[CANCER RESEARCH 63, 4533–4537, August 1, 2003]

Opposite Effects of Modifiers of the Apc^{Min} Mutation in Intestine and Mammary Gland[1]

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ABSTRACT

Patterns of tumor susceptibility in different organs are widely divergent in mouse strains: one strain may be highly susceptible to tumors in one organ but resistant in another organ, whereas another strain may exhibit the opposite pattern (P. Demant, Semin. Cancer Biol., *3:* 159–166, 1992). Therefore, susceptibility to tumors in different organs is assumed to be controlled by different sets of genes. On the other hand, many oncogenes and tumor suppressor genes are mutated in tumors from different organs, indicating that similar tumorigenic pathways operate in various tissues. To obtain insight into the interactions of susceptibility genes with one of such pathways, we compared tumorigenesis in intestine and mammary gland in recombinant congenic strains (RCSs) carrying the Apc^{Min} mutation, affecting the Wnt pathway. The presence of Apc^{Min} increased considerably the incidence of intestinal and mammary tumors. The individual RCSs differed in the number and latency of Apc^{Min}-induced intestinal and mammary tumors and histological type of the latter. Unexpectedly, the strain distribution of susceptibility to the intestinal and mammary tumors in the Apc^{Min}-bearing mice was opposite in the RCSs; the strains most susceptible for intestinal tumors were most resistant to mammary tumors and *vice versa*. This suggests that a set of genes controls the impact of the Apc^{Min} mutation in both organs but with opposite effects. Elucidation of the basis of the observed strain differences in organ-specific Wnt pathway-mediated tumorigenesis will help to understand the interactions between germ-line encoded allelic differences in susceptibility genes and the spectrum of somatic mutations in tumor cells.

INTRODUCTION

Colorectal cancer and breast cancer are collectively responsible for the largest part of cancer-related deaths. In humans, the genetic factors affect both the sporadic and hereditary forms of these cancers (1, 2). Identification of genes that modify the development of these cancers is important for the unraveling of the pathways of the neoplastic process, as well as for the understanding of the role of the host genotype for the propensity of cancer development and progression. The development of nonfamilial, "sporadic," cancer is influenced by many genes (3). In this respect is cancer similar to other common (nonfamilial) diseases, including cardiovascular, metabolic, and infectious diseases. With the infectious diseases, it has been shown that susceptibility is controlled by multiple loci, and for both Leishmaniasis (4, 5) and Lyme disease (6), it was demonstrated that different loci affect different aspects of the disease phenotype, thus permitting to link heterogeneity of the disease with the host genotype. Such associations between heterogeneity of the disease and genetic polymorphisms of the host are likely to apply to all types of diseases, including cancer. Obviously, the understanding of the genetic factors regulating different aspects of the cancer phenotype would be important for the assessment of future disease progression and selection of optimal therapy. The accumulation of specific somatic alterations in cancer cells is linked with the probability of malignant progression. However, it is not clear to what extent the genes of the host modify the effects of such alterations. Definition of the contribution of such genomic modifiers would considerably improve the interpretation of the genetic profiling of cancer.

