Most Lung and Colon Cancer Susceptibility Genes Are Pair-Wise Linked in Mice, Humans and Rats

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Abstract

Genetic predisposition controlled by susceptibility quantitative trait loci (QTLs) contributes to a large proportion of common cancers. Studies of genetics of cancer susceptibility, however, did not address systematically the relationship between susceptibility to cancers in different organs. We present five sets of data on genetic architecture of colon and lung cancer susceptibility in mice, humans and rats. They collectively show that the majority of genes for colon and lung cancer susceptibility are linked pair-wise and are likely identical or related. Four CcS/Dem recombinant congenic strains, each differing from strain BALB/cHeA by a different small random subset of ±12.5% of genes received from strain STS/A, suggestively show either extreme susceptibility or extreme resistance for both colon and lung tumors, which is unlikely if the two tumors were controlled by independent susceptibility genes. Indeed, susceptibility to lung cancer (Sluc) loci underlying the extreme susceptibility or resistance of such CcS/Dem strains, mapped in 226 (CcS-10×CcS-19)F2 mice, co-localize with susceptibility to colon cancer (Scc) loci. Analysis of additional Sluc loci that were mapped in OcB/Dem strains and Scc loci in CcS/Dem strains, respectively, shows their widespread pair-wise co-localization (P = 0.0036). Finally, the majority of published human and rat colon cancer susceptibility genes map to chromosomal regions homologous to mouse Sluc loci. 12/12 mouse Scc loci, 9/11 human and 5/7 rat colon cancer susceptibility loci are close to a Sluc locus or its homologous site, forming 21 clusters of lung and colon cancer susceptibility genes from one, two or three species. Our data shows that cancer susceptibility QTLs can have much broader biological effects than presently appreciated. It also demonstrates the power of mouse genetics to predict human susceptibility genes. Comparison of molecular mechanisms of susceptibility genes that are organ-specific and those with trans-organ effects can provide a new dimension in understanding individual cancer susceptibility.

Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide. Individual risk of sporadic cancer in populations varies greatly and is controlled by numerous low penetrance susceptibility genes [1]. Genome-wide association (GWA) studies have revealed common variants associated with risk of cancers of colon [2–10], lung [11–16], breast [17–23] and prostate [24–30], but these variants explain only a fraction of population risk [31] and their organ specificity is unknown. Lung and colon cancer are the first and second leading causes of cancer death in the United States, accounting for 28% and 9% of cancer deaths, respectively [32]. Here we report systematic tests involving three species that reveal genetic linkage and possible identity of most susceptibility genes for the two cancers.

We analyzed mouse colon and lung cancer susceptibility genes using recombinant congenic (RC) strains, which increase the power of mapping by reducing genetic heterogeneity [33]. The

RC strains were produced by two subsequent generations of backcrossing of a "donor" parental strain to a "background" parental strain, followed by twenty generations of brother-sister mating from randomly selected breeding pairs of mice. This generated a set of about 20 homozygous RC strains. Each RC strain carries a different, random set of 12.5% of "donor" strain genes and 87.5% of "background" strain genes (Figure 1) [33,34]. In this way, the number of segregating quantitative trait loci (QTLs) in crosses between an RC strain and its background strain is considerably reduced and the power to detect them increased [34]. RC strains also improve QTL mapping by locating the mapped loci to relatively short donor strain-derived regions that can be precisely demarcated. Previously, we mapped 15 *Susceptibility to colon cancer* (*Scc*) loci using CcS/Dem (CcS) RC strains, derived from the 'background' strain BALB/cHeA (BALB/c, resistant) and 'donor' strain STS/A (STS, susceptible) [35–39]. Independently, we mapped 30 *Susceptibility to lung cancer* (*Sluc*) loci using the OcB/Dem (OcB) RC strains, derived from the
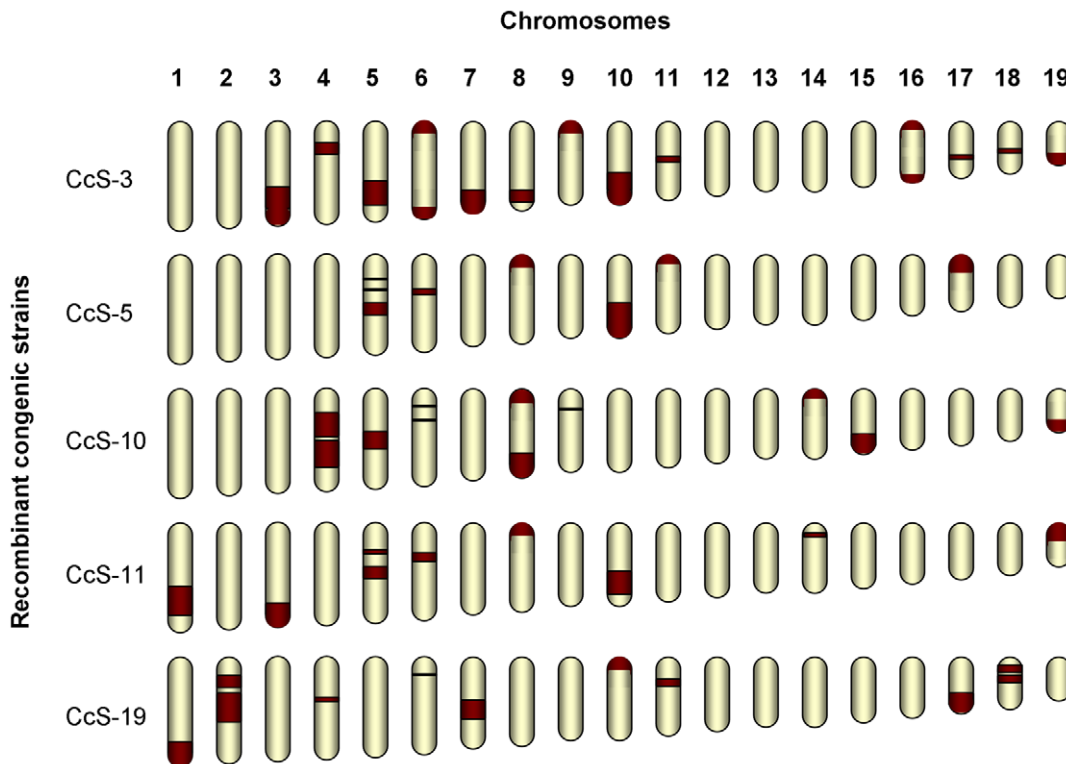
Figure 1. Schematic representation of the genetic composition of recombinant congenic (RC) strains. The major donor-strain regions of the CcS RC strains that were used to map colon or lung tumor susceptibility genes are shown based on real genotypes.
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'background' strain O20/A (O20, susceptible) and 'donor' strain B10.O20/Dem (B10.O20, resistant) [40–42].

If a sizeable proportion of cancer susceptibility genes for the two organs are identical or genetically related, a significant number of colon and lung cancer susceptibility loci would co-localize in the same chromosomal locations. Indeed, analysis of these loci strongly indicates that most susceptibility genes for lung and colon cancer are not genetically independent but are pair-wise linked. Moreover, this co-localization is observed also between mouse lung cancer susceptibility loci and colon cancer susceptibility loci in human and rat. We show five independent sets of data including interspecies homologies, which collectively strongly suggest that most susceptibility genes for lung and colon cancer are not genetically independent as has been believed but are linked pair-wise and probably functionally related or identical.

Materials and Methods

Ethics Statement

All animal experiments were approved by the IACUC committee at Roswell Park Cancer Institute (permit number IACUC M905).

Mice

Mice were maintained in ventilated filter top cages under a strict light-dark regimen and received acidified drinking water and a standard laboratory diet (LM-485, Harlan Teklad, U.S.) *ad libitum*. RC strains are inbred and form sets of about 20 strains derived from the same parental strains. Each OcB recombinant congenic (RC) strain has 87.5% of the genome from the O20 strain – the "background" strain that is relatively susceptible and 12.5% from

the B10.O20 strain – the "donor" strain that is relatively resistant to lung tumors. Each CcS recombinant congenic (RC) strain has 87.5% of the genome from the BALB/c strain – the "background" strain that is relatively resistant and 12.5% from the STS strain – the "donor" strain that is relatively susceptible to colon tumors (Figure 1) [33,34].

Lung tumor induction and analysis in the present study

Lung tumor induction in mice has been described previously [40]. Briefly, on day 17 of gestation, the pregnant (CcS-10×CcS-19)F1 females were given an intraperitoneal (i.p.) injection of 30 mg/kg body weight of the carcinogen N-ethyl-N-nitrosourea (ENU) dissolved in phosphate-buffered citric acid (pH 5.8) [40]. The offspring of carcinogen-injected F1 females were thus exposed to ENU transplacentally. This progeny were euthanized at the age of 16 weeks and their whole lungs were removed, fixed in 10% neutral buffered formalin and embedded in histowax. For tests of lung tumor susceptibility of CcS strains, we induced lung tumors in CcS-19, CcS-11, CcS-10 and CcS-20 mice. We also induced lung tumors in crosses of CcS-19, CcS-11, CcS-10 or CcS-20 female mice with (BALB/c×FVB)F1 male mice (due to the small number of available CcS mice). For linkage tests, lung tumors were induced in 226 F2 intercross mice produced between CcS-10 and CcS-19 mice.

The embedded lungs were sectioned semi-serially (5-μm sections at 100-μm intervals). In most cases, we obtained 30 to 35 sections per lung. All sections were stained with haematoxylin-eosin and examined microscopically at 50X and 400X magnifications. To distinguish unequivocally individual tumors, position of a tumor in the lung lobe in sequential sections, its shape and size, positional relation to bronchi and blood vessels, and characteristics of tumor

cells have been used. The tumors analyzed in this study represent a continuous histological spectrum from entirely benign adenomas (a minority) to adenocarcinomas of different degree of progression, characterized by extent of disorganization of the original organ architecture, large differences in cell morphology, pronounced nuclear pleomorphism, intra-nuclear cytoplasmic inclusions, extensive stromal areas and vascular recruitment. When allowed to develop for a longer time than in the present study, most of the tumors form advanced carcinomas with invasion of adjacent alveoli, and penetration into bronchi and blood vessels (unpublished observatins). Number of tumors, tumor size and tumor load were scored as described previously [40]. Briefly, tumor size was expressed as the sum of all measured surfaces (calculated using a grating in the ocular) in the semiserial sections where the tumor was present, and it corresponded to tumor volume. Tumors that did not exceed a diameter of 300 μm in any of the sections were not included in the data. Tumor load was calculated as the sum of the sizes (volumes) of all tumors in a mouse and it corresponded to the total tumor burden of the mouse.

