nessa publishers

Journal of Gynecology
Volume1| Issue 7

Research Article **Open Access**

Chimeric Antigen Receptor (CAR) with scFv Genes of Humanized Anti-GnRH Receptor for Immunotherapy of Gynecologic Cancer. Running title: GHR106-linked CAR-T

Gregory Lee[1*]

[1] UBC Centre for Reproductive Health, Vancouver, Canada

[*]**Corresponding author**: Dr. Gregory Lee, UBC Centre for Reproductive Health, Vancouver, Canada;

Email: leecyg@gmail.com

Citation: Gregory Lee (2017) Chimeric Antigen Receptor (CAR) with scFv Genes of Humanized Anti-GnRH Receptor for Immunotherapy of Gynecologic Cancer. Running title: GHR106-linked CAR-T: Nessa J Gynecology

Received: 6th October 2017, **Accepted:** 10th October 2017, **Published:** 15th November 2107

Abstract

GHR106 is one of numerous monoclonal antibodies which were generated against N1-29 peptide in the extra-cellular domains of human GnRH receptor. These antibodies were demonstrated to be bioequivalent to GnRH decapeptide analogs in many biological functions of GnRH receptor, except that the former has a much longer circulations half-life (5-21 day vs. min/hours). Besides anterior pituitary, GnRH receptor was also expressed in the limited extra-pituitary tissues, and in many malignant cells. However, the extra-pituitary GnRH receptor may play very different biological roles from those in the pituitary. Anti-proliferative or anti-cancer properties of GnRH or its peptide analogs are known for decades. GnRH peptide analogs have been shown to be effective in treating many types of hormone-sensitive cancers. Humanized forms of GHR106 have been made available and were shown to be bioequivalent to murine GHR106 in many biological studies. Therefore, hGHR106 with longer half-life than those of GnRH peptide analogs may be suitable for development of anti-cancer drug or anti-cancer treatment. Therefore, in this study, CAR (chimeric antigen receptor)-T cell therapy technology was employed to construct GHR106-linked CAR and it potential applications in cancer therapy demonstrated. ScFv (single chain variable domain fragment) of hGHR106 genes were incorporated into a CAR format with a lentiviral vector. Following validation assays, specific CAR-T cells can then be expanded in vitro and re-infused back to the patients to achieve the cytotoxic cell killing of cancer cells in vivo. In view of pan-cancer nature of GnRH receptor, GHR106-linked CAR-T cell therapy may have broad applications for clinical cancer treatments in humans.

***Keywords**: Anti-GnRH receptor monoclonal antibody, Gynecologic Cancer, GHR106, GHR106-linked CAR-T cell therapy, anti-cancer treatment, chimeric antigen receptor (CAR)*

Abbreviations used

ATCC: American Type Culture Collection

CAR: chimeric antigen receptor

CD28: cluster of differentiation 28

CD3: cluster of differentiation 3

CDC: complement-dependent cytotoxicity reactions

CHO: Chinese hamster ovary

CRO: contract research organization

E/T: effect / target

EIA: enzyme immunoassay

ELISA: enzyme-linked immunosorbent assay

GD2: a disialoganglioside expressed on tumors of neuroectodermal origin

GD3: an overexpressed tumor-derived ganglioside

GHR106: a monoclonal antibody generated against N1-29 peptide corresponding to one extracellular domains of GnRH receptor.

GnRH: gonadotropin releasing hormone

IFNγ: Interferon γ

IgG: Immunoglobulin G

IL-13R-a2: Interleukin-13 receptor subunit alpha-2, a membrane bound protein

IL2: Interleukin 2

IL7: Interleukin 7

LDH: Lactate dehydrogenase

MAGE-A1: Melanoma-associated antigen 1, a protein in humans encoded by the MAGEA1 gene.

