Identification and characterization of mammaglobin-A epitope in heterogenous breast cancers for enhancing tumor-targeting therapy

Zhiqiang Liu1, Xiqin Yang1, Cuimi Duan1, Jiangxue Li1, Rongsheng Tong2, Yuting Fan2, Jiannan Feng3, Ruiyuan Cao3, Wu Zhong3, Xiaoyan Feng4, Heqiu Zhang4 and Lulu Cai4

Although targeted therapy has been extensively investigated for breast cancers, a molecular target with broad application is currently unavailable due to the high heterogeneity of these cancers. Mammaglobin-A (Mam-A), which is overexpressed in most breast carcinomas, has been proposed as a promising target. However, the lack of specific targeting moieties due to uncertain binding epitopes hampers further translational study. Here, seven potential epitopes of Mam-A were disclosed, and a unique epitope was then identified in most types of breast cancers, despite the genotypic heterogeneity. With phage display technology, the epitope was determined to be N-terminal amino acids 42–51 of Mam-A (N42–51). Then, the N42–51 epitope-specific monoclonal antibody, mAb785, was conjugated to poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with therapeutic agents, thereby enhancing the drug uptake and therapeutic efficacy in different genotypes of breast cancers. The computer simulation of the N42–51 epitope and the mAb785 structures, as well as their interactions, further revealed the specific targeting mechanism of the mAb785-conjugated nanoparticles to breast cancers.

INTRODUCTION

Breast cancer is the leading cause of cancer-related death in women.1,2 Currently, the most classic molecular targets for breast cancers include human epidermal growth factor receptor 2 (HER-2/ neu), oestrogen receptor (ER), and progesterone receptor (PR).3,4 However, due to the heterogeneous molecular alterations in the breast cancer cells, patients with highly aggressive triple-negative breast cancer (TNBC) cannot benefit from HER-2/ER/PR-targeted therapies.3,5 The development of new targeting strategies, particularly for TNBCs, is urgently needed. Mammaglobin A (Mam-A) was reported to be highly specific to the mammary gland and upregulated in most mammary carcinomas of different genotypes, including TNBCs.5–7 and it has been proposed as a promising molecular target for breast cancer therapy.8,10 However, despite a few scattered studies, a robust targeting system, overcoming the tumor heterogeneity. First, a mAb pool against Mam-A was established, by which the potential epitopes of Mam-A were systemically screened. Secondly, several membrane-associated epitopes and CAR T cells for infection and cancer treatment.21–23

Herein, we established a novel targeting system for breast cancer nanomedicine, which was modified with a Mam-A-specific antibody, that could target the Mam-A immunoepitope. It acted like a “skeleton lock-and-key” system, overcoming the tumor heterogeneity. First, a mAb pool against Mam-A was established, by which the potential epitopes of Mam-A were systemically screened. Secondly, several membrane-associated epitopes and the corresponding mAbs were experimentally determined and one was revealed as the optimal epitope that was immunoactive

ARTICLE OPEN

Received: 20 January 2020 Revised: 16 April 2020 Accepted: 6 May 2020 Published online: 28 May 2020

1Beijing Institute of Basic Medical Sciences, 27 Taiping Road, Haidian District, Beijing 100850, China; 2Personalized Drug Therapy Key Laboratory of Sichuan Province, Department of Pharmacy, Sichuan Provincial People’s Hospital, University of Electronic Science and Technology of China, Chengdu 611731, China and 3Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Haidian District, Beijing 100850, China
Correspondence: Zhiqiang Liu (zhiqiangliu_amms@163.com) or Heqiu Zhang (zhangheqiu2004@126.com) or Lulu Cai (cailulu@med.uestc.edu.cn)
These authors contributed equally: Zhiqiang Liu, Xiqin Yang

© The Author(s) 2020
in most breast cancers. Through phage display, the epitope was determined to be located at the N-terminal amino acids 42–51 (N₂₄₋₅₁) of Mam-A. Furthermore, the strep epitope, as well as its interaction with the corresponding mAb, was confirmed by computer simulation. Mam-A presented a triangle-like fold, and N₂₄₋₅₁ aa was located at the most prominent apex of Mam-A, which was easily gripped by the mAb. Finally, the optimal mAb–epitope targetting strategy was applied to nanomedicine by decorating the mAb on the surface of PLGA NPs that had been approved by the FDA. The mAb-modified PLGA NPs could target four genotypes of breast cancers and be uptaken by the tumor cells in vitro, significantly enhancing drug delivery as well as the anti-cancer efficacy against multiple breast cancers in vivo.