Genotyping

More than 90 % of the genetic material from the "donor" strain in a RC strain is concentrated in 9 to 13 discrete contiguous chromosomal regions with intermediate length (5–25 cM), that are usually located on 7 to 11 different chromosomes [34]. We determined the positions and length of the majority of the donor-strain derived chromosomal regions in CcS and OcB RC strains with 855 and 716 microsatellite markers across the whole genome, respectively. Based on such information, the donor strain-derived regions segregating in 226 (CcS-10×CcS19) F2 mice were PCR-genotyped [40] using 23 microsatellite markers: D1Mit291, D1Mit155, D2Mit99, D2Mit156, D2Nds3, D4Mit53, D4Mit15, D5Mit68, D6Mit177, D7Mit105, D8Mit17, D8Mit36, D9Mit254, D10Mit28, D10Mit2, D11Mit316, D14Mit11, D15Mit16, D17Mit72, D17Mit123, D18Mit17, D18Mit124, D19Mit6 (http://informatics.jax.org). Each known segregating chromosomal region is represented by at least one marker. More markers have been tested in the longer donor chromosomal regions and the maximal distance between two markers was less than 10 cM.

Statistical analysis

a. Linkage and direction of allelic effects in (CcS-10xCcS-19)F₂ Mice. The dataset of this experiment was submitted to the PLoS One website as supplemental material (Dataset S1). The chromosomal regions affecting tumor load, size and number were determined by analysis of variance (ANOVA) with the use of individual microsatellite markers listed in "genotyping" above. The effects of each marker, sex and interaction between pairs (marker-marker and marker-sex) on the corresponding phenotypes were tested by the PROC GLM (general linear models) procedure of the SAS 9.1 statistical package for Windows (SAS Institute, Inc., Cary, NC). A backward-elimination procedure was followed to exclude statistically nonsignificant effects ($P > 0.05$). The P-values of the significant effects were then corrected for multiple testing using the method of Lander and Kruglyak [43] to construct the final model. All statistical tests were two-sided. Using least square (LS) means of each genotype from ANOVA we determined the number of main effects and interactions, where CcS-19-like genotypes were associated with susceptibility or resistance compared to CcS-10-like genotype (differences >30%).

b. Evaluation of tumor susceptibility pattern of RC strains. Colon tumor numbers and lung tumor loads or numbers were compared between the RC strains CcS-10, -11, -19, and -20 by the Wilcoxon (rank sums) two-sample tests using

the PROC NPAR1WAY procedure of the SAS 9.1 statistical package for Windows (SAS Institute, Inc., Cary, NC).

c. Analysis of previously published Sluc and Scc loci. We used the published mapping data on the Scc or Sluc loci without any pre-selection. We identified the overlapping STS and B10.O20 donor strain-derived chromosomal regions, and determined which of the 14 Scc (Scc1 and Scc10 are considered here a single locus because they are less than 1 cM apart) and 30 Sluc loci map into such overlapping STS-B10.O20 donor strain-derived chromosomal regions. We used the Poisson distribution with mean parameter (mScc and mSluc), which is equal to the total number of detected loci of each type divided by the size of the total length of genome tested for that type. The probability to observe at least one locus of a particular type k in a region of size s equals 1-exp(-mk*s). The probabilities that both Scc and Sluc, none of them, or only a Scc or a Sluc locus are present are {1-exp(-mScc*s)}*{(1-exp(-mSluc*s)}, exp(-mScc*s)*exp(-mSluc*s), {1-exp(-mScc*s)}*exp(-mSluc*s), and exp(-mScc*s)*{1-exp(-mSluc*s)}, respectively, assuming the loci of the two types are distributed independently over the genome. These values were then compared with the actual data by chi-square.

d. Co-localization of human colon and mouse lung tumor susceptibility genes. We used published information on human colon cancer susceptibility loci detected in genome wide association or linkage studies. Orthologous regions of these loci in the mouse were compared with known Sluc and Scc loci. We evaluated by the binomial distribution test possibility of the observed number of human colon cancer susceptibility loci, whose orthologous regions were polymorphic in the tested mice, within an average 3.3cM of the published Sluc loci.

Results

Correlated susceptibility to colon and lung tumors in CcS RC strains

At the first step, we selected two RC strains highly susceptible and two RC strains most resistant to colon tumors and tested their susceptibility to lung tumors. The RC strains CcS-19 and CcS-11 are highly susceptible and CcS-10 and CcS-20 are very resistant to colon tumors (p<0.0001, Figure 2B upper) [35,37] induced by repeated injections of carcinogens 1,2-dimethyl-hydrazine (DMH) or azoxymethane (AOM). We induced lung tumors in CcS-19 and CcS-20 or CcS-11 and CcS-10 in two independent experiments. We observed that, concordant with the colon tumor susceptibility or resistance, CcS-19 is highly susceptible to ENU-induced lung tumors compared to CcS-20 (p<0.0001, Wilcoxon test), and CcS-11 is highly susceptible to ENU-induced lung tumors compared to CcS-10 (p = 0.0012, Wilcoxon test) (Figure 2B lower). The extreme susceptibility or resistance to lung tumors observed in the CcS strains, concordant to colon tumor susceptibility, has been supported by results from crosses of CcS-19, CcS-11, CcS-10 and CcS-20 with (BALB/c×FVB)F₁ mice (tested due to the small number of available CcS mice) (Figure S1, Table S1). Mice of these crosses carry at each locus one allele of the pertinent RC strain and showed similar susceptibility pattern to the homozygous CcS mice. These data suggest that the small subsets of 12.5% STS genes received by these RC strains contain either predominantly susceptible (CcS-11, CcS-19), or predominantly resistant (CcS-10, CcS-20) alleles at most colon (Scc) and lung cancer (Sluc) genes, suggesting their pair-wise linkage or identity (Figure 2A upper). Otherwise, these RC strains would be extremely susceptible or extremely resistant to one type of tumor, but not likely to the other (Figure 2A lower).
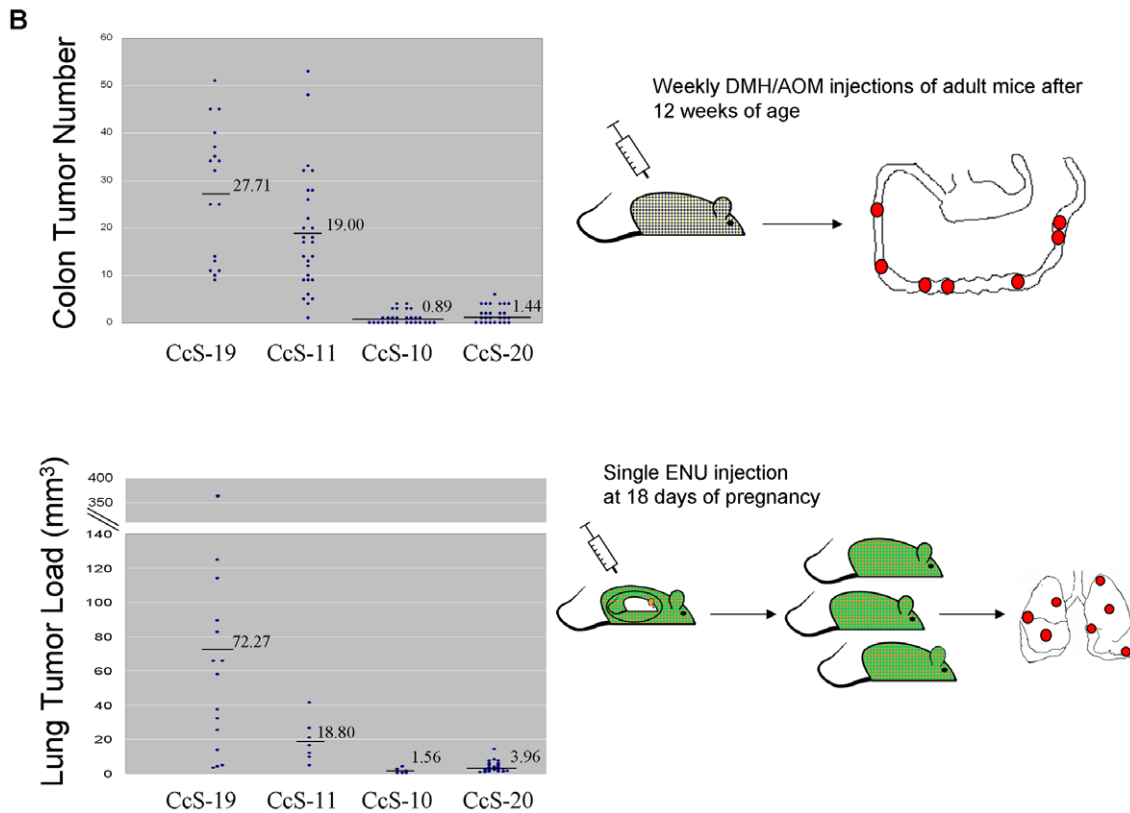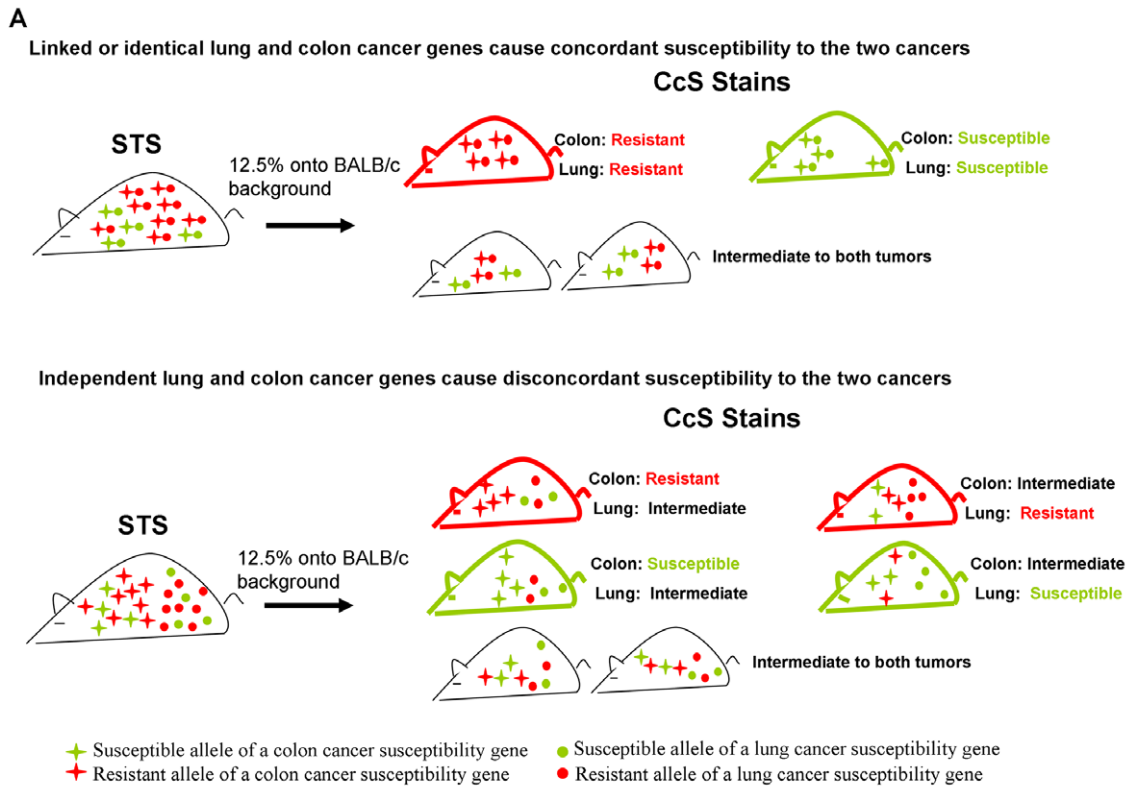
Figure 2. Correlated lung and colon cancer susceptibility in the CcS RC strains. A. Expected susceptibility to colon and lung tumors under different hypotheses. Concordant susceptibility or resistance to colon and lung tumors is expected when the majority of the susceptibility genes of the two cancers are closely linked or identical (upper panel); but not when the susceptibility genes of the two cancers are independent of each other (lower panel). **B.** Observed susceptibility to colon and lung tumors in the CcS RC strains with extreme susceptibility phenotype. Each dot represents a mouse. Mean tumor number of each strain is indicated. Upper panel: colon tumor numbers for CcS-19, CcS-11, CcS-10 and CcS-20 mice. Colon tumor number is directly proportional to colon tumor load, since in our experiments colon tumor sizes did not differ significantly among the CcS strains [35].

