Original Article

Iranian Journal of Immunology

December 2025; 22(4):

In Vitro Expansion of Dendritic Cells Pulsed with Placental Peptides for the Generation of Antigen-Specific T Cells with Antitumor Activity

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ABSTRACT

Background: Placental tissue contains a variety of tumor-associated antigens. Antigen-specific T cells generated by expanded dendritic cells (DCs) loaded with placental peptides have the potential to exert antitumor effects both in vitro and in vivo.

Objective: To investigate the immunotherapeutic potential of placental peptides as a novel source of tumor-associated antigens (TAAs). We hypothesized that DCs, expanded and matured in vitro and pulsed with placental peptide extracts, can effectively prime antigen-specific T cells (ASTs) with robust cytotoxic activity against various tumor cell lines and in vivo tumor models.

Methods: Mass spectrometry was used to identify tumor-related peptides within the placental extract. In vitro assays were employed to assess the expansion of DCs, their maturation following exposure to placental peptide preparations, and the subsequent activation of T cells.

Results: The cytotoxic activity of ASTs was assessed and showed strong tumor cell killing across three cancer cell lines, U87MG (glioblastoma), SH-SY5Y (neuroblastoma), and MCF-7 (breast cancer). In a neuroblastoma animal model, ASTs treatment significantly reduced tumor proliferation, indicating substantial therapeutic potential in vivo.

Conclusion: Our findings suggest that DCs pulsed with placental peptides can generate ASTs with potent antitumor activity in vitro and in vivo. Additional studies are needed to determine applicability across other tumor types, refine therapeutic parameters, and assess clinical potential.

Keywords: Antigen-specific T cells, cancer immunotherapy, Dendritic cell expansion, neuroblastoma, placental peptides

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Cite this article as:
Lin Y, Huang NP, He YB, Zuo PJ, Yang LQ, Huang FJ, Ye YQ. In Vitro Expansion of Dendritic Cells Pulsed with Placental Peptides for the Generation of Antigen-Specific T Cells with Antitumor Activity. *Iran J Immunol.* 2025; 22(4): , doi: 10.22034/IJI.2025.107705.3075.

Received: 2025-07-11
Revised: 2025-08-21
Accepted: 2025-09-02

INTRODUCTION

The immune system plays a critical role in the recognizing and eliminating of tumor cells. Dendritic cells (DCs), as professional antigen-presenting cells (APCs), are key initiators of immune responses. They capture, process, and present antigens to T cells, enabling the development of targeted adaptive immune responses (1, 2). Tumors, however, frequently evade immune surveillance by modulating immune responses, contributing to the complexity of cancer immunotherapy. Among current strategies, one promising approache is the use of DCs as therapeutic vaccines, in which DCs are loaded with tumor-associated antigens (TAAs) to stimulate tumor-specific T cells.

The human placenta has a long history as a medicinal substance in China and other East Asian countries, where it has served as a source of human-derived therapeutic agents (3, 4). Among these products, placenta-derived polypeptide injections, approved for clinical use in China and Japan, are manufactured from fresh placental tissue obtained from healthy mothers. Through in vitro chromatography, a variety of small-molecule peptides can be isolated from placental preparations. These peptides exhibit diverse therapeutic and preventive activities, including antiviral, anti-infective, anti-allergic, anti-mutagenic, anti-tumor, and endocrine-regulatory effects (5-9). These peptides are also widely applied in medical aesthetics (10-12). In oncology, placenta-derived polypeptide injections is are administered either as monotherapy or in combination with other therapeutic approaches, including surgery and chemotherapy. They have been reported to activate T cells, enhance immune responses, mitigate the adverse effects of chemotherapy and radiotherapy, and improve survival rates (13).

In this study, we investigated the therapeutic potential of placental peptides in cancer treatment by employing in vitro-expanded DCs loaded with placental peptides to induce the generation of ASTs. We assessed the cytotoxic activity of these T cells against tumor cell lines, including U87MG, SH-SY5Y, and MCF-7, and evaluated their therapeutic efficacy in a neuroblastoma animal model, with the goal of establishing a promising strategy for the treatment and prevention of cancer.

MATERIALS AND METHODS

Expansion of DCs from Hematopoietic Stem Cell (HSC)

The study was approved by the Ethics Committee of Fujian Provincial Maternal and Child Health Care Hospital (Approval No. 2021KRD024). Peripheral blood (PB) samples were obtained from three healthy donors after routine mobilization with granulocyte colony-stimulating factor (G-CSF, 10μg/kg/day for 4–5days), and informed consent was secured prior to collection. Mononuclear cells (MNCs) were isolated using Ficoll-Paque density gradient centrifugation and donor HLA genotyping was performed by BGI (Shenzhen China). $CD34^+$ hematopoietic progenitor cells were enriched using magnetic-activated cell sorting (Stemery, China) according to the manufacturer's instructions. The purified $CD34^+$ cells were seeded in 24-well plates at a density of 5×10^5 /mL in RPMI 1640 medium(ThermoFisher, USA) supplemented with 10% fetal bovine serum (FBS, ThermoFisher, USA), 50 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, PeproTech, USA), 50 ng/mL interleukin-4 (IL-4, PeproTech), and 100 ng/mL Flt3-ligand (FL, PeproTech). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2, and the medium was refreshed every two days with fresh cytokines. After 20–25 days of culture, immature dendritic cells (iDCs) were induced to mature by adding a maturation cocktail to the culture medium, consisting of 10 ng/mL lipopolysaccharide (LPS, Sigma-Aldrich, USA), 10 ng/mL tumor necrosis factor-alpha (TNF-α, PeproTech), and 1 μg/mL CD40

ligand (CD40L, PeproTech). The cells were incubated for an additional 48 hours under the same culture conditions. Morphological changes indicative of maturation were monitored using an inverted phase-contrast microscope. Dendritic cell phenotypes were analyzed by flow cytometry (Mindray, China). Cells were stained with fluorophore-conjugated monoclonal antibodies specific for the following markers: CD80, CD86, CD83, HLA-DR (BD Biosciences, USA).

