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The effect of immune checkpoint inhibitors on lung metastases of osteosarcoma

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ABSTRACT

Background/purpose: The prognosis of patients with metastases remains unsatisfactory in certain pediatric solid tumors. In this study, we evaluated the efficacy of immune checkpoint inhibitors against such metastases using a murine model of osteosarcoma.

Methods: Murine osteosarcoma LM8 cells were transplanted subcutaneously into C3H mice. The primary tumor lesion was surgically resected 11 days after transplantation. Two hundred micrograms of three antibodies (anti-PD-1, anti-PD-L1, and anti-OX-86) or an isotype antibody were administered intraperitoneally on post-transplantation days 11, 14, 18, and 21. Survival curves were plotted by the Kaplan-Meier method and compared with the log-rank test. Computed tomography (CT) scans were performed on day 11 after tumor transplantation (pre-therapy) and on day 25 (post-therapy). For pathology, 3 mice from each group were euthanized on days 11, 22, and 33 after tumor transplantation.

Results: The antibody-treated group had a significantly longer survival time compared with the control group ($p = 0.002$). Both the CT scan and pathological results revealed suppression of metastatic tumor proliferation in the treatment group as compared with the control group.

Conclusions: These results suggest that immune checkpoint inhibitors may be an innovative therapy for lung metastases of advanced pediatric solid tumors.

In the last 10 years, the outcome of pediatric solid tumors has improved significantly as the result of aggressive combination treatment with chemotherapy, radiation, and surgery. The current overall survival rate for non-metastatic pediatric solid tumors is 75 to 90%. However, 10 to 40% of all children with solid tumors present with lung metastases at the time of diagnosis, particularly for certain pediatric solid tumors including neuroblastoma, rhabdomyosarcoma, and osteosarcoma, and the prognosis of patients with metastases remains unsatisfactory [1,2].

Kuroda et al. suggested that the presence of circulating tumor cells and/or persistent micrometastasis may indicate a significantly higher risk of metastasis [3]. In that study, by using the reverse transcriptase–polymerase chain reaction, they found that the survival rate between patients with either circulating tumor cells or persistent bone marrow micrometastasis, and those with no detectable micrometastasis, were statistically significantly different in neuroblastoma. Therefore, metastases are thought to be a highly important prognostic factor in pediatric solid tumors. Furthermore, an improved therapeutic strategy targeting metastases is urgently needed to improve the outcomes of such tumors.

Immune checkpoint inhibitors have been used as innovative immunotherapies for adult solid tumors including melanoma, lung cancer, and renal carcinoma [4]. However, the efficacy of these agents for pediatric solid tumors, which might be poorly immunogenic, has been little examined. In this study, we evaluated the efficacy of immune checkpoint inhibitors against metastases of pediatric solid tumors by using a highly clinically relevant murine model of osteosarcoma, which is a common pediatric solid tumor in adolescents and young adults.

1. Material and methods

1.1. Mice

Male C3H/He mice (4 weeks old) were purchased from Sankyo Labo Service (Tokyo, Japan). Animal use was approved by the Laboratory Animal Center of Keio University School of Medicine.

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1.2. Cell lines and culture

LM8 was established as a murine osteosarcoma cell line by using eight repeated Fidler's procedures on murine Dunn osteosarcoma originating from the C3H/He mouse with high metastatic potential to the lung. LM8 cells were provided by RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Tumor cells were cultured in complete minimum essential medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 μg/mL of amphotericin B before cell suspensions were prepared and transplanted into mice. Cells were cultured at 37 °C in a fully humidified incubator under 5% CO_2.

1.3. Monoclonal antibodies (mAbs)

Therapeutic anti-PD-1 (clone RMT3–23; catalog #BE0115), anti-PD-L1 (clone 10F.9G2; catalog #BE0101), anti-OX40 (clone OX-86; catalog #BE0031), and control rat IgG2a (clone 2A3; catalog #BE0089) mAbs were purchased from BioXcell (West Lebanon, NH). For immunohistochemistry, purified anti-mouse mAbs specific for CD8α and CD279 were purchased from Biolegend (San Diego, CA).

1.4. Animal experiments

For the animal model with persistent lung metastases, mice were injected subcutaneously in the back with 5.0×10^6 cells in 200 μL of minimum essential medium. Surgical resection of the subcutaneous lesion was performed 11 days after transplantation (Fig. 1A). For the treatment group, 200 μg of three antibodies (anti-PD-1, anti-PD-L1, and anti-OX-86) were administered intraperitoneally on post-transplantation days 11, 14, 18, and 21. For the control group, 200 μg of control rat IgG2a mAb were administered on the same schedule (Fig. 1B).

