectrum and tumor-free survival until 18 months of age. At the population level, we observed a significantly increased pan-tumor incidence and tumor burden per mouse in THS-treated mice as compared to the control (p = 3.04E-06). Lung and liver tissues exhibited the largest risk of undergoing tumorigenesis after THS exposure. Tumor-free survival was significantly reduced in THS-treated mice compared to control (p = 0.044). At the individual strain level, we observed a large variation in tumor incidence across the 8 CC strains. CC036 and CC041 exhibited a significant increase in pan-tumor incidence (p = 0.0084 and p = 0.000066, respectively) after THS exposure compared to control. We conclude that early-life THS exposure increases tumor development in CC mice and that host genetic background plays an important role in individual susceptibility to THS-induced tumorigenesis. Genetic background is an important factor that should be taken into account when determining human cancer risk of THS exposure.

Keywords

Thirdhand smoke (THS); Collaborative Cross (CC) mice; Genetic susceptibility; Tumorigenesis; Pan-tumor incidence; Tumor burden; Tumor-free survival

1. Introduction

Thirdhand smoke (THS) is defined as residual tobacco smoke sorbed onto indoor surfaces and in dust after active smoking has ceased, some of the adsorbed constituents can be re-emitted into the gas-phase and/or react with environmental pollutants to form hazardous compounds (Matt et al., 2011a, 2011b; Jacob et al., 2017). Compared to secondhand smoke (SHS) that usually dissipates in hours, THS can last for a long time in indoor environments, ranging from days to months, even years, after smoking has ceased. Moreover, non-smokers can be exposed to THS pollutants through multiple routes, which include involuntary inhalation, ingestion, dermal adsorption, dermal gas-to-skin deposition, and epidermal nitrosation of nicotine (Matt et al., 2011a, 2011b; Jacob et al., 2017; Tang et al., 2022). Small children may be more susceptible to THS exposure than adults because their age-related behaviors bring them in close contact with surfaces and dust contaminated with THS toxins. In addition, their physiological characteristics, for example, lower toxin metabolic capacity and high toxin/body weight ratio, potentially make them more sensitive to adverse

health effects associated with THS exposure (Matt et al., 2011a, 2011b; Jacob et al., 2017; Hang et al., 2019a, 2019b).

Besides the most prevalent constituent in THS, nicotine, more than 100 chemical compounds have been identified in airborne or surface THS in recent years (Sleiman et al., 2014; Schick et al., 2014; Jacob et al., 2017; Tang et al., 2021; Tang et al., 2022; Borujeni et al., 2022). Of them, the products formed *de novo* from the reaction of nicotine with environmental pollutants such as nitrous acid (Sleiman et al., 2010a, 2010b) and ozone (Destaillats et al., 2006; Sleiman et al., 2010a, 2010b; Tang et al., 2021) have gained broad attention, as more hazardous compounds can be generated through the aging process of SHS. There is evidence that children have higher levels of THS constituents in urine such as nicotine and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, a metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)) in homes of parents who smoked or even if they never smoked inside their home than the children of nonsmokers (Matt et al., 2011a, 2011b; Northrup et al., 2016). Therefore, THS pollution represents a new challenge for public health, particularly for small children who may represent a group with higher health burden when exposed during childhood (Mahabee-Gittens et al., 2021).

An important question is whether THS exposure increases cancer risk in humans. Our group has been working to address this issue using molecular, *in vitro* and *in vivo* model systems. More than a hundred toxic chemicals have been identified thus far in THS, some of which have been identified by the International Agency for Research on Cancer (IARC) as known or possible carcinogens, including for example tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs) and benzene (IARC 2007; Jacob et al., 2017; Schick et al., 2014; Tang et al., 2022). Various investigations have shown that THS exposure causes a variety of molecular and cellular changes in cultured human cells at environmentally relevant doses including DNA strand-breaks, oxidized base lesions, bulky DNA adducts, and micronuclei (Hang et al., 2013; Hang et al., 2014; Hang et al., 2018; Bahl et al., 2016a, 2016b; Sarker et al., 2020; Tang et al., 2022; Sakamaki-Ching et al., 2022). Using mouse models, we previously observed that early age THS exposure impaired immune function in C57BL/6 mice (Hang et al., 2017) and increased lung cancer incidence in A/J mice (Hang et al., 2018; Hang et al., 2019a, 2019b). THS exposure did not increase tumor risk in similarly treated C57BL/6 mice (Hang et al., 2017; Dhall et al., 2016; Martins-Green, personal communication), suggesting that genetic background plays an important role cancer risk after THS exposure.

A critical knowledge gap remains in our understanding of the genetic basis for interindividual responses to THS. Population-based animal model systems can help fill this gap using well-controlled environments and exposures in a genetically tractable system. In recent years, we have used the genetically diverse Collaborative Cross (CC) mouse system, which contains a level of genetic and phenotypic diversity comparable to the human population, to study the role of genetic background on a wide array of phenotypic endpoings including, motor performance (Mao et al., 2015), memory and anxiety (Kim et al., 2019; Mao et al., 2020; He et al., 2021), gut microbiome composition (Jin et al., 2021; Snijders et al., 2016), and spontaneous cancer susceptibility (Wang et al., 2019). Through these studies

we have gained many insights into the interaction between environmental exposure and genetic background on health and disease.