Mass Spectrometry Analysis for Placental Peptides

Placenta polypeptide injection purchased from Guizhou Taibang Pharmaceutical. All samples were analyzed using an UltiMate 3000 RSLCnano system coupled online with a Q Exactive HF mass spectrometer through a Nanospray Flex ion source (ThermoFisher). MS raw data were processed with MaxQuant (version 1.6.6.0) using the Andromeda database search algorithm. Spectra were searched against the human protein sequence database downloaded from (Uniprot, No.20230619) using the following parameters: variable modification was set as Oxidation (M, +15.994915 Da); fixed modification was specified as carbamidomethylation (C, +57.021464 Da); enzyme digestion specificity was set to unspecific with a maximum of zero missed cleavages; peptide mass tolerance for the first and main searches were set to 20 ppm and 4.5 ppm, respectively; and fragment mass tolerance was set to 20 ppm. Proteins that could not be distinguished based on unique peptides were merged by MaxQuant into a single protein group. Search results were filtered at a 1% false discovery rate (FDR) at both peptide and protein levels. To explore potential correlations between identified peptides (proteins) and cancer, data were analyzed using the UALCAN database, following the procedures outlined in the tutorial provided on the website (http://ualcan.path.uab.edu.).

Cell Lines and Reagents

Human tumor cell lines, including U87MG, SH-SY5Y, and MCF-7, were obtained from the American Type Culture Collection (ATCC). These cell lines were selected due to their established relevance in cancer research and their distinct biological characteristics. For instance, U87MG glioblastoma cells are widely used in preclinical models of brain tumor, whereas SH-SY5Y and MCF-7 cells represent other major cancer types that exhibit resistance to conventional therapies. The K562 cell line was procured from Promocell (Wuhan, China). All cell lines were cultured and maintained according to the manufacturer's instructions.

Generation of ASTs

Immature DCs were incubated with 1mL of placental peptides for 24 hours to ensure efficient loading. Following a 48-hour incubation with DC maturation factors, the cells were thoroughly washed with RPMI-1640 medium to remove unbound proteins. The expression of surface markers, including CD80, CD83, CD86, and HLA-DR, was analyzed by flow cytometry (Mindray, China) to confirm DC maturation. To generate ASTs, naïve $CD8^+$ T cells were isolated using the Naïve CD8+T Cell Separation Kit (Stemery, China) and co-cultured with DCs from the same donor pulsed with placental peptides at a DC: T cell ratio of 1:4. Cultures were maintained in serum-free T cell medium (Stemery, China) supplemented with 100 U/mL IL-2, 10 ng/mL IL-21, 20 ng/mL IL-7, and 10 ng/mL IL-15 (EastMab, China). DCs were used to stimulate T cells weekly for a total of four to five stimulations with fresh medium added every 4-5 days to support optimal T cell expansion. Generation of ASTs using placental gp96 (Heat-Shock Bio, China) and synthetic peptides (GenScript, China) was performed following the same procedure used for placental peptide stimulation. In each stimulation cycle, DCs (1×10^7) were pulsed with 100 μg of gp96 and 100 μg of synthetic peptides.

Assessment of T Cell Proliferation

Cultured T cells were resuspended at a density of $1\text{-}2\times10^6$ cells/ml in RPMI-1640

medium supplemented with 10% FBS. Carboxyfluorescein succinimidyl ester (CFSE) was diluted to a final concentration of 1-5μM in DMSO and added to the cell suspension, followed by incubation for 10 minutes at 37°C. After labeling, the reaction was quenched with RPMI-1640 medium and the cells were washed twice by centrifugation (300 × g, 5 min). The CFSE-labeled T cells were then seeded at 1-2 × 10^6 cells/ml in culture medium, and maintained with IL-2 (1000 U/ml) every 2-3 days. Cells were harvested on day 3 and analyzed for proliferation by flow cytometry.

Cytotoxicity Assay

Tumor cell lines U87MG, SH-SY5Y and MCF-7 were used as target cells for cytotoxicity assays. T cells were co-cultured with target cells at varying effector-to-target (E: T) ratios of 1:1, 5:1, and 10:1 for 12 hours. Cytotoxicity was assessed using 7-Aminoactinomycin D (7-AAD) assay (Beyotime, China) analyzed by flow cytometry. Cell viability was determined by measuring 7-AAD staining and comparing the survival of target cells in the presence versus absence of T cells. Specifically, effector T cells and target tumor cells were co-incubated at the indicated E:T ratios in 24-well flat-bottom plates for 12 h at 37°C in a humidified 5% CO_2 atmosphere. Target cells cultured alone served as negative controls. After incubation, cells were collected, washed with ice-cold PBS, and stained with 100 μL of 7-AAD for 5 minutes in the dark. Samples were analyzed by flow cytometry. T cells cytotoxicity (%) was calculated as the proportion of 7-AAD-positive target cells /total CFSE-positive cells, after subtracting spontaneous lysis (%) observed in the negative control.

In vivo Studies

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice were purchased from GemPharmatech (Guangzhou, China) and maintained under specific pathogen-free conditions. To establish a humanized neuroblastoma mouse model, mice were first engrafted with human peripheral blood mononuclear cells (PBMCs). Briefly, 1× 10^7 freshly isolated PBMCs were intravenously injected into 6-8-week-old NSG mice. One week after engraftment, mice were subcutaneously inoculated with 1 × 10^6 SH-SY5Y human neuroblastoma cells suspended in 100 μL of PBS into the right flank. Tumor growth was monitored every 2–3 days. ASTs were generated by stimulating naïve T cells with peptide-loaded DCs and expanded in the presence of IL-2. When tumors reached an average volume of 100–150 mm^3, mice received adoptive transfer of in vitro-expanded ASTs via intravenous injection (1 × 10^7 cells per mouse). Mice were monitored regularly for tumor progression and overall health. At the study endpoint, mice were euthanized via intraperitoneal injection of pentobarbital sodium (100 mg/kg) followed by cervical dislocation. All animal procedures were conducted in accordance with institutional animal care and use guidelines.

Statistical Analysis

Data were analyzed using GraphPad Prism 9 (GraphPad Software, Inc., USA). One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, and Student's t-test was used for pairwise comparisons. A $p\text{-value}<0.05$ was considered statistically significant.

RESULTS

DCs Expansion and Maturation

HSCs derived from three healthy donors were expanded in vitro over approximately 25 days, yielding a total cell increase ranging from 812- to 2213-fold relative to the initial cell number (Fig. 1a). The expanded cells served as precursors for DC generation and exhibited high expression of the monocyte- associated marker CD14 (Fig. 1b). Following stimulation with DC maturation-inducing factors, including LPS, TNF-α and CD40L, the precursor cells differentiated into DCs displaying characteristic morphological changes (Fig. 1c).