PSMA: prostate-specific membrane antigen

qPCR: quantitative polymerase chain reaction

RPMI 1640: RPMI 1640 Medium for tissue culture use

scFv: Single-chain variable fragment of IgG

Introduction

GnRH (gonadotropin releasing hormone) is a decapeptide hormone and was discovered initially to react with GnRH receptor in the anterior pituitary. [1-3] This peptide hormone serves to stimulate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) for differentiation and maturations of reproductive functions. [1-4] Due to the relative short half-life of GnRH in circulations, numerous GnRH-relate peptide analogs were made available to increase the half-life of these GnRH analogs. [3-7]

GHR106 is one of numerous monoclonal antibodies which were generated against N1-29 peptide in the extracellular domains of human GnRH receptor. [6, 7] GHR106 and its humanized forms were demonstrated to be bioequivalent to GnRH peptide analogs in many biological actions to GnRH receptor,[1, 7, 8] except that the former has much longer half-life in circulations (days vs. hr/min). GnRH receptor in the extra-pituitary tissues and cancer cells was also identified, however, with different biological roles from those in the anterior pituitary. Anti-proliferative and anti-cancer properties of GnRH peptide analogs as well as GHR106 were known for decades. [3, 4, 6, 9] The former was also recommended for use in cancer therapy, but with limited success [9]. GHR106 with much longer half-life may be more feasible for future cancer therapy [6]. Since 2010, extensive biological and immunological studies have demonstrated that apoptosis can be induced in many cancer cells either by GHR106 or peptide analogs. [7, 8] However, complement-dependent cytotoxicity reactions (CDC) can be induced to cancer cells only with GHR106, but not with that of GnRH peptide analogs.

During the subsequent development of GHR106 as anti-cancer drugs or for anti-cancer treatments. [7, 8] Our effort has been focused on the CAR -T cell technology. [10-12] This technology combines gene therapy, cell therapy and immunotherapy for cancer treatments. Briefly, applications of GHR106-related CAR-T cell therapy can be best achieved by modifying cancer patient's T cells to express scFv genes of humanized GHR106 [13]. The resulting GHR106-CAR-T cells can recognize GnRH receptor on the cancer cell surface. The modified CAR-transfected T cells can be expanded in vitro and transfused back to the same patient in vivo. Cytotoxic cell killing of cancer cells can be induced through the actions of CAR-T cells in vivo. [10-12] The resulting efficacy of immunotherapy can be evaluated through the validation assays of GHR106-linked CAR-T cells. It remains to be demonstrated, if GHR106-linked CAR-T cells are more effective than treatments with GnRH peptide analogs. This technology may also be more feasible than infusion of gram or subgram quantity of humanized GHR106 antibody to cancer patients to achieve the same therapeutic efficacy.

In this communication, efforts were made to introduce scFv genes of humanized GHR106 to construct GHR106-linked CAR. The related CAR-T cells can then be prepared and tested by various in vitro validation assays. In view of the pan-cancer nature of GnRH receptor, the efficacy of GHR106-linked CAR-T cell therapy may be demonstrated for broader applications in routine cancer treatments.

Materials and Methods

Cell Lines

C33A is a cervical cancer cell line and was obtained from ATCC (American Type Culture Collection) (Rockville, MD). This cell line can be routinely cultured at 37 °C in humidified mixture of 95 % air and 5 % CO_2, and maintained in RPMI 1640 medium containing 10 % fetal calf serum and antibiotics according to supplier's instructions.

Humanized GHR106 Monoclonal Antibody and Construct of GHR106-linked CAR

GHR106 of mouse origin was humanized through collaborative CRO services by Lake Pharma Inc. (Belmont, CA). Details of humanization process and gene sequence have been previously reported. [13]

The clone of humanized GHR106 in a stable CHO cell line was expressed by a special vector system and confirmed by DNA sequencing. [13] The amino acid sequences were supplemented with human IgG1 constant region sequences to constitute full length antibody sequences. Protein sequence of the scFv chains including a heavy chain joined by a short linker with a light chain is presented as follows:

GHR106 Heavy chain

QVQLQESGPGLVKPSETLSLTCTVSGFSLSRYSVHWIRQPPGKGLEWIGMIWGGGSTDYNPSLKSRVTISKDNSK
SQVFLKMSSVTAADTAMYYCARGNDGYYSFAYWGQGTLVTVSS

GHR106 Light chain

DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFSGSGSG
TDFTLTISSLQAEDVAVYYCKQSYNLYTFGQGTKLEIK

The recombinant GHR106-linked lentiviral vector was digested with EcoR1-Xbal and 2079 bp fragment was generated.