Animal models provide the necessary versatility for such studies (7). One of them is the Apc^{Min} mutation in *Apc* gene, the mouse homologue of the human *APC* gene (8). C57BL/6J mice that carry Apc^{Min} mutation develop numerous intestinal tumors at an early age and rarely survive beyond 150 days of age (9). In addition, C57BL/6 *Min*/+ females tend to develop mammary tumors (9, 10). *Min* is a fully penetrant dominant mutation mapped to proximal chromosome 18 (11), and it is a homozygous lethal (12). The *Min*/+ mice are a good model for studies of both induction and prevention of inherited and sporadic cancers (9, 13). The development of adenomas in intestines of *Min*/+ mice is influenced by genetic background. The difference is probably attributable to several "modifier" genes, carried by different inbred strains. One of them is the semidominant *Mom1* (14–16) on chromosome 4. The second modifier *Mom2* on chromosome 18 is the result of a spontaneous mutation (17). Kohlhepp *et al.* (18, 19) identified in ENU-treated *Min*/+ mice a modifier effect on the resistance to mammary and intestinal tumors linked to ROSA26 insertion. As the modifier effect has been noted only in ENU-treated mice, it has not been formally ruled out that it affects the response of these tissues to ENU treatment. The multiplicity and invasiveness of intestinal adenomas of Apc^{Min} mice are enhanced besides *Mom1* susceptible allele also by *p53* deficiency (20). Genetic background affects also development of mammary tumors in *Min*/+ mice (21). It would be important for the understanding of the nature of modifier genes to compare their impact on the effects of the Apc^{Min} mutation in different tissues. We compared, therefore, the impact of the host genotype on tumor development in mammary gland and intestine of *Min*/+ mice, using the special tool of genetic dissection of multigenic traits, the RCSs (22, 23). In the series of homozygous OcB/Dem RCSs, each strain carries a different random portion of 12.5% of the genes of the strain B10.O20/Dem (B10.O20) on the genetic background of the O20/A (O20) strain. In this way, the different OcB strains contain different combinations of possible modifier genes and therefore could differently modify the tumorigenic effects of the Apc^{Min} mutation.

In the present study, we show that the mice of OcB strains exhibit in the presence of Min mutation different patterns of susceptibility to mammary and intestinal tumors. We also show that different OcB strains exhibit preferentially certain types of mammary tumors.

MATERIALS AND METHODS

Mice. RCSs of the OcB/Dem series (described in Refs. 22 and 23), B10.O20 mice, and the congenic strain BALB/c*Min*/+, produced by >15 backcrosses of the *Min* mutation from the C57BL/6 strain have been main-

Received 1/30/03; accepted 5/22/03.

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[1] Supported by European Commission Grant ERB IC 15 CT 98-317.

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[3] The abbreviations used are: *Min, multiple intestinal neoplasia*; *Mom, modifier of Min*; ENU, ethylnitrosourea; RCS, recombinant congenic strain.

tained at the breeding colony of P. Demant at the Netherlands Cancer Institute; breeding pairs for the experimental animals were shipped to Warsaw, Poland.

In OcB/Dem mice, the background strain is O20/A (*Mom 1*-resistant allele), and the donor strain is B10.O20 (*Mom 1* susceptible allele). OcB strains, with the exception of the strain OcB-9, carry the resistant *Mom 1* allele. *Min* mutation carried in the BALB/c*Min*/+ strain has been crossed into investigated OcB strains by one backcross [(OcB × BALB/c*Min*/+)F1*Min*/+ × OcB], and its effects have been followed by comparing *Min*/+ and +/+ mice in each OcB backcross. In the following text, such backcross mice are referred to as OcB*Min*/+ or OcB+/+ mice. In the same way, the BALB/c*Min*/+ mice were backcrossed to B10.O20 mice. Unfortunately, the mice of the "background" strain O20 could not be included into these experiments because of a considerable period of poor breeding performance at the time of initiation of these experiments. The effect of *Apc*^{Min} mutation was compared in 955 backcross mice: 85 of B10.O20 strain and 870 in backcrosses of nine OcB RC strains, males and females, *Min*/+ and +/+.

Mice were housed in semibarrier conditions and free from any commonly screened pathogens. Animals were allowed *ad libitum* access to standard pelleted diet (LABOFEED H) and water. All animals were maintained under a 12:12 h light/dark cycle at room temperature 21°C ± 2°C. The mice were sacrificed when visibly sick or at the age of animals of 900 days. The age of the mice at the date of sacrifice was recorded as their tumor age.

Tumors. The organs of mice were examined for the presence of mammary and intestinal tumors. The whole intestine was examined for the presence of macroscopically visible tumors, their number and localization in duodenum, jejuno-ileum, and colon. Tumors or suspected lesions were fixed in formol-alcohol-acetic acid, embedded in paraffin, sectioned, and stained with H&E. Sections were analyzed by light microscopy. The largest tumors were histopathologically classified according to Pathology of Tumors in Laboratory Animals (24, 25).

Genotyping. The presence of *Min* mutation in OcB and B10.O20 backcrosses was determined by PCR reaction as described (14). Genomic DNA was obtained from tails of mice (26) with minor modifications.

