Investigational New Drugs (2019) 37:1036–1043
https://doi.org/10.1007/s10637-018-00719-7

PRECLINICAL STUDIES

Bispecific anti-CD3 x anti-B7-H3 antibody mediates T cell cytotoxic ability to human melanoma in vitro and in vivo

Juan Ma[1,2,3] · Tengfei Shang[4] · Pan Ma[5] · Xin Sun[1,3] · Jin Zhao[1,2,3] · Ximing Sun[1,2] · Man Zhang[1,2,3]

Received: 8 November 2018 / Accepted: 25 December 2018 / Published online: 1 February 2019

Summary

Inhibition of the B7-H3 immune checkpoint is reported to limit the tumor growth of B7-H3$^+$ tumors. In this study, we demonstrated B7-H3 expression in human melanoma cells, including a primary culture and several cell lines. Furthermore, we investigated whether B7-H3 could serve as a target for T cell-mediated immunotherapy against melanoma. The cytotoxic capacity of activated T cells (ATCs) armed with an anti-CD3 x anti-B7-H3 bispecific antibody (B7-H3Bi-Ab) to melanoma cells was measured using a bioluminescent signal through a luciferase reporter on tumor cells. In contrast to unarmed ATCs, B7-H3Bi-Ab-armed ATCs exhibited increased cytotoxicity against melanoma cells at effector/target ratios from 1:1 to 20:1. Moreover, B7-H3Bi-Ab-armed ATCs secreted more interferin-gamma (IFN-γ), accompanied by higher levels of activating marker CD69 and CD25 expression. Infusion of B7-H3Bi-Ab-armed ATCs suppressed melanoma growth in a xenograft mouse model. Taken together, our results indicate that B7-H3Bi-Ab-armed ATCs may be a promising approach to immunotherapy for melanoma patients.

Keywords Melanoma · B7-H3 · Bispecific antibody · Immunotherapy

Introduction

Melanoma is a malignant tumor of skin cancer with increasing incidence and a poor prognosis [1]. Advanced melanoma is characterized by a broad spectrum of metastasis and the resistance to traditional treatment, including surgery, radiotherapy and chemotherapy. Efforts to develop new therapeutic approaches to improve the melanoma outcome are currently under investigation [2]. Adoptive T cell therapy and immune checkpoint blockade of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and PD-1 ligand (PD-L1) have shown great promise in recent years [3, 4].

Another promising approach is to block the immune checkpoint B7-H3, also known as CD276, a type I transmembrane glycoprotein that belongs to the B7/CD28 immunoglobulin superfamily [5]. Although receptor interaction with B7-H3 remains unknown, it is supposed to be a coinhibitory receptor in human T cells [6]. B7-H3 protein expression is lower in normal tissues but higher in a wide range of human malignancies; despite conflicting effects of B7-H3 observed in some tumors, most investigations have demonstrated that B7-H3 inhibits host immune cells, especially T cells, in cancer patients [7, 8]. In addition to immune evasion, B7-H3 has been implicated in tumor proliferation, apoptosis, adhesion, angiogenesis, invasion, and metastasis [9]. Overexpression of B7-H3 is usually associated with a worse clinical outcome and a poor prognosis [8, 9]. In melanoma patients, B7-H3 expression is significantly correlated with cancer progression and poor survival, and B7-H3 is involved in many metastasis-associated pathways [10, 11]. Furthermore, B7-H3 decreases the sensitivity to therapies, including dacarbazine chemotherapy and small-molecule inhibitors targeting the MAP kinase and AKT/mTOR pathways [12].

✉ Juan Ma
majuan96@sina.com

✉ Man Zhang
zhangman@bjsjth.cn

1 Clinical Laboratory Medicine, Beijing Shijitan Hospital, Capital Medical University, 10 Tieyi Road, Haidian District, Beijing 100038, China

2 Clinical Laboratory Medicine, Peking University Ninth School of Clinical Medicine, Beijing 100038, China

3 Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics, Beijing 100038, China

4 Medical Experimental Centre, China Academy of Chinese Medical Sciences, Beijing 100700, China

5 Key Laboratory of Protein and Peptide Pharmaceuticals, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Because the B7-H3 binding partner is unknown, no blocking mAbs against human B7-H3 are available. In tumor models, targeting B7-H3 with its specific mAb or inoculation of tumor cells into B7-H3 KO mice inhibits tumor development dependent on $CD8^+$ and NK cells [13]. Currently, a phase I trial of anti-B7-H3 antibody (MGA271) administration, through potent ADCC, is underway in the treatment of multiple B7-H3-positive refractory solid tumors, including melanoma [14]. Combining anti-B7-H3 with other biologics, the administration of anti-B7-H3-specific antibody with ^{131}I-radiolabeled 8H9 has demonstrated efficacy for the treatment of neuroblastoma patients [15]. These results indicate that B7-H3 in melanoma is an attractive target for immune-based antitumor therapies.

Bispecific antibodies (Bi-Abs), one arm recognizing a tumor-specific antigen (TSA) and the other binding to immune cells such as T cells and NK cells, have shown encouraging results in experimental and clinical studies [16, 17]. Recently, in a xenograft mouse model, an increase in the cytotoxic activity of T cells armed with an anti-CD3 x anti-B7-H3 bispecific antibody (B7-H3Bi-Ab) against lung cancer and cervix cancer has been observed [18]. Based on our previous results, considering advanced melanoma characterized with a broad pattern of metastasis, we wanted to investigate in more detail whether B7-H3 could serve as a target for T cell-mediated immunotherapy. In this study, human melanoma cells were demonstrated to express high levels of B7-H3 protein on the surface. Because adoptive T cell therapy is one of the best available treatments for patients with metastatic melanoma, activated T cells armed with B7-H3Bi-Ab were directed to kill melanoma cells in vitro and in vivo.