Construction of CAR in Lentiviral Vector

The full length of chimeric antigen receptor (CAR) was synthesized and subcloned into lenti-Puro vector. The insert was confirmed by Sanger sequencing. The GHR106-linked CAR construct was prepared by Qiagen plasmid maxi prep kit. The resulting protein sequence of the GHR106-linked CAR cassette is given as follows:

Signal peptide - GHR106 scFv-CD8-4-1BB-CD3Z-2A-IL7

AATMALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCTVSGFSLSRYSVHWIRQPPGKGLEWIGMI
WGGGSTDYNPSLKSRVTISKDNSKSQVFLKMSSVTAADTAMYYCARGNDGYYSFAYWGQGTLVTVSSGGGG
SGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESG
VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYNLYTFGQGTKLEIKTTTPAPRPPTPAPTIASQPL

SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE
DGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPREGRGSLLTCGDVEE
NPGPMFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHIC
DANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLK
EQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

Validation Assays of GHR106-linked CAR-T Construct

I. Validation by Cytotoxic Cell Killing Assay

Following the construction of GHR106-linked CAR, the resulting lentivirus vector was prepared and titrated. The human donor's primary T cells were prepared and cultured for 48 hours. The T cells were transduced with lentivirus (GHR106-linked CAR). Following transduction, the relevant CAR expression was validated by absolute quantitation with real-time qPCR. Copy number of GHR106-linked CAR in the lentivirus-transduced T cells (CAR-T) was determined.

The lysis of target tumor cells with GHR106-linked CAR-T cells was performed for validation. In this case, C33A cancer cells were cultured with two passages in logarithmic phase before the assay. The cancer cells were removed by trypsin digestion and cell density adjusted to 5 x 10^5 cells/ml. CAR-T cells were added and adjusted for different E/T (effect/target cells) ratios to a final volume of 100 µl/well. Meanwhile, four wells containing target cells without T cells with unrelated transfected T cells were reserved as the negative control of Maxi and Mini / lysis. Following 8 hours of co-incubation, LDH activity detection reagent was used to determine the percentages of target cells lysis. Averages of three repeated experiments were presented.

II. Validation of GHR106-linked CAR-T Cells by Cytokines Acitivity Release Assay

The activities of three cytokines, IL2 (Interleukin 2), IFNγ (interferon γ) and IL7 (Interleukin 7) were determined for the validation of GHR106-linked CAR-T cells following 8 hours of co-culturing with target C33A cancer cells. Briefly, 5 x 10^5 C33A cells in logarithmic growth phase were co-incubated with GHR106-linked CAR-T cells at three different E/T (Effect/Target cells) ratios (1, 5 and 10). Following co-incubation, the supernatant recovered by centrifugation was employed for determinations of activities of IL2, IFNγ and IL7 by using respective EIA assay kits. Averages of three experiments presented at three different E/T ratios.

Results

I. Construct of GHR106-linked CAR

Humanized GHR106 has been made available through previous CRO services.[13] Based on this information, the primary DNA sequence of humanized GHR106 genes can readily be used for the construction of GHR106-linked CAR with inserted scFv domain genes. Briefly, the full length of chimeric antigen receptor was synthesized and subcloned into lenti-Puro vector. GHR106-linked CAR construct was prepared by Qiagen plasmid maxi prep kit. The physical map of GHR106-linked CAR construct was diagrammatically illustrated in Figure 1. The resulting construct was validated by CAR expression and binding assays. The recombinant GHR106-linked CAR vector was digested with ECOR1-Xbal and 2079 bp fragment was generated. The lentiviral construct of GHR106-linked CAR is the third generation CAR design with additional co-stimulatory domain genes such as 4-1BB.[10-12]