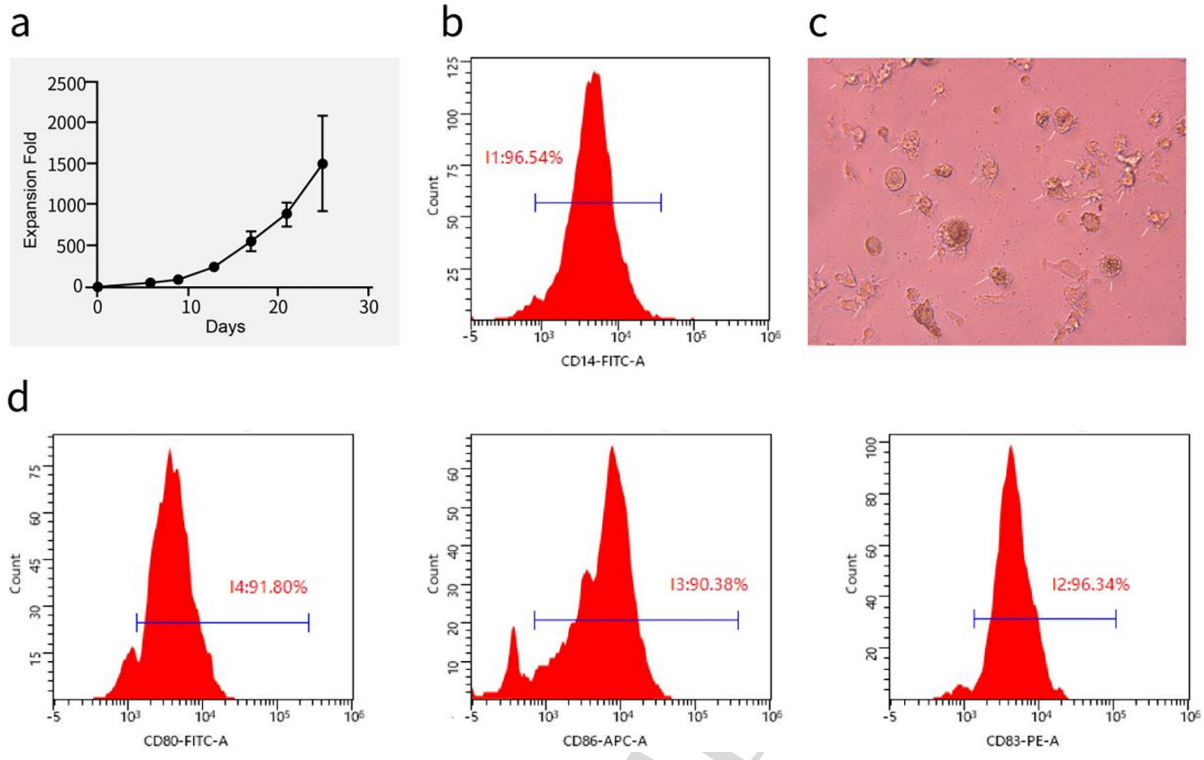

Fig. 1. In vitro expansion and maturation of dendritic cells (DCs). a. Fold expansion of HSC-derived cells relative to day 0. b. Expression of CD14 on DC precursors. c. Representative morphology of expanded DCs. d. Flow cytometric analysis of surface expression of the maturation markers CD80, CD83, and CD86 on expanded DCs.

The resulting mature DCs demonstrated a phenotype consistent with fully differentiated DCs, characterized by elevated expression of CD80, CD83, and CD86 (Fig. 1d).

Placental Peptides Information

A total of 27 peptides originating from 21 proteins were identified in the placenta polypeptide injection using mass spectrometry (Table S1).

Consistent with the well-recognized biological similarities between placental tissue and tumors, most genes corresponding to these peptides have been previously reported to be associated with tumors (Table 1).

A comparable trend was observed at the protein expression level. Several proteins corresponding to the identified peptide fragments exhibited significant differential expression between normal and tumor tissues. Notably, DSTN protein was markedly downregulated in tumor tissues, whereas RPL26L1, TIMELESS, POLR3C, and HNRNPA2B1 proteins were significantly upregulated (Fig. 2).

To further assess their immunogenic potential, the identified peptide fragments were analyzed using SYFPEITHI and the IEDB prediction tools to evaluate candidate T-cell epitopes (Table S2). Based on these analyses, ten dominant CTL epitopes were selected and synthesized for the generation of ASTs.

Generation of ASTs and Cytotoxicity Assay

DCs loaded with placental peptides were used to stimulate T cells. After five rounds of DC stimulation, the T cells exhibited robust proliferation. In all three samples, we successfully induced T cell expansion, with fold increases ranging from 14.5 to 36.5 times. The final cell population comprised CD4+ (20%-40%) and CD8+ (40%-55%) T cells, with 30%-55% of the cells displaying a memory phenotype (Fig. 3a). We also observed a significant acceleration in T cell proliferation beginning at the fourth stimulation. Synthetic peptides corresponding to identified CD8 epitopes were used to pulse DCs for T cell stimulation.

Table 1. Tumor association and functional roles of genes corresponding to identified placental peptides

Gene	Expression in Tumors	Role in Cancer	Reference
FGA	Overexpressed in pancreatic cancer and several other solid tumors.	Component of fibrinogen; associated with tumor progression, invasion, and metastasis.	(14)
BPTF	Upregulated in several cancers, including melanoma and lung cancer.	Chromatin remodeling factor; promotes tumor progression and is associated with poor prognosis.	(15)
H1-2	Dysregulated expression reported in certain cancers.	Histone H1 variant involved in chromatin organization; potential role in gene regulation during tumorigenesis.	(16)
WASL	Overexpressed in breast and colorectal cancers.	Regulates actin cytoskeleton dynamics; enhances cell motility, invasion and metastatic potential.	(17)
ITIH4	Elevated expression in hepatocellular carcinoma.	Acute-phase protein; implicated as a potential biomarker and may contribute to tumor progression and metastasis.	(18)
C11orf91	Cancer-associated expression patterns are poorly characterized.	Functional role in cancer remains unclear; further investigation is required.	(19)
LMTK3	Upregulated in breast cancer and gliomas.	Regulates estrogen receptor signaling; associated with therapeutic resistance and unfavorable clinical outcomes.	(20)
KNG1	Differentially expressed in prostate and renal cancers.	Involved in the kallikrein–kinin system and coagulation; may influence tumor angiogenesis and metastasis.	(21)
LMNA	Aberrant expression observed in colorectal and gastric cancers.	Encodes nuclear lamins; altered expression contributes to nuclear instability and cancer progression.	(22)
HMGN2	Overexpressed in certain leukemias.	Nucleosome-binding protein; modulates chromatin accessibility and gene transcription in cancer cells.	(23)
HNRNPA2B1	Elevated in lung and breast cancers.	RNA-binding protein; involved in mRNA splicing, stability and oncogenic transcript regulation.	
DSTN	Increased expression in hepatocellular carcinoma.	Actin-depolymerizing factor; may contribute to cytoskeletal rearrangements and enhanced tumor cell motility.	(25)
TMSB4X	Upregulated in various cancers, including gastric and prostate.	Promotes cell migration, angiogenesis and tumor progression.	(26)
KCNC3	Altered expression reported in gliomas.	Voltage-gated potassium channel; potential role in regulating tumor cell proliferation.	(27)
RAPH1	Overexpressed in breast cancer.	Mediates actin cytoskeleton dynamics and cell migration; associated with enhanced metastatic potential.	(28)

