Two Genetically Transmitted BALB/c Mouse Mammary Tumor Virus Genomes Located on Chromosomes 12 and 16

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We have examined *Eco*RI-restricted cellular DNA from BALB/c mouse-hamster somatic cell hybrids by blot hybridization for the presence of mouse mammary tumor virus-related sequences. Results of this analysis show that mouse mammary tumor virus-related proviral copies are located on chromosomes 16 (16-kilobase-pair fragment) and 12 (10.5- and 7.7-kilobase-pair fragments).

The mouse mammary tumor virus (MMTV) is transmitted either horizontally as an infectious agent in the milk of lactating mice or through the germ line as chromosomally integrated proviral genomes (1, 2, 4). All common inbred strains and some feral populations of *Mus musculus* contain genetically transmitted MMTV genomes in their cellular DNA (2, 5, 14, 16, 24). In the C3H/f and DBA/f inbred mouse strains, the genetically transmitted MMTV genome at the *Mtv-1* locus on chromosome 7 is associated with the appearance of viral proteins and the development of late mammary tumors (15, 21). In GR mice, the *Mtv-2* locus is associated with the expression of infectious virus and early mammary tumors in female mice and thymomas in aging males (10, 15, 22).

Among inbred strains, the BALB/c mouse has been widely used to study mammary neoplasia because of its low spontaneous incidence of late mammary tumors and its sensitivity to tumor induction by chemical carcinogens and infectious MMTV. BALB/c cellular DNA contains a partial MMTV proviral genome, designated unit I, and two complete MMTV proviral genomes, designated units II and III (4). The partial MMTV proviral genome is composed of primarily long-terminal repeat (LTR) sequences (4). Expression of these MMTV proviral genomes has been detected in various normal and neoplastic tissues. Low levels of MMTV genomic and envelope mRNA can be found in normal tissues of BALB/c mice (3). In the lactating mammary gland, a novel 1.6-kilobase-pair (kbp) MMTV-related RNA is expressed which hybridizes only with MMTV transcription leader and LTR sequences and therefore may be the product of the partial proviral genome (3, 23, 25). In neoplastic tissues, the 1.6-kbp MMTV RNA is uniquely expressed at high levels in BALB/c spontaneous and chemical carcinogen-induced mammary tumors, mammary hyperplastic alveolar nodules, and pristane-induced plasma cell tumors (3, 23, 25). Some of these tumors also express high levels of MMTV genomic and envelope mRNA (3, 23, 25).

To help further define, at the genetic level, the role of the BALB/c MMTV proviral genomes in neoplasia, we sought to identify their chromosomal location with a series of somatic cell hybrids. These cell hybrids were obtained by fusion of the Chinese hamster cell line E36 with peritoneal or spleen cells of BALB/c mice. The hybrids retain a full complement of Chinese hamster chromosomes and different subsets of mouse chromosomes. The isolation and characterization of

these hybrid lines for their mouse chromosome content has been described previously (13).

We have analyzed *Eco*RI-restricted cellular DNA from hamster, mouse, and hybrid cells by Southern blot hybridization with a recombinant cloned probe representing the MMTV genome. The enzyme *Eco*RI cleaves the MMTV proviral genome once internally, thus generating two cell-virus junction fragments for each complete provirus. Southern blot analysis of hamster and BALB/c mouse DNAs revealed five *Eco*RI MMTV-related fragments in BALB/c DNA (16, 10.5, 8.3, 7.7, and 6.6 kbp) and two different MMTV-related fragments in the Chinese hamster DNA (4.1 and 2.8 kbp) (Fig. 1, lane a). DNAs from 17 hamster-mouse hybrids all contained the two Chinese hamster fragments. The 16-kbp, the 10.5- and 7.7-kbp, and the 8.4- and 6.6-kbp BALB/c MMTV-related fragments segregated independently in these hybrids (Fig. 1; compare lanes b, c, and d),

FIG. 1. MMTV-related sequences in *Eco*RI-restricted cellular DNA from E36 Chinese hamster cells (lane a), BALB/c π liver tissue (lane b), the hamster-mouse hybrids BM12J (lane c), 8AZ2E (lane d), BM20 (lane e), and BALB/c π liver tissue (lane f). High-molecular-weight DNA was prepared as previously described (9). *Eco*RI-digested samples of DNA (10 μ g) were electrophoresed on 0.8% agarose gels, transferred to a nitrocellulose membrane, and hybridized under stringent conditions with 2×10^6 to 5×10^6 cpm/ml of 32 P-labeled MMTV proviral DNA prepared by nick translation of a recombinant clone of MMTV (C3H) LTR (2).

