Murine Model of Leptomeningeal Dissemination Using Human Medulloblastoma Cells

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Abstract

An experimental model of meningeal dissemination was developed by intracisternal inoculation of human medulloblastoma (ONS-76) cells into nude mice. All mice died within 65 days after inoculation of \(1 \times 10^7\) tumor cells. The median survival time was 56 days. Clinical signs and histological findings were similar to those in medulloblastoma patients with meningeal dissemination. Immunohistochemical studies showed that ONS-76 cells in the subarachnoid space expressed major histocompatibility complex (MHC) class I antigens until 20 days after inoculation. After 30 days, expression of MHC class I antigens decreased and cells began to proliferate rapidly. Expression of MHC class I antigens on tumor cells may result in effective recognition by the host immune system.

Key words: meningeal tumor, cell line, medulloblastoma, experimental model

Introduction

The 5-year survival among medulloblastoma patients has improved to 70% in the last 15 years.\(^\text{7,9,13}\) This dramatic improvement is mainly due to advances in radiation therapy.\(^\text{2,9,12}\) Medulloblastomas are certainly radiosensitive, but are not always radiocurable because dosages are limited by adverse effects on the whole neural axis.\(^\text{7}\) Eventually, tumor regrowth occurs and cerebrospinal fluid (CSF) metastasis often causes death in medulloblastoma patients.

To further advance the treatment of medulloblastoma, we have developed a murine model of leptomeningeal dissemination using human medulloblastoma cells. Here, we describe our histological results and the biology of disseminated cells in our murine model.

Materials and Methods

I. Cell line and animals

This study used a human medulloblastoma cell line (ONS-76). The establishment and biological characterization were described previously.\(^\text{16,18}\) The ONS-76 cells demonstrate neuron-like characteristics, expressing neuron-specific enolase and neurofilament proteins (145,200 kd). Flow cytometry showed that most ONS-76 cells continuously expressed major histocompatibility complex (MHC) class I antigens, and MHC class II antigens could be induced in vitro after coculture with recombinant human interferon-gamma (200 U/ml) for 48 hours.\(^\text{16}\)

ONS-76 cells were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum at 37°C in a humid incubator under 5% CO\(_2\), and passaged in vitro more than 100 times. Twenty-five female athymic nude mice (BALB/c, nu/nu, 8–12 weeks old; Japan SLC Inc., Hamamatsu, Shizuoka) were used.

II. Leptomeningeal dissemination model

ONS-76 cells were harvested and suspended in phosphate buffer saline (PBS) at concentrations of \(1 \times 10^7, 5 \times 10^6,\) and \(1 \times 10^6\) cells/100 \(\mu\)l. Each 100 \(\mu\)l of cell suspension or saline was percutaneously transplanted into the cisterna magna of nude mice (\(n = 5\)) under ether anesthesia.

The general condition and neurological signs were checked daily, and the median survival time (MST) calculated. The brain and spinal cord with the skull and vertebral column intact from dead mice were
fixed in 10% formalin. After decalcification, coronal, transverse, and longitudinal blocks were cut and embedded in paraffin. Histological sections were prepared from each block and stained with HE.

In another experiment, five mice inoculated with $1 \times 10^7$ tumor cells were sacrificed at 7, 20, and 30 days after inoculation to investigate the expression of MHC antigens on disseminated medulloblastoma cells. Animals were transcardially perfused with 100 ml PBS and the brains removed. Cryostat sections (8-10 µm) were cut and fixed with cold acetone for 10 minutes. MHC class I antigens on disseminated cells were evaluated using the indirect immunoperoxidase method with mouse monoclonal antibodies against human leukocyte antigens (HLA)-A, B, C (Serotec, Oxford, U.K.).

**Results**

I. Leptomeningeal dissemination model

Animals demonstrated severe anorexia and weight loss, and gradually became less active 30 days after tumor inoculation. Pareses of the hind limbs and urinary and rectal incontinence then developed. In the terminal stage, animals were prone with the back hyperflexed and the hind limbs extended.