The aim of this work was to investigate whether host genetic background influences the effect of THS exposure on tumorigenesis. CC mice were exposed to laboratory-generated THS material from 4 to 9 weeks of age and THS carcinogenic potential was assessed until 18-month of age.

2. Materials and methods

2.1. THS sample generation and characterization

The THS samples were generated with a controlled laboratory system at the University of California, San Francisco (UCSF), as previously described (Hang et al., 2013; He et al., 2021). Cotton terry cloth substrates were utilized as surrogates for indoor surfaces, onto which SHS chemicals could adsorb. Briefly, clean cotton terrycloth samples were repeatedly exposed to SHS in a 6-m^3 stainless steel chamber over the period of 520 days (the same material as used and described in He et al., 2021). During smoking, a total of 2804 mg of total particulate material was introduced into the steel chamber. This is equivalent to the smoke from 200 to 280 cigarettes over 520 days, or approximately half a cigarette per day. THS-laden cloths were then removed from the chamber, and vacuum-packed in Mylar film. All the samples were stored at − 20 °C until use.

To analyze the chemical constituents, we extracted the compounds in the above terrycloth samples with Dulbecco's Modified Eagle's Medium (DMEM) as previously described (Hang et al., 2013; He et al., 2021). Six known THS compounds in the DMEM samples were quantitatively measured following the procedures described before, using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Whitehead et al., 2015). Briefly, The samples were analysed on a Thermo Scientific Vantage LC–MS/MS with an Accela UPLC system using a Phenomenex Kinetex PFP column. An ammonium formate methanol solvent system was utilized for the analysis. The concentrations of the compounds measured were as follows: nicotine: 1.17 μg/ml, NNK: 2.30 ng/ml, NNN: 0.57 ng/ml, NAT: 0.49 ng/ml, NAB: 0.24 ng/ml, and nicotelline: 11.3 ng/ml.

2.2. CC mouse strains and maintenance

For this study, eight CC mouse strains, CC001, CC019, CC026, CC036, CC037, CC041, CC042 and CC051, were obtained from the Systems Genetics Core Facility at the University of North Carolina (UNC). Basic information of each CC mouse strain, including SNP marker genotypes, phenotypes and associated genetic disorders is available from the UNC website: https://csbio.unc.edu/CCstatus/index.py?run=AvailableLines.information. Mice were acclimated at the Lawrence Berkeley National Laboratory (LBNL) for eight weeks prior to the initiation of breeding. All animal experiments were performed at LBNL and the study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was approved by the Animal Welfare and Research Committee of LBNL (Protocol File Number 270024). Euthanasia of animals was by CO_2 exposure followed by cervical dislocation in

compliance with the AVMA guidelines for the euthanasia of animals (2020 edition). Mice were raised in groups whenever possible, fed a standard chow diet (PicoLab Rodent diet 5053), raised in individually ventilated cages in a light-controlled room with 12:12 h light/dark cycle.

2.3. THS exposure and experimentation

CC mice from each strain were randomly divided into THS-exposed and unexposed (control) groups immediately after weaning (Fig. 1). Mice were weighed every month, and physically examined. Mice were exposed to THS-laden terry cloth or control cloth from 4 to 9 weeks of age as follows, a 3.4 g (10 × 10 cm^2 swatch) of THS-exposed cloth or control cloth was added to the standard bedding in each cage, in lieu of standard enrichment, and the cloth swatches were replaced weekly during the standard cage change. The nicotine loading of the swatches was 23.4 μg/g, for a total of 79.6 μg. This level of nicotine is in the range of the estimated daily ingestion exposure of a toddler in a typical indoor THS exposure scenario (Bahl et al., 2014). The surface concentration of nicotine was ~ 8 mg/m^2, which is consistent with the high end of surface nicotine concentration recorded in a recent study of 220 homes in multiunit apartment buildings (Matt et al., 2020).

2.4. Gross examination and histopathology

When THS-treated or control mice reached their clinical endpoint or the endpoint of the study, a complete necropsy was performed immediately after euthanasia. Tumors were identified by visual inspection and any tumors found were counted and examined for tumor size, location and potential metastasis. In case mice were found dead in the cage (designated as FDIC) prior to reaching the end of the experiment, the time of death was recorded along with any tumors found during necropsy.

Organs were collected in 10% neutral buffered formalin (Azer Scientific). After 48 h at room temperature, fixed tissues were washed and immersed in 75% ethanol and stored at 4 °C until embedding. All tissues were embedded in paraffin, sectioned at 4 μm thickness, mounted on glass slides, and stained with hematoxylin and eosin (H&E) (Histology and Biomarkers Core, UCSF Mt. Zion Campus, San Francisco) for histopathologic examination.