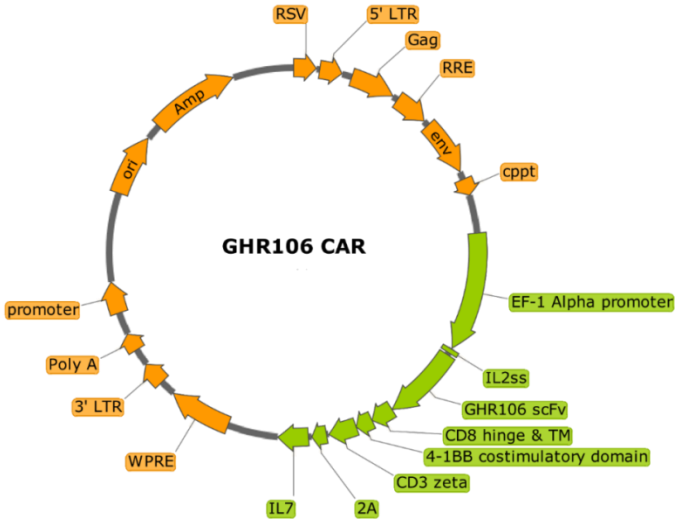


Figure 1: A diagram to reveal the physical map of a lentiviral construct of GHR106 (scFv)-linked CAR (Chimeric Antigen Receptor)

GHR106-linked CAR constructs

Abbreviations for CAR components

EF-1 alpha promoter: this is the promoter that is used to drive CAR expression in T cells.

IL2ss: interleukin 2 signaling sequence, used to direct CAR for cell membrane expression.

GHR106 scFv: single chain variable domain from GHR106 monoclonal antibody. scFv is a single peptide chain composed of chain variable domain and light chain variable domain. This is the **core** of the CAR construct, which renders CAR specificity.

CD8 hinge & TM: hinge and transmembrane domain from CD8 molecule that are used to connect extracellular scFv and intracellular costimulatory domain and signaling domain.

4-1BB costimulatory domain: a domain from 4-1BB molecule that costimulates T cells to improve CAR-T persistence *in vivo.*

CD3 zet : zeta subunit of CD3 (TCR) molecule that is responsible for signaling inside T cells.

2A: a short self-cleavage peptide sequence that is used to express two proteins by the same RNA. After translation, the long peptide that contain two proteins with self-cleave at the 2A sequence region.

IL7: a cytokine that is important for T cell development

Abbreviations for other components on the construct

WPRE: Woodchuck hepatitis virus post-transcriptional regulatory element. Sequence that stimulates the expression of transgenes via increased nuclear export.

3' LTR: Terminates transcription started by 5' LTR by the addition of a poly A tract just after the R sequence.

RSV: constitutive promoter

5' LTR: Acts as an RNA pol II promoter. The transcript begins, by definition, at the beginning of R, is capped, and proceeds through U5 and the rest of the provirus.

Gag: Precursor structural protein of the lentiviral particle containing Matrix, Capsid, and Nucleocapsid components.

RRE: Rev Response Element; sequence to which the Rev protein binds.

env: VSV-G envelope protein

cppt: Central polypurine tract; recognition site for proviral DNA synthesis. Increases transduction efficiency and transgene expression.

This plasmid is called the transfer plasmid that contains the insert of interest. It will be used with other three plasmids that encode different parts of lentivirus to transfect 293T cells to produce viruses.

II. Validation of GHR106-linked CAR Construct and CAR-T Cells

Generally, the validation of this GHR106 CAR construct was performed by several in vitro activity assays including (1). CAR expression test and binding assay, (2). Cytotoxic cell killing assay following Co-incubation of CAR-transfected T cells and cancer cells, (3). Proliferation/Apoptosis assays and (4). Cytokines release assay. The validation test results were confirmed to be in-line with those expected for GHR106-linked cassette and are assured for future clinical applications. Part of the validation tests are presented in this study.

II.1. Validation of GHR106-linked CAR-T Cells by Cytotoxic Cell Killing Assay of Cancer Cells

Following a successful construction of GHR106 (scFv genes) -linked CAR, lentiviral titration indicated that lenti-GHR106 -CAR was prepared with a high titer. A standard procedure was employed to isolate primary T cells from a given human donor. Average CAR gene copies per cell in CAR-T cells were determined to be 2.2/cell. The qPCR results showed that genes of the constructed GHR106-CAR were successfully transfected into T cells. These results indicate validation of the specific CAR expression.