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TABLE 1. Correlation between specific mouse chromosomes and the 16-kbp *Eco*RI MMTV-specific DNA fragment in 17 somatic cell hybrids

Mouse chromosomes	No. of hybrid clones (16-kbp MMTV <i>Eco</i> RI fragment/chromosome retention) ^a				% Discordant
	+/+	-/-	+/-	-/+	
1	3	7	2	5 ^b	41
2	3	9	2	3	29
3	1	12	4	0	24
4	2	10	3	2	29
5	2	12	3	0	18
6	2	8	3	4	41
7	4	5	1	7	47
8	1	12	4	0	24
9	2	10	3	2	29
10	1	11	4	1	29
11	0	12	5	0	29
12	3	6	2	6	47
13	3	11	3	1	24
14	2	11	3	1	24
15	4	6	1	6	41
16	5	12	0	0	0
17	4	8	1	4	29
18	3	12	2	0	12
19	1	8	4	4	47
X	3	11	1	1	12

^a The mouse chromosome content was determined by direct karyology with Giemsa-trypsin banding followed by staining with Hoechst 33258 (12).

^b Three hybrids contain MMTV and chromosome 1 (+/+); seven lack MMTV and chromosome 1 (-/-); seven contain only MMTV (+/-) or only chromosome 1 (-/+).

confirming previous studies which showed that these *Eco*RI MMTV fragments comprise two complete proviral copies and one MMTV proviral fragment (4).

The 16-kbp MMTV-related fragment was detected in 5 of the 17 hybrids tested. Examination of the mouse chromosome content of these 17 hybrids showed that only the 5 positive hybrids retained chromosome 16 (Table 1). One positive hybrid retained only chromosomes 5 and 16, but data from the other hybrids clearly rule out chromosome 5. All other mouse chromosomes also show discordant segre-

gation with this MMTV sequence. These data indicate that the 16-kbp proviral fragment is present on chromosome 16.

We have confirmed the assignment of the MMTV-related 16-kbp fragment to chromosome 16 and further localized its position by using a genetic cross between BALB/c π mice and mice of a *M. musculus* subsp. *domesticus* stock Centreville Lights (CL) from Centreville, Md. (18). *Eco*RI-restricted cellular DNA from CL mice contains MMTV-related fragments which are different from those in BALB/c π cellular DNA (Fig. 2, lane 1 and 2). Similarly, as shown previously (19) and in Fig. 2 (lanes 7 and 8), *Eco*RI-restricted cellular DNA from these mice also differs at the immunoglobulin lambda chain locus on chromosome 16 (6). *Eco*RI-restricted DNA from 33 (BALB/c $\times$ CL) F2 mice were examined by blot hybridization for cosegregation of the BALB/c 16-kbp MMTV and 8.6-kbp lambda chain-related fragments. An example of each observed F2 genotype is shown in Fig. 2 (lanes 3, 4, 5, 6, 9, 10, 11, 12). The results (Table 2) indicate that the lambda chain and MMTV loci are linked, with a recombination frequency of 0.16 ± 0.06 . Since the lambda chain locus is closely linked to the *Akv-2* ecotropic type C proviral locus at the centromeric end of chromosome 16 (R. Epstein and C. Buckler, personal communication), our data suggest that this MMTV-related sequence is also located at the centromeric end of the chromosome.

The 10.5- and 7.7-kbp MMTV-related *Eco*RI fragments in BALB/c cellular DNA were present in 7 of the 15 hybrids tested (Table 3). Several positive hybrid lines carried few chromosomes in addition to chromosome 12. One hybrid carried as its mouse complement only chromosomes 5, 7, 12, and 15, and another hybrid carried only chromosomes 3, 12, 15, 17, and X. Although chromosome 15 is present in both hybrids, there were three discordancies for this chromosome in other clones. We conclude that the proviral genome that generates the 10.5- and 7.7-kbp MMTV-related DNA fragments is present on chromosome 12.

The location of this MMTV proviral genome on chromosome 12 was confirmed by Southern blot analysis of the BALB/c congenic strain C4W which carries the chromosome 12-linked *Fv-4* locus of the wild Japanese mouse, *M. musculus* subsp. *molossinus* (11). Cellular DNA from *M. musculus molossinus* lacks the 10.5- and 7.7-kbp MMTV

FIG. 2. MMTV- and immunoglobulin lambda chain-related sequences in *Eco*RI-restricted cellular DNA from liver tissue of BALB/c π (lanes 1 and 7), CL (lanes 2 and 8), and F2 (BALB/c $\pi \times$ CL) mice no. 81 (lanes 3 and 9), no. 83 (lanes 4 and 10), no. 115 (lanes 5 and 11), and no. 80 (lanes 6 and 12). The restricted high-molecular-weight cellular DNAs were electrophoresed on 0.8% agarose gels, transferred to nitrocellulose membranes, and hybridized with 2×10^6 to 5×10^6 cpm/ml of 32 P-labeled MMTV (C3H) LTR DNA (lanes 1 to 6) or MOPC 104E C₁V₁ recombinant DNA (19) (lanes 7 to 12) as described in the legend to Fig. 1. The 10.0-kbp λ C₁V₁-related fragment in lanes 8 and 11 is the result of partial *Eco*RI restriction of the CL lambda chain cellular DNA. The restriction fragments monitored in this cross are indicated with arrows.