Using the entire sequence of the human Mam-A protein as an immunogen, we built tens of anti-Mam-A mAbs and screened 19 mAbs with superior affinity (Table S1). The epitope curve of Mam-A was mapped, and the possible distribution of epitopes within the protein was predicted using BioSun software. Based on this, Mam-A was cloned into four polypeptide fragments that were also evaluated, and only one (Thermo Scientific, Clone 304-1 A5, mAb304 for short) was found to react with polypeptide D, which was different from all the mAbs we had constructed. mAb304 was also the only one used for Mam-A targeting in previous publications. In total, seven different characteristics of the mAb–antigen reaction were observed, and, thus, all of these epitopes were identified within Mam-A.

For tumor targeting, it is essential for the intended molecular target to be accessible on the surface of the cancer cells. For this, immunofluorescent staining of the breast cancer cell ZR75.1, a Mam-A high-expressing cell line, was chosen to determine the mentioned above. ZR75.1 cells expressing green fluorescent protein (GFP) were cocultured with different breast cancer cells (Supplementary Fig. S1; polypeptides A, B, C, and D). Through the mAb–antigen reaction, six different epitopes in Mam-A and the corresponding mAbs were identified (Fig. S1b, Table S1). Meanwhile, several commercial mAbs against Mam-A were also evaluated, and only one (Thermo Scientific, Clone 304-1 A5, mAb304 for short) was found to react with polypeptide D, which was different from all the mAbs we had constructed. mAb304 was also the only one used for Mam-A targeting in previous publications. In total, seven different characteristics of the mAb–antigen reaction were observed, and, thus, all of these epitopes were identified within Mam-A.

To determine the targeting capacity, FITC-incorporated NPs (with and without mAb785 conjugation) were incubated with ZR75.1 and MCF-7 cells, respectively. As shown in Fig. S7, significant binding of mAb-NPs with breast cancer cells was detected. We further explored the feasibility of this targeting system for the delivery of doxorubicin (Dox) to four genotypes of breast cancer cells. Significantly higher Dox fluorescence was detected in mAb785-NP-Dox-treated cells than in NP-Dox-treated ones, indicating the active targeting of mAb785NPs to breast cancer cells. To determine the cellular uptake of NPs by breast cancer cell lines, it is also important for targeted nanomedicine. To determine the cellular uptake of mAb785-NPs, a phagocytosis indicator (pHrodo™ red conjugated Zymosan bioparticles) was mixed with NPs with or without mAb785 modification, respectively. Zymosan bioparticles were similar to nanoparticles in size and therefore, when they were mixed together for incubation with cells, they would be phagocytized together. pHrodo™ Red was a pH-sensitive fluorescent dye. Outside the cells where PH was neutral, nearly no fluorescence was observed; after internalization into cytoplasm where the PH was faintly acid, mild red fluorescence would be observed; When phagocytic vesicle was combined with lysosomes, where the acidity was further increased, relative strong red fluorescence would be observed. Thus, the fluorescent signal changes could be used as indicator of nanoparticle internalization (outside cells→ inside cells→ lysosome, Supplementary Fig. S9). As shown in Supplementary Fig. S10 and Fig. 3a, the mixed particles were incubated with four breast cancer cell lines. After incubation for 6 h and 12 h, mAb785-NP-treated cells were observed with significantly higher fluorescence than NP-treated cells, indicating the enhanced uptake of NPs through mAb785 binding, which was also verified by flow cytometry (Fig. 3b).
mild red fluorescence was detected in most cells, indicating that nanoparticles were phagocytized into cytoplasm and mainly in free state; after 12 h incubation, relative strong red fluorescence was detected in most cells, indicating that lots of phagocytized nanoparticles were combined by lysosome within cells. To directly determine the nanodrug uptake, coumarin with green fluorescence was encapsulated in NPs for labeling. Co-localization of green and red fluorescence was identified and quantified as internalized nanodrugs. As shown in Supplementary Fig. S11 and Fig. 3c, more internalized coumarin were detected in mAb785-NPs than the plain NPs group, providing further evidence that mAb785 modification significantly potentiated the uptake of nanodrugs by different genotypes of breast cancer cells.