2.5. Data analysis and statistics

As summarized in Table 1, in both THS-exposed and control groups, the number of mice with tumors (both benign and malignant), pan-tumor incidence (all the tumors found in a single CC strain treatment group), tumor burden per mouse (all the tumors found in the treatment group of a specific CC strain divided by the number of mice in that group), and specific tumor incidence were recorded or calculated.

Statistical analysis was performed using IBM SPSS software (version 24) and R (version 3.6.0). The Mann-Whitley *U* test was applied to compare the differences between the THS-exposed and control groups in tumorigenic phenotypes. A p-value less than 0.05 was used as the cut point for determining statistical significance. Odds ratios were calculated using fisher exact test to compare the relative odds or risk of the occurrence of tumor development

upon THS exposure. Differences in tumor-free survival were calculated using log-rank test and visualized using Kaplan-Meier plots.

3. Results

3.1. THS exposure increases overall pan-tumor incidence and tumor burden per mouse in exposed CC mouse cohort

To determine the effect of host genetic background on cancer susceptibility after THS exposure, we exposed mice from 8 CC strains to THS from 4 to 9 weeks of age and monitored for tumor development until mice reached 1.5 years of age (Fig. 1). The Collaborative Cross (CC) population-based mouse model is a genetically diverse model system to study how the host genetics influence the impact of exposures on health. The CC mouse system combines the genomes of eight genetically diverse founder strains – A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ and captures nearly 90% of the known variation present in laboratory mice (Leist & Baric, 2018). Each CC mouse strain is genetically unique and by investigating 8 CC strains, we are starting to approximate the genetic diversity present in the human population. A wide range of tumors were observed in the control and THS cohorts (summarized in Fig. 2, Table 1). Histopathological examples of select tumor types are shown in Fig. 2.

At the population level, combining all data across the eight strains, we observed a significant increase in pan-tumor incidence (p = 3.04E-6) and tumor burden per mouse (p = 0.002742) in the THS cohort compared to the control cohort (Fig. 3A and Table 1), indicating that THS exposure increases the overall tumor incidence in the exposed CC mouse cohort in this study (Odds ratio = 2.82, p = 4.39E-06). In addition, we observed a significant decrease in tumor-free survival in the THS exposed cohort compared to the control cohort (Fig. 3B). At the organ level, a significant increase in tumor incidence was observed for lung and liver (p less than 0.05). Increases were also observed in other tissues including stomach and lymphatic tissues (including thymus, spleen and abdominal lymph nodes) although these increases were not statistically significant (Fig. 3C).

3.2. Pan-tumor incidence and tumor burden per mouse in individual CC mouse strains upon THS exposure

We next analyzed the pan-tumor incidence at the strain level (Fig. 4). In the control cohort, tumor incidence ranged from 18% (CC051) to 41% (CC036). After THS exposure, all strains showed an increase in pan-tumor incidence. Significant increases in pan-tumor incidence were observed for CC036 (p = 0.0084) and CC041 (p = 0.000066). Meanwhile, CC051 (p = 0.095) and CC042 (p = 0.118) showed a borderline significant increase in pan-tumor incidence. In contrast, CC026, CC037, CC019 and CC001 showed no significant difference in pan-tumor incidence between control and THS treated cohorts. Tumor burden increased in all strains after THS exposure compared to control and ranged with a factor 1.5 for CC001 and CC037 to a factor 4.4 for CC041 (Table 1).

3.3. Variations of tumor spectrum in individual CC mouse strains upon THS exposure

We then investigated if THS exposure changed the tumor spectrum observed in individual CC strains. We first focused on strains which showed a significant increase in tumor incidence including CC036 and CC041 (Fig. 4). CC036 develops spontaneous gastric tumors and lymphomas (Wang et al., 2019). CC041 mice frequently developed spontaneous lymphomas of thymus and spleen. We found that THS exposure did not shift the tumor spectrum. CC036 mice exposed to THS developed gastric tumors more frequently and included both adenomas and adenocarcinomas in the pyloric region (Fig. 5). Similarly, CC041 mice exposed to THS developed thymic and splenic lymphomas more frequently (Fig. 5). These data indicate that THS exposure could accelerate gastric and lymphoma development in CC036 and CC041 mice, respectively. Interestingly, a few CC041 mice, developed other tumors not observed in control treated mice including lung, bone and kidney tumors.

Lung tumors were most frequently observed in CC042, CC019 and CC026, and were located in the peripheral regions, in the forms of adenoma and adenocarcinoma (e.g., small cell lung carcinoma, SCLC). No squamous cell carcinomas (LSCC) were discovered, which is strongly associated with cigarette smoking (Khuder, 2001). For tumors that occurred in the liver in CC051 and CC037, both hepatoma and hepatocellular carcinomas (HCCs) were identified, and the frequency of liver tumor development increased after THS exposure (Fig. 3). We also observed other types of tumors, benign and malignant, at low frequency, such as spindle cell carcinoma in the skin (CC042) and osteomas in CC041 (Fig. 5). However, it should be noted that these two tumors were only found in THS-treated mice.

