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DEMONSTRATION OF A NEW MOUSE MAMMARY TUMOR VIRUS LOCUS IN THE GENOME OF THE MAMMARY TUMOR PRONE STRAIN SHN MOUSE

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SUMMARY: Until this report, there have been no detailed analyses of the mouse mammary tumor virus (MMTV) loci in the mammary tumor prone strain SHN. Using a probe, which hybridizes to the ***env*** sequence of MMTV, Southern blotting of genomic DNA from the brain after digestion with EcoRI revealed 5 endogenous proviruses: *Mtv-1* (4.5kb), *Mtv-2* (11kb), *Mtv-8* (6.7kb), *Mtv-17* (8.3kb) and a newly-found 6.5-kb fragment. F1-hybrid mice (C3H/He female x SHN male) also possessed the 6.5-kb fragment. Thus, we conclude that the 6.5-kb fragment is unique to SHN mice. Genomic DNA from mammary tumors of SHN mice showed MMTV insertions, suggesting that activation of an oncogene(s) occurred in this strain.

Endogenous mouse mammary tumor virus (MMTV) integrates into the mouse genome at different loci (1). In accordance with differences in MMTV genotype, there is a wide variation of mammary tumor incidence among strains (2), suggesting that the endogenous MMTV genotype influences mammary tumor development. For example, C57BL/6 mice have *Mtv-8*, *Mtv-9*, and *Mtv-17* (2) and a very low mammary tumor incidence (3). In contrast, the GR mouse strain, which has a very high incidence of mammary tumors (4), is reported to have *Mtv-2*, *Mtv-3*, *Mtv-7*, *Mtv-8*, *Mtv-14*, and *Mtv-17* (2).

Female SHN mice are also known to have a very high incidence of mammary tumors (5-8). This mouse strain was established by Nagasawa and co-workers, starting from mice of a Swiss stock (5-6). In this strain, endogenous MMTV, rather than milk-transmitted MMTV (9), appears to be involved in mammary tumorigenesis since foster nursing does not decrease the

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incidence of early mammary tumors (10). Although the uniqueness of the endogenous MMTV genotype of this strain has been reported (10), a detailed analysis of the MMTV loci has not been performed.

The mechanism of tumorigenesis by MMTV has been well established in many strains of mice. MMTV activates cellular oncogenes located upstream or downstream of viral insertions (11). However, it is unknown whether these insertions are required for tumorigenesis in the SHN strain.

The major aim of the present study was to determine the endogenous MMTV loci in the SHN mouse strain. This information may be important, since this strain is reported not only to have a high incidence of mammary tumors but also other tumors and pathological lesions (12). A further aim of this study was to determine whether insertions occur in the mammary tumor DNA of this strain.

MATERIALS AND METHODS

Mice. Female mice of the SHN strain, originally given to us by Dr.H. Tanooka (National Cancer Institute, Tokyo, Japan), were maintained in our animal facility. C3H/He and C57BL/6 mouse strains (purchased from Nippon Clea ,Tokyo, Japan) were also maintained in our animal facility. Male SHN mice were mated with C3H/He females in our facility to obtain F1-hybrid C3H/SHN mice. These mice were housed in plastic cages with wood shavings at 23 °C, a relative humidity of 50%, and a 12-h photocycle.

DNA isolation from tissues. DNA was extracted from the tissues as described (13). The DNA was ethanol-precipitated and stored at -20° C until used for Southern blotting as described below.

MMTV probe preparation. A plasmid, derived from pBR322 and containing the MMTV genome (6kb) (14), was isolated from an *Escherichia coli* host according to Maniatis et al.(13). A 1-kb BamH1 fragment of the MMTV genome was used as a hybridization probe. The probe is known to detect only EcoRI fragments derived from the 3' portion of the MMTV provirus (11)(Fig.1) since this probe contained a part of the viral ***env*** sequence. The probe was ^{32}P-labeled by a random primer kit (Takara, Tokyo, Japan) to a specific activity of 1-3 x10^7 cpm /μg.