Materials and methods

Cell lines, preparation of activated T cells (ATCs), and mice

The following cell lines were cultured in RPMI 1640: a primary human melanoma cell culture, OCM-1, OMM-1, and 92–1 human melanoma, and the B16-F10-luc cell line (Shanghai Genomics Inc., Shanghai, China). The human melanoma cell lines Malme-3M, Mel 624, Mel 888 and SKMel 28 (were obtained from ATCC) were cultured in DMEM. Malme-3M-luc, a human melanoma cell line harboring a luciferase reporter gene, was generated by us previously [19]. The media were supplemented with 10% FCS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. All agents for cell culture were from Gibco Company (Gaithersburg, MD, USA). Peripheral mononuclear blood cells (PBMCs) were isolated using Ficoll density gradient centrifugation from healthy donors supplied by Beijing Blood Bank. Additionally, ATCs were obtained by the stimulation of PBMCs with anti-CD3 mAb (eBioscience, San Diego, CA, USA) and anti-CD28 mAb (eBioscience) in the presence of recombinant human IL-2 (Peprotech, Rocky Hill, NJ, USA) as described previously [18, 19]. SCID-Beige mice (8 to 10 weeks) were purchased from Vital River Laboratories (VRL, Beijing, China).

Synthesis of anti-CD3 x anti-B7-H3 bispecific antibody (B7-H3Bi-Ab) and arming of ATCs

An anti-B7-H3 mAb (R&D Systems, Minneapolis, MN, USA) was reacted with sulfo-SMCC, and anti-CD3 (OKT3, eBioscience) was reacted with Traut's reagent as described previously [18]. Cryopreserved ATCs were thawed and were usually armed with B7-H3Bi-Ab at a concentration of 50 ng/10^6 cells at room temperature for 30 min, followed by washing the cells to eliminate unbound antibodies. The combination of OKT3 (50 ng/10^6cells) with anti-B7-H3 mAb (50 ng/10^6cells) preincubated with activated T cells was used as control unarmed ATCs.

Flow cytometry analysis

To detect the expression of B7-H3 on the cell surface, melanoma cells were incubated with PE-labeled-anti-human-B7-H3 mAb (R&D Systems). To detect B7-H3Bi-Ab bound to $CD3^+$ cells, ATCs were incubated with B7-H3Bi-Ab or the combination of OKT3 and anti-human-B7-H3 (used as the negative control for B7-H3Bi-Ab), and then the bound CD3 moiety of B7-H3Bi-Ab to ATCs was evaluated using PE-labeled anti-mouse-IgG1 to detect the B7-H3 moiety of Bi-Ab. To detect CD69 and CD25 expression on ATCs, the floating cells from Malme-3M and ATC coculture were incubated with anti-CD3-FITC (UCHT1; eBioscience) and anti-CD69-PE (eBioscience) or anti-CD25-PE (eBioscience). The cells were washed three times and assayed using the Guava Easycyte flow cytometer (Guava Technologies, Hayward, CA), and the data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA). The mean fluorescence intensity (MFI) was calculated by dividing the MFI value stained with control IgG from the cells stained with specific Ab.

In vitro cytotoxicity assay

The cytotoxicity of T cells against target cells was measured using the luciferase quantitative assay. Briefly, Malme-3M-luc cells or B16-F10-luc cells were seeded (10^4/well) into 96-well round-bottomed microplates in triplicate cocultured with B7-H3Bi-Ab-armed ATCs or unarmed ATCs at various effector-to-target ratios (E/T)—1:1, 5:1, 10:1, and 20:1—for 18 h. A final concentration of 0.15 mg/ml of D-luciferin (SynchemChemie, Kassel, Germany) was added to each well, and the bioluminescence imaging signals were measured using the Xenogen IVIS system (Caliper Life Science). The specific cytotoxicity by B7-H3Bi-Ab-armed ATCs was determined in photons per second.

IFN-γ ELISA assay

Melanoma cells were seeded (10^4/well) into 96-well round-bottomed microplates in triplicate cocultured with B7-H3Bi-Ab-armed ATCs or unarmed ATCs at an E/T ratio of 10:1 for 18 h. Next, the supernatants were collected, and IFN-γ was quantified using a human IFN-γ ELISA kit (eBioscience) according to the manufacturer's instructions. The plate was read using an ELISA reader (ELx808; Bio-Tek Instruments Inc., Houston, TX) with a 450-nm filter. The absorbance obtained from the standards was plotted, and values were calculated.

In vivo antitumor effect of B7-H3Bi-armed ATCs in the mouse model

SCID-Beige mice were engrafted subcutaneously with Malme-3M-luc cells on day 0. The mice were then divided into two groups randomly and were treated with B7-H3Bi-Ab-armed ATCs or control unarmed ATCs subcutaneously on day 1, day 3 and day 5. In vivo bioluminescence imaging was performed on day 1, day 7, day 16 and day 20 after tumor inoculation using the Xenogen IVIS-100 imaging system and living image software. The mice were injected by i.p. with D-luciferin (15 mg/ml) suspended in 100 μl of PBS, and the exposure conditions (time, aperture, stage position, binning, and time after injection) were kept identical in all measurements.

Statistical analysis

All experiments were repeated at least twice and mostly three times. The data were analyzed using Graphpad Prism 5 software (Graphpad, La Jolla, CA) and were presented as means ± SD. Unpaired Student's *t* test (two-tailed) was used to compare two groups where appropriate. $P < 0.05$ was considered significant compared with the control group. Significance was denoted by an asterisk in the figures.

Results

B7-H3 expression in human melanoma cells

The surface expression of B7-H3 on human melanoma cells was assessed by FACS analysis. As shown in Fig. 1, the mean fluorescence intensity (MFI) values obtained using anti-human B7-H3 mAb staining divided by the mouse IgG1 isotype control staining were indicated in the upper right of the histogram, and high B7-H3 expression was detected in all human melanoma cells, including the primary culture and cell lines OCM-1, 92–1, OMM-1, Malme-3M, Mel 624, Mel 888, and SKMel 28. However, the anti-human B7-H3 mAb could not react with B7-H3 molecules on the surface of the mouse melanoma cell line B16-F10 (data not shown), indicating its binding specificity for human B7-H3 molecules.

