博士論文(要約)

Development of novel tumor antigen GPC1 specific chimeric antigen receptor
transduced T (CAR-T) cells and evaluation of
combination immunotherapy with anti-PD-1 Ab
(新規腫瘍抗原 GPC1 特異的 CAR-T 細胞の開発及び
抗 PD-1 抗体併用による複合免疫療法の検証)

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Contents

General introduction

Epidemiology and conventional treatments of cancer

The International Agency for Research on Cancer in World Health Organization reported that cancer is the leading cause of death in humans worldwide, with approximately 18.1 million new cases and 9.6 million deaths in 2018[A]. In Japan, approximately 55 % of all people are expected to be diagnosed as a certain kind of cancer in their lifetime and 5 years survival rate of all cancer patients was 62.1 % based on the data collected during 2006-2008, according to the data from Center for Cancer Control and Information Services, National Cancer Center Japan[B]. The conventional treatment options for cancers have been surgery, radiation therapy, chemotherapy, and target therapy. The tumor removal surgery was documented in ancient Egypt. The radiation therapy was developed in the late 19th Century and the chemotherapy and targeted therapy were developed in the 20th century[1]. Although the cancer treatments have been modified to increase effectiveness and survivability, still half of the cancer patients cannot be cured.

Cancer immunotherapies

Early 21st century, cancer immunotherapies, harnessing the immune system to attack tumors, showed robust clinical responses in human clinical trials. The major effective cancer

immunotherapies include immune checkpoint inhibitors using anti-programmed cell death protein 1 (PD-1)/programmed cell death-ligand protein 1 (PD-L1) antibody (Ab) or anti-cytotoxic T lymphocyte antigen 4 (CTLA4) Ab, and adoptive cell therapies (ACT) using tumor-infiltrating lymphocytes (TILs) and gene-engineered T cells. As the cancer immunotherapy targets, not the tumor cell itself but immune system, mechanism of treatment is entirely different from the conventional treatments. Therefore cancer immunotherapies can adopt various types of cancer patients. For example, the anti-PD-1/PD-L1 Ab have demonstrated durable clinical responses in various solid cancers, including melanoma[2, 3], lung cancer[3, 4], renal cell carcinoma[3, 5], bladder cancer[6], ovarian cancer[7], triple-negative breast cancer[8], and gastric cancer[9]. However, the response rates of these immunotherapies in the cancer patients were approximately 20%[10]. As these immunotherapies targets and reinvigorates tumor antigen-specific cytotoxic T cells (CTLs), these immunotherapies exert the antitumor effect in only patients with highly CTLs infiltrated tumor (T cell inflamed tumor) before treatments[11, 12]. Therefore, these immunotherapies alone are thought to be not effective in most patients with less CTLs infiltrated tumor (T cell non-inflamed tumor) due to low immunogenic tumors or immunosuppressive tumors[13].

Chimeric antigen receptor transduced T (CAR-T) cell therapies

For the patients with T cell non-inflamed tumors, direct administration of *ex vivo* cultured tumor antigen-specific T cells would show the robust antitumor response. Recently, genetic engineering technologies to confer tumor specificity on irrelevant T cells has been developed and enabled the efficient generation of antigen-receptor gene-engineered T cells such as chimeric antigen receptor transduced T (CAR-T) cells (Fig1). CAR-T cells are generated by viral transduction of CAR gene. A versatile class of CAR genes are generated by combining antigen-binding domains of a single-chain variable fragment (scFv) from a monoclonal antibody (mAb) that recognizes tum or antigen fused with intracellular signaling motifs that are capable of T cell activation[14].

Many clinical trials of CD19 specific CAR-T cell therapy targeting B cell malignancies demonstrated objective regression of cancer in patients with acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and various other types of B cell lymphoma. CAR-T cell therapy of pediatric and adult patients with ALL demonstrated a complete remission rate of approximately 90%, with sustained remission for up to 2 years[15]. Although many patients exhibit cytokine release syndrome after T cell infusion, these adverse effects can

be managed with aggressive supportive care or immunosuppression including steroids and cytokine-specific antibodies in most cases[16]. As CD19 specific CAR-T cell therapy has shown a favorable and longer lasting clinical outcome compared with conventional radio- or chemotherapy, the Food and Drug Administration (FDA) in the USA has approved CD19 specific CAR-T cell therapy for relapsed B cell malignancies since 2017 [C].

