Biochemical and Biophysical Research Communications 534 (2021) 134–140

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A novel human anti-TIGIT monoclonal antibody with excellent function in eliciting NK cell-mediated antitumor immunity

Dong Han [a, 1], Yinfeng Xu [b, 1], Xinping Zhao [b, e], Yunyun Mao [b], Qinglin Kang [b], Weihong Wen [c], Xiaoyan Yu [b], Lei Xu [b], Fujia Liu [b], Mengyao Zhang [b], Jiazhen Cui [b], Zhang Wang [b, d], Zhixin Yang [b, ***], Peng Du [b, **], Weijun Qin [a, *]

[a] Department of Urology, Xijing Hospital, Fourth Military Medical University, Xi'an, China
[b] Beijing Institute of Biotechnology, Beijing, China
[c] Institute of Medical Research, Northwestern Polytechnical University, Xi'an, China
[d] Hainan Medical University, Haikou, China
[e] PLA Center for Disease Control and Prevention, Beijing, China

ARTICLE INFO

Article history:
Received 16 November 2020
Accepted 4 December 2020
Available online 16 December 2020

Keywords:
Tumor
Checkpoint blockade immunotherapy
Phage-displayed antibody library
TIGIT
NK cell

ABSTRACT

TIGIT is an emerging novel checkpoint target that is expressed on both tumor-infiltrating T cells and NK cells. Some current investigational antibodies targeting TIGIT have also achieved dramatic antitumor efficacy in late clinical research. Most recently, the relevance of NK cell–associated TIGIT signaling pathway to tumors' evasion of the immune system has been clearly revealed, which endows NK cells with a pivotal role in the therapeutic effects of TIGIT blockade. In this article, we describe a novel anti-TIGIT monoclonal antibody, AET2010, which was acquired from a phage-displayed human single-chain antibody library through a cell panning strategy. With emphasis on its regulation of NK cells, we confirmed the excellent ex vivo and in vivo antitumor immunity of AET2010 mediated by the NK-92MI cells. Intriguingly, our work also revealed that AET2010 displays a lower affinity but parallel avidity and activity relative to MK7684, an investigational monoclonal antibody from MSD, implying a reasonable balance of potency and potential side effects for AET2010. Together, these results are promising and warrant further development of AET2010.

Introduction

Checkpoint blockade immunotherapies targeting the PD-1/PD-L1 axis and/or CTLA-4 have achieved dramatic clinical benefits in multiple malignancies by enhancing effector T cell function. However, some challenges still exist, such as the small proportion of patients exhibiting remarkable and durable responses and a subset of patients showing drug resistance, suggesting that some mechanisms must be limiting the antitumor immunity of T cells and that supplementary novel strategies and therapeutic targets urgently need to be explored.

The inhibitory receptor TIGIT is an emerging novel checkpoint target that is expressed on both T cells and NK cells [1]. Mechanistic studies suggest that TIGIT induces the suppression of immune cells through the binding of CD155, generally observed on antigen-presenting cells or target cells; CD155 is a ligand shared by TIGIT, CD226 and CD96 and shows the highest affinity for TIGIT [2–4]. The role of TIGIT/CD155 signaling in tumor immunotolerance is analogous to that of the PD-1/PD-L1 axis. In recent years, the association between tumor progression and elevated TIGIT expression in tumor-infiltrating immune cells, especially cytotoxic T lymphocytes, has been extensively demonstrated in preclinical models and tumor-bearing patients [4–6]. Most recently, work by researchers further revealed the critical role of NK cells in the therapeutic effects of TIGIT blockade and highlighted NK cells as emerging targets for checkpoint inhibition [7,8]. Their data demonstrated that blockade of TIGIT reverses the exhaustion of tumor-infiltrating NK

* Corresponding author. Department of Urology, Xijing Hospital, Fourth Military Medical University, 127 Changlexi Street, Xincheng District, Xi'an, 710000, China.
** Corresponding author. Beijing Institute of Biotechnology, 20 Dongdajie Street, Fengtai District, Beijing, 100071, China.
*** Corresponding author. Beijing Institute of Biotechnology, 20 Dongdajie Street, Fengtai District, Beijing, 100071, China.
E-mail addresses: yy_xiao@126.com (Z. Yang), dudedu@sina.com (P. Du), qinwj@fmmu.edu.cn (W. Qin).
[1] These authors contribute equally.

https://doi.org/10.1016/j.bbrc.2020.12.013
0006-291X/

Abbreviations

TIGIT	T cell immunoreceptor with Ig and ITIM domains
PD-1	programmed death-1
PD-L1	programmed death-ligand-1
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
SPR	surface plasma resonance
EC_{50}	50% effective concentration
IC_{50}	50% inhibitory concentration
INF-γ	interferon-γ
TNF-α	tumor necrosis factor-α

cells and boosts NK cell–mediated antitumor immunity. Moreover, unleashing NK cells via TIGIT blockade could prevent the exhaustion of $CD8^+$ T cells and improve the therapeutic effects of PD-1/PD-L1 antibodies. Thus, with respect to immunotherapy targeting TIGIT, its regulation of the function and activity of NK cells may underpin an additional mode of action and simultaneously unleash both innate (NK cell) and adaptive (T cell) antitumor immunity.