Tumor cells from a cell line of cervical carcinoma (ATCC), C33A was employed as target tumor cells and co-cultured with CAR-T cells at three different E/T ratios (1, 5 and 10) under standard culture conditions. Following 8 hours of co-culturing with both C33A tumor cells and CAR-T cells, the supernatant was harvested for determination of amount of LDH activity released under different culture conditions. The experiment was repeated three times. The averages of three repeats are presented in Figure 2. The percentages of net cell lysis were also presented as a function of E/T ratios ranging from 1 to 10. All were shown to be statistically significant with $P < 0.01$ to $P< 0.001$, when compared with those of the negative control with co-incubation of virus only-transfected T cells. The untransfected or virus only-transfected T cells were also shown to exhibit a low degree of cytolytic effect as compared to that of transfected T cells with GHR106-linked CAR. The observed high background could potentially result from the activation of the T cells transfected from lenti-viral vector only with non-specific interactiions in our assay system.

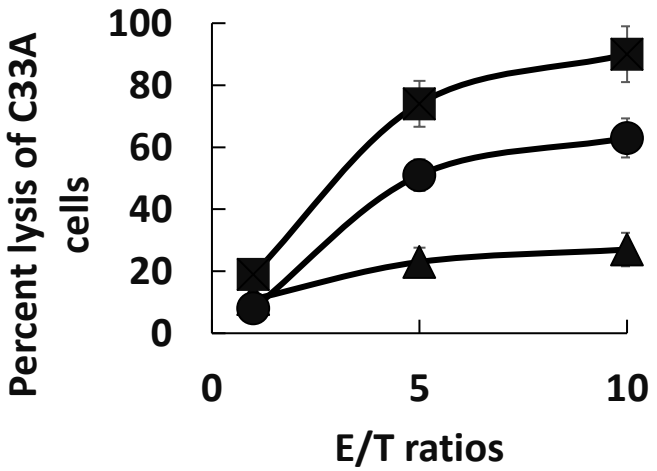
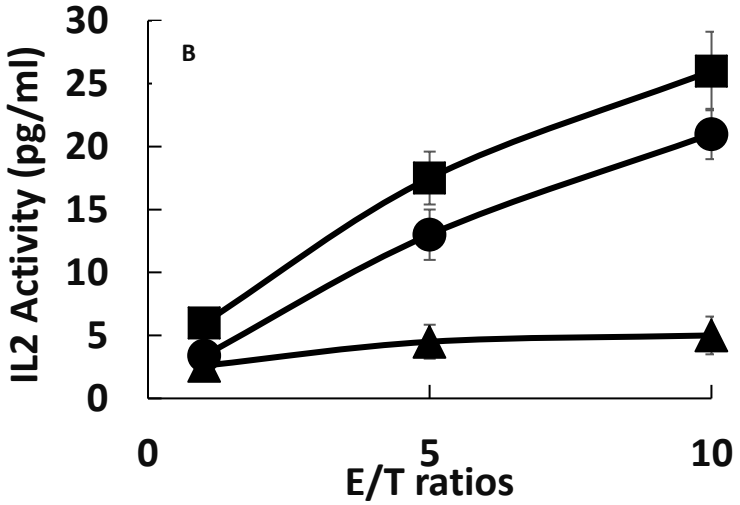


Figure 2: Percent cell lysis of targetC33A cancer cells (T) upon co-culturing with GHR106-linked CAR-T cells (E) at three different E/T (Effect/Target) ratios of 1, 5 and 10, respectively; where ▲ is negative control (with virus-only-transduced T cells for co-culturing); ■ not no is GHR106-linked CAR-T cells co-cultured with cancer cells; and ● is net tumor cell lysis due to the effect of GHR106-linked CAR-T cells.

II.2 Demonstration of Cytokine Release by GHR106-linked CAR-T Cells upon Co-culturing with C33A Cancer Cells

Upon co-culturing of GHR106-linked CAR-T cells with C33A cancer cells for 8 hours, the secretions of three different cytokines were determined by typical enzyme immunoassay (EIA) kits. Selected cytokines in this study including IL2, IFN-γ and IL7 were quantitatively determined and repeated three times. The averages of cytokines release as a function of three E/T ratios are presented in Figure 3A, 3B and 3C, respectively. The net release of each cytokine activity was also presented at three different E/T ratios. Compared to the negative control with T cells transfected with lentiviral vector only, the net cytokines release with co-culturing of GHR106-linked CAR-T cells, showed much higher activity releases in each case ($P < 0.001$) at all three different E/T ratios.