3.4. THS exposure led to reduced tumor-free survival in specific CC strains

Finally, we investigated strain specific changes in THS exposure effects on tumor-free survival (TFS). CC036 and CC042 showed a significant reduction in TFS after THS exposure ($p = 0.008$ and $p = 0.046$, respectively; Fig. 6). A borderline reduction in TFS was observed in CC041 ($p = 0.076$; Fig. 6). No significant different in TFS was observed in the remaining strains (p greater than 0.05).

4. Discussion

The carcinogenic potential of THS is a critical parameter in determining human risk, especially for young children who are among the most susceptible sub-population (Jacob et al., 2017). Over the last decade, we and others have reported many *in vitro* findings that revealed the genotoxic potential of THS exposure. Later, we reported the first *in vivo* evidence that early exposure to THS increases lung tumor risk in the A/J mouse model (Hang et al., 2018; 2019a). We, and others, also found that THS exposure affects the development of immunity in C57BL/6 mice (Hang et al., 2017; Martins-Green et al., 2014), which could be a contributing factor to the tumorigenesis in mice exposed to THS. A/J inbred mice are widely used as a model to test chemical carcinogens including tobacco carcinogens given their high sensitivity to chemically induced lung cancer (Hecht, et al., 1989; Hang, 2010; Hu et al., 2022). This system has also been used in several studies to test tobacco smoke such as secondhand smoke (SHS) for lung tumorigenesis

(Witschi, 2005). These studies demonstrated that although A/J mice were very susceptible to certain important classes of carcinogens identified in tobacco smoke, such as PAHs and nitrosamines (NNK and NNN), it was considered a weak model for tobacco smoke carcinogenesis (Witschi, 2005). Nevertheless, using this model system, we observed an increased lung tumor incidence after 3 weeks of early life (3 to 7 weeks) exposure to THS terrycloth at environmental dose ranges (Hang et al., 2018).

Our study focused around two aims: (1) to confirm whether THS increases cancer risk using the Collaborative Cross (CC) population-based mouse model; and (2) to use multiple genetically distinct CC strains to explore the role of genetic background on THS exposure associated cancer risk. CC mice are a large panel of inbred mouse strains derived from eight genetically diverse laboratory inbred strains. The CC captures 90% of the known genetic variations present in lab mice, which provides an excellent tool for the study of so-called “systems genetics” in a mammalian system (Churchill et al., 2004; Collaborative Cross, 2012). As mentioned before, we have successfully used this system to identify genetic variants associated with various phenotypes. Mouse models offer a distinct advantage for exposure research since host genetic background and the THS exposure are well controlled without confounding effects of SHS often present in human real-life studies of THS exposure. For this study, eight CC mouse strains, CC001, CC019, CC026, CC036, CC037, CC041, CC042 and CC051 were randomly chosen. As mentioned above, each CC strain contains a unqiue genomic contribution from the eight founder strains. While all strains spontaneously develop tumors, two strains were known to have relatively high baseline tumor incidence levels (CC036 and CC041). Moreover, one strain is known to develop hydrocephalus at high frequency (CC019). The purpose of including these strains was to observe whether pre-existing conditions, similarly present in the human population, would affect THS-induced tumorigenesis. Here we found that THS exposure increases overall pan-tumor incidence in the exposed CC mouse cohort. Our results indicate that the CC mouse model provides us with a qualitative and quantitative experimental system, where tumors in various organs develop after THS exposure on a background of varying spontaneous cancer risks, making the CC model a convenient tool for human risk assessment of tobacco smoke studies.

We observed a complex interaction between THS exposure and tumor incidence and organ spectrum in each strain. As expected, high baseline levels of spontaneous tumors were observed in specific strains. For example, CC36 and CC041, demonstrated high spontaneous levels of gastric tumors and lymphomas, respectively. THS exposure significantly increased the incidence of these types of tumors. In contrast, THS exposure was also associated with the development of other tumors including for example bone tumors in CC041, which were not observed in control animals of the same strain. Currently we do not have a clear mechanistic understanding of these strain specific responses to THS exposure, however, we believe that based on our data, genetic background plays an important role.

The interplay between genetic background and THS exposure effects on cancer risk is not well understood. In this proof-of-concept study, our results demonstrated that CC strains exhibited a tremendous variation in tumor susceptibility after THS exposure, indicating that CC mice are a valuable model system for studying the genetic variation-driven effects and

underlying mechanisms of THS exposure on tumorigenesis. Additional CC strains will be needed to add to this cohort to allow for robust genome-wide association analysis to identify individual genetic loci that contribute to THS exposure associated cancer risk. As mentioned before, we presented CC036 as a spontaneous laboratory mouse model for studying human gastric tumorigenesis (Wang et al., 2019). Our study further suggests that THS exposure could accelerate the development of gastric tumors in this model. Future studies will need to be conducted to identify the mechanisms.