Several investigational antibodies against human TIGIT, as a monotherapy or in combination with PD-1/PD-L1 antibodies, have demonstrated appreciable and excellent efficacy in some cancers, which makes the blockade of TIGIT a promising therapeutic approach. Among these agents, tiragolumab, developed by Roche/Genentech, is relatively advanced in clinical progression (phase III) and exerts notable antitumor effects in patients with nonsmall cell lung cancer when combined with atezolizumab (anti-PD-1 antibody) [9]. MK7684, a humanized antibody from MSD. that is in a phase II clinical trial, is the only candidate showing favorable efficacy as a monotherapy, displaying a 35% disease control rate in patients with advanced solid tumors [10]. However, given the limited knowledge about the pivotal role of NK cells in the early stage, almost all of the research on these investigational anti-TIGIT antibodies has focused on the immune responses of cytotoxic T cells within the tumor microenvironment.

Here, we describe a novel anti-TIGIT monoclonal antibody, named AET2010, acquired from a phage-displayed human single-chain antibody library through a cell panning strategy. With emphasis on its regulation of NK cells, we confirmed the excellent ex vivo and in vivo antitumor immunity of AET2010 mediated by the NK-92MI cell line, indicating a promising therapeutic application. Intriguingly, our work also revealed that AET2010 displays a lower affinity but parallel avidity and activity relative to MK7684, implying a reasonable balance of potency and potential toxicity for AET2010.

Materials and method

Construction and identification of a stable cell line with high TIGIT expression (CHO-TIGIT)

A human TIGIT cDNA ORF clone (SinoBiological, Beijing, China) was transfected into CHO cells (maintained in our lab) using TransIntro™ Transfection Reagent (TransGen, Beijing, China). Subsequently, the cells were cultured in RPMI-1640 medium (Gibco, NY, USA) containing 10% FBS (Gibco, NY, USA) and 200 μg/ml hygromycin-B (WWR Chemicals, CA, USA) for selection, which was renewed every 3–5 days. Single clone cells were resuspended and cultured on new cell plates until reaching approximately 90% confluence. The expression of TIGIT was determined via flow cytometry (BD Biosciences, NJ, USA) using APC-labeled anti-human TIGIT antibody (BioLegend, CA, USA).

Generation of monoclonal antibodies

The phage-displayed human single-chain (scFv) antibody library with a high capacity of 1.35×10^{10} (maintained in our lab) was first incubated with antigen-negative cells (CHO) to deplete nonspecific phage particles and then exposed to 4 rounds of selection with decreasing antigen-positive cell (CHO-TIGIT) density and increasing washing times. The light and heavy chain variable region genes of the acquired scFv antibodies were cloned into the expression vectors pABL and pABG1, respectively (constructed by our lab). FreeStyle™ 293-F cells (Invitrogen, CA, USA) were cotransfected with light and heavy chain vectors for simultaneous expression. Expression supernatants were collected and purified using a HiTrap™ MabSelect SuRe column (GE Healthcare, PA, USA).

Binding activity assay

For ELISA, 96-well microtiter plates precoated with HSA-hTIGIT-His (a fusion protein of human serum albumin, human TIGIT and histidine-tag prepared in our lab) were incubated with serial dilutions of AET2010 or MK7684 at 37 °C for 1 h and then incubated with secondary antibody (ZSGB-BIO, Beijing, China) for 30 min. The binding activity was detected via TMB chromogenic reaction at an OD of 450 nm with 595 nm as a reference. For cell-based ELISA, CHO-TIGIT or CHO cells were inoculated in 96-well cell culture plates at a density of 1×10^4 cells/well overnight. The remaining steps followed the ELISA procedure described above except that the temperature was adjusted to 4 °C. For flow cytometry, 5×10^5 CHO-TIGIT or CHO cells were incubated with serial dilutions of AET2010 or MK7684 at 4 °C for 2 h. Cells were then stained with FITC-labeled anti-human IgG antibody (OriGene, Beijing, China) at 4 °C for 1 h and analyzed by flow cytometry.

The affinity between antibodies and recombinant TIGIT-His (SinoBiological) was determined by multicycle kinetic analysis on the Biacore™ 3000 system. Purified AET2010 or MK7684 was captured on a CM5 chip handled with the Human Antibody Capture Kit (GE Healthcare). TIGIT-His at concentrations from 1.875 to 60 nM in HBS-EP + buffer was passed over the chip at a rate of 30 μl/min. The three (or two) minute association time was followed by a six (or eight) minute dissociation period. The sensorgram curves were fitted to a 1:1 binding model (Langmuir) using BIAevaluation software.