Gene	Expression in Tumors	Role in Cancer	Reference
RPLP0P6	Pseudogene; expression and role in cancer are not well-documented.	Functional relevance in cancer is unclear; Potential regulatory role on parental gene expression.	(29)
H2BC14	Limited data is available on its expression in cancers.	Histone protein; alterations may impact chromatin structure and gene expression in tumors.	(30)
POLR3C	Overexpressed in colorectal cancer.	Subunit of RNA polymerase III; may affect transcription of genes promoting tumorigenesis.	(31)
MMP17	Upregulated in gastric and breast cancers.	Matrix metalloproteinase; involved in extracellular matrix remodeling and metastasis.	(32)
RPL26L1	Limited data on its expression in cancers.	Ribosomal protein; potential role in altered protein synthesis during cancer progression.	N/A
TIMELESS	Upregulated in breast and colorectal cancers.	Participates in DNA replication and circadian rhythm regulation; linked to genomic stability in tumors.	(33)

In two donors, proliferation was comparable to that induced by natural placental peptides, whereas T cells from the third donor showed poor expansion across three independent experiments. These findings indicate that both placental peptides and synthetic analogs can drive T cell proliferation, while T cells without peptide stimulation fail to proliferate (Fig. 3b).

To further assess the specificity of the generated T cells, cytotoxicity assays were performed. Based on previous reports, placenta-derived gp96 contains multiple tumor-associated peptides and was therefore used as a positive control (34, 35). ASTs generated by DCs loaded with placental gp96 protein demonstrated potent antitumor activity. ASTs generated by DCs loaded with placental peptides showed comparable cytotoxicity, though slightly reduced against the MCF-7 cell line compared with the gp96 group (P=0.038). For U87MG, SH-SY5Y, and K562 cell lines, cytotoxicity was similar between groups. ASTs generated using DCs loaded with predicted dominant epitope peptides also exhibited strong antitumor activity, albeit weaker than that induced by gp96 or placental peptides. Across all conditions, cytotoxicity against K562 cells was consistently lower (Fig. 4).

HLA typing was performed for all three donors. Complete HLA profiles were obtained, except for one case in which DQRB1 could not be definitively identified (Table S3). Importantly, ASTs derived from DCs loaded with placental peptides displayed cytotoxic activity against multiple tumor cell lines regardless of donors HLA genotype. These results suggest that placental peptides may contain epitopes capable of presentation across diverse HLA alleles. However, given the limited donor pool (n=3), these findings should be considered preliminary. Future studies with larger, more diverse cohorts are required to confirm HLA-independence.

To verify MHC restriction, cytotoxicity assays were conducted against SH-SY5Y cells in the presence of anti-MHC antibodies. Addition of anti-HLA class I antibodies (HLA-ABC) significantly reduced AST cytotoxicity (Fig. 5), whereas anti-HLA class II antibodies had no effect (data not shown). These results demonstrate that the generated ASTs are predominantly MHC class I-restricted.

It has been reported that full-length overlapping peptide pools can broaden epitope coverage and stimulate ASTs independently of donor HLA background. Accordingly, we

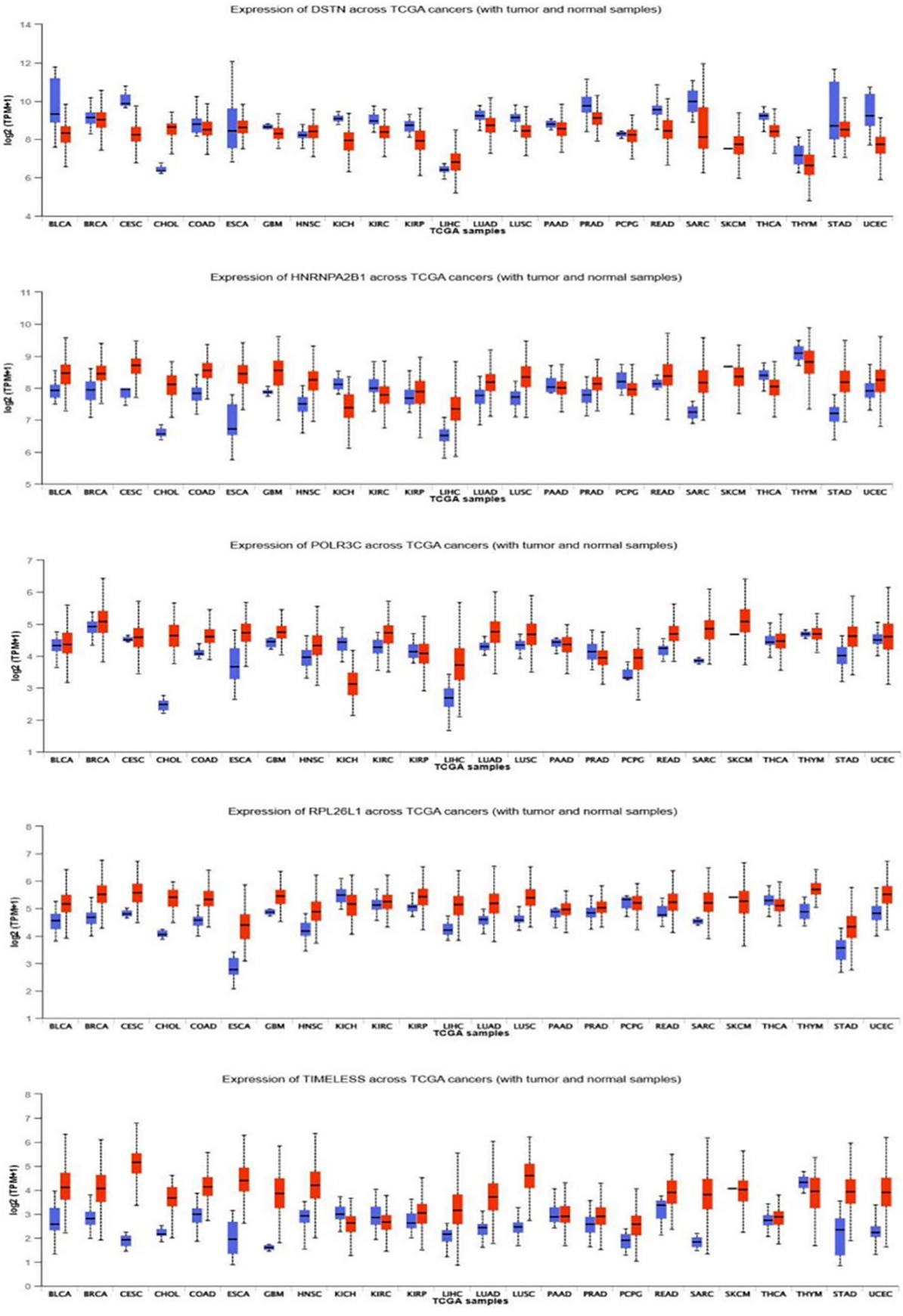

Fig. 2. Differential expression of placental peptide-associated proteins in normal and tumor tissues. Protein expression levels were analyzed using the UALCAN database. Blue bars represent normal tissue, and Red bars represent tumor tissue.

