Effects of dendritic cells transfected with full length wild-type p53 and modified by bile duct cancer lysates on immune response

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BACKGROUND: **Dendritic cells (DCs) are the most potent antigen-presenting cells and are actively used in cancer immunotherapy. Wild-type p53 can be recognized as an antigen and can induce specific cytotoxic T lymphocytes (CTLs) in the host body. The aim of this study was to investigate the effects of DCs transfected with full length wild-type p53 and modified by bile duct lysates on immune response.**

METHODS: **The wild-type p53 was transducted to DCs with adenovirus, which were modified by bile duct lysates (Lywtp53DC). The concentration of the surface molecules (B7-1, B7-2, MHC-I, MHC-II) of all DCs was detected with fluorescence activated cell sorter (FACS), and the ability of the DCs to induce efficient and specific immunological response in anti-^{51}Cr-labeled target cells was studied. BALB/c mice infected with the DCs and QBC939 were used. CTL response in mice immunized with Lywtp53DC and treatment of tumor-bearing mice with Lywtp53DC and CTL response in these mice were studied.**

RESULTS: **The surface molecules of Lywtp53DC had a high expression B7-1 (86.70%±0.07%), B7-2 (18.77%±0.08%), MHC-I (87.20%±0.05%), MHC-II (56.70%±0.07%) with FACS. The T lymphocytes had a specific CTL lysing ability induced by Lywtp53DC, with a CTL lysis rate of 81%. The immune protection of Lywtp53DC group was obvious, and the tumor diameter of the Lywtp53DC group was 3.10±0.31 mm, 2.73±0.23 mm, 3.70±0.07 mm on days 13, 16 and 19, smaller than those of any control groups (P<0.05), DC, wtp53DC and LyDC. On the other hand, the growth rate of tumor of the Lywtp53DC group was slower than that of any other groups (P<0.05).**

CONCLUSION: **Dendritic cells transfected with wild-type p53 and modified by bile duct lysates have specific CTL killing capability.**

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KEY WORDS: **lysate; bile duct cancer; dendritic cells; full-length wild-type p53; lymphocytes**

Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells that are actively utilized in cancer immunotherapy.[1-3] Wild-type p53 can be recognized as an antigen that can generate specific cytotoxic T lymphocytes (CTLs). It is an effective method to immunize the body with p53 in p53-overexpressing tumor cells.[4-7] p53-based immunization is an attractive approach to cancer immunotherapy due to the accumulation of p53 protein in bile duct cancer cells.[8,9] In this study, we detected the effects of dendritic cells transfected with full-length wild-type p53 and modified by bile duct cancer lysates on immune response and tried to make DC induce an efficient and specific anti-tumor immunological response.

Methods

Animals and cell lines

Six 8-week-old female BALB/c mice were purchased from Hubei Animal Center and raised in pathogen-free units of Tongji Hospital Experiment Centre. Bile duct cancer cells (QBC939) were obtained from the third Military Medical University, China and were cultured in complete culture medium (CCM) containing RPMI 1640 supplemented with 25 mmol N-2-hydroxyethyl piperazine ethanesulfonic acid (HEPES), 10mmol L-glutamine, 10% fetal calf serum (FCS), and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B). It was a relatively immunogenic tumor that carries a mutant endogenous p53 gene. P815 mastocytoma cells were obtained from American type culture collection (ATCC) and cultured in CCM. The

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cells were maintained at 37 ℃ in a humidified atmosphere of 5% CO_2.

Reagents

Adenovirus (Ad)-mp53 was constructed by cloning the 1.5 kb murine p53 cDNA (Sigma, USA) into pAd1/CMV containing cytomegalovirus (CMV) promoter and bovine growth hormone poly A signal sequence. This plasmid was cotransfected with pBHG10 into 293 cells. Recombinant adenovirus was selected after PCR analysis of individual plaques. Control adenovirus (Ad-c) was prepared by deletion of E1 region from adenovirus serotype.

