Transplantation of iPS-Derived Tumor Cells with a Homozygous MHC Haplotype Induces GRP94 Antibody Production in MHC-Matched Macaques

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Abstract

Immune surveillance is a critical component of the antitumor response in vivo, yet the specific components of the immune system involved in this regulatory response remain unclear. In this study, we demonstrate that autoantibodies can mitigate tumor growth in vitro and in vivo. We generated two cancer cell lines, embryonal carcinoma and glioblastoma cell lines, from monkey-induced pluripotent stem cells (iPSCs) carrying a homozygous haplotype of major histocompatibility complex (MHC, Mafα in Macaca fascicularis). To establish a monkey cancer model, we transplanted these cells into monkeys carrying the matched Mafα haplotype in one of the chromosomes. Neither Mafα-homozygous cancer cell line grew in monkeys carrying the matched Mafα haplotype heterozygously. We detected in the plasma of these monkeys an IgG autoantibody against GRP94, a heat shock protein. Injection of the plasma prevented growth of the tumor cells in immunodeficient mice, whereas plasma IgG depleted of GRP94 IgG exhibited reduced killing activity against cancer cells in vitro. These results indicate that humoral immunity, including autoantibodies against GRP94, plays a role in cancer immune surveillance. Cancer Res. 77(21): 6001-10. ©2017 AACR.

Introduction

The establishment of an experimental monkey model of cancer is important to develop new treatments for cancer. Cynomolgus macaques are genetically closer to humans than are mice, and the structures of biologically relevant molecules in monkeys and the organization of the hematopoietic system in macaques are similar to those in humans. Indeed, more than half of the antibodies against human molecules react to molecules of cynomolgus macaques (1, 2). Furthermore, recent genomic studies have revealed that several monkey cytochrome P450s, which work in drug metabolism, are apparently orthologous to human P450s (3). Therefore, a cancer model in cynomolgus macaques may be more useful than a cancer model in mice for preclinical experiments (4, 5). However, spontaneous neoplasms and malignant tumors in cynomolgus monkeys are uncommon (6). In addition, no cancer model in cynomolgus monkeys has been established by transplantation of tumor cell lines as in mouse cancer models because there is no inbred line in cynomolgus monkeys. To solve this problem, we established major histocompatibility complex (MHC) homozygous tumor cell lines of cynomolgus macaques for transplantation to MHC-matched heterozygous monkeys.

We established induced pluripotent stem cells (iPSCs) from fibroblasts of a cynomolgus macaque carrying homozygous MHC genes by introduction of the four Yamanaka factors (Oct3/4, Sox2, Klf4, and c-Myc; refs. 7, 8) and colonies of cynomolgus macaques carrying the identical MHC haplotype heterozygously (7, 9). From an immunological aspect, cells derived from MHC homozygous iPSCs are more acceptable than MHC-mismatched cells by hosts carrying identical MHC genes. This strategy enables the establishment of various types of tumor models transplantable to cynomolgus macaques as various somatic cells have been developed from iPSCs including neural cells, hematopoietic cells, and pancreatic cells (10-14).

We established cancer cell lines from iPSCs of a cynomolgus macaque carrying a homozygous MHC haplotype; however, MHC-matched hosts rejected these cell lines after transplantation. We found antibodies reacting to the cancer cells in plasma of the macaques that rejected transplanted cells. The plasma also reacted to other immortalized cells transduced with oncogenes that were not transplanted. The IgG in plasma recognized glucose-regulated protein (GRP) 94, a kind of heat shock protein that is considered as a target of tumor therapy. GRP94 is a common antigen of oncogene-transduced cells that we
Flow cytometry

Single-cell suspensions of tumor cells and other cells were immunostained with a purified mouse anti-CD30 antibody (Clone: J854, Immunotech #87939), purified rat anti-GRP94 (Clone: 9G10, Abcam #ab2791), purified mouse anti-GRP78 (Clone: C38, ebioscience #14-9768), and purified mouse anti-GRP75 (Clone: JG1, Abcam #ab2799) following by staining with fluorescein isothiocyanate (FITC)-conjugated goat polyclonal anti-mouse IgG (Nordic Immunological Laboratories #GAM/Fab/FITC), cyanine 3 (CY3)-conjugated goat anti-mouse IgG (Abcam #ab7035), and Texas Red (TR)-conjugated goat polyclonal anti-rat IgG (Abcam #ab6843) as secondary antibodies. Dead cells were labeled with 2 μg/ml propidium iodide (Sigma-Aldrich #PI470). In all of the flow cytometry experiments, isotype-matched antibodies corresponding to each antibody were used as controls. The samples were analyzed by a FACS Calibur instrument (Becton, Dickinson and Company) in the Central Research Laboratory of Shiga University of Medical Science.

Detection of antibodies in plasma of the monkeys after transplantation

After heat-inactivation, plasma was diluted 10 times with PBS. Fifty microliters of the diluted plasma was added to the cell suspension and the cells were incubated for 30 minutes on ice. After washing with PBS twice, the cells were incubated with 2 μl of FITC-labeled goat polyclonal anti-monkey IgG for another 30 minutes on ice. The cells were analyzed by flow cytometry after washing with PBS twice.