Statistical Analysis. The differences in tumor incidence between and within OcB strains (*Min*/+ and +/+ mice) and tumor latency (time between birth and appearance of tumor) and kinds of tumor were evaluated by ANOVA (PROC GLM statement, SAS 6.12 for Windows) and Spearman rank correlation coefficient.

RESULTS

Intestinal Tumors. We have not found intestinal tumors (or only in very low number) in +/+ mice of any strain. In *Min*/+ mice, the incidence of intestinal tumors depended on the strain used ($P < 0.001$ both in females and males). The most susceptible were B10.O20 mice, which developed intestinal neoplasms in 86.7% of females and 89.5% of males. These mice also had the highest number of tumors per mouse (Fig. 1) and at the earlier age of animals compared with OcB mice. In OcB9 mice, 59% of females and 75.8% of males developed intestinal tumors, what was significantly more than in other OcB strains (Table 1). The high susceptibility of B10.O20 and OcB-9 strains is most likely caused by the presence of *Mom1* susceptible allele (23).

The tumors were usually present through duodenum, jejuno-ileum,

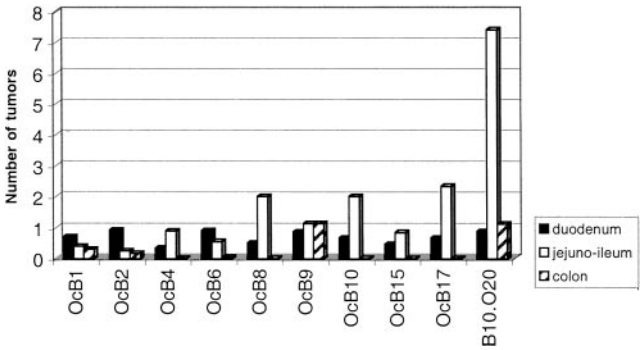

Fig. 1. The distribution and number of tumors per mouse in three regions of intestine in male mice of OcB and B10.O20 strains.

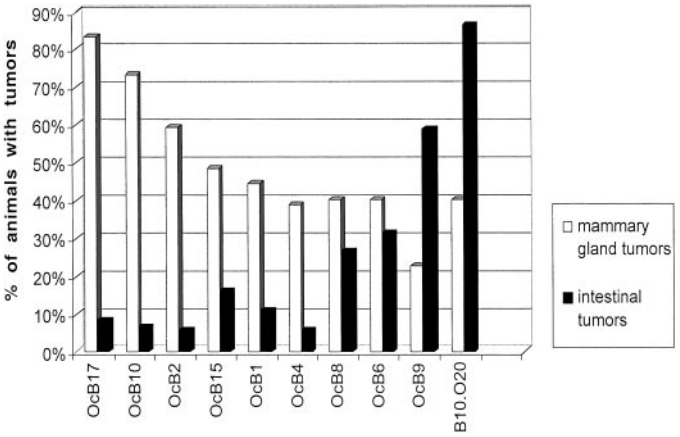

Fig. 2. Ranking of intestinal and mammary gland tumor incidence in females of OcB and B10.O20 mouse strains.

and colon, but their distribution was different in the OcB strains (Table 1 and Fig. 1). Of 101 histologically analyzed tumors (obtained in 84 mice from all investigated strains), two types of adenoma (tubular and villous; Ref. 24) represented 68% of all classified tumors (Fig. 3, *A* and *B*). Adenomas are the most common neoplastic lesions, comprising ~80% of all intestinal tumors in mice (24). Macroscopically, they appeared to be rounded or flattened lesions projecting into the intestinal lumen and frequently having a stalk of variable thickness. Many adenomas histologically showed a mixture of both tubular and villous growth patterns (Fig. 3*B*). Adenocarcinomas, invading the muscle layer (Fig. 3, *C* and *D*), represented in our material 32% of all classified tumors. With the limited number of tumors analyzed, the proportion of adenomas and adenocarcinomas did not appear to differ between the strains (data not shown).