FITC-labeled mouse anti-mouse p53 antibody and isotype mouse IgG2a were purchased from Serotec Company. FITC-labeled anti-mouse CD11c antibody and isotype mouse IgG2ak, PE-labeled anti-mouse I-Ad, anti-mouse B7-1, B7-2, MCH-Ⅰ, MCH-Ⅱ antibody and isotype mouse IgG2ak, as well as hamster anti-mouse CD40 (HM40-3) monoclonal antibody and anti-hamster IgM were purchased from Sigma Company, USA.

Generation of DCs

DCs were processed from bone marrow of naive syngeneic mice and then cultured in CCM supplemented with 20 ng/ml murine GM-CSF, 10 ng/ml IL-4, and 50 μmol 2-mercaptoethanol. The cells were cultured at 37 ℃ in a humidified atmosphere of 5% CO_2. Half of the medium was replaced 3 days later. After culture for 5 or 6 days, the cells were collected and enriched by centrifugation over a 13.5% metrizamide gradient. The purity of the DC fraction was higher than 80% as determined by FACS analysis of the expression of surface molecules (B7-1, B7-2, MCH-, MCH-Ⅱ).

Infection of cells with adenovirus

DCs were infected with Ad-c or Ad-p53 (10000 viral particles per cell) for 90 minutes in 0.5 ml serum-free RPMI 1640 medium supplemented with 20 ng/ml GM-CSF and 10 ng/ml IL-4 in 24-well plates followed by culturing in CCM with the same cytokines for 48 hours. This dose was selected after some preliminary experiments, and did not affect cell viability (95% cells viable after staining of trypan blue).

Preparation of lysate and DCs loaded with tumor cell lysate

DCs were washed twice in PBS and incubated with 14 μg/ml hamster anti-mouse CD40 monoclonal antibody for 25 minutes on ice, then washed in RPMI 1640 twice and cultured in 1 ml CCM supplemented with 3.5 μg/ml anti-hamster IgM, 20 ng/ml GM-CSF and 10 ng/ml IL-4 overnight. Four kinds of DC: DC, wtp53DC, LyDC, and Lywtp53 were collected and loaded with tumor cell lysates.

FACS analysis of four kinds of expression of DC surface molecules

DCs were estimated by intracellular staining and then flow cytometry was carried out. The cells were washed in PBS twice, fixed by 0.25% paraformaldehyde solution for 30 minutes on ice, washed in PBS, permeabilized by 0.2% Tween 20 for 15 minutes at 37 ℃, washed in PBS, incubated with FITC-labeled mouse anti-mouse p53 antibody or isotype mouse IgG2a for 25 minutes on ice, washed twice in PBS, and analyzed by FACS caliber.

To study the expression of surface molecules, DCs were washed twice in PBS, incubated with 1 μg/10^6 cells FITC-labeled anti-mouse CD11c antibody and PE-labeled anti-mouse I-Ad or anti-mouse B7-1, B7-2, MCH-Ⅰ, MCH-Ⅱ antibody for 25 minutes on ice, washed twice in PBS and analyzed by flow cytometry. Non-specific binding was estimated using FITC-labeled isotype mouse IgG2a-k and PE-labeled isotype mouse IgG2b-k.

Lywtp53DC of immune protection and treatment in mice

DCs were generated from bone marrow of BALB/c mice and infected with DCs as described above. Forty-eight hours later the cells were washed for three times in PBS and injected to BALB/c mice subcutaneously (2×10^5 DCs/mouse) three to four times with a 10-14-day interval. Seven days after the last immunization, QBC939 tumor cells (3.5×10^5 cells/mouse) were inoculated subcutaneously, and then the tumor size in the mice was observed.

The same tumor model was used to evaluate the effect of treatment. Five hundred thousand QBC939 cells were inoculated subcutaneously into the shaved backs of BALB/c mice. The treatment was started when tumors reached 4-6 mm in diameter (day 7). The mice were treated with these DCs prepared as described above. The treatment was repeated four times with a 5-6-day interval. Tumor sizes were measured every 3-5 days for 4 weeks.