Fig. 1 Screening and evaluation of membrane-associated anti-Mam-A mAbs. a ZR75.1 cells and tumor slides expressing green fluorescent protein (GFP) in the cytoplasm were immunostained by representative mAbs, targeting seven epitopes of Mam-A, and the DiI dye was used as a positive control of membrane staining. b Flow cytometry analyzing the fluorescent intensities of ZR75.1 cells immunostained by mAb785, mAb656 and mAb304. c Representative image of tissue chips stained by different mAbs and the total detecting rate. d Heat maps of Mam-A expression on breast cancer tissue chips stained by different mAbs. e Quantitative analysis of immunostained breast tumor samples on chips by different mAbs (*P < 0.05, **P < 0.01, ***P < 0.001, One way ANOVA /Tukey's multiple comparisons test was used for multiple group comparison). f Evaluation of mAb specificity by tissue chips, including non-breast cancer tissue and normal tissue chips. MOD, mean optical density; IOD, integrated optical density.
To determine the efficacy of the targeting system in breast cancer treatment, we prepared a multifunctional “Nanodrug” that encapsulated Dox inside itself and absorbed siRNAs (targeting surviving gene) outside itself (Supplementary Fig. S12) because Dox and siRNA could inhibit cancers synergistically (Supplementary Fig. S13). In addition, mAb785-NPs were coated with polyethyleneimine to improve the siRNA loading capacity (Supplementary Fig. S14). To determine the targeting capacity of

**Fig. 2** The efficacy of mAb785 as ligand mediating the targeting of NPs to multiple breast cancers. **a** The targeting of mAb785 to four genotypes of breast cancer cells. **b** Preparation and SEM image of mAb785-NPs, confirmation of the successful conjugation of mAbs onto the NPs by flow cytometry, and the determination of targeting capacity of mAb785-NPs to multiple breast cancer cells by fluorescent microscope (Bar = 100 μm, Two-tailed Student’s t test was performed for statistical analysis). **c** Multiple breast cancer cells (ZR75.1, MCF-7, SKBR3, and MDA-MB-231) were cocultured with non-breast cancer cells (control cells, GFP positive). mAb-NP-Dox (Red fluorescence) was incubated with cocultured cells and the relative red fluorescent intensities were measured along the white arrowheads (Bar = 50 μm). **d** Intensity distribution of red and green fluorescence which indicated the distribution of red fluorescent signals were opposite to that of green fluorescent signals, that was, GFP+ cells were detected with relative low red fluorescence while GFP− cells were detected with relative high red fluorescence (two-tailed Student’s t test was performed for statistical analysis).
siRNA-carrying NPs, Cy5-labeled siRNA was loaded onto mAb785-NPs or plain NPs. As shown in Supplementary Fig. S15, Cy5 imaging demonstrated the targeted binding of siRNA-loaded mAb785-NPs with breast cancer cells. Then, we determined the efficacy of gene silencing and found that mAb785-NPs loaded with siRNA were more efficient than plain NPs and commercial liposome (Supplementary Fig. S16). Furthermore, mAb785-NPs were incorporated with NH₃HCO₃ to acquire pH responsiveness so as to promote the intracellular release of Dox (Supplementary Fig. S17). To evaluate the targeting and uptake of the Nanodrug by...
multiple breast cancers, FAM-labeled siRNAs (green fluorescence) and Dox (red fluorescence) were co-loaded. After incubation with cancer cells, increased fluorescence (yellow as co-localization of red and green) was detected in mAb785–Nanodrug-treated cells (Fig. 3d, Supplementary Figs. S18, S19), indicating the enhanced delivery and uptake of Nanodrugs by mAb785 modification.