TIGIT blocking activity assay

In the ELISA, serial dilutions of AET2010-Fab or MK7684-Fab were mixed with CD155-Fc at 37 °C for 1 h and then transferred into 96-well microtiter plates precoated with HSA-hTIGIT-His. The remaining steps followed the ELISA procedure described above. In the flow cytometry assay, serial dilutions of AET2010-Fab or MK7684-Fab were mixed with CD155-Fc and then added to approximately 5×10^5 CHO-TIGIT cells at 4 °C for 2 h. The cells were stained with FITC-labeled anti-human IgG/Fc (BioLegend) at 4 °C for 1 h and then analyzed by flow cytometry. Isotype human IgG served as a blank control, and CD155-Fc alone served as a positive control. The inhibition rate was calculated as follows:

$$\%Inhibition\ rate = \left(1 - \frac{MFI\ sample - MFI\ blank}{MFI\ positive - MFI\ blank}\right) \times 100\%$$

Molecular modeling, docking and structure analysis

A homology model of the AET2010 variable region was built

using Discovery Studio 3.0 (Accelrys software, CA, USA) with the structure of PDB:6A3W as template, as the A and B chains of this protein share a high sequence similarity with the AET2010 variable region. The conformation of human TIGIT was taken from the crystal structure of PDB:3UCR. The docking simulation of AET2010 and TIGIT was executed by using the integrated ZDOCK algorithm, and a subsequent refining process (RDOCK) was performed. Ultimately, one structure model of the AET2010 and TIGIT complex was selected according to the ZDock score, ZRank score and inspection of the structure. The predicted interface residues were identified by Structure/Monitor panel. Molecular structure illustrations were generated using PyMOL Software.

Cell culture and in vitro functional assay

Target cells (A549, A431 or U251MG; maintained in our lab) were inoculated in 96-well cell plates at a density of 1×10^4 cells/well for 48 h. Effector cells (NK-92MI; ProCell, Wuhan, China) at different effector-target ratios (1:1 for A549, 2:1 for A431, 0.25:1 for U251MG) were coincubated with serial dilutions of AET2010 or MK7684 or isotype human IgG at 37 °C for 1 h and then transferred onto the cell plates for a 6-h incubation. For U251MG ($CD155^+$ $PD\text{-}L1^+$), nivolumab (anti-PD-1 antibody; a gift from Dr. Shuang Wang) was combined with AET2010 or MK7684 at equal radio or used alone and then coincubated with NK-92MI cells.

The cytotoxic factors secreted by NK-92MI cells, such as perforin, INF-γ and TNF-α, were quantified by ELISA. Supernatants were collected from the 96-well cell plates and ELISA assays were performed according to the manufacturer's recommendation (Elabscience, Wuhan, China).

The viability of the target cells was determined by the addition of CCK-8 (Dojindo Laboratories, Tokyo, Japan) and measurement of the OD at 450 nm after a 1.5-h incubation. The cytotoxicity of the NK-92MI cells was calculated as follows, setting the OD 450 nm in the presence of NK-92MI and antibodies as "As", NK-92MI alone as 'Ae', target cells alone as 'At' and medium alone as 'Ac':

$$\%\text{Cytotoxicity} = \left(1 - \frac{\text{As} - \text{Ae}}{\text{At} - \text{Ac}}\right) \times 100\%$$

Mice and animal experiment

BALB/c nude mice (male, 18–22 days old; Vital River, Beijing, China) were subcutaneously inoculated with 100 μL of A549 cells resuspended in PBS for a total of 8×10^7 cells/mouse. The tumor volumes were calculated weekly as follows: volume = long diameter × (short diameter)^2/2. The mice were randomized into four groups when the tumor volume reached approximately 300 mm^3. In the treatment group, mice were intravenously injected with NK-92MI cells (1×10^7 cells/mouse) combined with AET2010, MK7684 or isotype human IgG (each antibody: 300 μg/mouse) once every 5 days for 30 days. In the control group, mice were intravenously administered PBS (200 μl/mouse). Mouse weight and tumor volume were measured every 5 days. Mice were sacrificed when the tumor volume exceeded 2000 mm^3 or on the 30th day after the start of treatment. Once the mice were euthanized, the tumor tissue was separated and weighed.

This protocol was approved by the Animal Ethics Committee of the Academy of Military Medical Sciences (NO. IACUC-DWZX-2020-053). All procedures were in accordance with the approved guidelines of the National Institute of Biological Sciences Guide.

Statistical analysis

All quantitative data are presented as the mean ± standard deviations. The statistical significance of differences between groups was determined using one-way ANOVA or *t*-test. Differences were considered statistically significant at $p < 0.05$. All data were analyzed using GraphPad Prism 5.

Results

Generation of anti-human TIGIT antibody by using the phage-displayed human antibody library and cell panning

The recombinant TIGIT ectodomain protein has a small molecular weight (approximately 14 kDa) and shows unstable and heterogeneous characteristics, which makes it difficult to use to select antibodies from our phage library in precoated immunotubes, a commonly used high-throughput compatible method [11,12]. Instead, a cell panning strategy was established to obtain phage-displayed specific antibodies against TIGIT in this study. Due to their lack of endogenous expression of TIGIT and low expression of other unwanted surface proteins, CHO cells were chosen to construct a stable cell line with high expression of human TIGIT. Through transfection of the TIGIT gene and subsequent hygromycin screening, an antigen-positive cell line (CHO-TIGIT) was successfully generated in which the expression of human TIGIT reached 99%, as validated by flow cytometry (Fig. 1A). First, phage particles binding to these unwanted targets were depleted from the library via nontransfected cells (CHO cells). Then, the depleted library was further used for the selection steps on CHO-TIGIT cells. The four rounds of panning with progressively stringent conditions gave rise to fifteen positive phage clones with specific human single-chain antibody genes (Table S1), of which nine with distinct enrichment were cloned into a full-length human IgG1 expression vector and then expressed in the FreeStyle™ 293-F system. Subsequently, the top candidate antibody, named AET2010, was identified for its highest binding activity to human TIGIT (Fig. S1). Meanwhile, the excellent purity of AET2010 was also confirmed by SDS-PAGE and high-performance liquid chromatography assays (Fig. S2).