Cytotoxic effects of B7-H3Bi-Ab-armed ATCs on melanoma cells

Anti-human B7-H3 mAb was hetero-conjugated using the anti-human CD3 mAb (OKT3) chemically named B7-H3Bi-Ab, and the binding specificity of B7-H3Bi-Ab was tested with positive-stained cells detected in 98% of the ATC population (Fig. 2a). ATCs were incubated with B7-H3Bi-Ab or the mixture of OKT3 and anti-B7-H3 mAb as the control, and then the anti-B7-H3 moiety of the B7-H3Bi-Ab was confirmed using the PE-anti-mouse IgG1 mAb. The scheme for the flow cytometry-based binding assay for B7-H3Bi-Ab is demonstrated in Fig. 2b. Next, the amount of B7-H3Bi-Ab required to arm ATCs against melanoma cells was examined (Fig. 2c). ATCs were armed with B7-H3Bi-Ab ranging from 5 to 500 ng/10^6 cells, and the cytotoxic effect of B7-H3Bi-Ab-armed ATCs on Malme-3M-luc cells was tested in vitro. After 18 h of incubation with B7-H3Bi-Ab-armed ATCs, the bioluminescence imaging signal expressed in photons per second was converted into living cell numbers, and the cytotoxic assay was calculated at the E/T ratio of 5:1. The cytotoxicity was almost 60% at the concentration of 50 ng of B7-H3Bi-Ab/10^6 ATCs and did not increase significantly until the concentration of 500 ng of B7-H3Bi-Ab/10^6 cells. Considering that 50 ng and 500 ng/10^6 cells showed similar cytotoxicity, ATCs armed with 50 ng of B7-H3Bi-Ab/10^6 cells were determined for all subsequent experiments.

Next, the cytotoxic assay was performed at E/T ratios from 1:1 to 20:1 for 18 h, and ATCs mixed with individual OKT3 and anti-B7-H3 mAb were used as unarmed controls. As shown in Fig. 2d, an increasing E/T ratio was correlated directly with cytotoxicity both in B7-H3Bi-Ab-armed ATCs and unarmed-ATC effectors. The cytotoxicity with armed ATCs was significantly greater than that with unarmed effectors at each E/T ratio. Moreover, the B7-H3Bi-Ab-armed ATC-mediated cytolysis was blocked by anti-B7-H3 mAb, which confirmed B7-H3-specific killing. By contrast, no difference was found in the cytotoxicity against B16-F10-luc cells between B7-H3Bi-Ab-armed ATCs and unarmed ATCs (Fig. 2e).

Cytotoxic effects of B7-H3Bi-Ab-armed ATCs on melanoma cells with cytokine production

To analyze the levels of T cell-derived cytokines involved in cytotoxicity, cell supernatants were analyzed for IFN-γ