Given that THS refers to tobacco residue and stale or aged SHS (Jacob et al., 2017), positive previous human epidemiology data based on SHS exposure supports the biological plausibility that childhood THS exposure might contribute to cancer risk. Several studies showed that exposure to cigarette smoke, including SHS, during pregnancy or during infancy, was associated with an increased risk of cancer later in life (John et al., 1991; Sasco & Vainio, 1999; Benowitz et al., 2018). In support of these findings for SHS, the data shown in this study strongly suggests that early life exposure to THS is a risk factor for cancer development in humans. Future epidemiological studies focused on the contribution of THS to cancer risk are required to validate these findings.

In summary, under the laboratory conditions of THS exposure mimicking real-life exposure levels, we discovered a significantly increased pan-tumor incidence and tumor burden in THS-treated CC mice. Eight CC strains were included in our cohort and exhibited tremendous variation in tumor susceptibility to THS exposure. THS exposure also decreased tumor-free survival of CC mice in specific strains. These data support the idea that early life exposure to THS may cause cancer, and indicate that individual genetic background plays a critical role in THS exposure-induced cancer risk.

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Data availability

Data will be made available on request.

Abbreviations:

THS	thirdhand smoke
SHS	secondhand smoke
PAH	polycyclic aromatic hydrocarbon

TSNA	Tobacco-specific nitrosamine
CC	Collaborative Cross
TFS	Tumor-free survival
TLY	Thymic lymphoma
SLY	Splenic lymphoma
ALY	Abdominal lymphoma
SCLC	small cell lung carcinoma
HCC	Hepatic cell carcinoma
SNP	Single Nucleotide Polymorphism

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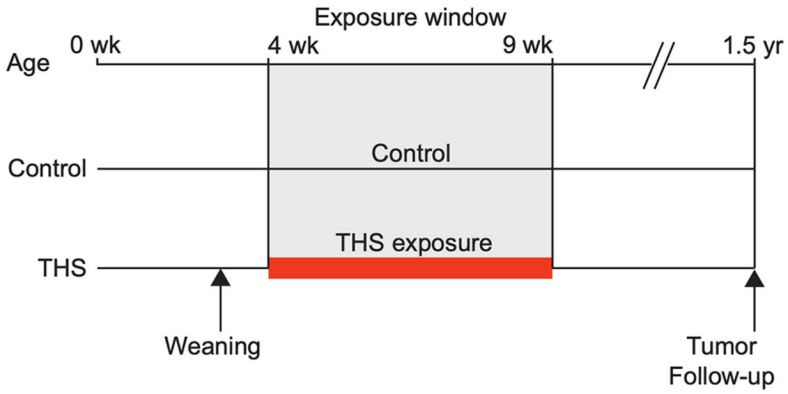

Fig. 1. Overview of experimental design.

Mice from eight CC strains were divided into THS-exposed and control cohorts. The mice were weaned at 3 weeks of age. THS-laden terrycloth and control terrycloth were placed in cages and changed weekly from week 4 to week 9 of age. Tumor development was monitored until mice reached 1.5 years of age.

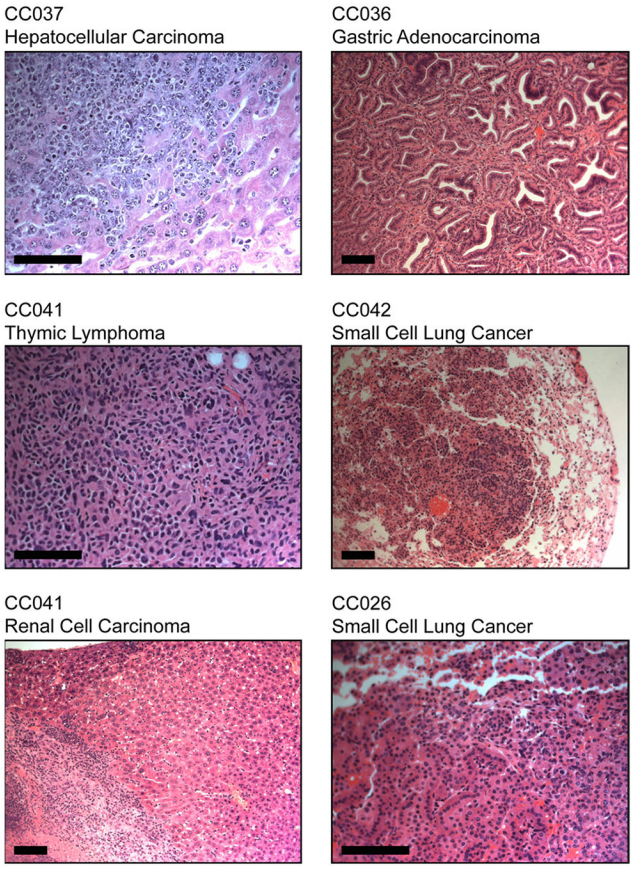

Fig. 2. Representative H&E stained histopathological images of tumors found in THS-treated CC strains.