AET2010 specifically binds to human TIGIT with high affinity

A panel of assays with the investigational monoclonal antibody MK7684 as a control was conducted to identify the binding activities of AET2010 to both recombinant TIGIT and natural TIGIT on the cell surface.

The results of an antigen-coated ELISA (Fig. 1B) revealed the dose-dependent interaction between AET2010 and recombinant human TIGIT. As shown, AET2010 displays avidity (two-arm binding power) comparable to that of MK7684. AET2010 specifically recognizes human TIGIT without cross reactivity to mouse TIGIT and other antigens (Fig. S3A). Additionally, the affinity constant of AET2010 to recombinant human TIGIT was examined through SPR analysis with immobilized antibody. The results (Table 1 and Fig. S4) show that AET2010 has a much lower affinity (single-arm binding power) for recombinant human TIGIT than does NK7684.

For further verification, cell-based ELISA and flow cytometry were performed to assess the binding capability of AET2010 to natural TIGIT on CHO-TIGIT cells (Fig. 1C and D). In agreement with the antigen-coated ELISA results, AET2010 binds to CHO-TIGIT cells in a dose-dependent manner and shows an EC_{50} equivalent to that of MK7684.

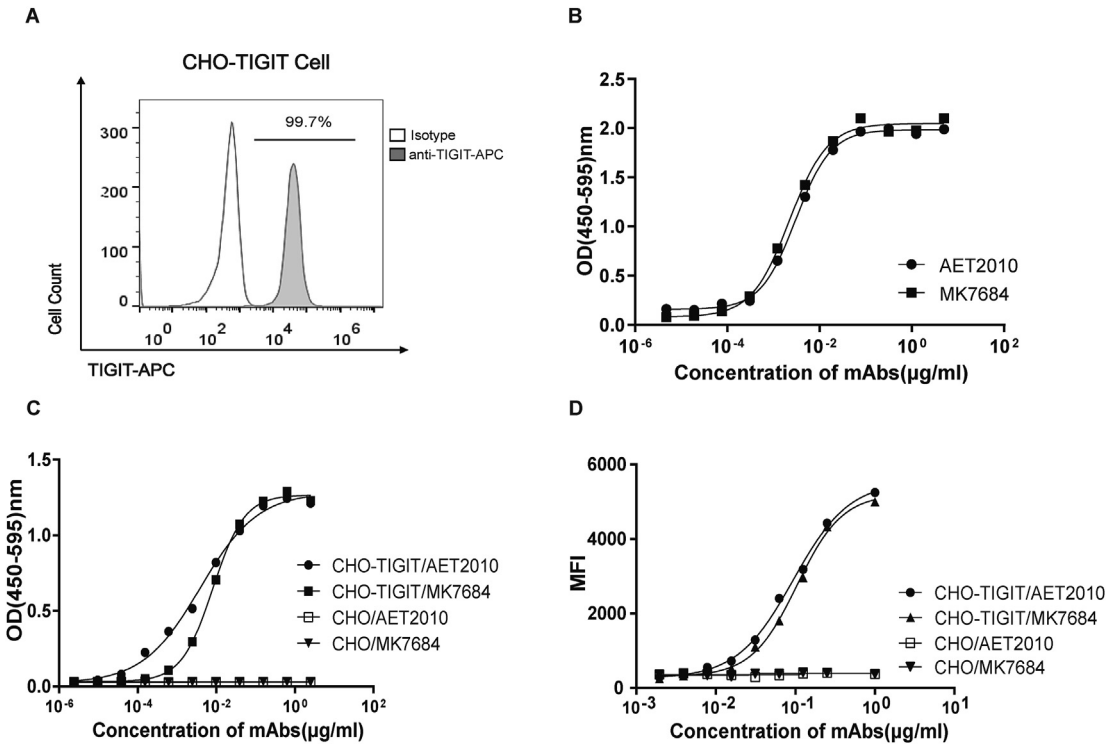

Fig. 1. Generation and characterization of the anti-TIGIT antibody AET2010. A Validation of CHO-TIGIT cells using flow cytometry. **B-D** The binding activities of AET2010 and MK7684 (positive control) to recombinant HSA-hTIGIT-His or TIGIT expressed on CHO-TIGIT cells determined via ELISA (**B**), cell-based ELISA (**C**) or flow cytometry (**D**).

Table 1
Affinity constant of AET2010 and MK7684.

Antibody	Mean ± Standard Deviations			
	k_a (10^5 $M^{-1}s^{-1}$)	k_d (10^{-3} s^{-1})	K_D (10^{-9} M)	χ^2 (RU^2)
AET2010	6.49 ± 0.27	2.30 ± 0.14	3.74 ± 0.12	0.29 ± 0.01
MK7684	30.37 ± 5.65	0.32 ± 0.08	0.11 ± 0.01	0.32 ± 0.15

k_a, association rate constant; k_d, disassociation rate constant; K_D, equilibrium disassociation constant. $K_D = k_d/k_a$ (1:1 binding). All values were determined by a Biacore™ 3000 system and represent the mean ± standard deviations from 3 separate assays.