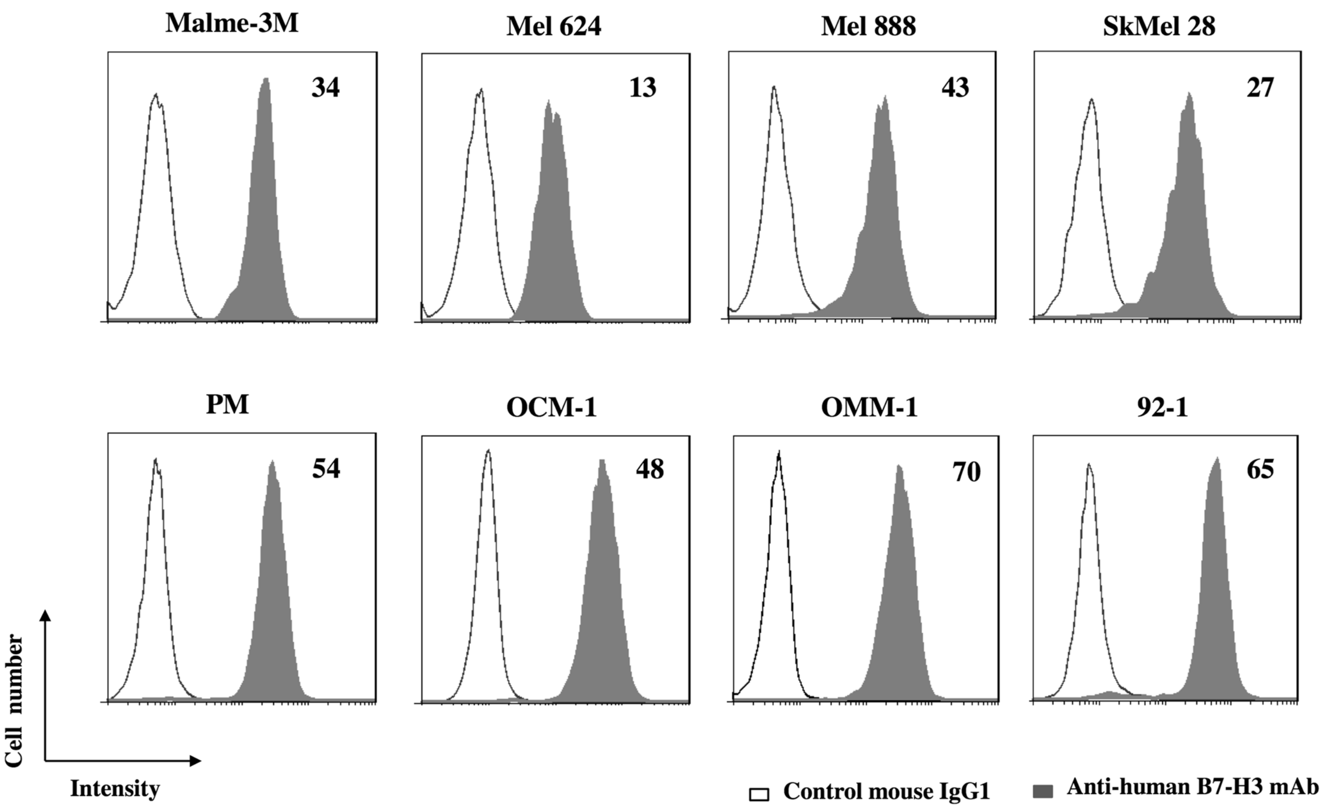

Fig. 1 Expression of B7-H3 on different human melanoma cells. Surface expression of B7-H3 was evaluated by flow cytometry on human melanoma cell lines (OCM-1, OMM-1, 92–1, Malme-3M, Mel 888, SKMel 28 cell, and Mel 624) and primary melanoma (PM) cells. Shaded histograms represent cells stained with anti-human B7-H3 mAb, and open histograms represent cells stained with isotype mouse IgG1 as the control. Mean fluorescence intensity (MFI) values obtained from anti-B7-H3 mAb staining divided by the control mouse IgG1 staining are indicated. A representative experiment is shown from the three experiments

production at an E/T ratio of 10:1. As shown in Fig. 3a, significantly more IFN-γ secretion was observed from B7-H3Bi-Ab-armed ATCs than from their unarmed control ATC counterparts when ATCs were cocultured with human melanoma cells, including the primary culture and OCM-1, 92–1, OMM-1, Malme-3M, Mel 624, Mel 888, and SKMel 28 cell lines. Meanwhile, as shown in Fig. 3b, real-time photographs demonstrated that B7-H3Bi-Ab-armed ATCs, but not unarmed control ATCs, aggregated with melanoma cells in culture, clustering around the edge of the target cell bulk, showing specific activation of B7-H3Bi-Ab-armed ATCs. Moreover, FACS analysis of B7-H3Bi-Ab-armed ATCs showed increased CD69 and CD25 expression over their unarmed ATC counterparts after 18 h of incubation with target Malme-3M cells, respectively. (Figure 3).

B7-H3Bi-Ab-armed ATCs inhibit melanoma growth in SCID-beige mice

To further determine whether B7-H3Bi-Ab-armed ATCs could suppress tumor growth in vivo, SCID-Beige mice were engrafted subcutaneously with Malme-3M-luc cells on day 0. The mice were then divided into two groups randomly and were treated with B7-H3Bi-Ab-armed ATCs or control unarmed ATCs subcutaneously on day 1, day 3 and day 5. The mice were monitored by bioluminescent imaging on the indicated day, and three representative mice of each group were shown (Fig. 4a). The mice treated with control unarmed ATCs showed stronger luminescence than those treated with B7-H3Bi-Ab-armed ATCs. After comparing the mean luminescence of the two groups, a significant difference in the inhibition of tumor growth was observed between them (Fig. 4b). Therefore, compared with unarmed control ATCs, B7-H3Bi-Ab-armed ATCs could inhibit tumor growth in vivo.

Discussion

One characteristic of advanced melanoma is the resistance to conventional cancer therapy. Cancer immunotherapy, which has shown great promise recently, is