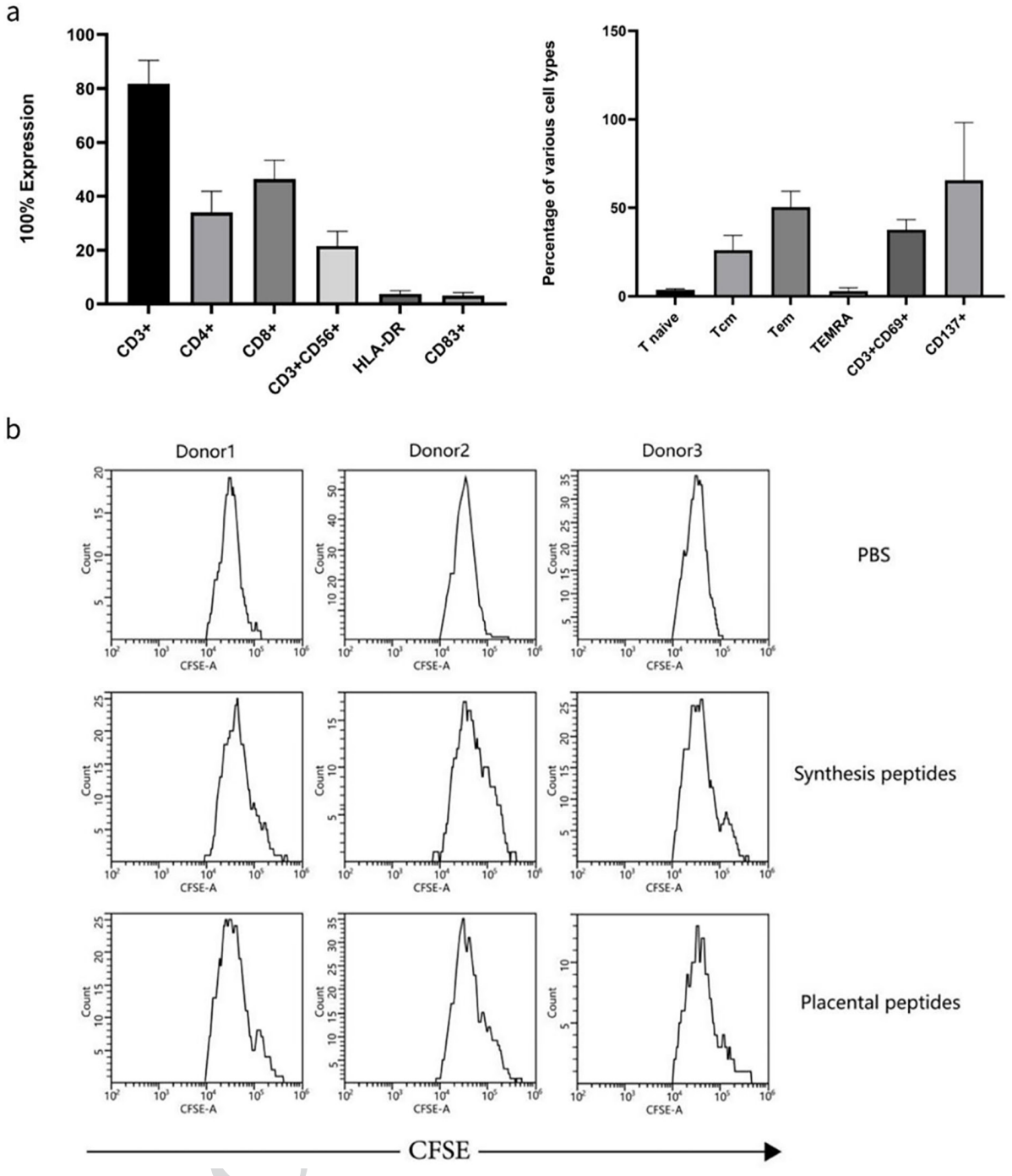

Fig. 3. Proliferation and composition of antigen-specific T cells (ASTs) generated by dendritic cells (DCs) loaded with placental peptides/proteins. a. Proportions of different cell subsets within AST populations generated by DCs pulsed with placental peptides. b. Proliferation of AST cells generated by DCs loaded with placental peptides or synthetic peptides.

synthesized overlapping peptide pools spanning MMP17, TIMELESS, RAPH1, LMTK3, FGA, LMNA, POLR3C, HMGN2 and WASL proteins. Among the four cell lines tested, ASTs induced with the overlapping peptide pools achieved 52.5% cytotoxicity against MCF-7 cells, comparable to placenta-derived peptides (58.7%), with no significant difference ($P>0.05$). In K562 cells, both peptide sources elicited similarly low cytotoxicity. In contrast, ASTs stimulated with overlapping peptide pools demonstrated reduced cytotoxic activity against U87MG and SH-SY5Y cells, with killing efficiencies of 47.4% and 22.5%, respectively, compared to 76.7% and 39.7% for placenta-derived peptide-induced ASTs ($P=0.023$).