AET2010 could effectively block the interaction of TIGIT and CD155

As mentioned above, CD155 is the high-affinity receptor for TIGIT and contributes most of the TIGIT inhibitory signals in the tumor microenvironment. Therefore, it is sensible to examine the TIGIT/CD155 interaction blockade effects of AET2010. First, AET2010-Fab, MK7684-Fab (the Fab region of antibody; Fig. S5) and CD155-Fc (fusion protein of CD155 ectodomain and antibody Fc region) were prepared. Then, the blockade assays were carried out by using ELISA and flow cytometry and revealed that both AET2010-Fab and MK7684-Fab could effectively and dose-dependently block the binding of CD155-Fc to either recombinant TIGIT protein or natural TIGIT on the cell surface (Fig. 2A and B).

Furthermore, we built a structural model of the variable region of AET2010 and its complex with TIGIT (Fig. 2C). The predicted interaction mode of the AET2010/TIGIT complex (Fig. 2D and Table S2) reveals that the binding epitope of AET2010 on TIGIT overlaps the CD155 recognition region, which was defined as lock-and-key binding pocket [13]. The plausibility of the AET2010/TIGIT complex model was also demonstrated by substitution of these interface residues with alanine and subsequent detection of binding activity changes using the ForteBio system (Table S3). To some extent, the predicted recognition mode of AET2010 to TIGIT explains the molecular mechanism of its blockade activity.

AET2010 promotes the cytotoxicity of NK-92MI cells towards malignant cells in vitro

As reported, NK-92MI is a cell line with biological activity analogous to that of activated NK cells and possesses cytotoxicity to a wide range of malignant cells [14]. In this research, we confirmed a certain extent of TIGIT expression on NK-92MI cells (Fig. S6), which may result in its cytotoxicity to tumor cells being suppressed by high CD155 expression, similar to the described exhaustion and dysfunction of NK cells in tumor-bearing mice and some cancer patients [7]. Consequently, blocking antibodies against TIGIT may have the ability to restore and promote the antitumor ability of NK-92MI.

Cytotoxicity assays were established to investigate the effect of TIGIT blockade on NK-92MI in vitro. Some kinds of adherent tumor cell lines with elevated expression of CD155, such as A549, A431 and U251MG (Fig. S7), were used as target cells, while suspended NK-92MI cells were used as effector cells. The viability of tumor cells after the killing process was detected. The levels of cytotoxic factors, such as perforin, INF-γ and TNF-α, secreted by NK-92MI cells were quantified in the medium. As shown in Fig. 3A-C, NK-92MI cells have limited killing ability for all three types of tumor cells, while their cytotoxicity is significantly enhanced by TIGIT antibodies, either AET2010 or MK7684. Additionally, the amounts of released cytotoxic factors are in good agreement with the killing rate of NK-92MI cells exposed to antibodies and display a direct association with antibody concentration (Fig. 3E–G). Moreover, when combined with nivolumab, both AET2010 and MK7684 exhibited considerable improvement in NK-92MI cytotoxicity to $CD155^+$ $PD\text{-}L1^+$ U251MG cells (Fig. 3D and H, S8B and S9B).