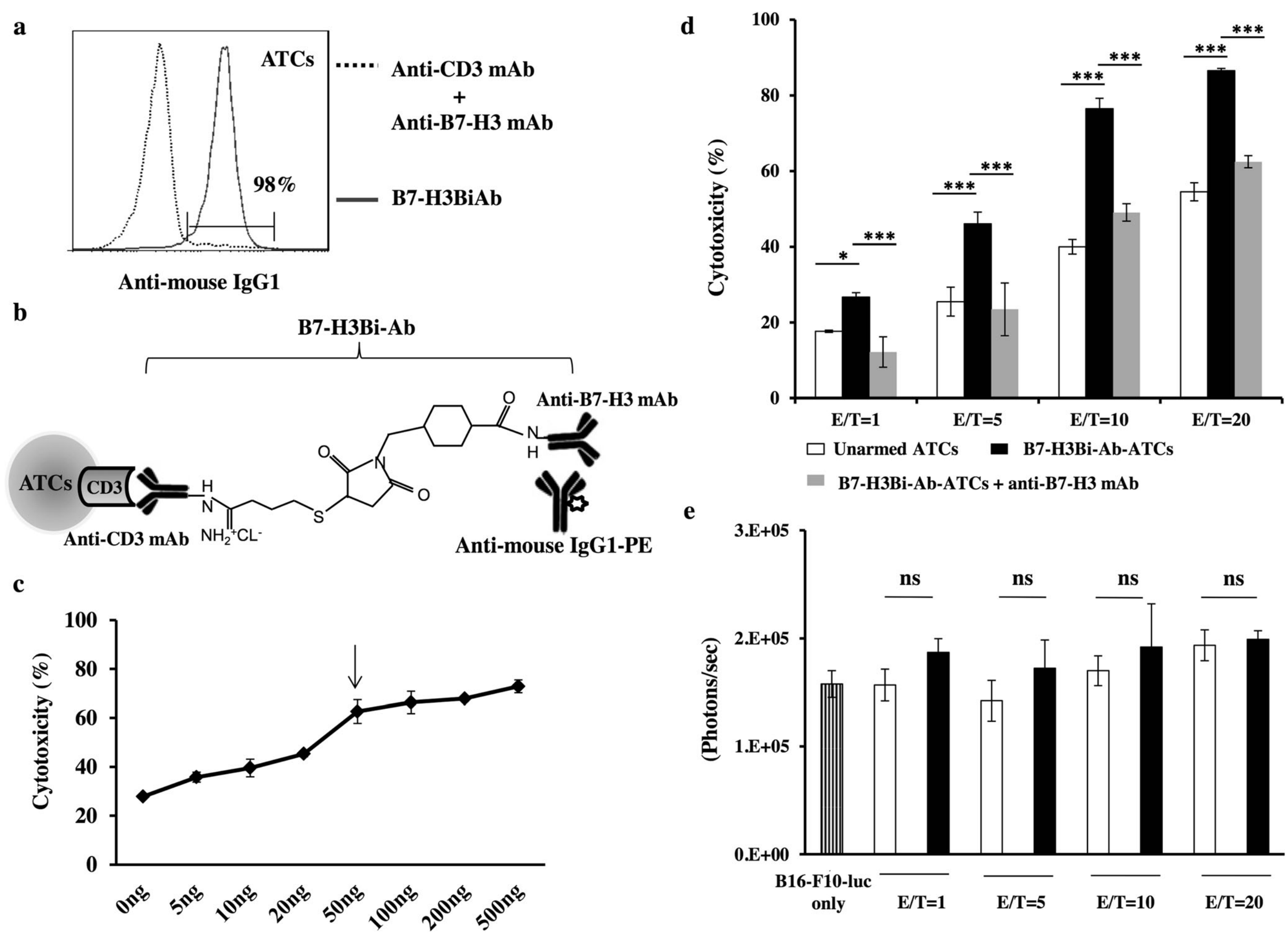

Fig. 2 Lytic activity of B7-H3Bi-Ab-armed activated T cells against melanoma cells. a Flow cytometry-based binding assay for B7-H3Bi-Ab. Activated T cells (ATCs) were incubated with B7-H3Bi-Ab or the combination of OKT3 and anti-human B7-H3 mAb (left histogram), and then B7-H3Bi-Ab binding was analyzed by flow cytometry using PE-labeled anti-mouse IgG1 to detect the anti-B7-H3 moiety of the B7-H3Bi-Ab. b Scheme of the flow cytometry-based binding assays for B7-H3Bi-Ab. c Measurement of the titer of B7-H3Bi-Ab-armed ATCs. ATCs were armed with B7-H3Bi-Ab ranging from 5 to 500 ng/10^6 cells at an effector/target (E/T) ratio of 5:1, and the cytotoxic effect of B7-H3Bi-Ab-armed ATCs on Malme-3M-luc cells were tested. After 18 h of incubation with B7-H3Bi-Ab-armed ATCs, bioluminescence imaging signals in photons per second were converted into the living cell number, and the cytotoxicity assay was measured at each concentration. d Lytic activity of B7-H3Bi-Ab-armed ATCs against Malme-3M-luc cells. After incubation with B7-H3Bi-Ab-armed ATCs or unarmed ATCs, the cytotoxicity assay was measured at the indicated E/T ratio. e Lytic activity of B7-H3Bi-Ab-armed ATCs against B16-F10-luc cells. For C to E, the data are the means ± SD of triplicate experiments. Statistical analysis was conducted using Student's *t* test. A representative experiment of at least three is shown. *$P < 0.05$, *** $P < 0.001$, B7-H3Bi-Ab-armed ATCs compared with unarmed ATCs under similar conditions

dependent on the presence of effective antitumor lymphocytes to recognize and kill cancer cells in vivo [20]. Bi-Abs, usually comprising an anti-CD3 mAb heteroconjugated to a tumor-specific mAb, will empower T cells to retarget tumor cells [17, 21]. Because of the production of ATCs ex vivo efficiently and availability of mAbs specific for tumor surface protein, the strategy for arming ATCs with Bi-Ab has shown encouraging anti-tumor effects in recent years [17, 22, 23]. B7-H3 is involved in many metastasis-associated pathways in melanoma, and adoptive T cell therapy is effective for patients with metastatic melanoma [3, 11, 12]. Therefore, modulation of ATCs retargeting to B7-H3 appears to be a reasonable way to treat melanoma.

In this study, we examined B7-H3 expression on human melanoma cells and exploited luciferase as a bioluminescence reporter for cell viability. Our study made new findings in targeting B7-H3 against melanoma. The expression of B7-H3 on several melanoma cell lines was confirmed by FACS analysis. In vitro cytotoxicity assay showed that B7-H3Bi-Ab-armed ATCs exhibited significant cytotoxic activity against melanoma cells. B7-H3Bi-Ab-armed ATCs secreted significantly higher levels of IFN-γ and expressed higher levels of activating