To determine the anti-cancer efficacy, two breast cancer cell lines, ZR75.1 and MCF7, were evaluated in vitro. The CCK8 assay and TUNEL staining consistently showed that mAb785–Nanodrugs significantly inhibited cell proliferation compared with the Nanodrug alone (Fig. 3e, f, Supplementary Fig. S20). Meanwhile, to determine the specificity of the mAb785-Nanodrug to breast cancers, an irrelevant cancer cell A549 which does not express Mam-A was used to evaluate anti-cancer efficacy in vitro. CCK8 assay demonstrated slight inhibition of A549 cells by nanodrugs, which may be due to the non-specific adsorption of nanodrugs to the cells (Supplementary Fig. S21). No significant difference was detected between nanodrug-treated cells and mAb785–Nanodrug-treated ones, indicating the lack of specific response to mAb785–Nanodrug for the irrelevant cancer cell. For in vivo evaluation, nude mice bearing breast tumors were prepared using ZR75.1 and MCF-7 cells. After injection through the caudal vein, the targeting of mAb785–Nanodrug to tumors in vivo was confirmed by NIR imaging of Cy5-labeled siRNAs in the tissues (Fig. 4a) and fluorescent microscopy of tumor sections (Fig. 4b). RT-PCR analysis of the tumors also demonstrated that the survivin gene was significantly inhibited in mAb785–Nanodrug-treated animals (Supplementary Fig. S22). Bioluminescent imaging showed that MCF-7 tumor (labeled with luciferase) growth was significantly inhibited in the mAb785–Nanodrug group compared to the control, mAb785 alone, and the Nanodrug groups (Fig. 4c and Supplementary Fig. S23a). Measurement of tumor growth was consistent (Fig. 4d) with bioluminescent imaging. At the end of the experiment, weighing of tumors also demonstrated the significant tumor inhibition by mAb785–Nanodrug (Fig. 4e). The anti-cancer efficacy of mAb785–Nanodrug was also confirmed in models bearing the ZR75.1 tumors (Supplementary Fig. S23b).

Meanwhile, the body weight was measured during the experiment and it demonstrated no significant difference among different groups (Fig. 4f), which may suggest that no significant toxicity of mAb785–Nanodrug existed. To further explore the potential toxicity of mAb785–Nanodrug, several main organs including heart, liver, spleen, lung, and kidney were analyzed by H&E staining, and no significant damage was observed in any organs (Supplementary Fig. S24), providing additional evidence for the little toxicity of the Nanodrug.

To further confirm the efficacy of the targeting system in multiple breast cancers, TNBC models were prepared using MDA-MB-231 cell line. 24 h after delivery, the distribution of Cy5-labeled Nanodrugs in TNBC tumors was detected by fluorescence microscope. As shown in Supplementary Fig. S25, obviously more mAb-conjugated Nanodrugs were detected in tumor sections than control Nanodrugs, indicating the in vivo targeting of mAb-conjugated nanodrugs to TNBC tumors, which was consistent to in vitro results. The results suggested that using mAb785 antibody could also enhance the drug delivery into TNBC tumors. However, in the following experiment, no significant inhibition of TNBC tumors was detected in vivo (Data not shown). We supposed that it may be due to the following reasons: (1) the expression level of Mam-A was lower in MDA-MB-231 cells than those in ZR75.1 and MCF-7 cells. Thus, the bond nanodrug dose may be lower in TNBC tumors in vivo than those in ZR75.1 and MCF-7 tumors, which was confirmed too by in vitro experiments (Figs. 2b, 3c); (2) MDA-MB-231 cells may be more tolerant to drugs used in the study (doxorubicin and siSurvivin) than ZR75.1 and MCF-7 cells. We supposed that significant efficacy on TNBC using the present targeting system may be achieved by employing highly toxic or TNBC-specific antitumor drugs, which deserved further investigation in future.