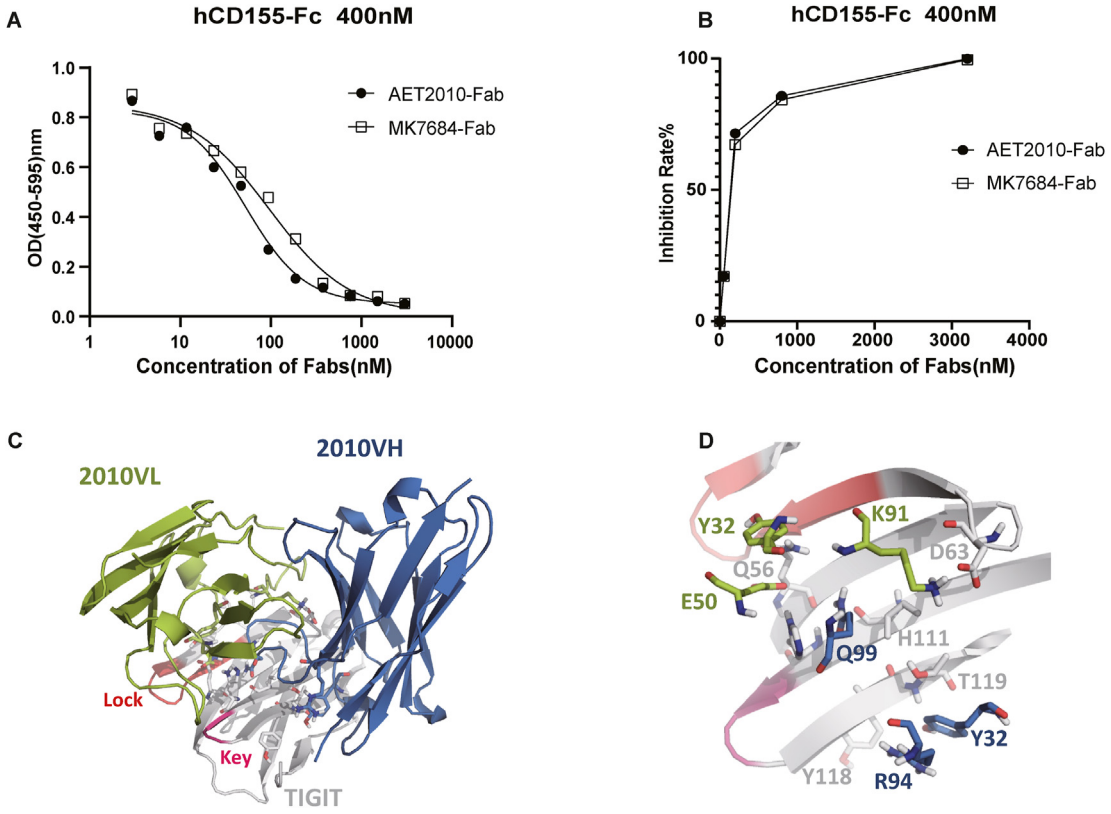

Fig. 2. AET2010 could effectively block the interaction of TIGIT and CD155-Fc. A-B The blocking activities of AET2010-Fab and MK7684-Fab (positive control) validated via competitive ELISA (**A**) or flow cytometry (**B**). **C** The AET2010/TIGIT complex structure model. **D** The predicted interaction interface between AET2010 and TIGIT. Variable region of AET2010 light chain (VL): blue, variable region of heavy chain (VH): green, TIGIT: light gray, CD155-recognized lock-and-key pocket on TIGIT: red and pink. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

AET2010 shows enhanced antitumor activity in a xenograft model with NK-92MI injection

The in vivo antitumor efficacy of AET2010 was tested in an A549 cell xenograft nude mouse model, which is highly sensitive to NK-92MI cells (Fig. 3). At the same time, considering the non-crossreactivity of AET2010 to mouse TIGIT (Fig. S3B), an adoptive injection of NK-92MI cells was performed concomitant with antibody treatment. As shown in Fig. 4A-B, the mouse group receiving AET2010 treatment exhibited significantly decreased tumor volume and tumor weight compared with the isotype IgG group at the experimental endpoint. No significant difference was observed between the AET2010 and MK7684 groups. In line with the ex vivo assay, the blockade of TIGIT via antibodies in vivo also promoted the activity of NK-92MI and resulted in enhanced antitumor efficacy in the A549 xenograft model with NK-92MI injection.

Discussion

NK cells are essential antitumor innate lymphocytes and suffer functional exhaustion in tumor microenvironments [7,15]. Unleashing tumor-infiltrating NK cells might provide a direct attack on escaped tumor cells with downregulated expression of MHC class I and prevent the exhaustion of $CD8^+$ T cells, which is considered beneficial for the therapeutic effects of PD-1/PD-L1 antibodies [7,8]. As a promising checkpoint target expressed on both NK cells and T cells, TIGIT has been proven to be closely associated with tumor progression [16,17]. Some current investigational antibodies targeting TIGIT have also achieved dramatic efficacy in late clinical research [9]. In this study, we developed a novel candidate therapeutic antibody, AET2010, which was acquired from a phage-displayed human single-chain antibody library via a cell panning strategy. AET2010 specifically recognizes human TIGIT and effectively blocks its interaction with CD155, resulting in a significant promotion of the cytotoxicity of NK-92MI cells towards multiple types of malignant cells in vitro. Additionally, AET2010 also enhances the antitumor activity of NK-92MI injection in an A549 xenograft mouse model. Together, these results are promising and warrant further development of AET2010.

In our research, the application of a cell panning strategy was executed for the selection of anti-TIGIT antibodies and to avoid the need for preparation of high-quality recombinant target proteins, which is usually considered critical but very challenging. This cell panning strategy would be a valuable approach for other drug targets, such as G-protein coupled receptors.

According to our results (Table 1 and Fig. S4), both AET2010 and MK7684 bind robustly to TIGIT with apparently higher affinity than its main receptor CD155, whose previously reported affinity is approximately 3.2 μM [18]. Considering the predicted antigen epitope of AET2010 (Fig. 2C and D), AET2010 could effectively block the interaction of TIGIT and CD155, which was also confirmed by our research (Fig. 2A and B). Moreover, AET2010 shows much lower affinity (approximately 34 times, Table 1) but similar avidity (Fig. 1B–D) and activity (Figs. 3–4) to MK7684, implying that AET2010 may occupy a preferable antigen epitope. More importantly, the lower affinity, and especially the fast disassociation rate, might assign AET2010 a poor interaction with normal cells with little expression of TIGIT but considerable binding power to exhausted NK cells or T cells with high-expressed TIGIT (avidity), which may result in a lower risk of potential side effects but