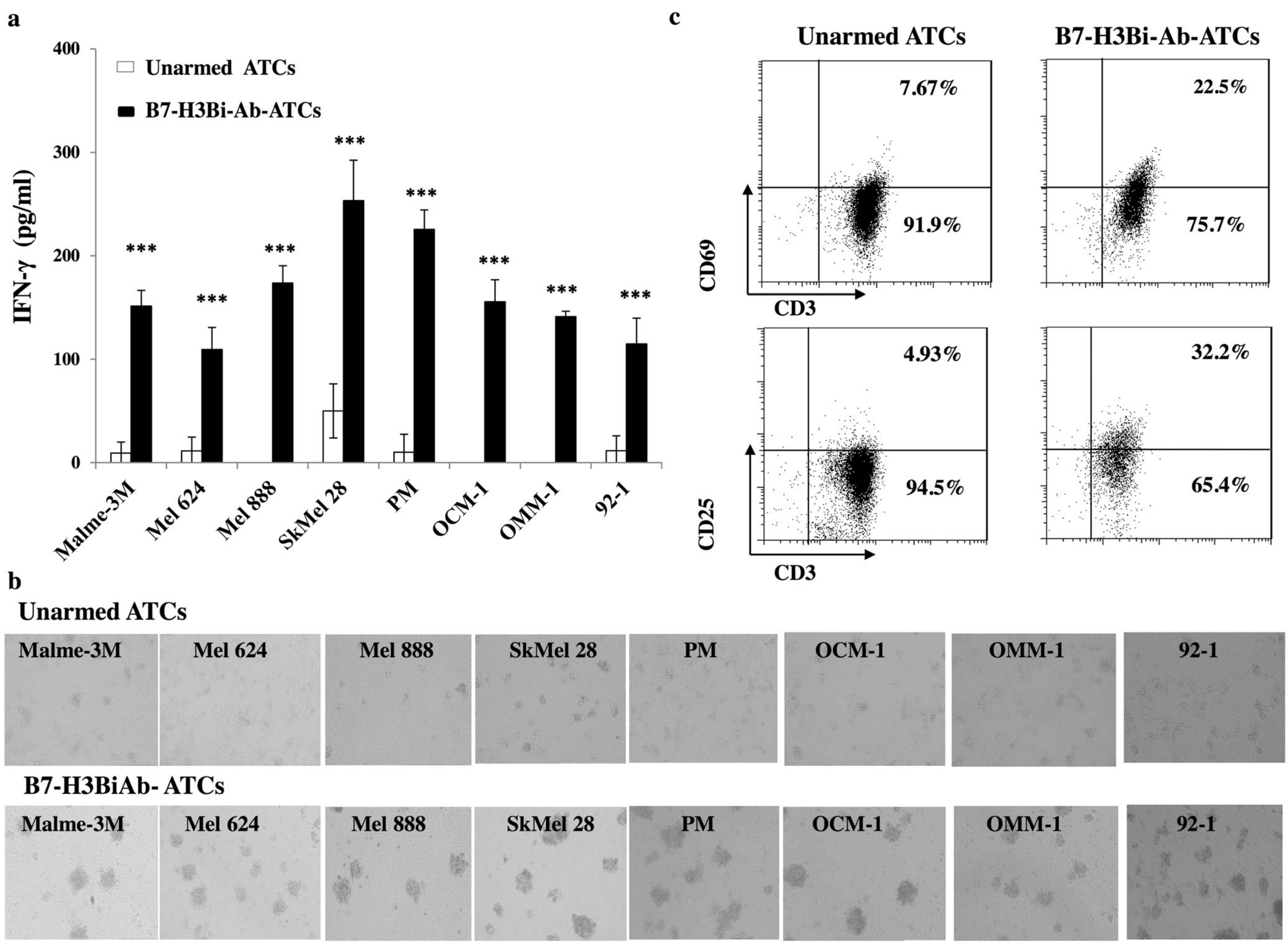

Fig. 3 Cytokine production and T cell activation of B7-H3Bi-Ab-armed ATCs by melanoma cells. Melanoma cells were incubated with B7-H3Bi-Ab-armed ATCs (50 ng/B7-H3Bi-Ab/10^6 ATCs) for 18 h at an effector/target ratio of 10:1 in a 96-well microplate. The combination of OKT3, anti-B7-H3 mAb with ATC served as unarmed control ATCs. a IFN-γ secretion by B7-H3Bi-Ab-armed ATCs against melanoma cells. The supernatants of cocultures were harvested and analyzed using a specific ELISA Kit for IFN-γ production. b Real-time photographs were taken at 200× magnification. c Expression of CD69 and CD25 on B7-H3Bi-Ab-armed ATCs was detected by flow cytometry. For A, the data are the means ± SD of triplicate determination. Statistical analysis was conducted using Student's *t* test. A representative experiment from the three experiments is shown. *** $P < 0.001$, B7-H3Bi-Ab-armed ATCs compared with unarmed control ATCs under similar conditions

marker CD69 and CD25. Moreover, infusion of B7-H3Bi-Ab-armed ATCs suppressed melanoma growth in a xenograft mouse model.

To analyze the effector's ability to target tumor cells accurately, we used a previously established Malme-3M-luc cell line that stably expressing a high level of luciferase. There was good correlation between the bioluminescence intensity and number of living Malme-3M cells. Consequently, cytotoxicity was successfully measured using the luciferase quantitative assay. We also applied a previously generated xenograft mouse model to evaluate the antitumor capacity of B7-H3Bi-Ab-armed ATCs because it supplied a sensitive model to monitor and treat human melanoma in vivo [18, 19, 24]. Our study demonstrated that, compared with control unarmed ATCs, those armed with B7-H3Bi-Ab exhibited strong cytotoxic the ability against melanoma cells both in vitro and in vivo. These results indicated that T cell cytotoxicity was dependent on the engagement of B7-H3 via Bi-Ab bridge. At the E/T ratio of 1:1, 50 ng of Bi-Ab per 10^6 ATCs showed remarkable cytotoxicity against tumor cells after an 18-h interaction whereas anti-B7-H3 antibody had no inhibitory effect at the concentration of 10 μg/ml after a 72-h incubation (data not shown). Moreover, our study demonstrated that B7-H3Bi-Ab-armed ATC-mediated cytolysis was blocked by anti-B7-H3 mAb, further supporting B7-H3-specific killing. Additionally, B7-H3Bi-Ab-armed ATCs could not kill B16-F10 cells, also exhibiting the specificity of B7-H3Bi-Ab.

Our results indicated that ATCs armed with B7-H3Bi-Ab generated higher levels of IFN-γ when interacted with melanoma cells. The increased cytokine production suggested that ATCs gained reactivation when encountering tumor cells. IFN-γ secreted by Bi-Ab-armed ATCs