Scc-Sluc linkage in (CcS-10×CcS19)F2 hybrids

To elucidate the concordant extreme susceptibility of CcS-19 and resistance of CcS-10 mice to both colon and lung tumors, we mapped *Sluc* loci in ENU-treated intercross (CcS-10×CcS-19)F$_2$ mice. We compared locations of these *Sluc* loci with locations of *Scc* loci previously detected in the CcS strains.

Mapping of Sluc loci. We detected 1191 lung tumors in 226 (CcS-10×CcS-19)F$_2$ mice. The 21 STS-derived regions segregating in the cross (about 23.5% of the genome) were defined in the CcS strains using 855 microsatellite markers (data not shown) and scanned in F2 hybrids with 23 microsatellite markers spaced on average 10cM apart. Mapping data of all significant linkages is in Figure 3, including p values corrected for genome-wide testing [43] and least-square means of susceptibility phenotypes of each locus. We detected 15 *Sluc* loci that affect tumor size, load and number (Figure 3A&B, Table S2). Eight of these loci had individual effects (Figure 3A) and seven loci were detected only in inter-locus interactions. We found 17 pair-wise inter-locus interactions (Figure 3B), in which the effect of one *Sluc* locus depends on the genotype of a second interacting *Sluc* locus [40,42]. Five of these 15 *Sluc* loci are novel loci: *Sluc31 – Sluc35*, linked to D2Mit99, D17Mit72, D5Mit68, D15Mit16 and D19Mit6, respectively (Figure 3A&B, Table S2). The other 10 *Sluc* loci are very close (0–1.5 cM, five loci) or relatively close (6–12 cM, five loci) to the positions of previously published *Sluc* or *Pas* loci (Table S2), so we could not rule out that they are duplicate detections rather than novel loci and did not assigned them novel symbols.

Effects of alleles of these loci support the hypothesis that they are responsible for the high susceptibility and resistance, respectively, of CcS-19 and CcS-10, because with few exceptions the allele obtained from CcS-19 confers a higher susceptibility than that from CcS-10 (Figure 3C). Similarly, the combination of alleles in interacting pairs of *Sluc* loci that is present in CcS-19 is more susceptible than that present in CcS-10 (Figure 3D).

Scc-Sluc co-localization. We compared the map location of the 15 *Sluc* loci detected in the (CcS-10×CcS-19)F$_2$ hybrids with location of *Scc* loci detected previously in crosses of CcS strains -3, -5, -11, -19, with BALB/c (Table S3)[36,38,39]. Seven of the 15 *Sluc* loci, linked to D1Mit291, D2Mit99, D8Mit17, D10Mit28, D11Mit316, D17Mit72 and D18Mit17, mapped to regions that had been previously tested for colon cancer susceptibility. Without exception, they all co-localized with *Scc* loci and formed linked pairs of *Scc3/Sluc5, Scc1/Sluc31, Scc8/Sluc20, Scc14/Sluc29, Scc15/Sluc4, Scc4/Sluc32* and *Scc5/Sluc14* (Figure 4, Table S2). Five of these *Sluc* loci mapped less than 1cM from the paired *Scc* locus; one locus 2 cM and one 5 cM. The other eight newly detected *Sluc* loci are located in regions that were not yet tested for colon tumor susceptibility in RC strains and could pair with presently unknown *Scc* loci. These data show that the *Scc* and *Sluc* loci underlie the concordant extreme susceptibility or resistance to colon and lung tumors and are pair-wise clustered.

Scc-Sluc linkage in independent strains

We performed an analysis of map locations of *Scc* loci and *Sluc* loci that were previously mapped in two completely independent projects using crosses of four CcS strains to map *Scc* genes [36,38,39] and five OcB strains to map *Sluc* genes [40–42], respectively (Table S3). The donor strain chromosomal regions of the CcS strains tested for colon tumor susceptibility comprised

together about 40–50% of the genome, which is similar to the total proportion of the genome of the OcB strains tested for lung tumor susceptibility. The 'overlap' regions screened for both lung and colon tumor susceptibility can be used to evaluate the possible co-localization of *Scc* and *Sluc* genes, whereas the 'non-overlap' regions were studied for only one tumor type and hence are not informative (Figure 5A). We identified 23 'overlap' chromosomal regions with a total length of 430 cM ('overlap' in Table 1), containing 9 *Scc* loci and 14 *Sluc* loci (Table 1). We found that the "concordant 'overlap' regions" containing either both a *Scc* and a *Sluc* locus (n = 9) or none of them (n = 11) outnumber vastly the "discordant 'overlap' regions" that contain either only a *Scc* (n = 0) or only a *Sluc* locus (n = 3) (Table 1). These observed frequencies are significantly different from the expected frequencies assuming independent distribution of *Scc-Sluc* loci, as calculated from Poisson distribution based on length of the overlap regions (P = 0.0036, modified 2×2 test–Materials and Methods). These data indicate that the genetic relatedness between a large number of colon and lung cancer susceptibility genes that we observed in the CcS RC strains is likely common in other mouse strains as well. In fact, the p value 0.0036 likely underestimates the actual significance of the co-localization, because in the nine overlap regions that contain both a *Scc* locus and a *Sluc* locus, the distances between the markers for these loci are about 75% shorter than the length of the overlap regions (derived from Table 1, Figure 5B).

Several laboratories carried out productive searches for colon [44–46] and lung [47–52] cancer susceptibility genes. However, we could not include these published cancer susceptibility loci into the present analyses because the candidate regions of most of them cannot be defined as precisely as the donor-strain regions in RC strains, so the extent of their overlaps cannot be evaluated statistically. Also, the detection of co-localization depends on the power of the mapping test, which is less in whole genome crosses than in RC crosses [34]. Nevertheless, these data showed that the colon cancer susceptibility locus *Ccs1* detected in ICR×C57Bl/6 backcross [44], maps only 1cM from the lung cancer resistence locus *Par3* detected in SMXA×A backcrosses [50] on mouse chromosome 12.

Mouse Sluc loci co-localize with mouse orthologues of human and rat colon cancer susceptibility loci.

Co-localization of colon and lung cancer susceptibility genes in mouse suggests that many of them may be related or identical. We therefore investigated possible parallels of this finding in humans and rats (Figure 6A).