In summary, compared to T cell transfected wit virus only in the negative control, GHR106-linked CAR-T cells showed significantly high percentages of cytotoxic cell killing in a dose-dependent manner upon co-culturing with C33A cancer cells. At the same time, much more cytokine release was observed when cancer cells were co-cultured with GHR106-linked CAR-T cells. These experimental results are summarized in Figure 3A, 3B and 3C, respectively to indicate consistency in cytokine activity detected upon co-incubation with cancer cells.



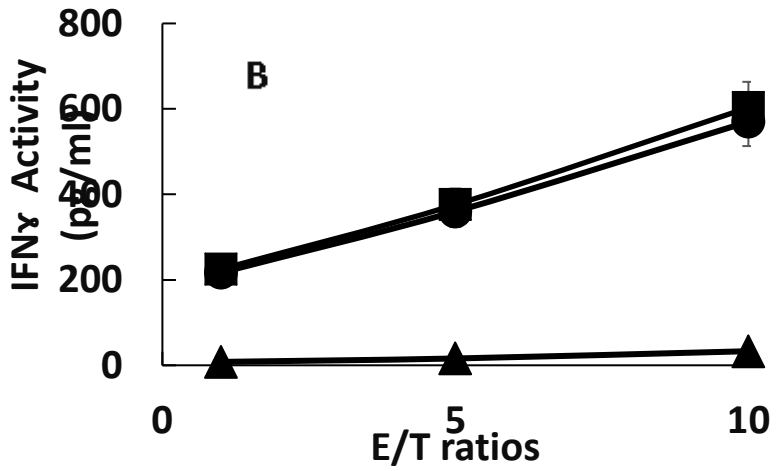


Figure 3 Cytokine activity release as determined by ELISA following co-culturing of C33A cancer cells and GHR106-CAR-T cells:

A. Interferon 2 (IL2) activity (pg/ml) released upon co-culturing of GR106-linked CAR-T cells (E) and target C33A cancer cells (T) at three different E/T ratios of 1, 5 and 10, respectively; where is negative control (with virus-only-transduced T cells for co-culturing); no is GHR106-linked CAR-T cells co-cultured with cancer cells; and is net IL2 activity in pg/ml due to the effect of GHR106-linked CAR-T cells.

B. Interferon γ (IFNγ) activity (pg/ml) released upon co-culturing of GHR106-linked CAR-T cells (E) with target C33A cancer cells (T) at three different E/T ratios of 1, 5 and 10, respectively; where is negative control (with virus-only-transduced T cells for co-culturing); no is GHR106-linked CAR-T cells co-cultured with cancer cells; and is net IFNɤ activity (in pg/ml) release due to the effect of GHR106-linked CAR-T cells.

C. Interferon 7 (IL7) activity (pg/ml) released upon co-culturing of GHR106-linked CAR-T cells (E) and target C33A cancer cells (T) at three different E/T ratios of 1, 5 and 10, respectively; where is negative control (with virus-only-transduced T cells for co-culturing); no is GHR106-linked CAR-T cells co-cultured with cancer cells; and is net IL7 activity in pg/ml due to the effect of GHR106-linked CAR-T cells..

Discussion

Several biological and immunological studies have been performed to reveal that GHR106 and its humanized forms of monoclonal antibody are bioequivalent to GnRH peptide analogs. The latter have been used clinically for decades in cancer treatments.[1-3] Given the fact that GHR106-related antibodies have a much longer half-life than that of the decapeptide analogs (days *vs.* hours), GHR106 may be a better choice than the corresponding decapeptide analogs. [1, 4-6] Chimeric antigen receptor T cell therapy (CAR-T) has recently been developed for adoptive anti-tumor treatments.[10-12]

By employing this technology, the tumor cells can be localized and eliminated through interactions of CAR-T cells which express specific scFv genes to target tumor-associated antigens on the tumor cell surface. [12-15]

In the CAR-T cell technology for cancer therapy, the most important step is to insert scFv gene fragment of anti-cancer gene (GHR106 in this case) to format a lentiviral construct of chimeric antigen receptor (CAR).[11-13] The resulting CAR construct with scFv genes of humanized GHR106 was verified by appropriate validation assays including additional sequencing and molecular analysis. So far, the verification test results were confirmed to be in-line with those expected for GHR106-linked CAR cassette. This verified and validated CAR construct should be assured for consistency of future clinical applications.