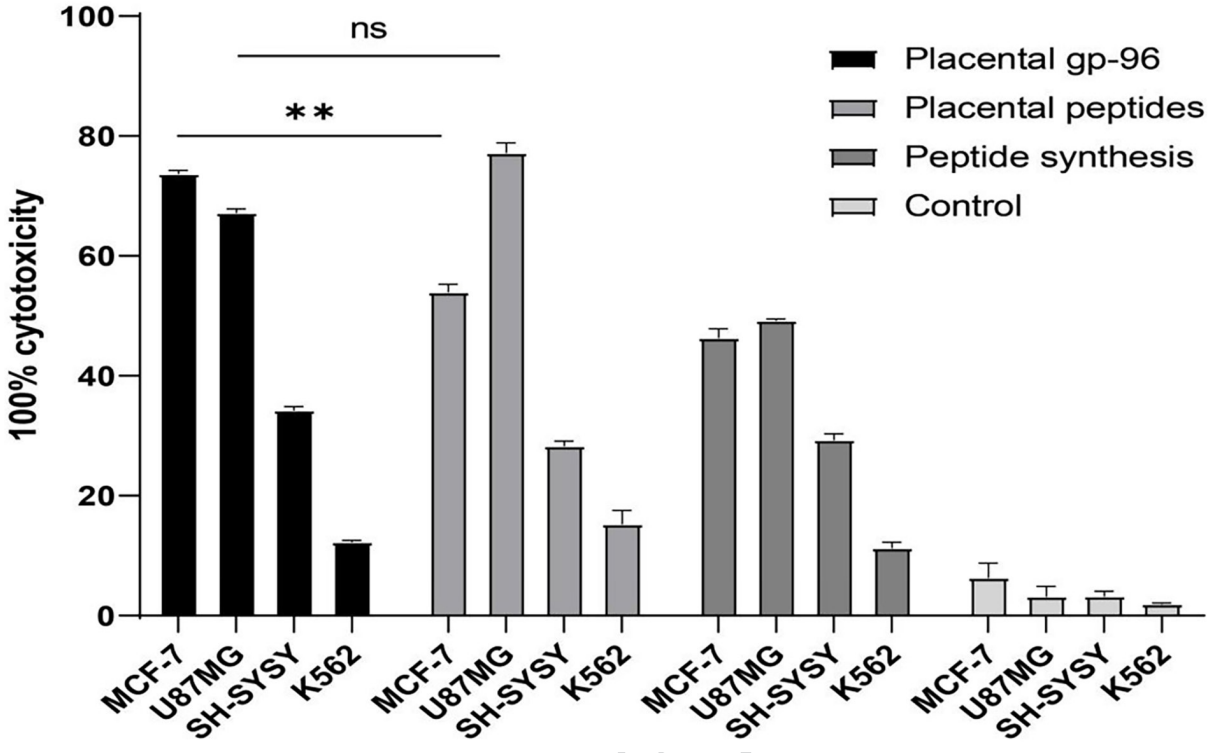

Fig. 4. Cytotoxic activity of antigen-specific T cells (ASTs) generated by dendritic cells (DCs) loaded with placental proteins/peptides on various tumor cell lines.

This reduced activity may reflect preferential overexpression of proteins such as MMP17, TIMELESS, RAPH1, LMTK3, and WASL in breast cancer cells, enhancing antigen processing and presentation in MCF-7 but not in other tumor types (Fig. 6**)**.

ASTs Generated from DCs Pulsed with Placental Peptides In vitro Effectively Suppress Neuroblastoma Growth In vivo.

To evaluate the therapeutic efficacy of ASTs generated by peptide-loaded DCs, we established a subcutaneous neuroblastoma mouse model. Mice were treated with T cells primed using three peptide strategies: an overlapping peptides pool, placental peptides, and an irrelevant peptide control (CMV pp65). Tumor growth was monitored longitudinally. As shown in Fig. 7a–d, mice receiving T cells stimulated with placental peptides exhibited the most pronounced inhibition of tumor growth, followed by the overlapping peptides pool group, whereas the control group displayed the most rapid tumor progression. Fig. 7b demonstrates that tumor volume increased significantly more slowly in the placental peptide group compared with the other groups, particularly after day 10 post-tumor implantation. Consistently, Fig. 7d demonstrates that tumor weights were significantly lower in the placental peptide group compared with the control group ($P<0.01$) and the overlapping peptides pool groups. Notably, both AST-treated groups exhibited superior tumor suppression compared with the control.

DISCUSSION

The placenta and tumors share several biological characteristics, particularly with respect to immune evasion, rapid cellular proliferation, and angiogenesis. Both tissues employ mechanisms to escape immune recognition: the placenta expresses immune checkpoint molecules such as PD-L1 to prevent maternal immune rejection, while tumors often exploit similar pathways to evade immune surveillance.