Mammary Tumors. The incidence of mammary gland tumors was significantly higher in *Apc*^{Min} mice of all OcB strains. Moreover, there was a difference between OcB strains ($P < 0.01$). In *Min*/+ females of OcB2, OcB10 and OcB17 mammary tumors developed more often (in 59.6, 73.3, and 83.3% of mice, respectively) than in OcB strains, OcB4, OcB6, OcB8, OcB9, and B10.O20 (22.7–40%). We found a negative correlation between the number of mammary tumors and intestinal neoplasms ($P = 0.07$). In OcB strains with higher incidence of mammary tumors, the incidence of intestinal neoplasms was lower (OcB2, OcB10, and OcB17; Table 1 and Fig. 2). This difference is not a result of a shorter life span of females caused by mammary tumor development, because this phenomenon was seen also in strains with very similar average age of mammary tumors and intestinal tumors (OcB4, OcB10, OcB17, and B10.O20).

The histological type of 126 mammary gland tumors was classified according to Squartini and Pingitore (25). Analyzed tumors (23.8%) were adenocarcinomas of type A (Fig. 3*E*) or B, 51.5% were adenoacanthomas (Fig. 3*F*), and 24.6% were mixed, *i.e.*, adenocarcinomas with few small focuses of keratinic material. The histopathological type of tumor is influenced by the strain (Table 2). In the strains where more than a few tumors were analyzed, adenocarcinoma was observed seldom in OcB2 (in 6.6% of all analyzed tumors) in OcB15 (16.6% of all analyzed tumors) strains, whereas in OcB10 and OcB17 strains, 40–44% of tumors were adenocarcinomas. The highest proportion of adenoacanthomas developed in OcB2 and OcB15 animals. Adenocarcinomas developed at the animal age of 409–669 days, and adenoacanthomas developed at 235–551.6 days.

DISCUSSION

The findings presented in this study demonstrate the complex effects of genetic background of the host on development of tumors, which have been initiated by a single mutation in the *Apc* gene. We