The detailed location and sequence of the epitope were determined by phage display technology (Supplementary Fig. S26). Three phage colonies (P731, P734, and P733) were screened with a higher affinity to mAb785 (Supplementary Table S5). The pre-blocking test in the ELISA assay showed that the mAb785–antigen reactivity could be partially blocked by P731 and P734, while it could be completely blocked by P733 (Supplementary Fig. S27). The results were further confirmed by immunostaining of breast cancer sections (Fig. 5a), suggesting that the peptides carried by P733 may be the target epitope of mAb785. The peptides were sequenced as YAELLKEFVDPV (Supplementary Table S5). Sequence alignment demonstrated that the first 10 amino acids of the peptide (total 12 aa) were mostly the same as the N$_{32-51}$ aa of Mam-A. More importantly, three different pairs of amino acids (AAs) were similar in property and they were mutually substitutable (Fig. 5b), suggesting that the N$_{32-51}$ aa of Mam-A could be the target epitope of mAb785. For verification, we synthesized Peptide 733 (carried by P733) and Peptide 733-3R (three substitutable AAs were replaced with the same AAs in the primary Mam-A). Pre-blocking with the Peptide 733 and Peptide 733-3R both totally blocked mAb785 immunostaining of the breast cancer sections (Fig. 5c). To determine whether the last two AAs (PV) were necessary for the reaction with mAb785, a truncated peptide without PV was synthesized (Peptide 733-3RE$_2$). It was found that Peptide 733-3RE$_2$ and Peptide 733-3R blocked mAb785 in the same way, suggesting that the last two amino acids (PV) were unnecessary for the epitope (Fig. 5d). To determine the epitope sequence more precisely, we further truncated the first two AAs from Peptide 733-3RE$_2$ and obtained peptides 733-3RE$_2$F$_2$ (Fig. 5e), indicating that the first two AAs in Peptide 733-3RE$_2$ were essential for the epitope. The above results were further verified by the ELISA assay (Supplementary Fig. S28). Collectively, the epitope of mAb785 was determined as YKELQEFID and located at N$_{32-51}$ within the Mam-A protein (Fig. 5f). Based on the location and sequence, the structure of the epitope within Mam-A was computationally reconstructed. The variable region of mAb785 was also computationally reconstructed after antibody sequencing (Supplementary data: Antibody sequences of mAb785). Furthermore, the interaction between mAb785 and the epitope was computationally simulated (Fig. 6a). Mam-A presented as a triangle-like fold, and the N$_{32-51}$ aa was located at the most salient and stable vertex of Mam-A, which could make the epitope easily exposed and recognizable by its antibodies. The variable region of mAb785 folded into a puller-like structure and...
perfectly fit with the N42–51 epitope. The computer-assisted simulation of the N42–51 epitope and mAb785 structures, as well as their interactions, could explain the mechanism underlying the specific targeting of the mAb785-NPs to breast cancers, as illustrated in Fig. 6b.

The study successfully screened a specific targeting system for heterogeneous breast cancers based on the Mam-A epitope. When applied to nanomedicine, this system allowed for the active targeting of antibody-conjugated NPs to multiple breast cancers. Importantly, the intracellular uptake of NPs by multiple breast cancer cells was significantly enhanced due to the epitope–antibody interaction. Consequently, the anti-cancer efficacies of the drug-loaded NPs were significantly augmented against different genotypes of breast cancers. Furthermore, the
precise targeting site was determined as the N42–51 AAs of Mam-A. The structures and interactions of the epitope with its optimal antibody were further revealed by computer simulation. Understanding the location and sequences of the epitope could extend the epitope-based approach for targeted cancer therapy, e.g., developing new CART cells. Collectively, the immunoepitope–mAb785 acted like a "skeleton lock-and-key" system, opening the doors between nanomedicine and multiple breast cancers, which could potentially be a tool for targeted drug delivery to heterogeneous breast cancers.

MATERIALS AND METHODS
Preparation of anti-human mammaglobin-A (Mam-A) monoclonal antibodies
The procedures for the immunization, preparation, and selection of hybridomas cells, as well as the collection and purification of monoclonal antibodies (mAbs) are described in our previous report.11 Based on this, Mam-A was cloned into four polypeptide fragments that could contain different epitopes (polypeptides A, B, C, and D; see Fig. S1). Indirect ELISA was performed to screen effective mAbs that could react with Mam-A or the polypeptides. Immunofluorescent staining and flow cytometry Cells were fixed with cold methanol/acetone and incubated with mAbs. For laser scanning confocal microscopy, AF594-labeled anti-mouse IgG antibodies (Abcam, USA) were used as secondary antibodies. Nuclei were stained by DAPI. DiI staining was performed according to the manufacturer's instructions (Invitrogen). For flow cytometry, AF647-labeled anti-mouse IgG antibodies were used as secondary antibodies. The stained cells were analyzed using a flow cytometer (FACSCalibur, BD, USA).