Tumor tissues were formalin fixed and paraffin embedded. Histological sections were H&E stained and photographed. Note that the scale bars in pictures represent 100 μm.

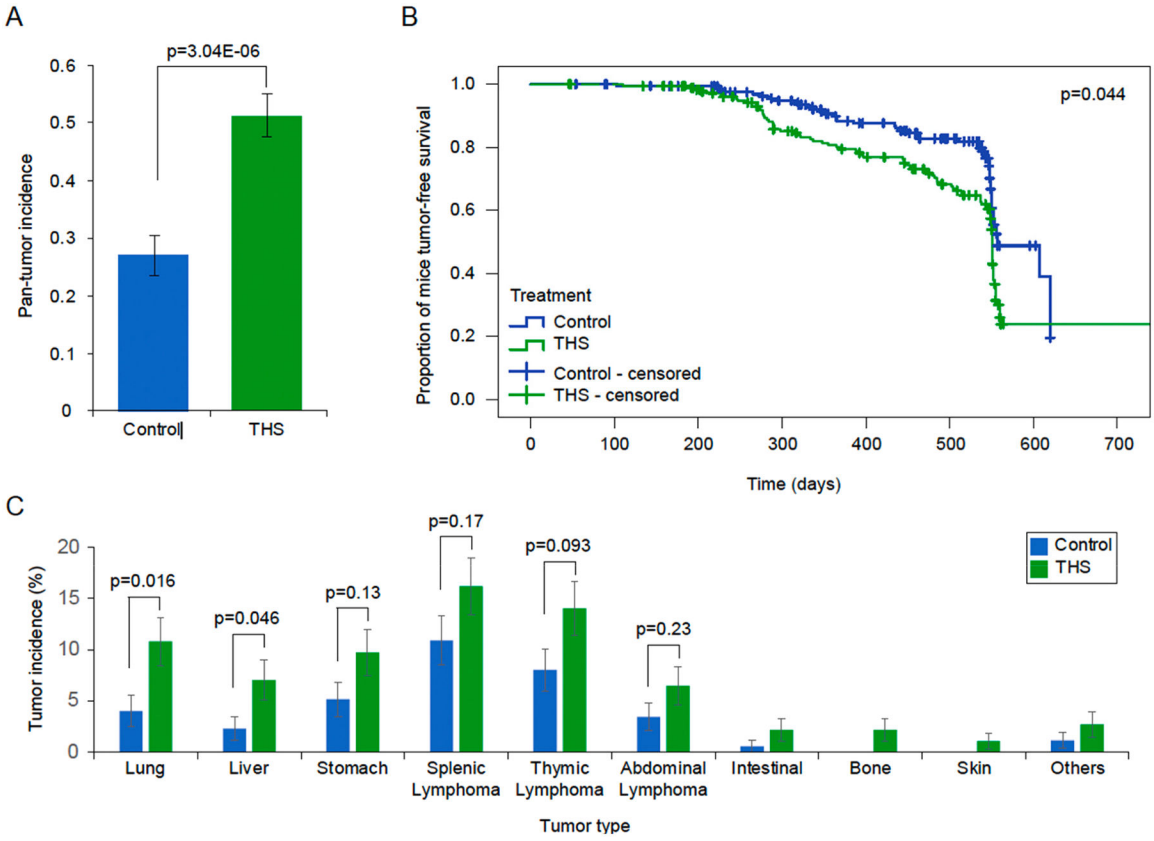

Fig. 3. Pan-tumor incidence, tumor-free survival and tumor spectrum in control and THS-treated CC mice.

A. Pan-tumor incidence in 174 control mice and 185 THS-treated mice. **B.** Tumor-free survival (TFS) in control (blue) and THS-treated (green) mice. P-value was obtained using log-rank test. Note that Some mice were found dead in cage without any abnormality found in any organs, which were defined as censored for tumor development. **C.** Tumor incidence for individual tumor types between control (blue) and THS-treated (green) CC mice. P-values were obtained using Mann-Whitney *U* test. Error bars indicate standard error.