Human colon cancer susceptibility loci. Genome-wide association and linkage studies in humans detected 13 susceptibility loci for colorectal cancer and four for lung cancer [2–10]. The orthologous regions of 11 out of 13 human colon cancer susceptibility loci are polymorphic in the OcB or CcS strains that were tested for lung tumor susceptibility. Surprisingly, nine of these 11 regions are close to previously detected *Sluc* loci (Figure 6B). Six are very close (0–2.5 cM): 8q24/*Sluc25*, 9q22.2–31.2/*Sluc23*, 10p14/*Sluc16*, 3q21–24/ *Pas1c*-D6mit177 (or *Sluc11*), 14q22.2/*Sluc13* and 6q23–25(both colon and lung)/*Scc14*/ *Sluc29* [2,3,5,6,8,10,11,39,42,51]; Three are relatively close(5–7cM): 8q23.3/*Sluc25*, 16q22.1/*Sluc9* and 19q13.1/*Sluc30* [8,10,42]. No *Sluc* locus was detected near orthologues of 15q13 or 20p12.3 [7,8]. The fact that nine of the 11 human colon cancer susceptibility loci, whose orthologues have been tested in mouse

A. Main Effects

% deviation from the mean tumor load, number or size

	Genotype			Effects	P value
	S/S	S/C	C/C		
Sluc14 (D18Mit17)	129.90	16.37	-125.86	Size Male	5x10⁻⁶
Sluc31 (D2Mit99)	70.14	124.63	-46.01	Size Male	0.0001
Sluc5 (D1Mit291)	126.81	-81.28	-95.43	Load Male	0.0001
Sluc29 (D10Mit28)	82.76	-98.23	48.95	Load Male	0.003
Sluc10 (D9Mit254)	18.29	-53.34	20.30	Num. Female	0.005
Sluc33 (D5Mit68)	-95.96	115.66	13.78	Load Male	0.001
Pas9 (D4Mit15)	-58.47	-43.93	53.41	Load All	0.001
Pas9 (D4Mit15)	-25.37	-10.33	68.32	Size All	0.004
Sluc9 (D8Mit36)	-54.22	-28.35	103.00	Size Male	0.001

Effects column P values restated with LaTeX notation: Size Male 5×10^{-6}.

B. Interactions

Genotype of Locus A†										Effects	P value
Locus A† / Locus B†	S/S	S/C	C/C	S/S	S/C	C/C	S/S	S/C	C/C		
Pas9 (D4Mit15) / Sluc4 (D11Mit316)	108.28	15.29	-191.30	-89.00	1.15	2.43	89.10	-15.46	-179.95	Load Male	0.0007
Pas9 (D4Mit15) / Sluc9 (D8Mit36)	-37.81	-6.63	-31.66	-46.58	-27.43	6.92	66.60	154.43	-16.08	Size All	0.04
Pas9 (D4Mit15) / Sluc10 (D9Mit254)	-28.33	-48.02	13.43	4.39	20.26	35.83	293.67	-54.94	-174.98	Size Male	2x10⁻⁸
Pas9 (D4Mit15) / Sluc14 (D18Mit17)	-29.87	-13.79	43.39	42.94	2.07	-27.41	43.16	-3.74	-28.31	Number All	0.01
Pas9 (D4Mit15) / Sluc29 (D10Mit28)	26.17	-25.77	-76.48	-40.80	-35.58	9.30	175.77	6.32	22.84	Size All	0.04
Pas9 / Sluc29 (D10Mit28)	-102.79	3.06	36.80	-30.27	35.78	54.98	308.28	-149.89	-94.65	Size Male	7x10⁻⁵
Sluc31 (D2Mit99) / Pas9 (D4Mit15)	-37.13	29.81	-136.85	17.46	10.04	263.73	-43.25	20.62	-63.13	Size Male	0.007
Pas9 / Pas1c (D6Mit177)	-72.98	-13.12	23.16	38.96	-1.21	22.75	140.34	148.42	-225.00	Size Male	1x10⁻⁵
Sluc4 (D11Mit316) / Sluc14 (D18Mit17)	61.11	129.27	-82.01	-36.81	-6.93	44.72	-62.35	-103.04	156.47	Load Male	0.006
Sluc20 (D6Mit177) / Sluc4 (D11Mit316)	206.02	30.66	-49.21	-189.04	-62.21	174.55	91.39	32.53	-134.26	Load Male	1x10⁻⁶
Sluc10 (D9Mit254) / Sluc14 (D18Mit17)	-44.20	75.47	189.21	-23.30	-43.05	120.76	37.20	13.49	-225.14	Load Male	0.002
Sluc10 (D9Mit254) / Sluc29 (D10Mit28)	159.25	-29.47	-3.86	-11.91	-6.44	-38.73	13.80	-19.12	-1.74	Size All	0.05
Sluc10 (D9Mit254) / Sluc35 (D19Mit0)	-29.41	39.98	18.21	39.98	-10.03	30.17	118.47	-34.68	46.44	Num. Male	0.007
Sluc32 (D17Mit72) / Sluc29 (D10Mit28)	83.04	-21.97	16.31	-10.68	38.07	16.94	-59.00	17.36	-85.15	Num.Female	0.008
Sluc33 (D5Mit68) / Sluc29 (D10Mit28)	-66.90	-221.56	0.58	299.52	44.14	3.32	15.65	-117.29	142.97	Load Male	0.001
Sluc29 (D10Mit28) / Sluc34 (D15Mit16)	-18.48	-17.59	65.05	3.85	-2.85	-17.81	35.79	-31.21	11.45	Number All	0.004
Sluc29 (D10Mit28) / Sluc34 (D15Mit16)	-51.43	26.10	155.08	26.10	3.13	-2.37	43.81	-22.23	41.42	Num. Male	0.006
Sluc33 (D5Mit68) / Sluc24 (D15Mit10)	-96.44	65.68	-37.45	-86.40	-25.11	26.56	98.31	52.92	-29.29	Num. Female	1x10⁻⁵

Genotype of Locus B† : S/S, S/C, C/C

Legend: smaller than average — 3-4x, 2-3x, 1.5-2x, 1-1.5x | bigger than average — 1-1.5x, 1.5-2x, 2-3x, 3-4x

C.

%Deviation from the means

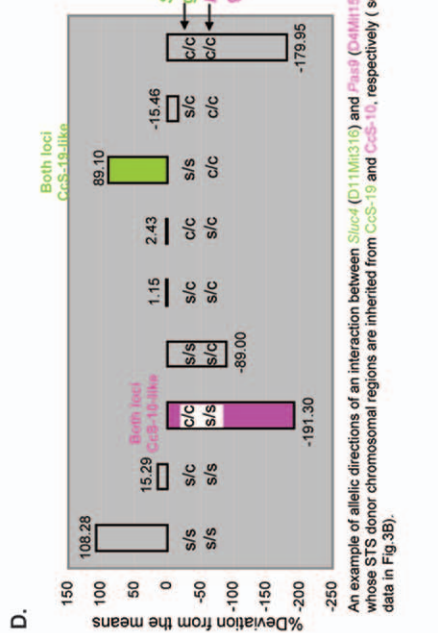CcS-19-like genotype — S/S 126.81
CcS-10-like genotype — S/C -81.28, C/C -95.43

The STS allele of *Sluc5* (D1Mit291), whose STS donor region is inherited from CcS-19, is susceptible to lung tumors (see data in Fig.3A).

CcS-19-like genotype — C/C 53.41
CcS-10-like genotype — S/S -58.47, S/C -53.93

The STS allele of *Pas9* (D4Mit15), whose STS donor region is inherited from CcS-10, is resistant to lung tumors (See data in Fig.3A).

D.

%Deviation from the means

Both loci CcS-10-like — s/s s/s 108.28; s/c s/s 15.29
s/c s/c 1.15; c/c s/c 2.43
Both loci CcS-19-like — s/s c/c 89.10; s/c c/c -15.46
s/s s/c -89.00; c/c c/c -179.95
Both loci CcS-10-like — s/s c/c -191.30

Sluc4 genotype / *Pas9* genotype

An example of allelic directions of an interaction between *Sluc4* (D11Mit316) and *Pas9* (D4Mit15), whose STS donor chromosomal regions are inherited from CcS-19 and CcS-10, respectively (see data in Fig.3B).

Figure 3. Linkage data and the estimated effects of lung cancer susceptibility (*Sluc*) loci in (CcS-10×CcS-19)F2 mice. Eight main effects (**A**) and 17 interactions (**B**) were detected. The microsatellite markers with linkage are listed below each locus. Markers in pink represent donor chromosomal region from CcS-10 and markers in green represent donor regions from CcS-19. The data are presented as percent deviations from the means of tumor load, number and size for each genotype or genotypic combination (for interactions) of the corresponding loci in female, male or both sexes (all), respectively, adjusted for the remaining markers in the model (least-squares means from the ANOVA output). The means (±SEM) of tumor number in the whole cross are: females, 4.78(±0.37); males, 4.18(±0.29); all mice, 4.48(±0.24). The means (±SEM) of tumor size per mouse (mm^3) are: females, 2.05(±0.25); males, 2.26(±0.28); all mice 2.15(±0.19). The means (±SEM) of tumor load per mouse (mm^3) are: females, 10.57(±1.60); males, 10.04(±1.30); all mice, 10.30(±1.03). †Loci A and B are interacting; s/s, homozygous STS; c/c homozygous BALB/c; s/c heterozygous; **C**. Examples are shown that the STS alleles of the *Sluc* loci are susceptible when they are inherited from the CcS-19 parental mice (*Sluc5*) and resistant when they are inherited from the CcS-10 parental mice (*Pas9*). **D**. Example is shown that in interactions, the genotypic combinations that are similar to the CcS-19 parental mice (CcS-19-like) are susceptible compared to the genotypic combinations that are similar to the CcS-10 parental mice (CcS-10-like). In the example, one of the interacting loci *Pas9* is inherited from CcS-10 and the other locus *Sluc4* is inherited from CcS-19.

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RC strains, map on average to 3.3 cM from a *Sluc* locus, suggests that lung cancer and colon cancer susceptibility loci are also significantly clustered in humans ($P = 0.0015$, binomial distribution test). Orthologues of the colon cancer susceptibility loci at 18q21 [4] and 11q23 [9] and the lung cancer susceptibility loci at 5p15, 6p21 and 15q25 [12–14], are in regions that were not tested for *Sluc* or *Scc* loci.

Rat colon cancer susceptibility loci. Ten rat colon cancer susceptibility loci were reported [53]. The orthologous regions of seven of them are polymorphic in OcB or CcS strains tested for lung

cancer susceptibility. Five of these seven regions co-localize with mouse lung cancer susceptibility loci within a distance of 2–8 cM, forming pairs of *rCcr2/Sluc13*, *rCcr3/Sluc14*, *rCcr5/Sluc12*, *rCcr7/Sluc23* and *rCcs1/Sluc6* (Figure 6B). No *Sluc* locus was detected in the region orthologous to rat *rCcr6* or *rCcr8*. The orthologues of *rCcr1*, *rCcr4* and *rCcr9* are in regions not tested for *Sluc* or *Scc* loci.

