学 位 論 文 の 要 旨

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学 位 論 文 名	Expression of SV40 T antigen gene in the oligodendroglia induced primitive neuroectodermal tumor-like tumors in the mouse brain
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論 文 内 容 の 要 旨

INTRODUCTION

Primitive neuroectodermal tumors (PNET) are classified as the embryonal tumors developed in the brain, except for the cerebellum. These types of tumors are characterized with primitive histological features resembling neuroepithelial stem cells of the embryonic neural tube or their immature progeny. However, some tumors tend to acquire the embryonal characteristics following the neoplastic changes and their progressions. Thus, it has been often difficult to determine the type of the cell from which embryonal tumors, particularly PNET, are derived. To understand the origin or pathogenesisof PNET, several transgenic or related studies have been carried out. In this study, we report that expression of SV40-Tag by an oligodendroglia-specific promoter in the transgenic mice repeatedly induced PNET-like tumors in the brain stem.

MATERIALS AND METHODS

For oligodendrocyte-specific expression of SV40-Tag intransgenic mice, a 1.3-kb mouse myelin basic protein (MBP) gene promoter was ligated to the SV40-Tag gene, rabbit β -globin intron and poly(A) addition signals derived from the rabbit β -globin and SV40 early gene, respectively. Tail DNA of each mouse was screened by DNA blot analysis for identification of transgenic individuals. Total RNA from one-sided hemisphere of the mouse brains were hybridized with the appropriate fragment of various marker-gene cDNA, including mouse MBP cDNA, mouse proteolipid protein (PLP) cDNA, and mouse neurofillament-light chain (NF-L) cDNA. Portions of mouse PLP cDNA (8–454) and NF-L cDNA(522–908) were obtained from mouse brain RNA by reverse transcription-polymerase chain reaction (RT-PCR).

The paraffin sections of the mouse brain were stained with hematoxylin–eosin HE and in part with Klüver-Barrera's staining, and histopathologically inspected. Immunostaining was carried

out with the antibodies against the marker proteins of the CNS or SV40-Tag. The antibodies used were as follows: anti-SV40-Tag, anti-mouse MBP, anti-human glial fibrillary acidic protein (GFAP), anti-neurofillament (NF), and anti-S-100 protein.

RESULTS AND DISCUSSION

Three founder mice (MBT3-19L, MBT9-14L and MBT11-3L) showed abnormal behavior including tremor and ataxia. One transgenic mouse (MBT9-14L) with mild tremor gave rise to progeny (MBT transgenic line). In three of the 29 transgenic offspring of MBT9-14L, ataxia and/or mild tremor were also observed after 6–12 months. Two founder mice (MBT3-19L and MBT11-3L) and 29 transgenic offspring derived from MBT9-14L were further analyzed.

In two founder mice (MBT3-19L and MBT11-3L) and three of 29 transgenic offspring (no. 17, 18, 23), brain or spinal cord tumors were identified. All of these mice showed the abnormal behaviors. Macroscopically, all brain tumors were found in the brain stem.

These tumors showed almost identical histopathological features as follows: homogenous oval or polygonal cell shape, chromatin-rich oval nucleus, frequent mitosis, poor cytoplasm and extensive invasion into the normal brain tissue. In the three enlarged tumors (no.17, 18, 23), scattered roset-like structures were observed. Brain tumors in MBT3-19L and MBT11-3L, which showed severe tremor at their early postnatal days, were localized dominantly at the dorsal side of the pons. They showed the same cell type as found in the enlarged tumors. Spinal cord tumors were identified at the lower cervical level in MBT3-19L, MBT11-3L, and no. 23. They showed undifferentiated characters that were indistinguishable from the tumors in the brain stem. No apparent sign of demyelination was identified in the MBT transgenic mice. Neural or glial markers, including GFAP, NF, MBP and S-100 protein, were not detected except for one tumor (no. 18). One tumor in no. 18 contained MBP-positive cells, which were predominantly observed at the central region of the tumor mass. This tumor also contained S-100 protein-positive cells. MBP-positive cells and S-100 protein-positive cells showed similar distribution in the central region of the tumor mass. In the marginal zone of the tumor, MBP- or S-100 protein-positive cell was not observed.