Surface plasmon resonance
Surface plasmon resonance was performed using a Biacore 3000™ instrument (GE Healthcare, USA). CM5 sensor chips, a thiol ligand coupling kit, and HBS−EP + 10× running buffer (0.1 mol/L Hepes, 1.5 mol/L NaCl, 30 mmol/L ethylene diamine tetraacetic acid [EDTA], and 0.5% [v/v] p20) were all purchased from GE Healthcare.
Cell targeting and uptake assay
To evaluate cell targeting, cells were incubated with NPs for 2 h and then washed with PBS. The binding of the NPs to the cancer cells was observed under a fluorescent microscope according to the carried fluorescent tag. For the cell uptake assay, breast cancer cells were incubated with a mixture of phagocytosis indicator pHrodo™ Red conjugated Zymosan bioparticles (Thermo Fisher Scientific, P35364) and prepared NPs. The cellular uptake was evaluated according to the fluorescent signal of the phagocytosis indicator. The quantification of fluorescent signals was performed using flow cytometry or Image-Pro Plus software.

Cell viability and apoptotic assay
The cell viability and apoptosis were evaluated using CCK-8 staining (CCK-8 staining kit, Dojindo, Japan) and TUNEL staining (TUNEL staining kit, Key GEN INC., China), respectively, using commercial kits according to the manufacturers’ instructions.

Phage display screening
A phage display library containing $10^{11}$ random peptides (12 amino acids) was used (Biolabs, Lot:0411702). Monoclonal antibodies were coated onto an ELISA plate to capture phages; uncaptured phages were removed by washing. Captured phages were eluted for expansion. The expanded phages were used for the second capture by antibodies. With the same protocol, a total of 3 phage expansion-capture cycles were repeated to enrich high-frequently captured phages. Then, the phages were sequenced to determine the peptide sequences.

Pre-blocking test, antibody sequencing, and computer-assisted structure analysis
For the pre-blocking test, antibodies were pre-incubated with phages or peptides for blocking and then used for the ELISA reaction or immunostaining. Antibody variable domain sequencing was performed by GenScript Inc. Based on the epitope and antibody sequences, the immunoreactive structure of the epitope in Mam-A and the mAb variable region were computationally calculated and simulated using InsightII 2000 software.

Preparation and characterization of the nanodrugs
Poly (lactic-co-glycolic) acid (PLGA, MW = 100,000; lactide/glycolide = 50:50) was purchased from Shandong Institute of Medical Instrument (Jinan, China). PLGA NPs were prepared using the emulsion–evaporation method. For details, see the supplementary material.

![Diagram](image_url)
Animal experiments
All experimental protocols related to animals were approved by the Animal Care Committee of the Beijing Institute of Basic Medical Sciences and the Ethics Committee of Sichuan Provincial People’s Hospital, China. To prepare nude mice bearing breast tumors in situ, 5 × 10^4 breast cancer cells in 100 µL of PBS were injected into the mammary fat pad of the nude mice. Intraperitoneal injection of oestradiol benzoate (100 µg) was performed twice a week. One week after cell injection, the animals were randomly divided into different treatment groups. The control group was injected with 100 µL of PBS into the caudal vein. Nanodrugs containing 20 µg of Dox and 100 pmol of siRNA in 100 µL of PBS were injected through the caudal vein four days after cell seeding. The length (L) and width (W) of the tumors were measured with a calliper, and the tumor size was calculated as L × W × W/2, as in previous reports. 10

Non-invasive optical imaging
Bioluminescent and near-infrared imaging with a highly sensitive charge-coupled device (CCD) camera (IVISSO, Xenogen, USA) was performed. For bioluminescent imaging, D-Luciferin (150 mg/kg body weight) was intraperitoneally injected into nude mice, and then the animals were anesthetised by inhaling isoflurane (2–3%). The imaging was performed for 1–10 min until the maximum signals were obtained. Near-infrared imaging of Cy5 was performed to detect the distribution of NPs in animals and tissues.