The orthologous regions of most human and rat colon cancer susceptibility loci are not polymorphic in the mouse strains that were tested for colon cancer susceptibility. Therefore their co-localization with *Scc* loci could not be assessed.
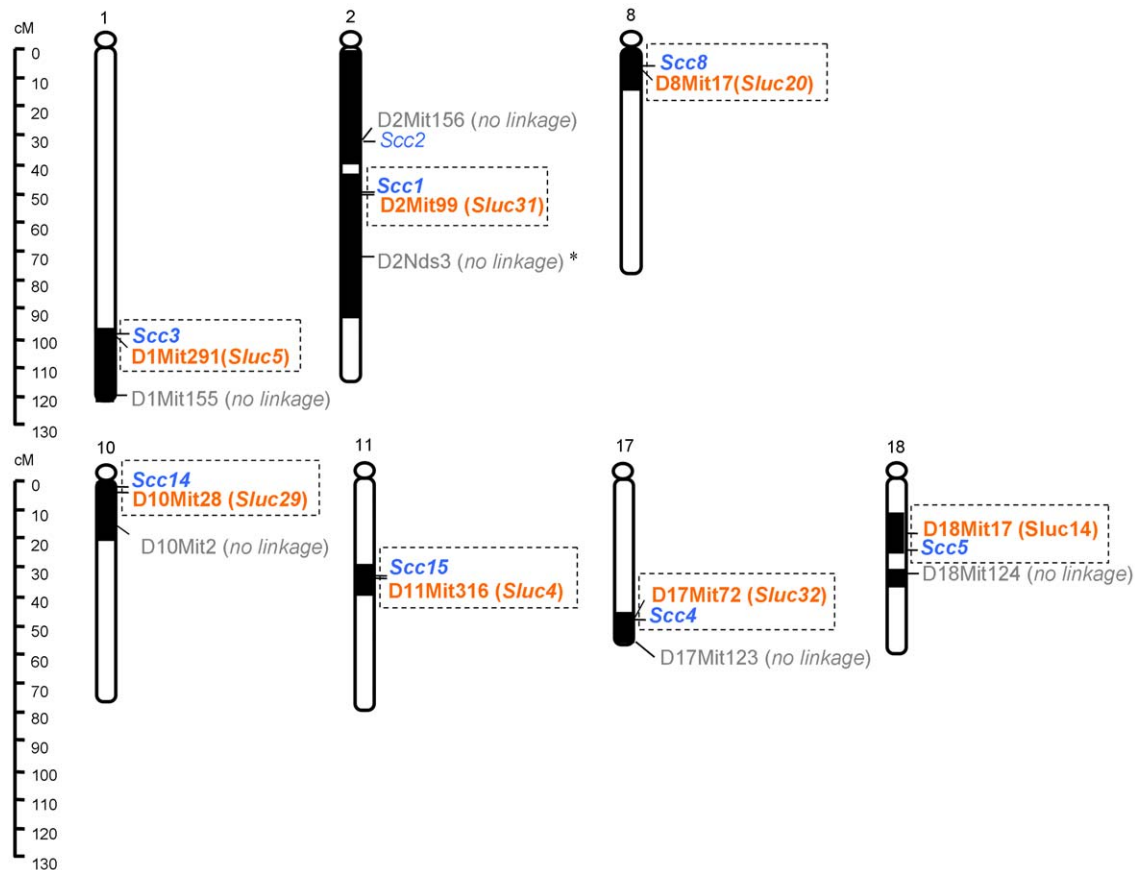
Figure 4. *Sluc* loci detected in (CcS-10×CcS-19)F$_2$ mice co-localize with previously detected *Scc* loci. The seven *Sluc* loci in the chromosomal regions that have been tested for colon cancer susceptibility previously are shown. All the seven *Sluc* loci co-localize with *Scc* loci. Markers with linkage are highlighted in orange and the corresponding *Sluc* loci are listed. Additional markers tested on the same donor chromosomal regions that did not show linkage can help to limit the candidate regions and are shown in grey color. Previously detected *Scc* loci are highlighted in blue. Detailed information of each locus is listed in Suppl. Table 2 and Suppl. Table 3.

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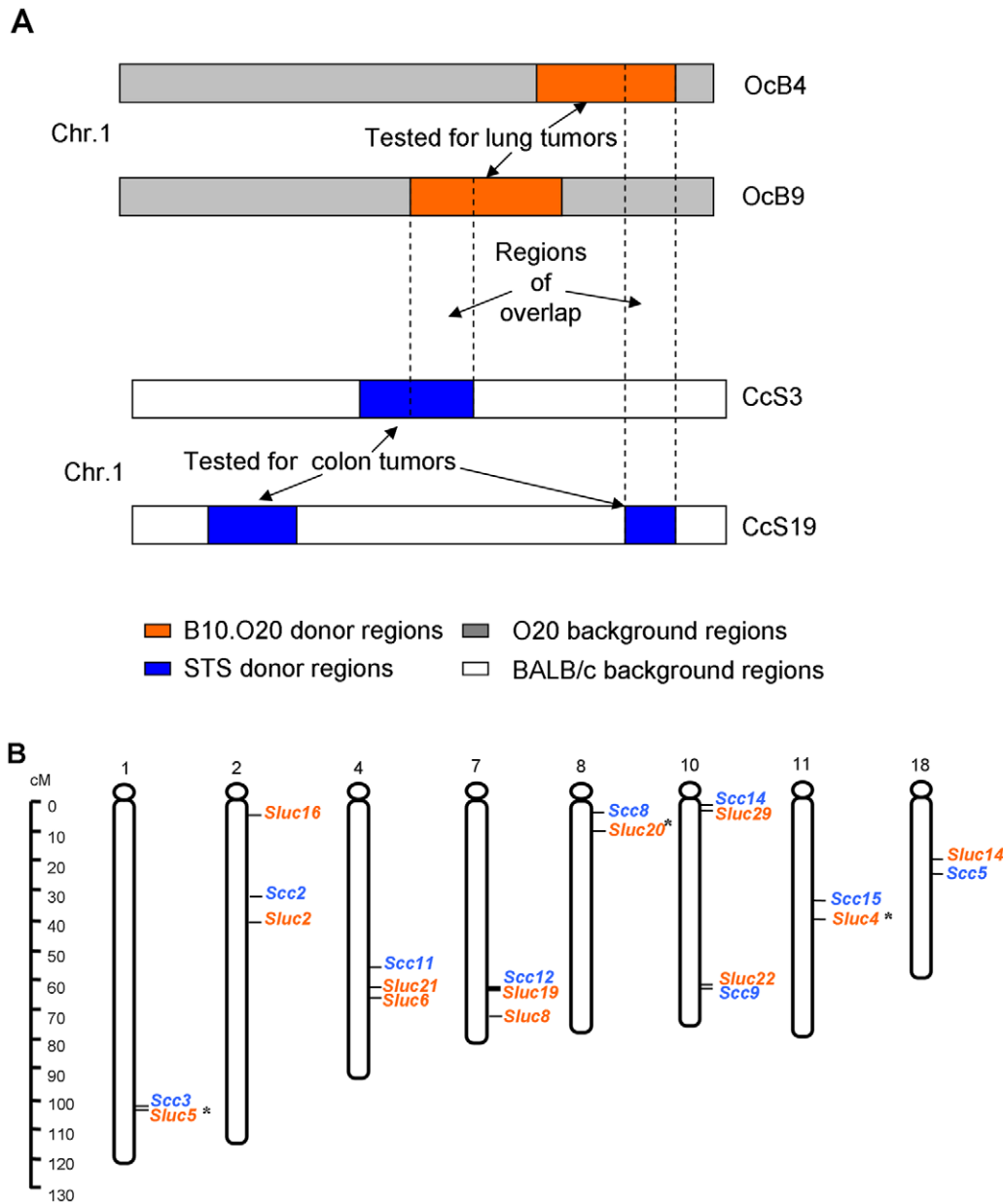
Figure 5. Co-localization between *Sluc* loci mapped in the OcB RC strains and *Scc* loci mapped in the CcS RC strains. A. Schematic representation of overlapping donor chromosomal regions between the CcS and OcB RC strains (regions tested for both colon and lung cancer susceptibility loci). Such regions are informative and we used them to test whether *Sluc* and *Scc* loci are more frequently located together in the same donor chromosomal region. Part of a chromosome is shown as example. **B**. Frequent co-localization between *Sluc* and *Scc* loci identified independently in OcB and CcS RC strains, respectively (See also Table 1 for detailed locations). *Map locations of these *Sluc* loci are slightly different from the locations of the same loci shown in Figure 4, since they are mapped in different RC strains using different microsatellite markers.
doi:10.1371/journal.pone.0014727.g005

Discussion

Colon and lung cancer susceptibility genes are related or identical

The five independent sets of presented data contradict the notion of independent genetic control of colon and lung tumor susceptibility and suggest that the two classes of susceptibility genes are functionally or genetically related, or identical: **i.** Concordant high susceptibility or high resistance to both tumors in several CcS strains suggests linkage of *Scc* and *Sluc* genes received from STS (Figure 2, Figure S1, Table S1). **ii.** Indeed, in F2 hybrids between

highly susceptible CcS-19 mice and highly resistant CcS-10 mice, each segregating *Scc* locus is linked with a *Sluc* locus (Figure 4). **iii.** Most *Scc* loci that were detected in crosses of CcS strains are pairwise linked with the *Sluc* loci that were detected in crosses of OcB strains (Figure 5). **iv–v.** Most mouse orthologues of human and rat colon cancer susceptibility loci co-localize with the mouse *Sluc* loci (Figure 6). Overall, 12/12 mouse *Scc* loci, 9/11 human and 5/7 rat colon cancer susceptibility loci are close to a *Sluc* locus or its homologous site, forming 21 clusters of lung and colon cancer susceptibility loci from one, two or three species. Importantly, lung and colon cancer susceptibility loci from 15 of

Table 1. *Sluc* and *Scc* loci identified independently in OcB and CcS strains frequently co-localize in the same donor chromosomal region.