The oligodendrocyte-specific expression of the transgene was confirmed in transgenic brains immunohistochemically using MBT11-3L and transgenic offspring of MBT9-14L line. In the brains of the founder mice that exhibited severe tremor, expression of SV40-Tag was mosaic in oligodendrocytes, and predominantly located in the white matter tracts. However, in the transgenic offspring of the MBT9-14L line exhibiting mild tremor, SV40-Tag-positive cells were limited to the white matter of the cerebellum. Mosaic and relatively condensed expression of MBP was observed in the transgenic brains in contrast to the ubiquitous and extensive staining in the control. RNA blot analysis was performed for the brains of transgenic or control mice using SV40-Tag gene and three oligodendrocyte marker gene probes. Expression of SV40-Tag mRNA was observed in the transgenic samples, but not in the non-transgenic controls. However, expression of MBP and PLP were approximately 3-fold and 1.5-fold lower in the transgenic samples than in the controls, respectively. The expression of NF-L mRNA appeared unaltered in the transgenic brain, although the transgenic samples exhibited slightly decreased signals. These results suggested that expression of the SV40-Tag transgene was oligodendroglia-specific, and the transgene expression caused the reduction of MBP and PLP expression. Several studies

showed that MBP synthesis was inhibited by the overexpression of SV40-Tag at various levels. SV40-Tag expression was thus immunohistochemically detected in all the brain tumors examined. These results clearly indicate that tumor development in the transgenic mice is caused by the SV40-Tag transgene expression. All tumors had quite undifferentiated features, and showed no expression of CNS specific markers except for a part of one tumor. These tumors did not develop in the cerebellum. Tumors exhibited a rosetlike structures, however, did not have an apparent honeycomb-structure. These findings support that the tumors which developed in the MBT transgenic mouse brains are indistinguishable from PNET.

In the MBT transgenic mice, expression of SV40-Tag gene is designed to be driven by an oligodendroglia-specific MBP promoter. The activation of MBP promoter in the cell implicates that the cell is in the stage of differentiation toward the oligodendroglia. Therefore, synthesis of SV40-Tag should occur only in the differentiating oligodendroglia.

The site and timing of tumor development also correlated well with those of MBP expression during normal development. MBP expression normally begins to increase after birth and at first at the dorsal side of the brain stem. In this present study, all PNET-like brain tumors developed at the brain stem, and the appearance of behavioral abnormalities and development of tumor mass in the newborn founder mice suggested that expression of the transgene occurred at the early postnatal period. Although two of the three brain tumors showed no glial differentiation, a few cell populations of the other one tumor showed oligodendroglia-like characteristics. According to these lines of evidences, at least, some of tumors which are indistinguishable with PNET developed in the MBT transgenic mice, might arise from the primitive oligodendroglia.

Our results suggest that primitive oligidendroglia may be one candidate for the origin of PNET-like tumor. Our transgenic line (MBT9-14L line) is thus quite a unique strain that develops PNET-like tumor reproducibly at the restricted region of the brain.

In this present study, hypomyelination were suggested in MBT13-9L and MBT11-3L, which showed severe tremors in their early postnatal days in spite of the small tumor masses. In contrast, MBT9-14L and its offspring, which showed relatively mild tremors, no apparent hypomyelination was observed whereas some of them developed the enlarged PNET-like tumor. These results suggest that higher expression of SV40-Tag causes hypomyelination during early postnatal days and induces the individual death before the tumor progression. Low incidence of the brain tumor (10%) in the MBT strain also suggests that the 'appropriate' level of SV40-Tag expression is required for the tumor progression.

CONCLUSION

The tumors developed in the present transgenic mice were indistinguishable from PNET, and one of them showed oligodendroglia-like characteristics. This transgenic line is therefore a useful animal model to study the pathogenesis of undifferentiated tumor.