Statistical analysis
Student’s t test has been used for two groups’ comparison, and the One way ANOVA has been used for more than two groups’ comparison. All data are expressed as the mean ± SD. Statistical analysis was performed using SPSS 17.0, GraphPad Prism7, and OriginPro8. P < 0.05 was considered statistically significant. Multiple software packages were used for plotting, including Excel2007, OriginPro8, and GraphPad Prism7.

Acknowledgements
This research was supported by the National Nature Science Foundation of China (81672607 and 81500077), the National Key Specialty Construction Project of Clinical Pharmacy (30303003668), the Nature Science Foundation of Beijing (7192143), the Beijing Nova Program (Z171100001117115), the Special Foundation for State Major Research Program of China (2016YFC0106603), and the Research Funding of Sichuan Provincial People’s Hospital (2017L0YB). We would like to thank Dr Peng Du and Professor Zhizei Sun for their technical support.

Authors Contributions
Z.L and X.Y. contributed equally to this work. L.C, H.Z., and Z.L. conceived and designed the research. C.D., J.L., J.F., and R.C. performed the experiments. R.T., Y.F., and W.Z. synthesized the nanomaterials. X.F. and L.C. performed some work for the revision of the manuscript. H.Z. and Y.F. analyzed the results. Z.L. and L.C. wrote the manuscript.

Additional Information
The online version of this article (https://doi.org/10.1038/s41392-020-0183-1) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