CTL assay

Splenocytes (effector cells) freshly isolated or restimulated for 7 days with 4 kinds of DCs were mixed with different Cr-labeled target cells: QBC939 cells preincubated for 48 hours with 5 ng/ml murine IFN-γ or p815 cells infected with Ad-p53 or Ad-c. After 6-hour incubation and harvesting of supernatants, the radioactivity was measured by a gamma-counter. To estimate the maximum ^{51}Cr-release, 1% triton X-100 was used. The percentage of cell lysis was calculated (experimental release-spontaneous release/maximum release-spontaneous release) ×100%.

Table 1. Expression of surface molecules in 4 kinds of DCs after modification with lysate (%)

Surface molecules	DC	wtp53DC	LyDC	Lywtp53
B7-1	36.70±0.07	61.77±0.08	67.20±0.05	86.70±0.07 *
B7-2	5.27±0.01	9.90±0.04	9.13±0.03	18.77±0.08 *
MCH-I	44.03±0.04	69.13±0.05	68.10±0.03	87.20±0.05 *
MCH-II	17.13±0.12	31.10±0.31	32.73±0.23 *	56.70±0.07 *

*: $P<0.05$. Lywtp53 group compared with other groups

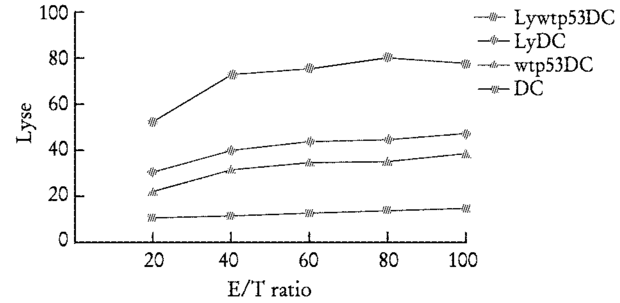

Fig. The lyse ability of DCs.

Table 2. Immune protection of DCs (tumor size: mm)

Group	day 10	day13	day16	day19
Control	3.72±0.01	6.70±0.02	7.10±0.09	9.90±0.02
DC	3.70±0.01	5.70±0.08	6.10±0.04	9.70±0.08
wtp53DC	3.07±0.01	4.90±0.04	5.13±0.03	6.22±0.08
LyDC	4.03±0.04	4.13±0.05	6.10±0.03	7.77±0.05
Lywtp53DC	3.13±0.02	3.10±0.31	2.73±0.23	3.70±0.07 *

*: $P<0.05$. On day 16, 19, the Lywtp53 compared with other groups.

Table 3. Immune treatment of DCs (tumor size: mm)

Group	day7	day12	day15	day18
Control	5.72±0.02	7.88±0.09	9.20±0.11	11.90±0.09
DC	4.70±0.03	7.71±0.08	9.02±0.04	10.70±0.68
wtp53DC	5.03±0.09	6.90±0.09	7.13±0.11	8.29±0.05
LyDC	5.06±0.01	6.13±0.08	7.10±0.05	8.77±0.03
Lywtp53DC	5.19±0.09	5.10±0.39	6.73±0.66	6.79±0.77 *

*: $P<0.05$. On day 18, Lywtp53DC compared with other groups.

Results

The increased expression of surface molecules of lywtp53DC was detected with fluorescence activated cell sorter (FACS): B7-1 (86.70%±0.07%), B7-2 (18.77%±0.08%), MCH-I (87.20%± 0.05%), MCH-II (56.70%± 0.07%) (Table 1).

To induce an immune response against the wild-type p53, we started with three immunizations of BALB/c mice with DCs generated from bone marrow progenitors of syngeneic mice and transduced with Ad-p53 loaded with lysates as described in the section of Methods. A statistically significant difference was found between mice immunized with Lywtp53 DCs and control groups in tumor formation, and there was no difference between wtp53DC and LyDC (Fig.).