1.5. Survival analysis

Survival rates of the two groups (10 mice/group) were analyzed using the Kaplan–Meier method and compared with the log-rank test.

1.6. Evaluation of lung metastases

1.6.1. Imaging study

Computed tomography (CT) scan examinations were performed on days 11 (pre-therapy) and 25 (post-therapy) after tumor transplantation.

1.6.2. Pathological study

Three mice of each group were euthanized on days 11, 22, and 33 after tumor transplantation, and their lungs were resected. Lung tissue was fixed in 4% formaldehyde, embedded in paraffin, and evaluated by hematoxylin and eosin staining.

1.6.3. Immunohistochemistry

Lung tissue on day 33 after tumor transplantation was frozen rapidly using OCT Tissue Tek (Sakura Finetek, Tokyo, Japan) and fixed in acetone. Endogenous peroxidases were blocked in 0.3% H_2O_2. Microwave antigen retrieval was performed in Blocking One (Nacalai Tesque, Kyoto, Japan). Then, sections were incubated with primary antibodies against CD8α and CD279 (1:100 dilution) on slides overnight at 4 °C in a moist chamber, followed by incubation for 1 h with an anti-rat secondary antibody conjugated to horseradish peroxidase (1:2000 dilution).

1.7. Statistical analyses

All statistical analyses were performed using SPSS 23.0 (IBM, Chicago, USA). Student's t test was used to compare the statistical difference between two groups. P values less than 0.05 were considered statistically significant.

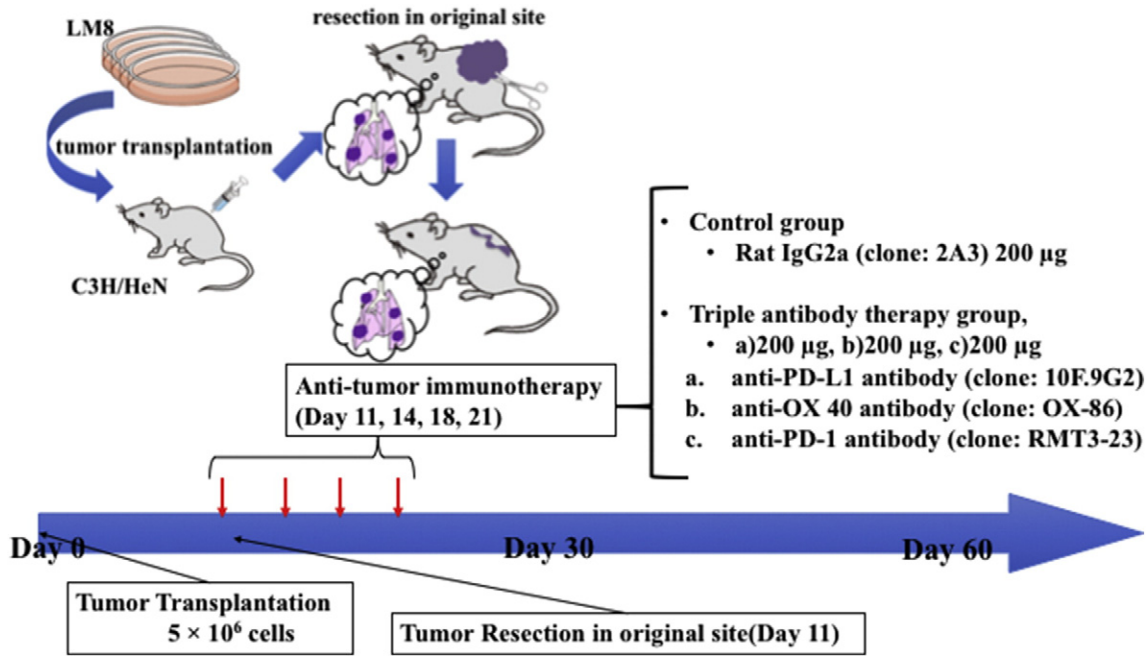

Fig. 1. (A) Preparation of an animal model with persistent lung metastases from a pediatric solid tumor. C3H/HeN mice were injected subcutaneously in the back spaces with 5.0×10^6 LM8 cells in 200 μL of minimum essential medium. The subcutaneous lesion was resected surgically 11 days after transplantation. (B) Triple antibody treatment of persistent lung metastases model mice. For the treatment group, 200 μg of three antibodies (anti-PD-1, anti-PD-L1, anti-OX-86) were administered intraperitoneally on post-transplantation days 11, 14, 18, and 21. For the control group, 200 μg of an isotype antibody were administered.