All five mice died within 65 days of intracisternal inoculation of $1 \times 10^7$ ONS-76 cells (Fig. 1). MST was 56 days. Only one of the five mice receiving $5 \times 10^6$ cells had died 50 days after tumor inoculation. No mouse inoculated with $1 \times 10^6$ ONS-76 cells died within 3 months.

**Fig. 2** Macroscopic appearance of coronal section of the brain from experimental animals, which died of meningeal dissemination. The whole ventricular system including the fourth ventricle (arrow) was severely dilated. There was a tumor mass at the cranial base (arrowheads).

II. Histological findings

Macroscopic examination found various degrees of hydrocephalus and leptomeningeal thickening at the cranial base in all models (Fig. 2). Microscopic examination revealed tumor layers and nodules within the subarachnoid or epidural spaces from the cerebral hemisphere to the cauda equina. In particular, thick tumor layers occurred at the skull base involving major cranial nerves, such as the optic (Figs. 3A and 4A) or trigeminal nerves (Fig. 3B). Heavy infiltration of tumor cells was observed especially in the cistern around the brainstem. The spinal root ganglions were also preferentially involved (Fig. 4B). The tumor cells extended into the perivascular space of the penetrating vessels (Virchow-Robin’s space) (Fig. 4C). Many mitotic figures were observed in the infiltrating cells (Fig. 4D). These histological findings were similar to those in medulloblastoma patients.

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**Fig. 1** Survival curves for murine model after intracisternal inoculation of ONS-76 cells (n = 5). All mice inoculated with $1 \times 10^7$ cells (○) died within 65 days of inoculation. MST was 56 days. Only one mouse receiving $5 \times 10^6$ (●) or less ($1 \times 10^6$, △) tumor cells died within 3 months. □: mice injected with saline.

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III. MHC class I antigen expression on inoculated ONS-76 cells

MHC class I antigens (HLA-A, B, C) were detected on transplanted cells until 20 days after tumor inoculation (Fig. 5A, B). About 30 days after inoculation, expression of MHC class I antigens became almost undetectable (Fig. 5C).

Discussion

Tumor cell dissemination along the CSF pathways is nearly always fatal in medulloblastoma patients despite improved irradiation techniques. The development of an effective therapeutic strategy to prevent meningeal dissemination of medulloblastoma requires animal models to investigate the pathophysiology. However, few animal models for meningeal dissemination using human medulloblastoma cells have been developed because of the difficulties in establishing a medulloblastoma cell line. Present culture techniques can now culture human medulloblastoma cells in vitro, and several useful cell lines
have been reported.\textsuperscript{4,5,10,18} Friedman et al.\textsuperscript{4,6} reported experimental models for human medulloblastoma using TE-671 cells, which, however, were demonstrated to be a subline of human rhabdomyosarcoma.\textsuperscript{15} In 1988, Friedman et al.\textsuperscript{4} reported the first animal models with meningeal involvement using human medulloblastoma cell line (D341 Med). We have further developed murine models for meningeal dissemination using human medulloblastoma (ONS-76) cells. The neurological signs and histological findings in our models were very similar to those in medulloblastoma patients.

ONS-76 cells expressed MHC class I antigens on their cell surface, and also in the subarachnoid space. MHC class I antigens were present until 20 days after tumor inoculation, but expression markedly decreased after 30 days. Around this time, animals began to demonstrate anorexia and weight loss. This suggests that the inoculated tumor cells proliferated rapidly when autologous MHC antigen expression was reduced. In contrast, ONS-76 cells developed with difficulty as subcutaneous xenografts in athymic nude mice. ONS-76 cells easily propagated in the subarachnoid space, which is thought to be an immunologically privileged site. These results suggest that expression of MHC class I antigens results in effective recognition of the disseminating tumor cells by the host immune system,\textsuperscript{1,8,14,17} although immunological reactions in the subarachnoid space may be different from systemic host reactions.\textsuperscript{11}

There may be a relationship between the tumor cell phenotype, such as MHC antigen expression, and occurrence of meningeal metastasis. This model should be useful in investigating the clinical outcome of human medulloblastoma with meningeal dissemination.

References


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