References
1. Garber, K. Targeting copper to treat breast cancer. Science 349, 128–129 (2015).
2. Rosenberg, L. H. et al. Therapeutic targeting of casein kinase 1 delta in breast cancer. Sci. Transl. Med. 7, 318ra202 (2015).
3. Lang, T. et al. Cocktail strategy based on spatio-temporally controlled nano device improves therapy of breast cancer. Adv. Mater. 31, e1806202 (2019).
4. Yao, Y. D. et al. Targeted delivery of PLK1-siRNA by ScFv suppresses Her2+ breast cancer growth and metastasis. Sci. Transl. Med. 4, 130ra48 (2012).
5. Li, L. et al. Actively targeted deep tissue imaging and photothermal-chemo therapy of breast cancer by antibody-functionalized drug-loaded x-ray-responsive bismuth sulfide@mesoporous silica core-shell nanoparticles. Adv. Funct. Mater. 28, 1704623 (2018).
6. Chen, F. & Ma, K. Ultrasound targeted nanoparticles with engineered antibody fragments for imaging detection of HER2-overexpressing breast cancer. Nat. Commun. 9, 4141 (2018).
7. Kodack, D. P., Askoxylakis, V., Ferraro, G. B., Fukumura, D. & Jain, R. K. Emerging strategies for treating brain metastases from breast cancer. Cancer Cell 27, 163–175 (2015).
8. Villanueva, M. T. Targeted therapies: THRESA trial, overcoming hurdles in breast cancer. Nature reviews. Clin. Oncol. 11, 379 (2014).
9. Most, S., Sharief, M. S., Crown, J. & Hennessy, B. T. Overcoming resistance and restoring sensitivity to HER2-targeted therapies in breast cancer. Ann. Oncol. 23, 3007–3016 (2012).
10. Tafreshi, N. K. et al. A mammaglobin-A targeting agent for noninvasive detection of breast cancer metastasis in lymph nodes. Cancer Res. 71, 1050–1059 (2011).
11. Duan, C. et al. Generation of monoclonal antibodies against MGA and comparison of their application in breast cancer detection by immunohistochemistry. Sci. Rep. 5, 13073 (2015).
12. Span, P. N. et al. Mammaglobin is associated with low-grade, steroid receptor-positive breast tumors from postmenopausal patients, and has independent prognostic value for relapse-free survival time. J. Clin. Oncol. 22, 691–694 (2004).
13. Zuo, L., Li, L., Wang, Q., Fleming, T. P. & You, S. Mammaglobin as a potential molecular target for breast cancer drug delivery. Cancer Cell Int. 9, 8 (2009).
14. Senapati, S., Mahanta, A. K., Kumar, S. & Maiti, P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct. Target Ther. 3, 7 (2018).
15. Youn, Y. S. & Bae, Y. H. Perspectives on the past, present, and future of cancer nanomedicine. Adv. Drug Deliv. Rev. 130, 3–11 (2018).
16. Coty, J. B. & Vauthier, C. Characterization of nanomedicines: a reflection on a field under construction needed for clinical translation success. J. Control Release 275, 254–268 (2018).
17. Yan, L. et al. A safe-by-design strategy towards safer nanomaterials in nanomedicines. Adv. Mater. 31, e1805391 (2019).
18. Agrahari, V. & Agrahari, V. Facilitating the translation of nanomedicines to a clinical product: challenges and opportunities. Drug Discov. Today 23, 974–991 (2018).
19. Liu, X. et al. Vesicular antibodies: a bioactive multifunctional combination platform for targeted therapeutic delivery and cancer immunotherapy. Adv. Mater. 31, e1808294 (2019).
20. Feng, Y. et al. Antibodies@MOFs: an in vitro protective coating for preparation and storage of biopharmaceuticals. Adv. Mater. 31, e1805148 (2019).
21. Xu, K. et al. Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1. Nat. Med. 24, 857–867 (2018).
22. Chauhan, V., Rungta, T., Goyal, K. & Singh, M. P. Designing a multi-epitope based vaccine to combat Kaposi Sarcoma utilizing immunoinformatics approach. Sci. Rep. 9, 2517 (2019).
23. Raschka, S. et al. Identification of potential small-molecule protein-protein inhibitors of cancer metastasis by 3D epitope-based computational screening. J. Physical Chem. Phys. 69, 255–263 (2018).
24. Sette, A. & Fikes, J. Epitope-based vaccines: an update on epitope identification, vaccine design and delivery. Curr. Opin. Immunol. 15, 461–470 (2003).
25. Correa, B. E. et al. Proof of principle for epitope-focused vaccine design. Nature 507, 201–206 (2014).
26. Feng, X. et al. Enhanced serodiagnostic utility of novel Mycobacterium tuberculosis polysaccharides. J. Infect. 66, 366–375 (2013).
27. Bjornmalm, M. & Thurecht, K. J. Bridging bio-nano science and cancer nano-dicine. ACS nano 11, 9594–9613 (2017).
28. Zafrakas, M. et al. Expression analysis of mammaglobin A (SCGB2A2) and lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. BMC Cancer 6, 88 (2006).
29. Al Joudi, F. S. Human mammaglobin in breast cancer: a brief review of its clinical utility. Indian J. Med. Res. 139, 675–685 (2014).
30. Li, C. & Zhang, T. Human mammaglobin: a specific marker for breast cancer prognosis. J. Buon. 21, 35–41 (2016).
31. O’Brien, N. A. et al. Mammaglobin A in breast cancer: existence of multiple molecular forms. Int. J. Cancer 114, 623–627 (2005).
32. Kishimoto, T. K. Improving the efficacy and safety of biologic drugs with tolerogenic nanoparticles. Nat. Nanotechnol. 11, 890–899 (2016).
33. Liu, Q. et al. pH-responsive poly(DL-lactic-co-glycolic acid) nanoparticles with rapid antigen release behavior promote immune response. ACS Nano 9, 4925–4938 (2015).
34. Xu, X. & Hou, S. Precision-guided nanospears for targeted and high-throughput intracellular gene delivery. ACS Nano 12, 4503–4511 (2018).
35. Jin, Q., Deng, Y., Chen, X. & Ji, J. Rational design of cancer nanomedicine for simultaneous stealth surface and enhanced cellular uptake. ACS Nano 13, 954–977 (2019).

36. He, C., Lu, K., Liu, D. & Lin, W. Nanoscale metal-organic frameworks for the co-delivery of cisplatin and pooled siRNAs to enhance therapeutic efficacy in drug-resistant ovarian cancer cells. J. Am. Chem. Soc. 136, 5181–5184 (2014).

37. Xiong, X. B. & Lavasanifar, A. Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin. ACS Nano 5, 5202–5213 (2011).

38. Li, L., Gu, W., Chen, J., Chen, W. & Xu, Z. P. Co-delivery of siRNAs and anti-cancer drugs using layered double hydroxide nanoparticles. Biomaterials 35, 3331–3339 (2014).

39. Mitsunaga, M. et al. Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules. Nat. Med. 17, 1685–1691 (2011).