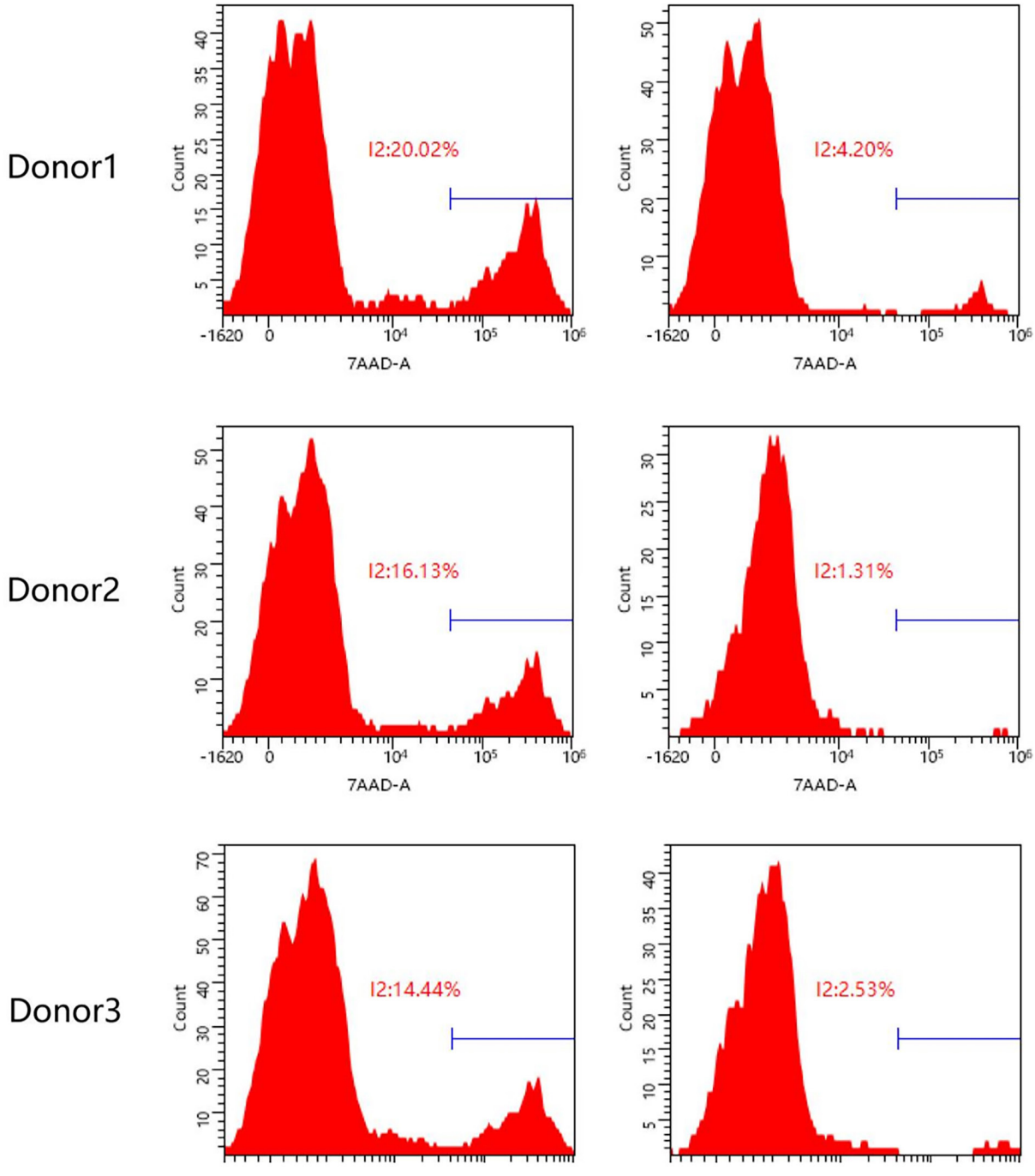

Fig. 5. HLA class I-restriction of AST cytotoxicity. Antigen-specific T cells (ASTs) generated by dendritic cells loaded with placental peptides were tested for their ability to kill SH-SY5Y cells in the presence or absence of an anti-HLA-class I blocking antibody.

Additionally, both exhibit rapid growth—placental expansion supports fetal development, whereas tumor growth results from deregulated cell proliferation. Angiogenesis is also a critical feature of both tissues, driven by factors such as VEGF, to ensure adequate blood supply (36-38). Despite these similarities, a key distinction between the placenta and tumors is that the placenta is a transient organ that detaches from the maternal host after approximately 10 months, whereas tumors typically persist and grow uncontrollably. Investigating whether principles derived from placental biology can be leveraged to inform novel cancer immunotherapies represents an intriguing area of future research. In this study, we identified multiple peptide species derived

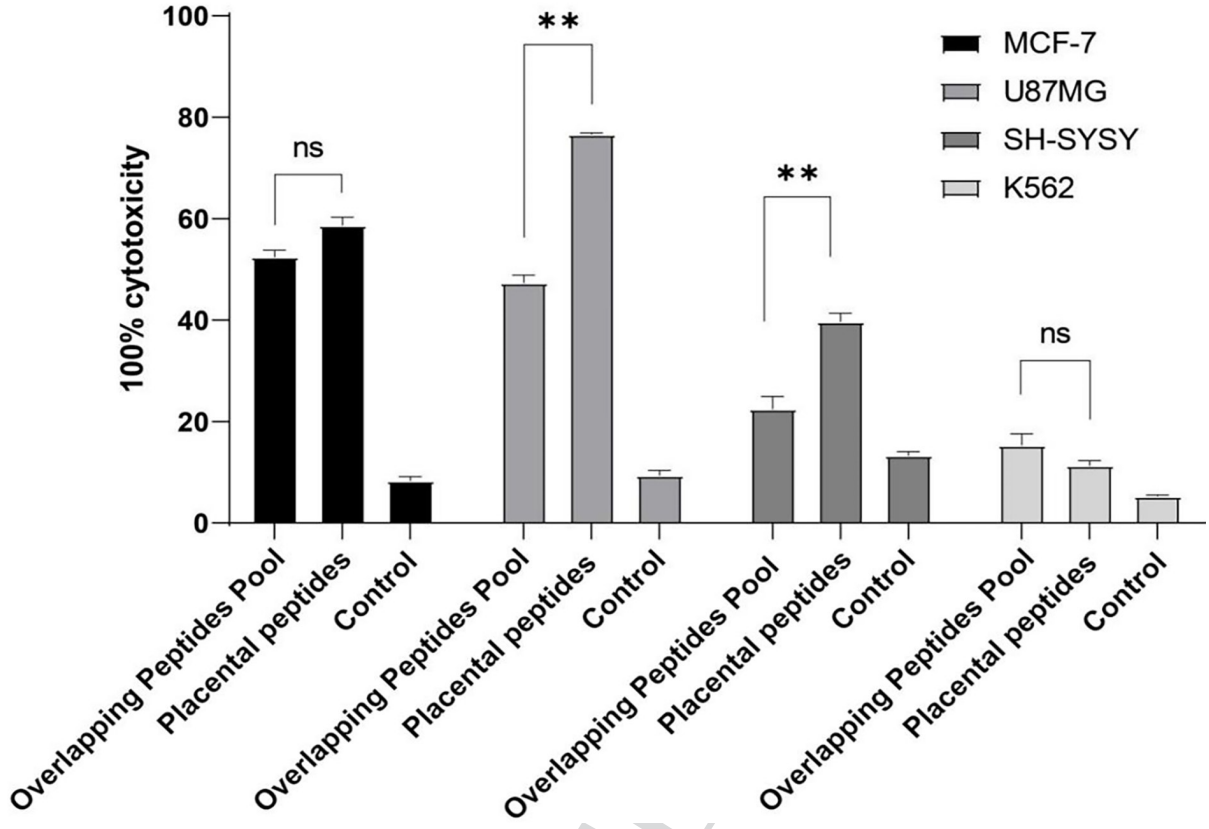

Fig. 6. Cytotoxic activity of ASTs generated by dendritic cells loaded with a full-length protein overlapping peptide pool on MCF-7, U87MG, SH-SY5Y, and K562 cell lines.

from placental tissue and demonstrated their association with tumors. Furthermore, we generated ASTs by loading DCs with placental peptides and validated their antitumor activity both in vitro and in vivo. Importantly, by predicting dominant antigenic epitopes from these identified placental peptides and synthesizing overlapping peptide libraries corresponding to full-length proteins, we propose a feasible strategy to replace placental peptide preparations for the induction of tumor-specific ASTs.

The use of placental-derived substances as immune-activating antigens has been previously reported. For example, placental gp96, which has been used as a vaccine adjuvant, carries naturally chaperoned tumor-associated antigen peptides, including GPC3 and HER2, and is capable of inducing a strong antitumor immune responses both in vitro and in vivo (35). In the present study, we utilized placental-derived peptides and demonstrated their capacity to generate ASTs with potent cytotoxic activity against multiple tumor cell lines. This antitumor efficacy is comparable to the previously reported induction of ASTs using placenta-derived gp96, suggesting that, placental peptides, similar to gp96, may encompass a diverse repertoire of tumor-associated epitopes. Collectively, these findings indicate that placental peptides can serve as a broad-spectrum antigen source for the induction of effective antitumor immune responses.