Chr	Colon Tumors – tested in CcS strains			Overlap*	Lung Tumors – tested in OcB strains		
	Scc locus (cM)	Tested Regions (cM)	Regions with Linkage (cM)		Regions with Linkage (cM)	Tested Regions (cM)	*Sluc* locus (cM)
1	none	32.8–41		32.8–41		0–59	none
1	*Scc3* (101.5)	81.6–127	100–127	**81.6–127**	81.6–127	81.6–127	*Sluc5* (100†)
2	*Scc2* (32)	5–41.4	5–41.4	**5–41.4**	5–42.7	0–47.5	*Sluc2* (41)
2				0–10		0–47.5	*Sluc16* (5)
2	none	45–95.5		69–95.5		69–114	none
3	none	6.7–11.2		6.7–11.2		0–45.2	none
4	none	20.8–40		20.8–40		0–81	none
4	*Scc11* (57.4)	56.5–62.3	56.5–62.3	**56.6–62.3**	0–62.3	0–81	*Sluc21* (62.3)
4					56.6–81	0–81	*Sluc6* (67)
5	none	61–78		64–78		64–92	none
6	none	0–20.5		2.8–20.5	2.8–16	2.8–26.5	*Sluc7* (6)
6	none	42–61.4		42–48.7		36.5–48.7	none
6	none			58.6–61.4	58.6–63.6	58.6–63.6	*Sluc3* (61.2)
6	none	62.5–75		67–75		67–75	none
7	none	8–74		8–15		0–15	none
7	*Scc12* (63.5)	8–74	60–74	**28.7–74**	51.8–66	28.7–74	*Sluc19* (63.5)
7					28.7–74	28.7–74	*Sluc8* (72.0)
8	*Scc8* (4)	0–19.5	0–16	**0–19.5**	0–31.5	0–31.5	*Sluc20* (10)
8	none	41–67		53–67	53–73	53–73	*Sluc9* (59)
10	*Scc14* (2)	0–21	0–21	**0–21**	2–36	0–36	*Sluc29* (4)
10	*Scc9* (63)	49–77	62–77	**51.5–77**	56–77	51.5–77	*Sluc22* (61)
11	*Scc15* (33.9)	20–40	30–40	**27.9–40**	27.9–40	27.9–40	*Sluc4* (40)
16	none	0–28.2		0–27.6		0–27.6	none
18	*Scc5* (25)	2–26	5–26	**2–26**	0–24	0–41	*Sluc14* (20)
18	none	31–37		31–37		31–37	none
19	none	4.5–41		4.5–41		4.5–53	none

*The 'overlap' chromosomal regions that have been tested for both colon cancer susceptibility in CcS RC strains and lung cancer susceptibility in OcB RC strains are listed. The regions containing a *Scc* gene as well as a *Sluc* gene are highlighted in bold.
†Lung tumor susceptibility *Sluc5* has been mapped in separate experiments at 87 and 100 cM, respectively (Tripodis et al. 2001); the position at 100 cM is used for the analysis.
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these clusters mapped within 2.5 cM of each other (Figure 6). This evidence is hardly compatible with genetic independence of most of colon and lung tumor susceptibility loci.

Multi-organ specificity of cancer susceptibility genes is also supported by epidemiological studies in humans that revealed familial aggregations of different types of cancers, which do not correspond to known cancer syndromes [54,55]. Although no aggregations of colon and lung cancer were found, this could be due to the distinct environmental etiology of lung cancer in humans, or because aggregation between tumors of other organs is even stronger. We also compared the map locations of *Sluc/Scc* loci with the location of 16 published skin cancer susceptibility (*Skts*) loci [56–60]. It seems that the genetic control of skin cancer might also be related to that of lung and colon, but to a much lesser extent (data not shown).

Possible mechanisms underlying the co-localizing colon and lung cancer susceptibility genes

Molecular interpretation of our data is limited because the candidate genes for most colon and lung cancer susceptibility loci are not known. A co-localization alone need not necessarily indicate relatedness or identity. However, we have demonstrated statistically significant pair-wise co-localization of a large majority of colon and lung cancer susceptibility loci, which is incompatible with their complete or extensive independence. The nature of the relatedness need not be the same for each *Scc-Sluc* locus. In some *Scc-Sluc* loci a single gene can affect susceptibility to both tumors. The genes that affect common cell autonomous pathways might affect susceptibility to several types of cancer [61]. The co-localizing *Sluc4/Scc15* are linked to *Trp53*; *Sluc32/Scc4* are linked to mismatch repair genes *Msh2* and *Msh6*; and *Sluc30* is linked to base pair repair gene *Xrcc1*. These genes are associated with both colon and lung cancer risk [62–66]. However, such linkage should be interpreted with caution. For example, lung cancer susceptibility locus *Pas1* is linked to *Kras*, but other genes in the region are more likely candidates [67,68]. As both colon and lung derive from primordial gut, cellular regulatory pathways in the two organs may partly involve the same genes. Systemic influences on tumorigenesis might also affect several tumor types, as might be the case for genes that modify such systemic reactions. Two *Scc/*
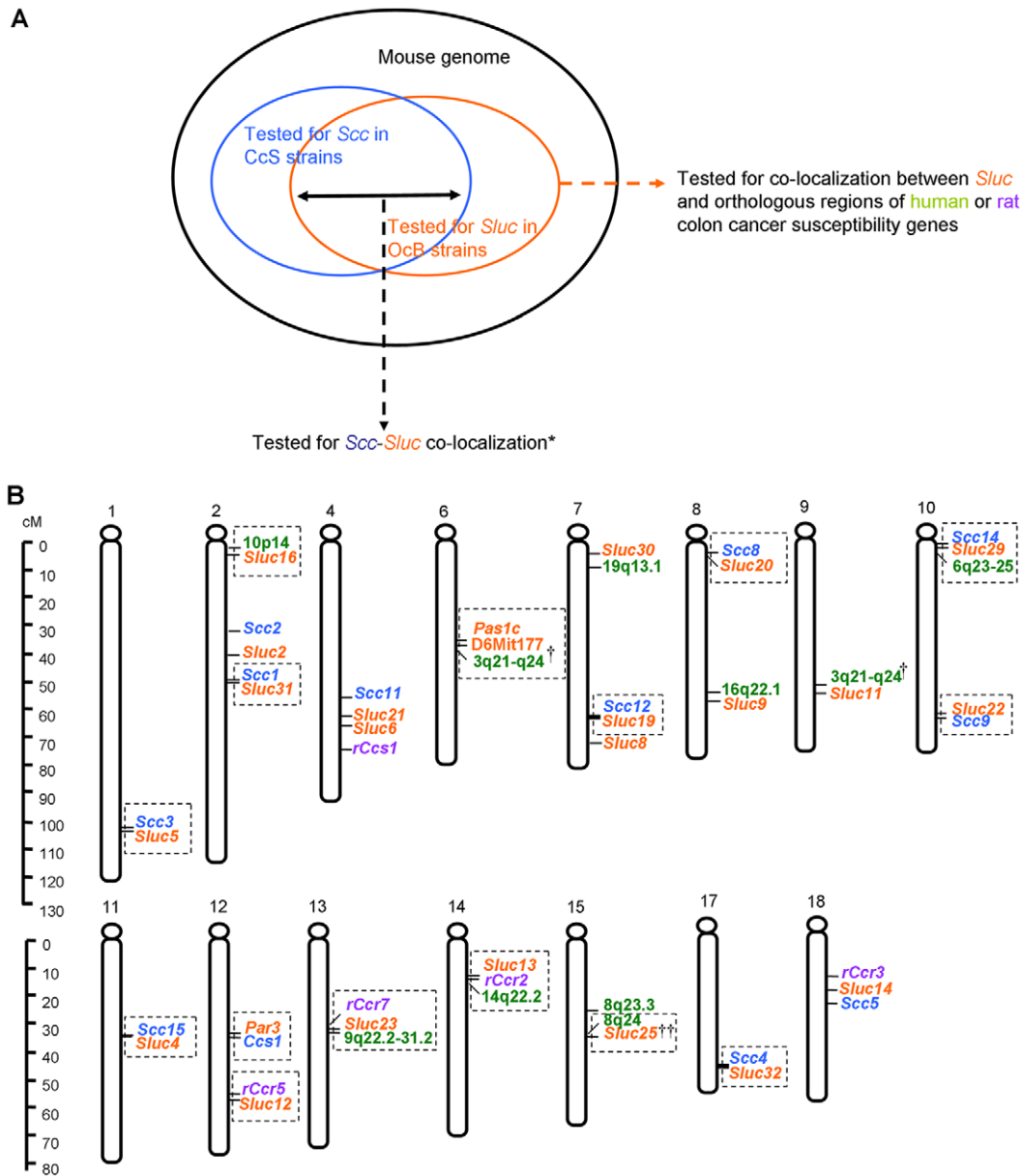
Figure 6. Interspecies correlation between colon and lung cancer susceptibility loci. A. Schematic representation of the part of genome used for the co-localization analyses. The *Sluc* loci analyzed here included also 2 *Sluc* loci identified in (CcS10 XCcS19)F2 mice and 1 *CcS* locus [44] and 1 *Par* locus [50] identified by other group. **B.** Interspecies correlation between colon and lung cancer susceptibility loci. This figure summarizes all 21 clusters of colon and lung cancer susceptibility loci mapped in mouse RC strains (orange for lung, blue for colon), human colon (green) and rat colon (purple). Clusters in which the lung and colon cancer loci mapped within 2.5cM of each other are highlighted in squares. Most colon and lung cancer susceptibility loci co-localize, with the exception of human 15q13 and 20p12.3 (colon), and rat *rCcr6* and *rCcr8* (colon). Orthologous regions of human 18q21, 11q23 (colon) and 5p15, 6p21 and 15q25 (lung), and rat *rCcr1, rCcr4* and *rCcr9* (colon) are not informative since they were not tested for lung or colon cancer susceptibility in mouse RC strains. †*Pas1c* has also been detected in our (CcS-10×CcS-19)F2 cross at D6Mit177. Human colon cancer locus 3q21-q24 is mapped to an 18Mb region and orthologous to two mouse chromosomal regions: Chr.6 (*Pas1c*) and Chr.9 (*Sluc11*), respectively. †† Two human colon cancer susceptibility loci co-localize with a *Sluc* locus.
doi:10.1371/journal.pone.0014727.g006