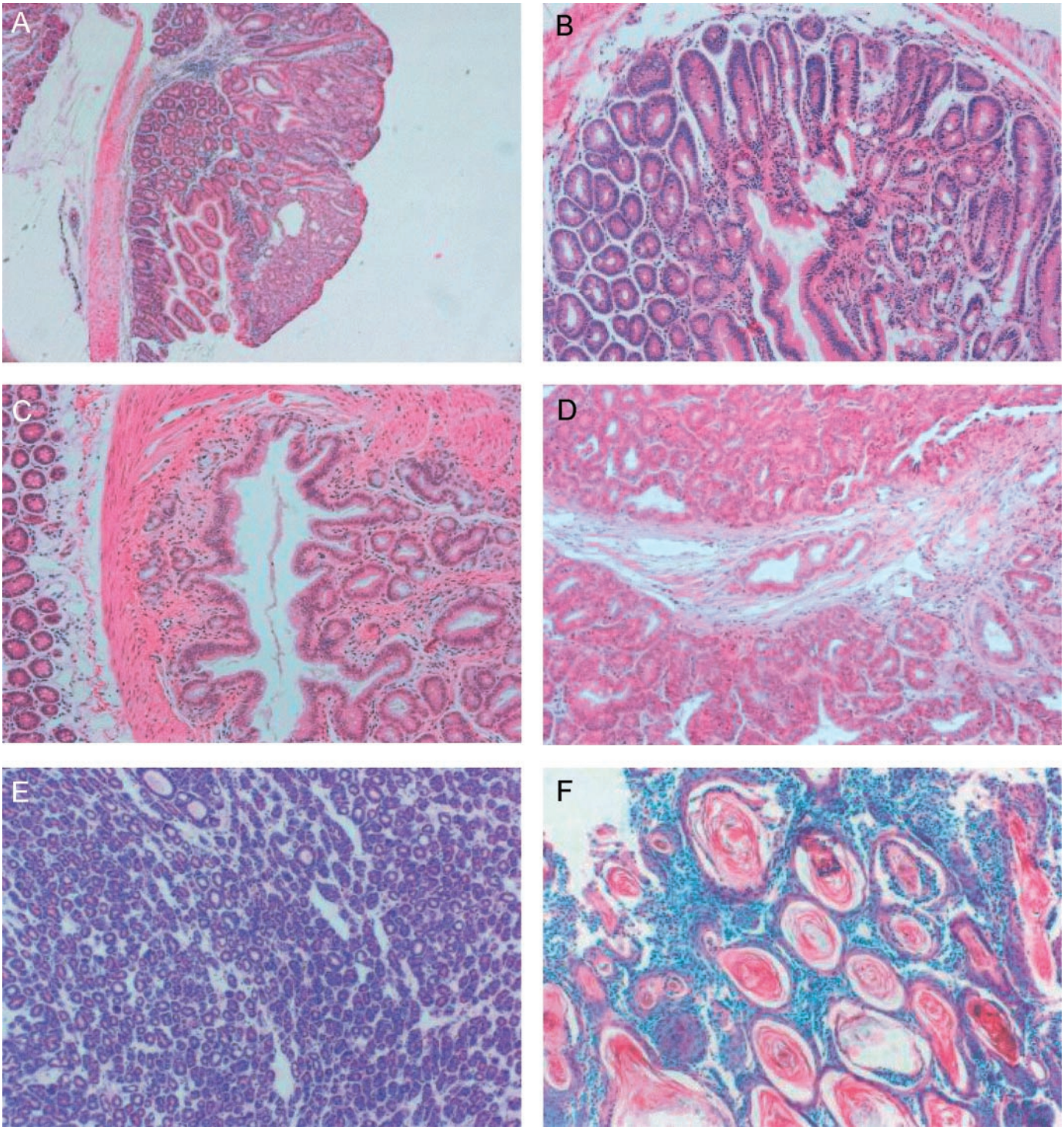

Fig. 3. *A*, intestinal tumor. Tubular adenomatous polyp composed of number of glandular structures. H&E, ×100. *B*, intestinal tumor. Tubular adenoma. H&E, ×200. *C*, intestinal tumor. Adenocarcinoma, invasion into the muscle layer. H&E, ×200. *D*, intestinal tumor. Adenocarcinoma, invasion into the muscle layer. H&E, ×200. *E*, mammary gland tumor. Adenocarcinoma type A. H&E, ×200. *F*, mammary gland tumor. Adenoacanthoma. The gland epithelium undergoes squamous change, and the lumina are filled with keratinic material. H&E, ×200.

have seen considerable differences between the individual RCSs into which the mutation was crossed. This happened although all these strains contained a considerable proportion of a random mix of BALB/c genes introduced together with the mutation. This obviously weakened the effects of the genes of individual OcB strains, so the differences which we found are an underestimate of the real differences, which would have been observed if the mutation was present in these strains without admixture of unrelated genes. One obvious finding is the predominant effect of *Mom1* gene in the development of intestinal tumors. The two strains with susceptible *Mom1* allele, namely B10.O20 and OcB-9, were much more susceptible than the rest of the OcB strains, which carry the resistant *Mom1* allele. Nevertheless, there is a very large difference in susceptibility between the strain B10.O20 and OcB9, although they carry the same susceptible *Mom1* allele, indicating the difference between the background strain O20 and B10.O20 contains many more genes influencing susceptibil-