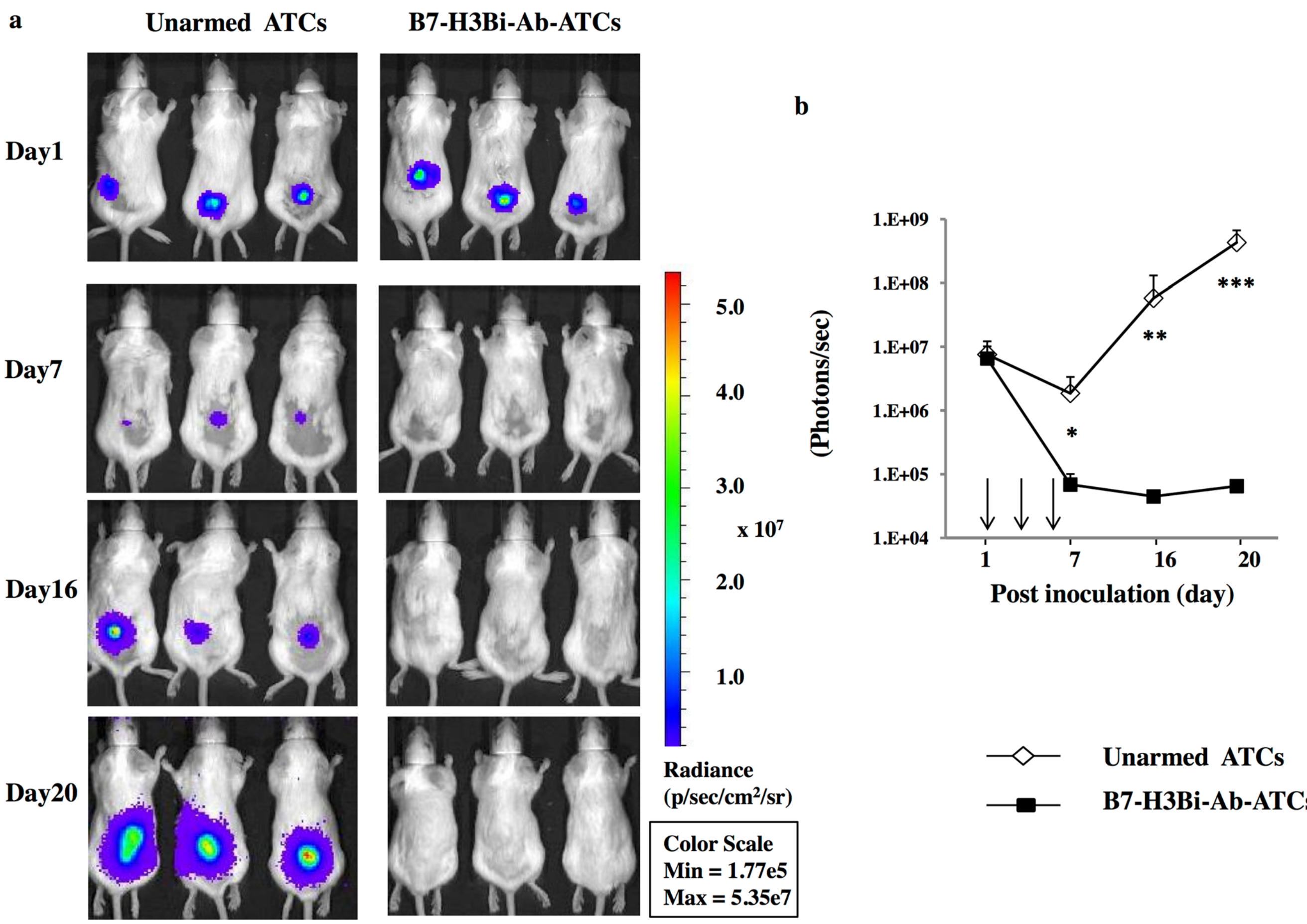

Fig. 4 In vivo cytotoxicity of B7-H3Bi-armed ATCs against Malme-3M-luc cells. SCID-Beige mice were inoculated with Malme-3M-luc cells on day 0 and then were treated with B7-H3Bi-Ab-armed ATCs or unarmed ATCs randomly on day 1, day 3 and day 5 (Each group contains $n = 6$ mice). a Bioluminescence images of three representative mice per group are shown on day 1 (before ATC treatment), day 7, day 16 and day 20. b Images were analyzed using Living Image software, and tumor values were represented as total flux measurements in photons/s; the mean values of tumor growth curves are shown. This experiment was repeated twice. The significance of the B7-H3Bi-Ab-armed ATC group and unarmed ATC group was compared by the Mann-Whitney rank sum test. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, B7-H3Bi-Ab-armed ATCs compared with unarmed control ATCs under similar conditions

exerted a tumor-killing effect and could also stimulate effectors in vivo, counteracting tumor-derived suppression. Furthermore, B7-H3Bi-Ab-armed ATCs expressed higher levels of CD69 and CD25 than unarmed-ATC controls. Both CD69 and CD25 are markers of early T cell activation, playing a costimulatory role in promoting T cell responses following TCR engagement [25]. Moreover, real-time photographs displayed that armed ATCs, but not unarmed counterparts, aggregated with melanoma cells in culture and gathered around the edge of the target cell population, denoting the specific activation of Bi-Ab-armed ATCs. PBMCs from metastatic patients administered HER2Bi-Ab-armed ATCs showed an increase in IFN-γ ELiSpot responses in clinical trials, suggesting armed ATCs induced an effective immune response in vivo [22, 23].

In conclusion, B7-H3 appears to be a suitable target for T cell-mediated immunotherapy against melanoma in vitro and in vivo. B7-H3Bi-Ab-armed ATCs may provide a promising approach for melanoma in the future.

Funding This work is funded by grants from the National Nature Science Foundation of China (No.31400754) and Beijing Municipal Administration of Hospitals' Ascent Plan (DFL20150701).

Compliance with ethical standards

Conflicts of interest Juan Ma, Tengfei Shang, Pan Ma, Xin Sun, Jin Zhao, Ximing Sun, and Man Zhang declare that they have no conflicts of interest.

Ethical approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. The animal study complied with the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, and the protocol was approved by the Ethics Committee of Beijing Shijitan Hospital of Capital Medical University.