While our study presents promising results, several limitations should be acknowledged. First, the therapeutic efficacy of this approach was evaluated in only three tumor cell lines and a single in vivo tumor model. Whether placental peptide–induced ASTs exert broad-spectrum antitumor activity requires further validation using a wider range of tumor types and additional animal models. Second, the conclusion regarding HLA-independent cytotoxicity requires confirmation in a larger donor cohort, as the current observations are based on samples from only three donors. Finally, variability in the composition

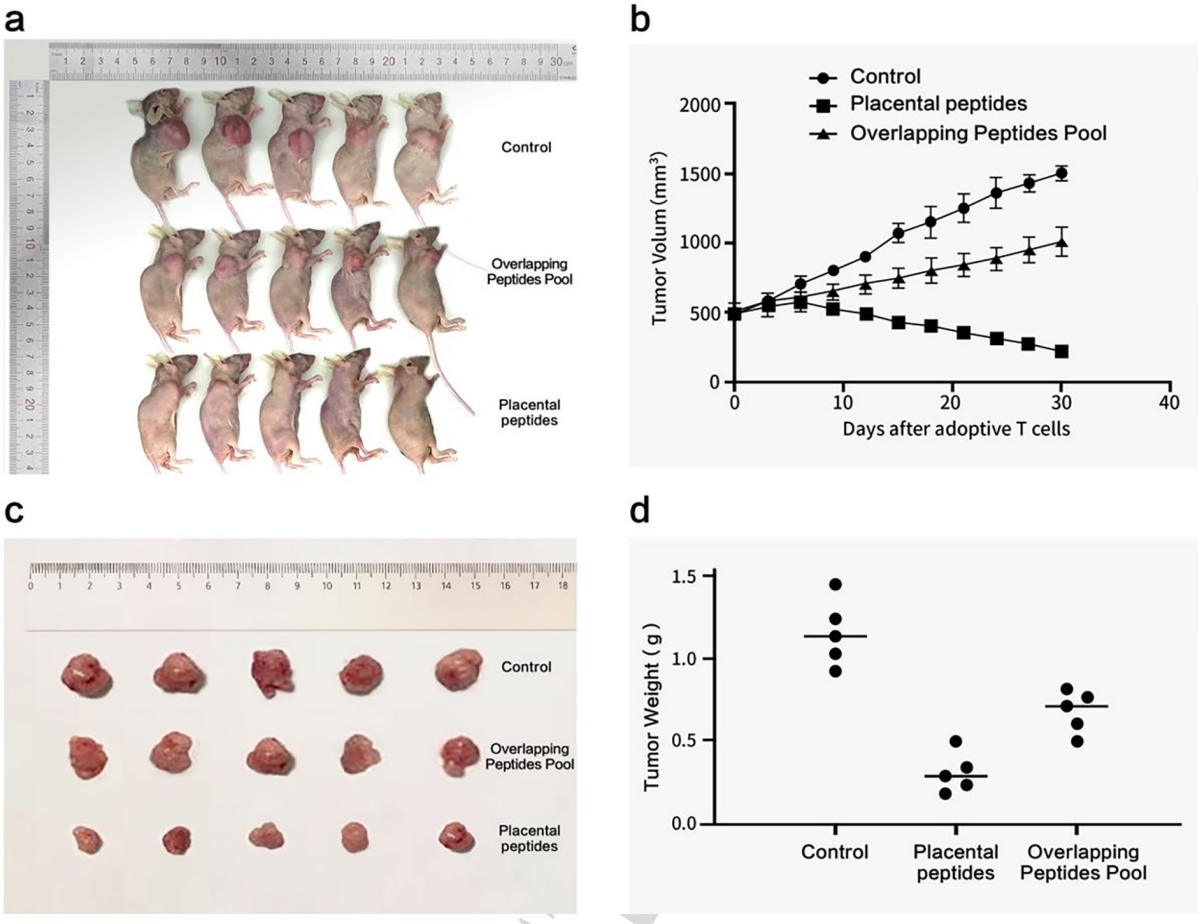

Fig. 7. Therapeutic effect of ASTs generated by placental peptides in a neuroblastoma mouse model. a. Tumor volume in mice on day 30 following AST therapy. b. Tumor growth curves showing changes in tumor volume over the course of AST treatment. c. Volume of excised tumors measured on day 30 post-treatment. d. Tumor weight determined on day 30 after AST therapy.

and consistency of placental polypeptide injections remains a significant challenge. To address batch-to-batch variability, the China National Medical Products Administration (NMPA) and several research groups have established quality-control strategies, including HPLC fingerprinting, to ensure the consistency of placenta polypeptide injection. Huang et al. (39) analyzed ten production batches by HPLC fingerprint analysis and identified nine highly consistent peaks with nearly identical intensities across all batches. The similarity coefficients ranged from 0.992 to 1.000, indicating excellent batch-to-batch reproducibility and product quality. In the present study, we also included representative mass spectra from three independent batches in the Supplementary Materials (Fig. S1), further supporting the relative consistency of the placental peptide composition across batches. This observation is consistent with the findings reported by Huang et al. (39). Notably, placental peptides comprise sequences homologous to tumor-associated proteins as well as peptides derived from proteins expressed in normal tissues. Consequently, the use of placental peptides may carry a potential safety risk by inducing immune responses against normal tissues. Although placenta polypeptide injections have been approved by the China NMPA have been used clinically for more than two decades with a favorable safety profile, their tissue-specific targeting mechanisms are not yet fully understood. To better characterize this potential risk, we are currently conducting in vitro autoimmunity screening using PBMCs from donors with a predisposition to autoimmune diseases. Further mechanistic studies and preclinical

models that more accurately recapitulate human immune responses are required. Additionally, well-designed clinical trials will be essential to comprehensively evaluate the safety, efficacy, and translational feasibility of this therapeutic strategy in human patients.

CONCLUSION

In conclusion, this study provides initial evidence supporting the use of placental peptide-pulsed dendritic cells (DCs) to generate antigen-specific T cells (ASTs) with potent antitumor activity against glioblastoma, neuroblastoma, and breast cancer cell lines in vitro, as well as in an in vivo neuroblastoma model. These findings highlight the potential of placental peptides as a novel source of tumor-associated antigens for cancer immunotherapy. Further investigations are warranted to determine the generalizability of these this approach across additional malignancies, optimize treatment protocol, assess clinical translatability, and explore combination strategies with other immunotherapeutic modalities.

ACKNOWLEDGEMENT

The authors thank all participants and technical staff who contributed to this study. The project was supported by Fujian Provincia Natural Science Foundation of China (Fund No. 2021J01418).

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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