Southern blotting. Isolated genomic DNA was digested with an excess amount of EcoRI (Takara, Tokyo, Japan), electrophoresed in a 1% agarose gel and then transferred to a nylon membrane. Lamda DNA digested with HindIII was used as a molecular weight marker and was visualized under UV light after ethidium bromide staining.

RESULTS AND DISCUSSION

The SHN mouse strain was previously reported to possess *Mtv-4* (10), based on the restriction fragment length polymorphism of the MMTV loci. In that study, the digestion pattern of MMTV with EcoRI was compared with that of the GR strain and, because of the absence of two fragments, the authors reported that the genotype of the SHN strain should be named *Mtv-4*. However, accumulated evidence on the role of MMTV in mouse mammary tumor development has revealed that some loci are actively transcribed, and thereby result in tumor formation, while others are not (11). This indicates that the individual MMTV loci are more important than the overall restriction fragment pattern for understanding the roles of MMTV in pathological events. The new standardized nomenclature for endogenous MMTV is based on this concept (2). As shown (Fig.2), five fragments of 11, 8.3, 6.7, 6.5 and 4.5 kb were detected in SHN mice. The restriction fragment pattern of the SHN mouse strain found in the present study appears to be slightly different from that previously reported (10). We consider that this difference is due to the differences in the probes. We used a DNA fragment that hybridized exclusively to the ***env*** sequences (Fig.1), while in the previous report, they used the whole MMTV which contained LTR. Thus, our probe hybridized to the 3' portion of the endogenous MMTV fragment after digestion with EcoRI, while their probe hybridized to both the 5' and 3' portions of

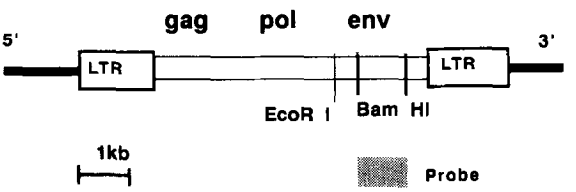
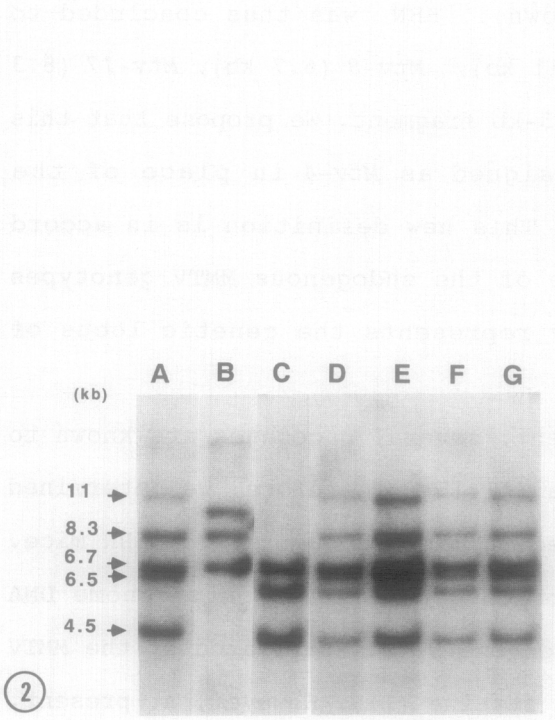


Fig.1. Restriction map of the MMTV provirus, and the fragment used as the probe in the present study.

MMTV. We tentatively assigned these fragments as *Mtv-1* (4.5 kb), *Mtv-2* (11 kb), *Mtv-8* (6.7 kb) and *Mtv-17* (8.3 kb) by comparison with the known restriction fragments of C3H/He, C57BL/6 and GR (2). However, the 6.5-kb fragment appears to be unique to the SHN