Sluc pairs co- localize with functional polymorphisms of the immune system that may affect host-tumor interactions: *Scc8/ Sluc20* are linked to *Marif2*, *Cinda5*, and *Lynf4*, which control macrophage and lymphocyte activation and lymphocyte infiltration of tumors, respectively, and *Scc15/Sluc4* are linked to *Cinda1* [69–71]. Four *Scc/Sluc* pairs, *Scc3/Sluc5*, *Scc1/Sluc31*, *Scc11/Sluc21* and *Scc15/Sluc4*, co-localize with microRNA genes [72], some of which could play an essential role in tumorigenesis [73]. In other

Scc-Sluc pairs the two loci may represent duplicated genes whose function diverged into regulating tumorigenesis in the two organs. Still others may contain linked regulatory elements with tissue specific effects, such as the human 8q24 gene desert region that modifies susceptibility to five tumor types [74]. Some instances of *Scc-Sluc* clustering may reflect the phenomenon of non-random distribution of genes and the presence of clusters of functionally related or co-regulated genes in the genome [75], including

functionally related QTLs [76–78]. Finally, susceptibility QTLs upon close analysis may turn out to be complex. Therefore the co-localizing colon and lung cancer susceptibility loci described here may be consisting of multiple closely linked genes, with some overlap between the two sets. The dual specificity of *Scc-Sluc* loci is unlikely due to carcinogen processing, as the two tumors were induced by different carcinogens acting in different ways and at different differentiation stages. DMH and AOM are metabolically activated in liver and primarily excreted into the intestine through bile, where they act locally to mutagenize intestinal epithelial cells causing G→A transitions [79]. On the other hand, ENU acts by directly modifying DNA without any prior enzymatic activation causing a wider spectrum of alterations [80,81]. Prenatal treatment of mice with ENU at day 18 of pregnancy causes predominantly lung tumors but no colon tumors. Moreover, relative strain susceptibility to lung tumors is largely independent on the carcinogen used [82], and susceptibility loci for urethane-induced lung tumors also co-localize with *Sluc* and *Scc* loci [34] (Figure 3).

Mouse genetic mapping predicts chromosomal location of human cancer susceptibility genes

GWA studies of cancer susceptibility [83] confirmed the genetic basis of common cancer [84] and enabled uncovering novel pathways of tumorigenesis [85,86]. However, GWA data cannot yet identify high risk individuals, explain familial cancer clusters [87], nor identify the responsible genes, so the loci cannot be experimentally validated [31]. It has been shown previously that individual QTLs identified in rodent cancer susceptibility studies may provide a strong guide to identification of cancer susceptibility genes in humans [59,88,89]. The present data extend this potential by showing that the orthologous regions of most susceptibility genes for one class of cancer (lung cancer) identified in mice could systematically predict susceptibility genes for another class of cancer (colon caner) in human. This potential contribution is enhanced by several characteristics of mouse crosses: **i.** detection of polymorphic susceptibility alleles is independent on gene frequency of their human homologues and hence can reveal rare alleles in humans; **ii.** the power of mouse RC crosses is very high (1 locus per 29 tested F2 mice [34]) resulting in detection of large numbers of loci; **iii.** gene-gene interactions can be readily detected [37,40,42,90]; **iv.** susceptibil-

ity genes can be molecularly identified [59,67,68,91] and **v.** a hypothesis free 'candidate region' approach can improve detection power in human studies by strongly diminishing the multiple testing penalties.

In conclusion, this is to our knowledge the first systematic study of organ specificity of cancer susceptibility. It indicates that many lung and colon cancer susceptibility genes are linked and possibly identical. Consequently, the presently prevailing organocentric approach to cancer susceptibility may be enriched by comparing systematically the organ-specific pathways with those active in several organs. Application of this finding may also enhance effectiveness of GWA studies of cancer susceptibility in humans.

Supporting Information

Table S1 Supplementary Table 1.
Found at: doi:10.1371/journal.pone.0014727.s001 (0.03 MB DOC)

Table S2 Supplementary Table 2.
Found at: doi:10.1371/journal.pone.0014727.s002 (0.05 MB DOC)

Table S3 Supplementary Table 3.
Found at: doi:10.1371/journal.pone.0014727.s003 (0.10 MB DOC)

Figure S1 Supplementary Figure 1.
Found at: doi:10.1371/journal.pone.0014727.s004 (0.28 MB TIF)

Dataset S1 The mapping spreadsheet for linkage analysis.
Found at: doi:10.1371/journal.pone.0014727.s005 (0.10 MB XLS)

References

1. Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, et al. (2002) Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 31: 33–36.
2. Wiesner GL, Daley D, Lewis S, Ticknor C, Platzer P, et al. (2003) A subset of familial colorectal neoplasia kindreds linked to chromosome 9q22.2–31.2. Proc Natl Acad Sci U S A 100: 12961–12965.
3. Kemp Z, Carvajal-Carmona L, Spain S, Barclay E, Gorman M, et al. (2006) Evidence for a colorectal cancer susceptibility locus on chromosome 3q21-q24 from a high-density SNP genome-wide linkage scan. Hum Mol Genet 15: 2903–2910.
4. Broderick P, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, et al. (2007) A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. Nat Genet 39: 1315–1317.
5. Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, et al. (2007) A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nat Genet 39: 984–988.
6. Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, et al. (2007) Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nat Genet 39: 989–994.
7. Jaeger E, Webb E, Howarth K, Carvajal-Carmona L, Rowan A, et al. (2008) Common genetic variants at the CRAC1 (HMPS) locus on chromosome 15q13.3 influence colorectal cancer risk. Nat Genet 40: 26–28.
8. Houlston RS, Webb E, Broderick P, Pittman AM, Di Bernardo MC, et al. (2008) Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. Nat Genet 40: 1426–1435.
9. Tenesa A, Farrington SM, Prendergast JG, Porteous ME, Walker M, et al. (2008) Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. Nat Genet.
10. Tomlinson IP, Webb E, Carvajal-Carmona L, Broderick P, Howarth K, et al. (2008) A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. Nat Genet.
11. Bailey-Wilson JE, Amos CI, Pinney SM, Petersen GM, de Andrade M, et al. (2004) A major lung cancer susceptibility locus maps to chromosome 6q23–25. Am J Hum Genet 75: 460–474.
12. Amos CI, Wu X, Broderick P, Gorlov IP, Gu J, et al. (2008) Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet 40: 616–622.
13. Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M, et al. (2008) A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452: 633–637.
14. Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, et al. (2008) A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature 452: 638–642.
15. Wang Y, Broderick P, Webb E, Wu X, Vijayakrishnan J, et al. (2008) Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet 40: 1407–1409.
16. McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, et al. (2008) Lung cancer susceptibility locus at 5p15.33. Nat Genet 40: 1404–1406.
17. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, et al. (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447: 1087–1093.

18. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, et al. (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 39: 870–874.

19. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, et al. (2007) Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 39: 865–869.

20. Gold B, Kirchhoff T, Stefanov S, Lautenberger J, Viale A, et al. (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. Proc Natl Acad Sci U S A 105: 4340–4345.

21. Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, et al. (2008) Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 40: 703–706.

22. Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, et al. (2009) Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. Nat Genet 41: 585–590.

23. Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, et al. (2009) A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet 41: 579–584.

24. Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, et al. (2006) A common variant associated with prostate cancer in European and African populations. Nat Genet 38: 652–658.

25. Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, et al. (2007) Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat Genet 39: 977–983.

26. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, et al. (2007) Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nat Genet 39: 645–649.

27. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, et al. (2008) Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40: 316–321.

28. Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, et al. (2008) Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet 40: 281–283.

29. Sun J, Zheng SL, Wiklund F, Isaacs SD, Purcell LD, et al. (2008) Evidence for two independent prostate cancer risk-associated loci in the HNF1B gene at 17q12. Nat Genet 40: 1153–1155.

30. Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, et al. (2008) Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 40: 310–315.

31. Manolio TA (2010) Genomewide association studies and assessment of the risk of disease. N Engl J Med 363: 166–176.

32. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2009) Cancer Statistics, 2009. CA Cancer J Clin. 2009; 59: 225–249.

33. Demant P, Hart AA (1986) Recombinant congenic strains—a new tool for analyzing genetic traits determined by more than one gene. Immunogenetics 24: 416–422.

34. Demant P (2003) Cancer susceptibility in the mouse: genetics, biology and implications for human cancer. Nat Rev Genet 4: 721–734.

35. Moen CJ, van der Valk MA, Snoek M, van Zutphen BF, von Deimling O, et al. (1991) The recombinant congenic strains—a novel genetic tool applied to the study of colon tumor development in the mouse. Mamm Genome 1: 217–227.

36. Moen CJ, Snoek M, Hart AA, Demant P (1992) Scc-1, a novel colon cancer susceptibility gene in the mouse: linkage to CD44 (Ly-24, Pgp-1) on chromosome 2. Oncogene 7: 563–566.

37. van Wezel T, Stassen AP, Moen CJ, Hart AA, van der Valk MA, et al. (1996) Gene interaction and single gene effects in colon tumour susceptibility in mice. Nat Genet 14: 468–470.

38. van Wezel T, Ruivenkamp CA, Stassen AP, Moen CJ, Demant P (1999) Four new colon cancer susceptibility loci, Scc6 to Scc9 in the mouse. Cancer Res 59: 4216–4218.

39. Ruivenkamp CA, Csikos T, Klous AM, van Wezel T, Demant P (2003) Five new mouse susceptibility to colon cancer loci, Scc11-Scc15. Oncogene 22: 7258–7260.

40. Fijneman RJ, de Vries SS, Jansen RC, Demant P (1996) Complex interactions of new quantitative trait loci, Sluc1, Sluc2, Sluc3, and Sluc4, that influence the susceptibility to lung cancer in the mouse. Nat Genet 14: 465–467.

41. Fijneman RJ, Jansen RC, van der Valk MA, Demant P (1998) High frequency of interactions between lung cancer susceptibility genes in the mouse: mapping of Sluc5 to Sluc14. Cancer Res 58: 4794–4798.