TABLE 2. Linkage of BALB/c lambda chain locus and the 16-kbp *Eco*RI MMTV-related fragment in (BALB/c × CL) F2 mice

Genotype	MMTV (16-kbp BALB/c fragment)	Observed genotype (lambda chain) ^a		No. of mice
		6.0- and 5.6-kbp CL fragments	8.6-kbp BALB/c fragment	
1	+	—	+	3
2	+	+	+	20
3	—	+	—	7
4	—	+	+	3

^a The genotypes of 33 (BALB/c × CL) F2 mice were determined as described in the legend to Fig. 2. Characteristic restriction patterns for each genotype are shown in Fig. 2 (genotype 1, lanes 3 and 9; genotype 2, lanes 4 and 10; genotype 3, lanes 5 and 11; genotype 4, lanes 6 and 12). The recombination frequency (genotype 4) is 0.16 ± 0.06 as determined by the method of maximum likelihood (8).

*Eco*RI fragments as does cellular DNA from the BALB/c C4W congenic mice (Fig. 3, lanes G and H, respectively). These congenics do, however, contain the 16-kbp and the 8.3- and 6.6-kbp MMTV fragments in their cellular DNA. C4W mice carry the wild mouse allele of the *Pre-1* locus on chromosome 12 (H. Ikeda, personal communication), but *Hind*III- and *Eco*RI-restricted BALB/c C4W cellular DNAs show identical patterns of immunoglobulin heavy-chain-related sequences (Fig. 3, lanes B and C and lanes E and F). Since the gene order of these loci is centromere-heavy-chain-*Pre-1*-*Fv-4* (11), we conclude that the 10.5- and 7.7-kbp MMTV-related *Eco*RI fragments are located distal to the heavy-chain locus on chromosome 12.

TABLE 3. Correlation between specific mouse chromosome and the 10.5- and 7.7-kbp MMTV-specific fragments in 17 somatic cell hybrids

Mouse chromosomes	No. of hybrid clones (10.5- and 7.7-kbp MMTV <i>Eco</i> RI fragments/chromosome retention) ^a				% Discordant
	+/+	-/-	+/-	-/+	
1	4	6	5	2 ^b	41
2	3	6	6	2	47
3	0	7	9	1	59
4	3	7	6	1	47
5	0	6	9	2	64
6	3	6	6	2	47
7	7	5	2	3	29
8	1	8	8	0	47
9	3	7	6	1	41
10	1	8	8	0	47
11	0	8	9	0	53
12	9	8	0	0	0
13	2	6	7	2	53
14	2	7	7	1	47
15	8	6	1	2	18
16	4	6	5	2	41
17	4	4	5	4	53
18	2	7	7	1	47
19	1	7	8	1	53
X	3	6	6	2	47

^a The mouse chromosome content was determined by direct karyology with Giemsa-trypsin banding followed by staining with Hoechst 33258 (12).

^b Four hybrids contain MMTV and chromosome 1 (+/+); six lack MMTV and chromosome 1 (-/-); seven contain only MMTV (+/-) or only chromosome 1 (-/+).

FIG. 3. Restriction endonuclease digests of *M. musculus* subsp. *molossinus* (lanes A, D, and G), BALB/c π (lanes B, E, and H), and BALB/c C4W congenic (lanes C, F, and I) cellular DNAs. The cellular DNA in lanes A to C and G to I was digested with *Eco*RI and with *Hind*III in lanes D to F. The restricted cellular DNAs were electrophoresed and transferred to nitrocellulose membranes and were hybridized with 2×10^6 to 5×10^6 cpm/ml of 32 P-labeled P107 α R5 recombinant DNA (7) of the S107 immunoglobulin heavy chain mRNA (lanes A to F) or recombinant MMTV (C3H) LTR DNA (lanes G to I) as described in the legend to Fig. 1.

The 8.3- and 6.6-kbp MMTV-related DNA fragments were present in only 1 of the 17 somatic cell hybrids examined (Fig. 1, lane e). Although chromosome 18 shows the best correlation with these sequences (data not shown), several other chromosomes could not be definitely excluded. Currently, we are analyzing genetic crosses to identify the chromosomal location of this MMTV proviral genome.

Previous genetic studies have assigned specific MMTV proviral genomes to chromosomes 1, 4, 6, 7, and 18 in different mouse strains (15, 17, 20, 21). The genetic mapping of two BALB/c MMTV proviral genomes to chromosomes 12 and 16 provides additional evidence that MMTV proviral genomes are located at nonallelic sites and on different chromosomes of inbred mice. More importantly, the known chromosomal assignments for these MMTV loci make it possible to explore more extensively the role of these proviruses in the etiology of neoplasia. In particular, the location of two MMTV-related sequences near the immunoglobulin loci on chromosomes 12 and 16 is interesting in light of the specific 12:15 chromosomal translocations found in many plasma cell tumors (18). These translocations result in aberrant immunoglobulin gene rearrangements and altered transcriptional activity of the genes located near the chromo-

somal breakpoints. Thus, the increased expression of the MMTV loci in these tumors may be a direct result of this tumor-specific chromosomal abnormality (3). Whether this also reflects some etiological role for these sequences in the induction or maintenance of plasma cell tumors is under study.

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