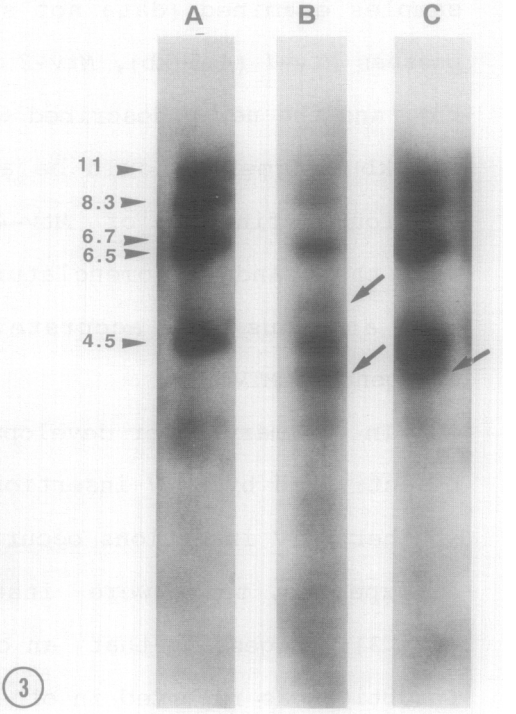


Fig.2. Representative EcoRI restriction patterns of endogenous MMTV in genomic DNA isolated from the brain and other tissues. Approximately 10 μg of cellular DNA was isolated from tissues of four different strains. The DNA was digested to completion with the restriction enzyme EcoRI, subjected to electrophoresis in a 1.0% agarose gel, transferred to a nylon membrane, hybridized to a ^{32}P-labeled MMTV probe (***env***) and autoradiographed for 72 hrs. The membrane was washed as previously reported (11). Molecular sizes of the fragments in this gel and those shown in Fig.3 were based on molecular weight standards of lamda DNA digested with HindIII.
A: Brain DNA from SHN strain. B: Brain DNA from C57BL/6 strain. C: Brain DNA from C3H/He strain. D: Brain DNA from C3H/SHN. E: Liver DNA from C3H/SHN. F: Lung DNA from C3H/SHN.
G: Spleen DNA from C3H/SHN.

Fig.3. Representative EcoRI restriction patterns of MMTV specific proviral DNA in genomic DNA isolated from mammary tumors of SHN mice. Approximately 10 μg cellular DNA isolated from mammary tumors from SHN mice was digested to completion with the restriction enzyme EcoRI and then subjected to electrophoresis. Southern blotting was conducted as described in the "MATERIALS AND METHODS" section and in Fig.2. The arrows indicate the MMTV insertions.
A. SHN brain : B. SHN mammary tumor : C. SHN mammary tumor.

mouse strain (Fig.2). To further confirm this, we analyzed the MMTV loci in the F1-hybrid mice of C3H/SHN. The restriction fragments of the resulting F1-hybrid revealed 6 loci (Fig.2) corresponding to *Mtv-1* (4.5 kb), *Mtv-2* (11 kb), *Mtv-8* (6.7 kb), *Mtv-11* (5.8 kb), *Mtv-17* (8.3 kb), and the new locus (6.5 kb). Genomic DNA digested with BamH1 yielded, as predicted, a 0.8-kb DNA fragment in all samples examined (data not shown). SHN was thus concluded to possess *Mtv-1* (4.5 kb), *Mtv-2* (11 kb), *Mtv-8* (6.7 kb), *Mtv-17* (8.3 kb), and the newly described 6.5-kb fragment. We propose that this 6.5-kb fragment should be assigned as *Mtv-4* in place of the previous definition of *Mtv-4*. This new definition is in accord with the standard nomenclature of the endogenous MMTV genotypes (2), and thus more accurately represents the genetic locus of endogenous MMTV.

In mammary tumor development, several oncogenes are known to be activated by MMTV insertions (11,15). Therefore, we determined whether MMTV insertions occurred in mammary tumors from SHN mice. As expected, there were insertions of MMTV DNA into genome DNA (Fig.3), suggesting that an oncogene(s) was activated by the MMTV insertions as reported in other strains (11). However, at present, it is unknown whether a new or known oncogene(s) is activated by the MMTV insertions in the SHN strain.

Congenic mouse lines of the new locus of MMTV in the C57BL/6 or C3H/He background appear to be promising for analyzing the roles of this locus in tumorigenesis. It is reported that female C3H/SHN mice actively produce MMTV viruses in normal mammary glands (16). Research on the role of this new locus in mammary tumorigenesis and oncogene(s) involvement is now underway.

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