The functional validations of GHR106-linked CAR-T cells can be performed by established standard procedures. Following successful transfections with isolated donor's T cells, cytotoxic cell killing assay was initially performed to demonstrate the lysis of cancer cells upon co-culturing with GHR106-linked CAR-T cells. Based on the assay results presented in Figure 2, percentages of the net lysis of target cancer cells increase significantly with increasing E/t ratios from 1 to 10 ($P < 0.01 – 0.001$) in a dose-dependent manner.

The results of this validation assay clearly demonstrated that GHR106-linked CAR-T cells can interact with target cancer cells upon co-culturing and lead to the lysis of C33A cancer cells in vitro. This result has become an important basis in the target-specific CAR-T cell therapy technology. Therefore, it can be assumed that scFv genes of humanized GHR106 are expressed by the transfected T cells which in turn react with GnRH receptor on the surface of target cancer cells.

GHR106-linked CAR-T cells can also be validated with cytokine activity assay upon co-incubation with the target cancer cells. Cytokine activity release was determined by EIA kits at all three different E/T ratios for three different cytokines, Il2, IFNɣ, and IL7 upon co-culturing of CAR-T cells and target cancer cells. The results of three repeated experiments are averaged and presented in Figures 3A, 3B and 3C, respectively. Parallel relationships were observed consistently between the cytotoxic cell killing (percent cell lysis) of target cancer cells and cytokine activity release of CAR-T cells under three different E/T ratios in a dose-dependent manner.

Therefore, in this study, successful construction of GHR106-linked CAR has been demonstrated upon transfection to T cells, the resulting GHR106-linked CAR-T cells were functionally validated by both cytotoxic cell killing assays of target cancer cells as well as the cytokine release of GHR106-linked CAR T cells upon co-culturing of cancer cells. The results of these two validation assays are also consistent with our previous studies of GHR106 in inducing apoptosis to culturing cancer cells.[8, 13]

Previous studies with GHR106 have revealed widespread expressions of GnRH receptors among many types of cancer cells in humans.[1-4] The cancer with high incidence of GnRH receptor expressions are given as follows: breast, ovary, endometrium, prostate, melanoma renal, pancreases NH lymphoma, brain and liver.[8] Therefore, GHR106-linked CAR construct may have unlimited potential applications for individualized clinical treatments of these cancers in humans by using CAR-T cell therapy technology. [10-12]

Success of employing CAR-T cell technology in treatments of B cell malignancy has been reported.[10-12] However, difficulties still remain with respect to those of solid tumors.[10, 14, 15] Regarding the solid tumor targets, several tumor-associated antigens have been selected for clinical studies via CAR-T cell technology.[10, 12] Among those are MAGE-A1/GD2/GD3 for neuroblastoma and melanoma, IL-13R-a2 for glioblastoma, and PSMA for prostate cancer.[10, 14-17]

With the application of this technology, the tumor cells can be localized and eliminated through interactions of CAR-T cells which express specific scFv genes to target tumor-associated antigens on the cancer cell surface.[16-18] Treatments of B cell malignancy with CAR-T cells have been reported with high remission rates(60-90 %).[17, 19, 20] The introduction of GHR106-linked car construct in this study will certainly provide a new opportunity to explore its efficacy in the treatments of many solid tumors in humans.

Acknowledgement

This research project was supported in parts by NSERC and NRC-IRAP programs (#073951 and 090601) and contract research collaborations with Lakepharma Inc. (Belmont, CA) and Creative Biolabs Inc (Shirley, NY). Technical supports by Dr. Yunyu Han, Dr. Bixia Ge and Dr. Suefay Liu are acknowledged.

Conflict of Interest

Gregory Lee is a co-founder of Vancouver Biotech Ltd, and the sole patent holder of GHR106-linked CAR.