Informed consent Informed consent was obtained from all individual participants included in the study.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68(1):7–30
2. Dillman RO, CornforthAN DC, McClay EF, Amatruda TT, de Leon C, Ellis RE, Mayorga C, Carbonell D, Cubellis JM (2012) Tumor stem cell antigens as consolidative active specific immunotherapy: a randomized phase II trial of dendritic cells versus tumor cells in patients with metastatic melanoma. J Immunother 35(8):641–649
3. Ullenhag GJ, Sadeghi AM, Carlsson B, Ahlström H, Mosavi F, Wagenius G, Tötterman TH (2012) Adoptive T-cell therapy for malignant melanoma patients with TILs obtained by ultrasound-guided needle biopsy. Cancer Immunol Immunother 61 (5):725–732
4. Larkin J, Chiarion-Sileni V, Gonzaler R, Grob JJ, Cowey CL, Lao CD, Schadedof D, Dummer R, Smylie M, Rutkowki P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horek C, Hodi FS, Wolchok JD (2015) Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373(1):23–34
5. Steinberger P, Majdic O, Derdak SV, Pfistershammer K, Kirchberger S, Klauser C, Zlabinger G, Pickl WF, Stöckl J, Knapp W (2004) Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. J Immunol 172(4):2352–2359
6. Leitner J, Klauser C, Pickl WF, Stöckl J, Majdic O, Bardet AF, Kreil DP, Dong C, Yamazaki T, Zlabinger G, Pfistershammer K, Steinberger P (2009) B7-H3 is a potent inhibitor of human T-cell activation: no evidence for B7-H3 and TREML2 interaction. Eur J Immunol 39(7):1754–1764
7. Wang L, Kang FB, Shan BE (2014) B7-H3-mediated tumor immunology: Friend or foe? Int J Cancer 134(12):2764–2771
8. Picarda E, Ohaegbulam KC, Zan g X (2016) Molecular pathways: targeting B7-H3 (CD276) for human cancer immunotherapy. Clin Cancer Res 22(14):3425–3431
9. Castellanos JR, Purvis IJ, Labak CM, Guda MR, Tsung AJ, Velpula KK, Asuthkar S (2017) B7-H3 role in the immune landscape of cancer. Am J Clin Exp Immunol 6(4):66–75
10. Wang J, Chong KK, Nakamura Y, Nguyen L, Huang SK, Kuo C, Zhang W, Yu H, Morton DL, Hoon DS (2013) B7-H3 associated with tumor progression and epigenetic regulatory activity in cutaneous melanoma. J Invest Dermatol 133(8):2050–2058
11. Tekle C, Nygren MK, Chen YW, Dybsjord I, Nesland JM, Maelandsmo GM, Fodstad O (2012) B7-H3 contributes to the metastatic capacity of melanoma cells by modulation of known metastasis-associated genes. Int J Cancer 130(10):2282–2290
12. Flem-Karlsen K, Tekle C, Andersson Y, Flatmark K, Fodstad Ø, Nunes-Xavier CE (2017) Immunoregulatory protein B7-H3 promotes growth and decreases sensitivity to therapy in metastatic melanoma cells. Pigment Cell Melanoma Res 30(5):467–476
13. Lee YH, Martin-Orozco N, Zheng P, Li J, Zhang P, Tan H, Park HJ, Jeong M, Chang SH, Kim BS, Xiong W, Zang W, Guo L, Liu Y, Dong ZJ, Overwijk WW, Hwu P, Yi Q, Kwak L, Yang Z, Mak TW, Li W, Radvanyi LG, Ni L, Liu D, Dong C (2017) Inhibition of the B7-H3 immune checkpoint limits tumor growth by enhancing cytotoxic lymphocyte function. Cell Res 27(8):1034–1045
14. Loo D, Alderson RF, Chen FZ, Huang L, Zhang W, Gorlatov S, Burke S, Ciccarone V, Li H, Yang Y, Son T, Chen Y, Easton AN, Li JC, Rillema JR, Licea M, Fieger C, Liang TW, Mather JP, Koenig S, Stewart SJ, Johnson S, Bonvini E, Moore PA (2012) Development of an Fc-enhanced anti–B7-H3 monoclonal antibody with potent antitumor activity. Clin Cancer Res 18(14):3834–3845
15. Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Smith-Jones P, Zanzonico P, Humm JL, Xu H, Wolden SL, Souweidane MM, Larson SM, Cheung NK (2010) Compartmental intrathecal radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma. J Neuro-Oncol 97(3):409–418
16. Lum LG, Thakur A (2011) Targeting T cells with bispecific antibodies for cancer therapy. BioDrugs 25(6):365–379
17. Bhutani D, Lum LG (2015) Activated T cells armed with bispecific antibodies kill tumor targets. Curr Opin Hematol 22(6):476–483
18. Ma J, Ma P, Zhao C, Xue X, Han H, Liu C, Tao H, Xiu W, Cai J, Zhang M (2016) B7-H3 as a promising target for cytotoxicity T cell in human cancer therapy. Oncotarget 7(20):29480–29491
19. Ma J, Han H, Liu D, Li W, Feng H, Xue X, Wu X, Niu G, Zhang G, Zhao Y, Liu C, Tao H, Gao B (2013) HER2 as a promising target for cytotoxicity T cells in human melanoma therapy. PLoS One 8(8): e73261
20. Fousek K, Ahmed N (2015) The evolution of T-cell therapies for solid malignancies. Clin Cancer Res 21(15):3384–3392
21. Yu S, Li A, Liu Q, Yuan X, Xu H, Jiao D, Pestell RG, Han X, Wu K (2017) Recent advances of bispeicific antibodies in solid tumors. J Hematol Oncol 10(1):15
22. Lum LG, Thakur A, Al-Kadhimi Z, Colvin GA, Cummings FJ, Legare RD, Dizon DS, Kouttab N, Maizel A, Colaiace W, Liu Q, Rathore R (2015) Targeted T-cell therapy in stage IV breast cancer: a phase I clinical trial. Clin Cancer Res 21(10):2305–2314
23. Vaishampayan U, Thakur A, Rathore R, Kouttab N, Lum LG (2015) Phase I Study of Anti-CD3 x anti-Her2 bispecific antibody in metastatic castrate resistant prostate cancer patients. Prostate Cancer 2015:285193
24. Tiffen JC, Bailey CG, Ng C, Rasko JE, Holst J (2010) Luciferase expression and bioluminescence does not affect tumor cell growth in vitro or in vivo. MolCancer 9:299
25. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJ, Bickham KL, Lerner H, Goldstein M, Sykes M, Kato T, Farber DL (2013) Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. Immunity 38(1): 187–197