The presence of QBC939-specific CTLs was evaluated in mice immunized with activated Ad-p53-transduced DCs. Six-hour standard CTL assay was performed one month and a week after the last immunization (one month after inoculation of QBC939 cells into the immunized mice). Freshly isolated splenocytes were mixed with ^{51}Cr-labeled QBC939 cells at different ratios. Splenocytes from mice injected with Ad-c-infected DCs demonstrated no ability to lyse target cells, whereas those from mice immunized with Ad-p53-transduced DCs demonstrated low, but clearly significant cytotoxicity against QBC939 cells (Tables 2 and 3).

Treatment of tumor-bearing mice with Ad-p53-transduced DCs and CTL response in those mice showed that in the Lywtp53DC group, the developing speed of tumor was slower than that in other groups.

Discussion

p53 protein is an attractive target for immunotherapy of cancer.[10-12] Normal cells have a very low level of p53, whereas accumulation of this protein because of mutations or functional inactivation is observed in 50% of human malignancies. This provides, in theory, potential targets for CTLs that recognize 8-9 amino acid, class I MHC-bound epitopes.[12-14]

In this study, we have demonstrated that activated DCs transduced with full-length wild-type p53 loaded with lysates are able to break tolerance to this self-protein and induce potent antitumor response with no detectable autoimmune abnormalities. Wild-type, p53-derived, self-MHC-self-peptide complexes expressed by bone marrow-derived cells in the thymus cause negative selection of immature thymic T cells with a high avidity for such complexes.[15,16] This results in deletion of T cells with sufficient avidity to recognize natural wild-type p53 epitopes presented by MHC class I molecules on tumor cells, thus preventing immune response. Only CTLs with a low avidity survive the induction of self-tolerance.[17-20]

We suggest here another method of immunotherapy based on the use of full-length, wild-type p53. This approach may be devoid of many limitations of peptide-based immunization and would provide a valuable option for clinical trials. Overexpression of the wild-type p53 in antigen-presenting cells would allow for presentation of several different epitopes.[21-23] The feasibility of such an approach was shown previously in model experiments where each of the different minimal epitopes combined to a single fusion protein can be presented separately on the cell surface and be recognized by specific CTLs.[24,25]

This study demonstrated that the wild-type p53 was transducted to DCs with adenovirus, and the DCs were modified by bile duct cancer lysate. T lymphocytes have a specific CTL lyse ability induced by Lywtp53DC loaded with lysate, with a CTL lyse rate of 81%. The surface molecules of Lywtp53DC showed high expressions of B7-1 (86.70%±0.07%), B7-2 (18.77%±0.08%), MCH-I (87.20%±0.05%), MCH-II (56.70%±0.07%) with FACS. There were significance between the diameter of tumor of the Lywtp53DC group and that of any other group. In the Lywtp53DC group, the developing speed of tumor was slower than that in any other group ($P < 0.05$). These findings showed that Lywtp53DC has an ability to provide immune protection and treatment in mice, especially when loaded with lysate.

Because of a polyclonal nature of T cells generated after two rounds of stimulations with p53DC, it is possible that some level of cytotoxicity against tumor cells could be mediated by alloreactivity.[26,27] When the DC was loaded with lysate of tumor, the lysate will be presented to antigen presenting cell (APC), and the process can increase the immune ability in the body. T cells recognize and induce CTLs, while enhancing the specificity of CTLs.[28-30]

In conclusion, these data indicate that the DCs transduced with full-length wild-type p53 loaded with lysate are able to generate a CTL response specific for tumors with p53 overexpression. This response can be induced not only in healthy volunteers but also in patients with advanced cancer. These findings demonstrate that this approach may overcome the tolerance to self-protein and may serve as a valuable option for cancer immunotherapy.

Competing interest

The author or authors do not choose to response to the statements listed in Instructions for Authors.

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It is impossible to be a good surgeon if one is not familiar with the foundations and general rules of medicine [and] it is impossible for anyone to be a good physician who is absolutely ignorant of the art of surgery.

Henri de Mandeville (c1260-c1320)