Table 1 *The incidence of intestinal and mammary gland tumors in OcB mice*

Bc with line	Sex	Min	No. of mice		Mice with tumor		No. of intestinal tumors			
					Mammary gland	Intestine	All	Duodenum	Ileum	Colon
OcB1	Females	Min/+	27	No. of mice (%)	12 (44.4)	3 (11.1)	6	4	2	0
				Av. Age[a] ± SD	429.3 ± 183	703.3 ± 14.6				
		+/+	28	No. of mice (%)	1 (3.5)	0				
				Av. age ± SD	254					
	Males	Min/+	36	No. of mice (%)	3 (8.3)	10 (27.8)	14	7	4	3
				Av. age ± SD	484.7 ± 210	686.4 ± 61.7				
		+/+	31	No. of mice (%)	0	0				
				Av. age ± SD						
OcB2	Females	Min/+	52	No. of mice (%)	31 (59.6)	3 (5.8)	4	4	0	0
				Av. age ± SD	402.9 ± 150	576.3 ± 107				
		+/+	8	No. of mice (%)	0	0				
				Av. age ± SD						
	Males	Min/+	39	No. of mice (%)	0	12 (30.8)	16	11	3	2
				Av. age ± SD		697.5 ± 60				
		+/+	13	No. of mice (%)	0	0				
				Av. age ± SD						
OcB4	Females	Min/+	18	No. of mice (%)	7 (38.9)	1 (5.6)	1	0	0	1
				Av. age ± SD	361.9 ± 149	276.0				
		+/+	39	No. of mice (%)	1 (2.6)	2 (5.1)	2	1	1	0
				Av. age ± SD	285.0	707.5 ± 29				
	Males	Min/+	25	No. of mice (%)	0	9 (36)	11	3	8	0
				Av. age ± SD		714.7 ± 41.5				
		+/+	22	No. of mice (%)	0	1 (4.5)	2		2	
				Av. age ± SD		718.0				
OcB6	Females	Min/+	57	No. of mice (%)	23 (40.4)	18 (31.6)	28	16	12	0
				Av. age ± SD	564.0 ± 212	709.3 ± 113.9				
		+/+	39	No. of mice (%)	2 (5.1)	0				
				Av. age ± SD	643.0 ± 271					
	Males	Min/+	47	No. of mice (%)	6 (12.8)	20 (42.6)	30	18	11	1
				Av. age ± SD	753.7 ± 110	756.3 ± 94.3				
		+/+	12	No. of mice (%)	0	0				
				Av. age ± SD						
OcB8	Females	Min/+	15	No. of mice (%)	6 (40)	4 (26.7)	15	2	11	2
				Av. age ± SD	440.2 ± 204	693.5 ± 132				
		+/+	23	No. of mice (%)	0	0				
				Av. age ± SD						
	Males	Min/+	7	No. of mice (%)	0	4 (57.1)	10	2	8	0
				Av. age ± SD		633.3 ± 58.9				
		+/+	21	No. of mice (%)	2 (9.5)	0				
				Av. age ± SD	684.5 ± 31.8					
OcB9	Females	Min/+	22	No. of mice (%)	5 (22.7)	13 (59.1)	49	17	24	8
				Av. age ± SD	348 ± 135	645.7 ± 106				
		+/+	11	No. of mice (%)	0	0				
				Av. age ± SD						
	Males	Min/+	20	No. of mice (%)	0	15 (75)	47	13	17	17
				Av. age ± SD		599.9 ± 62.7				
		+/+	9	No. of mice (%)	0	0				
				Av. age ± SD						
OcB10	Females	Min/+	30	No. of mice (%)	22 (73.3)	2 (6.7)	2	2	0	0
				Av. age ± SD	513.2 ± 193	531.5 ± 94				
		+/+	24	No. of mice (%)	5 (20.8)	1 (4.2)	2	1	1	0
				Av. age ± SD	588 ± 165.8	746.0				
	Males	Min/+	38	No. of mice (%)	2 (5.3)	3 (7.9)	8	2	6	0
				Av. age ± SD	859.5 ± 43	719.7 ± 133				
		+/+	25	No. of mice (%)	0	0				
				Av. age ± SD						
OcB15	Females	Min/+	37	No. of mice (%)	18 (48.6)	6 (16.2)	11	7	3	1
				Av. age ± SD	353.1 ± 180	672.2 ± 78				
		+/+	10	No. of mice (%)	0	0				
				Av. age ± SD						
	Males	Min/+	32	No. of mice (%)	0	13 (40.6)	17	6	11	0
				Av. age ± SD		657 ± 118.3				
		+/+	6	No. of mice (%)	0	0				
				Av. age ± SD						
OcB17	Females	Min/+	12	No. of mice (%)	10 (83.3)	1 (8.3)	1	1		
				Av. age ± SD	529.4 ± 109	522.0				
		+/+	14	No. of mice (%)	2 (14.3)	0				
				Av. age ± SD	633 ± 38.2					
	Males	Min/+	13	No. of mice (%)	1 (7.7)	3 (23.1)	9	2	7	0
				Av. age ± SD	694.0	681.3 ± 206.3				
		+/+	8	No. of mice (%)	0	0				
				Av. age ± SD						
B10.O20	Females	Min/+	15	No. of mice (%)	6 (40)	13 (86.7)	103	23	80	0
				Av. age ± SD	313.0 ± 157	388.2 ± 153.6				
		+/+	25	No. of mice (%)	0	0				
				Av. age ± SD						
	Males	Min/+	19	No. of mice (%)	0	17 (89.5)	160	15	126	19
				Av. age ± SD		455.9 ± 140.7				
		+/+	26	No. of mice (%)	0	0				
				Av. age ± SD						