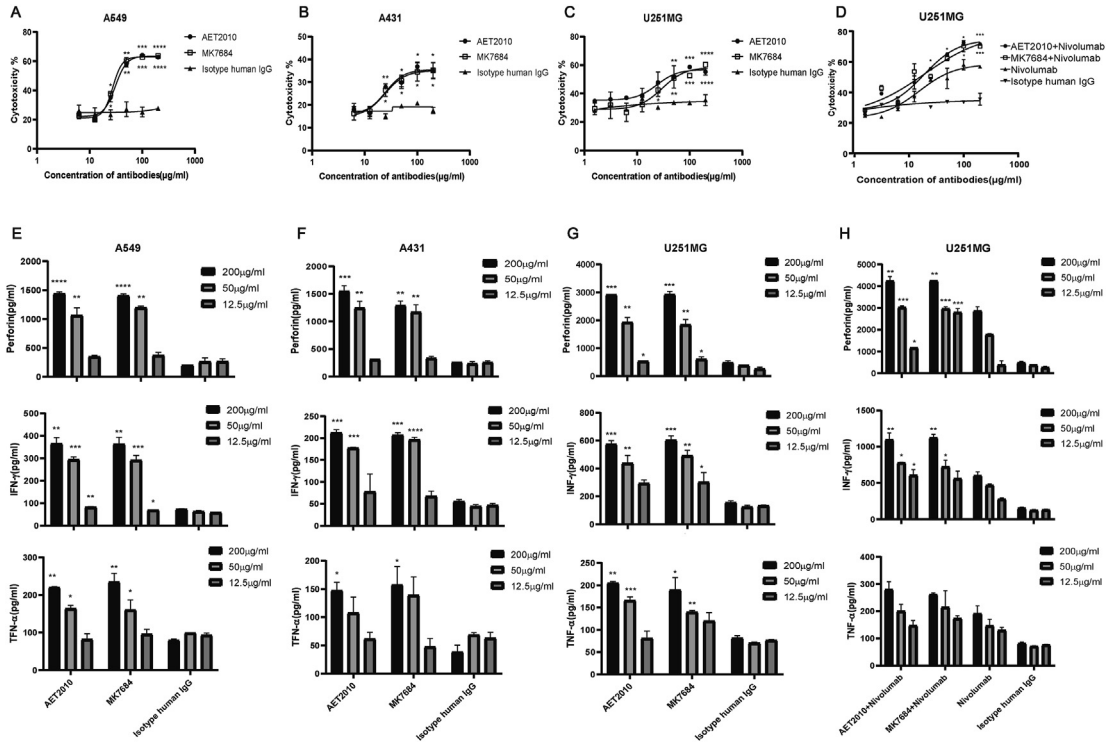

Fig. 3. AET2010 enhanced the cytotoxicity of NK-92MI cells towards malignant cells in vitro. A-C The cytotoxicity of NK-92MI cells towards A549 (**A**), A431 (**B**) and U251MG (**C**) cells after blockade with AET2010 or MK7684, as detected by CCK8. **E-G** The levels of perforin, INF-γ and TNF-α in the supernatant of A549 (**E**), A431 (**F**) and U251MG (**G**) cells measured via ELISA. **D, H** The cytotoxicity of NK-92MI cells towards U251MG cells (**D**) and the levels of perforin, INF-γ and TNF-α (**H**) after dual blockade of nivolumab and AET2010 (or MK7684). Isotype human IgG served as a negative control. P values were derived using the *t*-test. *p < 0.05, **p < 0.01, ***p < 0.001. ****p < 0.0001.

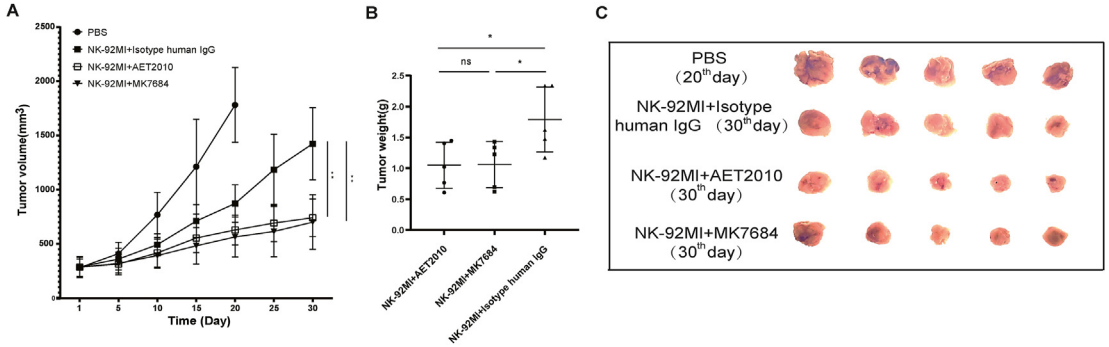

Fig. 4. AET2010 enhanced the antitumor activity of NK-92MI injection in an A549 xenograft mouse model. BALB/c nude mice subcutaneously transplanted with A549 cells were treated with NK-92MI cells combined with AET2010, MK7864 or isotype human IgG or administered PBS every five days. **A** Tumor volumes in mice were measured at various times after challenge. **B** The mice were euthanized on the 30th day, and then the tumor weights of the mice were assessed. **C** Images of tumor masses from different groups. P values were derived using ANOVA (**A**) or *t*-test (**B**). *p < 0.05, **p < 0.01, ****p < 0.0001.

undiminished efficacy.