In vitro killing assay by complement-dependent cytotoxicity

Fifty microliters of each of the inactivated plasma samples diluted at various concentrations or 100 μl of IgG in plasma of monkeys rejecting tumors or 5 μl of monoclonal rat anti-GRP94 (Clone: 9G10, final concentration of 0.05 mg/ml, Abcam) was added to 2 × 10⁶ PTY cells suspended in 100 μl of PBS and incubated on ice for 30 minutes. After washing twice, PTY cells were treated with 500 μl of guinea pig complement (Gibco #19195-015) or normal monkey serum, which were diluted 5 times in a complete medium for 45 minutes at 37°C. Dead cells were labeled with 2 μg/ml propidium iodide (Sigma-Aldrich), and live cells and dead cells were counted using flow cytometry.

ELISPOT assay

Heparinized peripheral blood was collected from the cancer-rejecting monkeys before and after transplantation. After lysing red blood cells, 5 × 10⁵ cells per well were cultured in an ELISPOT plate with PTY cell lysate, which was made from 5 × 10⁶ PTY cells by a freeze and thaw method. The experiments were usually performed in triplicate wells for each condition, but duplicate culture was used when the number of peripheral blood cells was not sufficient. After culture for 3 days, the number of interferon (IFN)-γ spots was counted by the analyzer Immunospot (Cellular Technology Limited). Stimulation indices (SI) were calculated by the following formula: number of spots in culture of the blood cells plus tumor lysis/ number of spots in culture of the blood cells only.

Purification of IgG fraction in plasma of cancer-rejecting monkeys

The IgG fraction in plasma of cancer-rejecting monkeys was purified using protein G-Sepharose (GE Healthcare UK Ltd. #28903134). Briefly, monkey plasma diluted in PBS at one hundred times was passed through protein G-Sepharose after treatment with a binding buffer (20 mmol/L sodium phosphate, pH 7.0). Trapped IgG fractions were recovered by an elution buffer (0.1 M glycine-HCl, pH 2.7), and eluted IgG fractions were immediately neutralized by 400 μl of 1 M Tris-HCl, pH 9.0. The eluted buffer was exchanged with PBS by dialysis in cellulose membrane tubes (EDTA Co. Ltd. #UC27-32-100).

SDS-PAGE and Western blot analyses

A plasma membrane protein extraction kit (101Bio, ICCP3031) was used to purify plasma membrane proteins from PTY cells according to the manufacturer’s instructions. Recombinant human GRP94 protein (ProSpec-Tany TechnoGene Ltd. #1HSP091), recombinant GRP78 protein (StressMarq BioScience Inc. #SPR-107), and PTY plasma membrane proteins precipitated by plasma IgG of the cancer-rejecting monkeys were loaded into 10% SDS slab gels at a concentration of 0.1 mg/ml in SDS-PAGE sample buffer (60 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue, 1% mercaptoethanol).

After separation by 6% to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, proteins were electrotransferred to Immobilon-P Transfer Membranes (Millipore #IPW0010) by a wet transfer apparatus. The membranes were blocked in Tris-buffered saline containing 5% nonfat milk and 0.01% Tween-20 at room temperature for 1.5 hours and then incubated with the plasma IgG of the cancer-rejecting monkeys, mouse monoclonal anti-GRP78 antibody (clone C38), or mouse monoclonal anti-GRP74 antibody (clone 21H3, Abcam #ab63469) overnight at 4°C. After washing the membranes were incubated at room temperature for 1 hour with HRP-conjugated goat polyclonal anti-monkey IgG (Nordic Immunological Laboratories #GANon/IgG(FG)PO) at 1:1,000 dilution in RIA buffer supplemented with 5% milk powder. HRP activity on the membranes was developed with Western Blotting Luminal Reagent (Millipore #WBLR0500) according to the manufacturer’s instructions.

Detection of an antibody specific for GRP94 and GRP78 by ELISA

Recombinant GRP94 protein (0.5 μg/ml) or recombinant GRP78 protein (0.5 μg/ml) was seeded in 96-well flat-bottom assay plates (Coaster #3368). After blocking with 3% BSA in PBS, diluted monkey plasma or the IgG fraction in the plasma of monkeys rejecting tumors was added. After 2-hour incubation at room temperature, the plates were washed 6 times with PBS containing 0.05% Tween 20. HRP-conjugated goat polyclonal anti-monkey IgG was incubated for 2 hours at room temperature. After washing 6 times with PBS containing 0.05% Tween 20, HRP activity was assessed using 3, 3′, 5, 5′-tetramethyl benzidine substrate (100 μl). The reaction was stopped by the addition of 1 M hydrogen chloride (100 μl). Optical density (OD) was measured at 450 nm and 620 nm. Results are shown after subtraction of OD at 620 nm from OD at 450 nm. The experiments were performed in triplicate wells for each condition.

Absorption of an antibody specific for GRP94 from the IgG fraction in plasma of monkeys rejecting tumors

Fifty microliters of recombinant GRP94 protein (10 mg/ml) was seeded in 96-well flat-bottom assay plates (Coaster #3368). PBS was used for a control instead of recombinant GRP94.