[a] Average age when autopsied.

Table 2 *Histological type of mammary gland tumors*

OcB strain	No. of analyzed mammary gland tumors	Adenocarcinoma No. (%) Av. age[a] (days)	Adenocarcinoma + adenoacanthoma No. (%) Av. age (days)	Adenoacanthoma No. (%) Av. age (days)
OcB1	8	2 (25) 458.5	1 (12.5) 438	5 (62.5) 270.8
OcB2	30	2 (6.6) 587	11 (35.6) 471.6	17 (56.6) 331.1
OcB4	5	1 (20) 507	1 (20) 262	3 (60) 259
OcB6	19	7 (36.8) 669	5 (26.3) 519	7 (36.8) 451.5
OcB8	6	0	3 (50) 622	3 (50) 254.6
OcB9	5	2 (40) 409	1 (20) 452	2 (40) 235
OcB10	21	9 (42.8) 590.6	4 (19) 662	8 (38.1) 351.2
OcB15	18	3 (16.6) 449.6	2 (11.1) 503.5	13 (72.2) 305.1
OcB17	9	4 (44.4) 538.5	2 (22.2) 514.5	3 (33.3) 511.6
B10.O20	5	0	1 (20) 187	4 (80) 368

[a] Average age when autopsied.

ity to intestinal tumors than *Mom1* only. This finding is analogous to the original observation of Dietrich *et al.* (14) that *Mom1* is strong but definitely not the only gene on the C57BL background causing susceptibility to intestinal tumors in mice with the *Min* mutation. Development of mammary tumors has been also influenced by genetic background of the OcB strains. This happened at three levels: (*a*) some OcB strains developed mammary tumors in much higher frequency than others. This difference is highly significant; (*b*) there are differences in the type of mammary tumors, which develop in individual strains. In some strains, there is a high proportion of adenoacanthomas; in other strains, the adenocarcinomas predominate; and (*c*) there appears to be an intriguing negative correlation between development of intestinal and mammary tumors. There appears to be no obvious trivial reason for this. Although we have no data explaining this finding, the most logical hypothesis is that some modifier genes segregating in OcB strains have an opposite modifying effect in the epithelium of intestine and mammary epithelium. This suggests that the modifying genes might have tissue-specific effects

In conclusion, as the *Min* mutation influences not only intestinal neoplasia but also mammary tumors, we were able to describe the impact of genetic polymorphisms on several effects of this gene: (*a*) numbers of intestinal tumors; (*b*) numbers and histological type of mammary tumors; and (*c*) the inverse relationship of the incidence of these two types of tumors. This opens way for identification of modifiers for the various aspects of *Min*-related tumor phenotype. Identification of such genes would likely be important also for human cancer, because *APC* gene mutations in humans play an important role in the initiation step of colorectal carcinogenesis in both familial adenomatous polyposis patients and nonfamilial adenomatous polyposis patients (27–29) and because somatic *APC* gene mutations were also found in some breast cancers (27), where the *APC* mutation spectrum appears to be different from that of colorectal cancers. *APC* is a component of the Wnt pathway, which operates both in intestinal and mammary/breast tumors. The OcB *Apc*^{Min} model with the differential modification of *APC* effects on mammary and intestinal tumors is therefore an interesting tool for analysis of molecular and cellular biology of these tumors.

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