To our knowledge, our experiments are the first to use a xenograft model with adoptive injection of NK-92MI to assess the in vivo antitumor activity of TIGIT inhibitors. Based on this model, we confirmed a significant antitumor efficacy of AET2010 directly mediated by NK-92MI cells (Fig. 4), which was in accordance with the results of the in vitro cytotoxicity assay (Fig. 3A). Nevertheless, this model is not sufficient for the precise evaluation of anti-TIGIT antibodies because of its boosting of NK cells and neglect of the modulating effect of NK cells on T cells. Published studies have shown that humanized mice, such as hTIGIT mice or hPD-1/hTIGIT mice from Beijing Biocytogen Co., Ltd., are a useful in vivo platform for the pharmacodynamic analysis of immune checkpoint inhibitors [19]. According to our results (Figs. 3–4), we anticipate promising pharmacodynamic effects of AET2010 as a monotherapy or in combination with PD-1 antibody in TIGIT or PD-1 humanized mouse models. Such experiments are planned.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dr. Shuang Wang (Beijing Kohnoor Science & Technology Co., Ltd., Beijing, China) for the gift of nivolumab. We are

also grateful to Prof. Junjie Yue for his support of computational design workstation. We further thank Prof. Zhiwei Sun, Qiang Sun, Weiyi Qiu and Yunzhou Yu for their expert technical help.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2020.12.013.

References

[1] X. Yu, K. Harden, L. C Gonzalez, et al., The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells, Nat. Immunol. 10 (2009) 48–57.
[2] N.A. Manieri, E.Y. Chiang, J.L. Grogan, TIGIT: a key inhibitor of the cancer immunity cycle, Trends Immunol. 38 (2017) 20–28.
[3] N. Stanietsky, H. Simic, J. Arapovic, et al., The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity, Proc. Natl. Acad. Sci. Unit. States Am. 106 (2009) 17858–17863.
[4] W.C. Dougall, S. Kurtulus, M.J. Smyth, et al., TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy, Immunol. Rev. 276 (2017) 112–120.
[5] S. Liu, H. Zhang, M. Li, et al., Recruitment of Grb2 and SHIP1 by the ITT-like motif of TIGIT suppresses granule polarization and cytotoxicity of NK cells, Cell Death Differ. 20 (2013) 456–464.
[6] N. Joller, J.P. Hafler, B. Brynedal, et al., Cutting edge: TIGIT has T cell-intrinsic inhibitory functions, J. Immunol. 186 (2011) 1338–1342.
[7] Q. Zhang, J. Bi, X. Zheng, et al., Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity, Nat. Immunol. 19 (2018) 723–732.
[8] A. Stojanovic, A. Cerwenka, Checkpoint inhibition: NK cells enter the scene, Nat. Immunol. 19 (2018) 650–652.
[9] https://www.roche.com/media/releases/med-cor-2020-05-14.htm.
[10] https://www.merck.com/news/early-phase-1-data-from-mercks-oncology-pipeline-for-investigational-anti-lag-3-therapy-mk-4280-and-anti-tigit-therapy-mk-7684-to-be-presented-at-sitcs-33rd-annual-meeting/.
[11] P. Du, L. Xu, W. Qiu, et al., A fully human monoclonal antibody with novel binding epitope and excellent neutralizing activity to multiple human IFN-α subtypes: a candidate therapy for systemic lupus erythematosus, mAbs 7 (2015) 969–980.
[12] D. Zeng, Q. Sun, A. Chen, et al., A fully human anti-CD47 blocking antibody with therapeutic potential for cancer, Oncotarget 7 (2016) 83040–83050.
[13] K.F. Stengel, K. Harden-Bowles, X. Yu, et al., Structure of TIGIT immunoreceptor bound to poliovirus receptor reveals a cell-cell adhesion and signaling mechanism that requires cis-trans receptor clustering, Proc. Natl. Acad. Sci. U. S. A 109 (2012) 5399–5404.
[14] J.H. Gong, G. Maki, H.G. Klingemann, Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells, Leukemia 8 (1994) 652–658.
[15] Q.-F. Zhang, W.-W. Yin, Y. Xia, et al., Liver-infiltrating CD11b-CD27- NK subsets account for NK-cell dysfunction in patients with hepatocellular carcinoma and are associated with tumor progression, Cell. Mol. Immunol. 14 (2017) 819–829.
[16] C. Kandoth, M.D. McLellan, F. Vandin, et al., Mutational landscape and significance across 12 major cancer types, Nature 502 (2013) 333–339.
[17] R.J. Johnston, L. Comps-Agrar, J. Hackney, et al., The immunoreceptor TIGIT regulates antitumor and antiviral CD8 + T cell effector function, Canc. Cell 26 (2014) 923–937.
[18] F.A. Deuss, B.S. Gully, J. Rossjohn, et al., Recognition of nectin-2 by the natural killer cell receptor T cell immunoglobulin and ITIM domain (TIGIT), J. Biol. Chem. 292 (2017) 11413–11422.
[19] Z. Shuang, Z. Min, W. Weiwei, et al., Preclinical characterization of sintilimab, a fully human anti-PD-1 therapeutic monoclonal antibody for cancer, Antibody Therap. 2 (2018) 65–73.