References

1. Conn PM, Crowley WF Jr. Gonadotropin-releasing hormone and its analogs. Annu Rev Med 1994; 45: 391-405.
2. Millar RP. GnRHs and GnRH receptors. Anim Reprod Sci 2005; 88: 5-28.
3. Limonta P, Moretti RM, Montagnani Marelli M, Motta M. The biology of gonadotropin hormone-releasing hormone: role in the control of tumor growth and progression in humans. Front Neuroendocrinol 2003; 24: 279-95.
4. Montagnani Marelli M, Moretti RM, Januszkiewicz-Caulier J, Motta M, Limonta P. Gonadotropin-releasing hormone (GnRH) receptors in tumors: a new rationale for the therapeutical application of GnRH analogs in cancer patients? Curr Cancer Drug Targets 2006; 6: 257-69.
5. Harrison GS, Wierman ME, Nett TM, Glode LM. Gonadotropin-releasing hormone and its receptor in normal and malignant cells. Endocr Relat Cancer 2004; 11: 725-48.
6. Lee G, Chow SN, Chien CH, Liu S. Anti-GnRH receptor monoclonal antibodies, first-in-class GnRH analog, J Gynecol Res 2015; 1(1): 102-11.
7. Lee CY, Ho J, Chow SN, Yasojima K, Schwab C, et al. Immunoidentification of gonadotropin releasing hormone receptor in human sperm, pituitary and cancer cells. Am J Reprod Immunol 2000; 44: 170-7.
8. Lee G, Zhang H, Yi T. Anti-GnRH receptor monoclonal antibodies as bioequivalent analogs of GnRH. In: Sills ES, editor. : Gonadotropin-Releasing Hormone (GnRH): Production, Structure and Function. Nova Biomedical, Los Angeles 2013; 10: 157-174.
9. Nagy A, Schally AV. Targeting of cytotoxic luteinizing hormone-releasing hormone analogs to breast, ovarian, endometrial, and prostate cancers. Biol Reprod 2005; 73: 851-9.
10. Wang Z, Wu Z, Liu Y, Han W. New developoment in CAR-T cell therapy. J Hemato. Oncol. 2017; 10: 53.
11. Mount N, Sharpe M. Genetically modified T cells in cancer therapy: opportunities and challenges. Dis Model. Mech. 2015; 8(4): 337-350.
12. Yu S, Li A, Li Q, Li T, Yuan X, Han X, Wa K. Chimeric antigen receptor T cells: a noval therapy for solid tumors, J Hermato. Oncol. 2017; 10:78.
13. Lee G, Huang C-Y, Ge B. Two distinct humanized monoclonal antibodies for immunotherapy of ovarian cancer. J Cancer Sci Therapy 2014; 6:110-116.
14. Grupp SA, Kalos M, Barrett D, Alpene R, Portro DL, Rheingold SR, Techey DT, Chew A, Hauck B, Wright JF et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Eng J Med, 2013; 368:1509-1518.
15. Dai H, Wang Y, Lux, Han W. Chimeric antigen receptors modified T-cells for cancer therapy. J Nat. Cancer Inst. 2016; 108(7): 439.
16. Feng K, Guo Y, Dai H, Wang Y, Li X, Jia H, Han W. Chimeric antigen receptor-modified T cells for immunotherapy of patients EGFR-expressing advanced relapsed/refratory non-small all lung cancer. Sci China Lif Sci 2016; 59(5): 468-79.

17. posy J AD, Schwab RD, Boesteanu AC, Steentoft C, Mandel U, Engel B, et al. Engineered CART cells targeting the cancer-associated Tn-glycoform of the membrane mucin MUC1 control adenocurcinoma. Immunity 2016; 44(16): 14444-54.
18. Lee DW, Kochenderfer JN, stetler-Stevenson M, Cui YK, delbrovk C, Feldman SA, Fry TJ, ea al. T cells expressing CD19 chimeric antigen receptors for cute lymphoblastic leukemia in children and young adults: a phase 1 dose-esclation tual. Lancet (London, England) 2015; 385: 517-528.
19. LIpowska-Bhalla G, Gilhman DE,Hawkins RE, Rothwell DG. Target immunotherapy of cancer with CAR-T cell:"Achievements and Challenges" Cancer Immunol. Immunother. 2012; 61(7):953-962.
20. Sadelain M, Gilhman DE, Hawkins RE, Rothewell DG. The promise and potential pitfals of chimeric antigen receptors. Current Opin. Immunol. 2009; 21(2); 215-223.