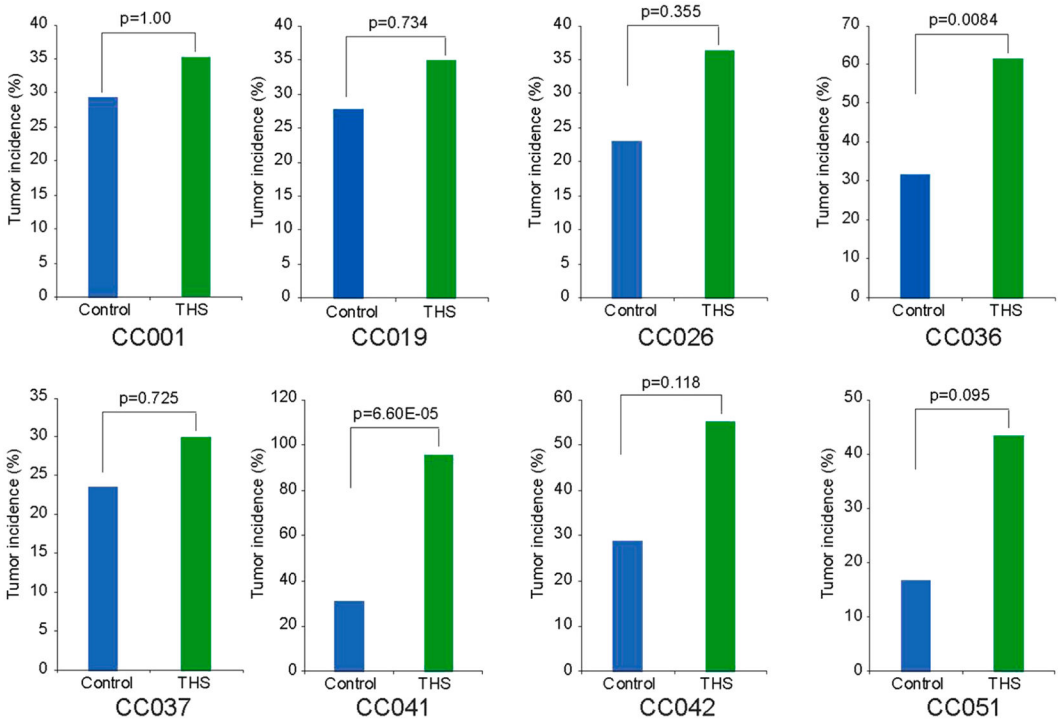

Fig. 4. THS exposure increases tumor incidence in specific CC strains.

Pan-tumor incidence (%) across 8 strains in control (blue) and THS exposed (green) mice. P-values were obtained using Mann-Whitney *U* test. The numbers of mice for each strain is given in Table 1.

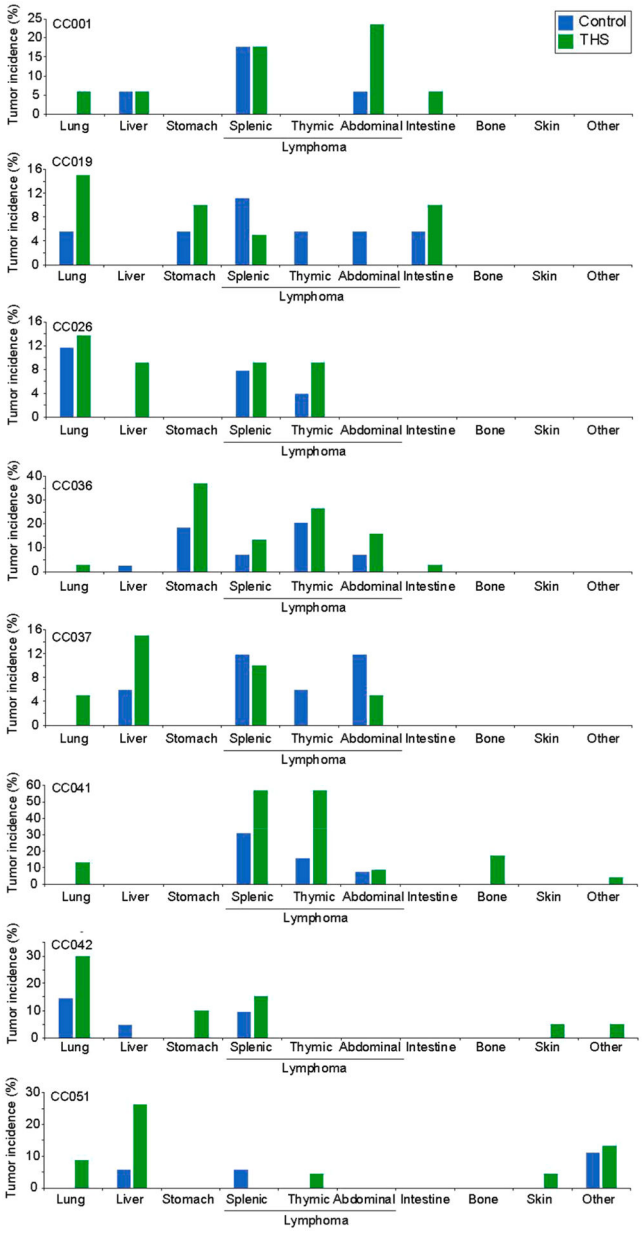

Fig. 5. Tumor spectrum across control and THS treated CC strains.

The tumor incidence for individual tumor types is shown for each CC strain in control (blue) and THS exposed (green) cohorts.