42. Tripodis N, Hart AA, Fijneman RJ, Demant P (2001) Complexity of lung cancer modifiers: mapping of thirty genes and twenty-five interactions in half of the mouse genome. J Natl Cancer Inst 93: 1484–1491.

43. Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11: 241–247.

44. Jacoby RF, Hohman C, Marshall DJ, Frick TJ, Schlack S, et al. (1994) Genetic analysis of colon cancer susceptibility in mice. Genomics 22: 381–387.

45. Angel JM, Popova N, Lanko N, Turusov VS, DiGiovanni J (2000) A locus that influences susceptibility to 1, 2-dimethylhydrazine-induced colon tumors maps to the distal end of mouse chromosome 3. Mol Carcinog 27: 47–54.

46. Meunier C, Cai J, Fortin A, Kwan T, Marquis JF, et al. (2010) Characterization of a major colon cancer susceptibility locus (Ccs3) on mouse chromosome 3. Oncogene 29: 647–661.

47. Gariboldi M, Manenti G, Canzian F, Falvella FS, Radice MT, et al. (1993) A major susceptibility locus to murine lung carcinogenesis maps on chromosome 6. Nat Genet 3: 132–136.

48. Manenti G, Gariboldi M, Elango R, Fiorino A, De Gregorio L, et al. (1996) Genetic mapping of a pulmonary adenoma resistance (Par1) in mouse. Nat Genet 12: 455–457.

49. Obata M, Nishimori H, Ogawa K, Lee GH (1996) Identification of the Par2 (Pulmonary adenoma resistance) locus on mouse chromosome 18, a major genetic determinant for lung carcinogen resistance in BALB/cByJ mice. Oncogene 13: 1599–1604.

50. Pataer A, Nishimura M, Kamoto T, Ichioka K, Sato M, et al. (1997) Genetic resistance to urethan-induced pulmonary adenomas in SMXA recombinant inbred mouse strains. Cancer Res 57: 2904–2908.

51. Festing MF, Lin L, Devereux TR, Gao F, Yang A, et al. (1998) At least four loci and gender are associated with susceptibility to the chemical induction of lung adenomas in A/J×BALB/c mice. Genomics 53: 129–136.

52. Liu P, Wang Y, Vikis H, Maciag A, Wang D, et al. (2006) Candidate lung tumor susceptibility genes identified through whole-genome association analyses in inbred mice. Nat Genet 38: 888–895.

53. De Miglio MR, Virdis P, Calvisi DF, Mele D, Muroni MR, et al. (2007) Identification and chromosome mapping of loci predisposing to colorectal cancer that control Wnt/beta-catenin pathway and progression of early lesions in the rat. Carcinogenesis 28: 2367–2374.

54. Thomas A, Cannon-Albright L, Bansal A, Skolnick MH (1999) Familial associations between cancer sites. Comput Biomed Res 32: 517–529.

55. Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, et al. (2004) Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS Med 1: e65.

56. Nagase H, Bryson S, Cordell H, Kemp CJ, Fee F, et al. (1995) Distinct genetic loci control development of benign and malignant skin tumours in mice. Nat Genet 10: 424–429.

57. Nagase H, Mao JH, Balmain A (1999) A subset of skin tumor modifier loci determines survival time of tumor-bearing mice. Proc Natl Acad Sci U S A 96: 15032–15037.

58. Angel JM, DiGiovanni J (1999) Genetics of skin tumor promotion. Prog Exp Tumor Res 35: 143–157.

59. Ewart-Toland A, Briassouli P, de Koning JP, Mao JH, Yuan J, et al. (2003) Identification of Stk6/STK15 as a candidate low-penetrance tumor-susceptibility gene in mouse and human. Nat Genet 34: 403–412.

60. Mao JH, Saunier EF, de Koning JP, McKinnon MM, Higgins MN, et al. (2006) Genetic variants of Tgfb1 act as context-dependent modifiers of mouse skin tumor susceptibility. Proc Natl Acad Sci U S A 103: 8125–8130.

61. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57–70.

62. Sun Y (2006) p53 and its downstream proteins as molecular targets of cancer. Mol Carcinog 45: 409–415.

63. Jiang J, Zhang X, Yang H, Wang W (2009) Polymorphisms of DNA repair genes: ADPRT, XRCC1, and XPD and cancer risk in genetic epidemiology. Methods Mol Biol 471: 305–333.

64. Lynch PM (2009) The hMSH2 and hMLH1 genes in hereditary nonpolyposis colorectal cancer. Surg Oncol Clin N Am 18: 611–624.

65. Kouso H, Yoshino I, Miura N, Takenaka T, Ohba T, et al. (2008) Expression of mismatch repair proteins, hMLH1/hMSH2, in non-small cell lung cancer tissues and its clinical significance. J Surg Oncol 98: 377–383.

66. Wang Y, Yang H, Li H, Li L, Wang H, et al. (2009) Association between X-ray repair cross complementing group 1 codon 399 and 194 polymorphisms and lung cancer risk: a meta-analysis. Cancer Lett 285: 134–140.

67. Zhang Z, Futamura M, Vikis HG, Wang M, Li J, et al. (2003) Positional cloning of the major quantitative trait locus underlying lung tumor susceptibility in mice. Proc Natl Acad Sci U S A 100: 12642–12647.

68. Manenti G, Galbiati F, Gianni-Barrera R, Pettinicchio A, Acevedo A, et al. (2004) Haplotype sharing suggests that a genomic segment containing six genes accounts for the pulmonary adenoma susceptibility 1 (Pas1) locus activity in mice. Oncogene 23: 4495–4504.

69. Fijneman RJ, Vos M, Berkhof J, Demant P, Kraal G (2004) Genetic analysis of macrophage characteristics as a tool to identify tumor susceptibility genes: mapping of three macrophage-associated risk inflammatory factors, marif1, marif2, and marif3. Cancer Res 64: 3458–3464.

70. Lipoldova M, Havelkova H, Badalova J, Demant P (2005) Novel loci controlling lymphocyte proliferative response to cytokines and their clustering with loci controlling autoimmune reactions, macrophage function and lung tumor susceptibility. Int J Cancer 114: 394–399.

71. Kakarlapudi N, Vernooy JH, Quan L, Fijneman RJ, Demant P (2008) Control of lymphocyte infiltration of lung tumors in mice by host's genes: mapping of four Lynf (lymphocyte infiltration) loci. Cancer Immunol Immunother 57: 217–225.

72. Sevignani C, Calin GA, Nnadi SC, Shimizu M, Davuluri RV, et al. (2007) MicroRNA genes are frequently located near mouse cancer susceptibility loci. Proc Natl Acad Sci U S A 104: 8017–8022.

73. Huppi K, Volfovsky N, Mackiewicz M, Runfola T, Jones TL, et al. (2007) MicroRNAs and genomic instability. Semin Cancer Biol 17: 65–73.

74. Ghoussaini M, Song H, Koessler T, Al Olama AA, Kote-Jarai Z, et al. (2008) Multiple loci with different cancer specificities within the 8q24 gene desert. J Natl Cancer Inst 100: 962–966.

75. Hurst LD, Pal C, Lercher MJ (2004) The evolutionary dynamics of eukaryotic gene order. Nat Rev Genet 5: 299–310.

76. Santos CA, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Genet Genomics 268: 122–129.

77. Xie X, Jin F, Song MH, Suh JP, Hwang HG, et al. (2008) Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an Oryza sativa×O. rufipogon cross. Theor Appl Genet 116: 613–622.

78. Bilusic M, Bataillard A, Tschannen MR, Gao L, Barreto NE, et al. (2004) Mapping the genetic determinants of hypertension, metabolic diseases, and related phenotypes in the lyon hypertensive rat. Hypertension 44: 695–701.

79. Wali RK, Skarosi S, Hart J, Zhang Y, Dolan ME, et al. (1999) Inhibition of O(6)-methylguanine-DNA methyltransferase increases azoxymethane-induced colonic tumors in rats. Carcinogenesis 20: 2355–2360.

80. Shibuya T, Morimoto K (1993) A review of the genotoxicity of 1-ethyl-1-nitrosourea. Mutat Res 297: 3–38.

81. Cordes SP (2005) N-ethyl-N-nitrosourea mutagenesis: boarding the mouse mutant express. Microbiol Mol Biol Rev 69: 426–439.

82. Demant P, Oomen LC, Oudshoorn-Snoek M (1989) Genetics of tumor susceptibility in the mouse: MHC and non-MHC genes. Adv Cancer Res 53: 117–179.

83. Hunter DJ, Chanock SJ (2010) Genome-wide association studies and "the art of the soluble". J Natl Cancer Inst 102: 836–837.

84. Easton DF, Eeles RA (2008) Genome-wide association studies in cancer. Hum Mol Genet 17: R109–115.

85. Ahmadiyeh N, Pomerantz MM, Grisanzio C, Herman P, Jia L, et al. (2010) 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. Proc Natl Acad Sci U S A 107: 9742–9746.

86. Wright JB, Brown SJ, Cole MD (2010) Upregulation of c-MYC in cis through a large chromatin loop linked to a cancer risk-associated single-nucleotide polymorphism in colorectal cancer cells. Mol Cell Biol 30: 1411–1420.

87. Ioannidis JP, Castaldi P, Evangelou E (2010) A compendium of genome-wide associations for cancer: critical synopsis and reappraisal. J Natl Cancer Inst 102: 846–858.

88. Lesueur F, Pharoah PD, Laing S, Ahmed S, Jordan C, et al. (2005) Allelic association of the human homologue of the mouse modifier Ptprj with breast cancer. Hum Mol Genet 14: 2349–2356.

89. Samuelson DJ, Hesselson SE, Aperavich BA, Zan Y, Haag JD, et al. (2007) Rat Mcs5a is a compound quantitative trait locus with orthologous human loci that associate with breast cancer risk. Proc Natl Acad Sci U S A 104: 6299–6304.

90. Schork NJ, Nath SP, Lindpaintner K, Jacob HJ (1996) Extensions to quantitative trait locus mapping in experimental organisms. Hypertension 28: 1104–1111.

91. Ruivenkamp CA, van Wezel T, Zanon C, Stassen AP, Vlcek C, et al. (2002) Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers. Nat Genet 31: 295–300.