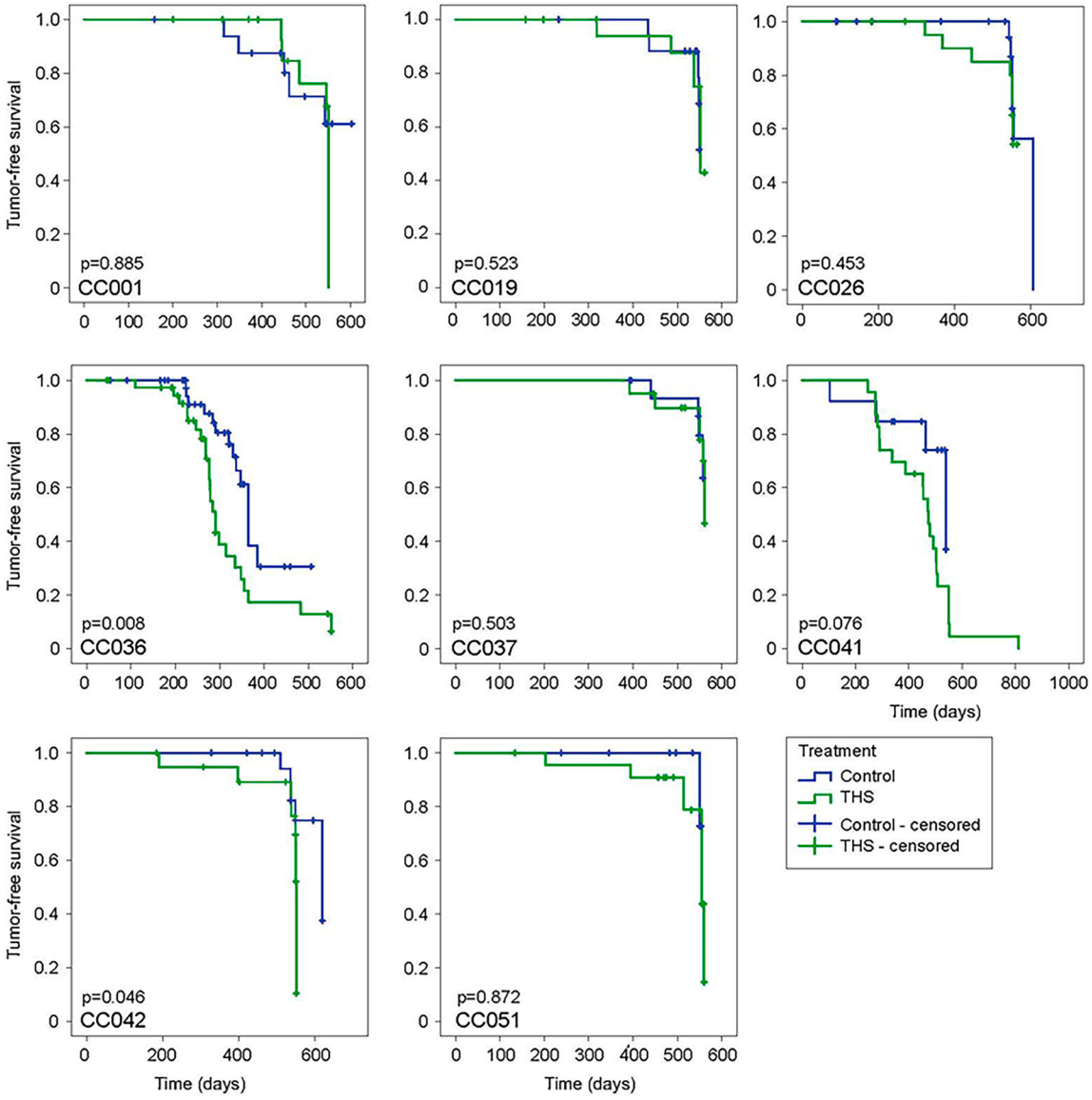

Fig. 6. Tumor-free survival rates across control and THS-treated CC strains.

Tumor-free survival (TFS) in control (blue) and THS-treated (green) mice. P-value was obtained using log-rank test.

Table 1

Tumor incidence and types of tumors in THS-exposed and control CC mouse strains.

CC Strain	Treatment	No. mice	No. mice with tumor	Total number tumors	Tumor burden /mouse
CC001	THS	17	6	9	0.529
	Control	17	5	6	0.353
	Odds ratio		1.30		
CC019	THS	17	7	9	0.529
	Control	16	5	5	0.313
	Odds ratio		1.52		
CC026	THS	22	8	8	0.318
	Control	26	5	5	0.192
	Odds ratio		2.36		
CC036	THS	37	24	31	0.838
	Control	44	18	23	0.523
	Odds ratio		2.63		
CC037	THS	19	6	7	0.368
	Control	16	4	4	0.250
	Odds ratio		1.37		
CC041	THS	24	23	29	1.20
	Control	11	3	3	0.273
	Odds ratio		49.53		
CC042	THS	22	12	13	0.59
	Control	21	5	5	0.24
	Odds ratio		3.71		
CC051	THS	23	10	13	0.565
	Control	17	3	4	0.235
	Odds ratio		3.48		