Application of CAR-T cell therapy to solid tumors

As CAR-T cell therapies for hematological malignancies targeting CD19 have shown robust clinical outcomes, the application of CAR-T cell therapy to solid tumors is expected as a promising strategy. Some clinical trials of CAR-T cell therapy using several tumor antigens expressed on tumor cells have been performed in human patients with solid tumors. Although it was a rare case, CAR-T cell therapy caused lethal adverse effects due to the recognition of target antigen expressed on normal tissues (on-target/off-tumor lethal toxicities) in some patients. For example, a lethal adverse effect was reported in first in human study of HER2-specific CAR-T cells expressing scFv generated from the humanized mAb trastuzumab, which is used for various kinds of HER2 overexpressed tumors. The patient received HER2-specific CAR-T cells resulted in fatal respiratory failure because administered CAR-T

5

cells recognized HER2 expressed on normal lung epithelial cells[17]. In contrast, CAR-T cell

therapy showed no significant clinical responses without apparent adverse effects in most cases

because of insufficient activation of CAR-T cells in tumor tissues in those trials[18]. In these

clinical trials, the major reason why CAR-T cells were not activated sufficiently in solid tumor

tissues is suspected to be immunosuppressive tumor microenvironment (TME). The solid TME

may strongly inhibit activation of CAR-T cells due to the presence of immunosuppressive

molecules such as immune checkpoint molecules (e.g. PD-1/PD-L1. Lag-3/LSECtin,

TIGIT/CD155) and immunosuppressive cells (e.g. Treg, Tumor-associated macrophage (TAM),

cancer-associated fibroblast (CAF))[14, 19, 20].

These two problems, severe adverse effects and insufficient activation of CAR-T cells in

the tumor tissues, should be solved to apply the CAR-T cell therapy to the patients with the

solid tumor safely and the immunotherapy exerts the dramatic effect as hematopoietic tumor. It

is important to identify highly tumor-specific target antigens and to develop novel strategies for

activation of CAR-T cells in TME.

Glipican-1

Glypican-1 (GPC1) is a cell-surface heparansulphate proteoglycan, which expresses in fetal and tumor tissues. The major function of GPC1 is involved in the development of the brain in the fetal phase. GPC1 knockout mice show the only mild reduction of brain volume without physiological abnormality[21]. It suggests that GPC1 does not have a critical function in healthy adult bodies. On the other hand, overexpression of GPC1 has been reported in many tumors including esophageal[22] and pancreatic cancer[23, 24], glioma[25], and mesothlioma[26]. GPC1 expression has also been linked with cancer malignancy such as cell-cycle promotion and enhanced metastatic potential[27]. GPC1 expression was reported to be one of the prognostic factors in some cancers[22][23]. These findings suggest that GPC1 is an attractive target for the novel CAR-T cell therapy for patients with GPC1 positive solid tumors.

Immune checkpoint blockade therapies and the CAR-T cell therapy

As described above the immunosuppressive status in the TME is one of the major problems in cancer immunotherapies including the CAR-T cell therapy because it inhibits the antitumor effects of immunotherapies. As anti-PD-1 Ab therapy showed dramatic antitumor effects in some human clinical cases, one of the major inhibitory molecule in the solid TME is the PD-1 expressed on activated T cells, which critically inhibits T cell activation[28]. PD-1

would also be expressed on activated CAR-T cell in TME, therefore a combination therapy of

CAR-T cell therapy with anti-PD-1 Ab may demonstrate robust antitumor responses against

solid tumors.

Purpose of this study

The purpose of this study was to develop GPC1 specific CAR-T cells and evaluate its

efficacy and safety and evaluate the synergistic antitumor effects of combination

immunotherapy with anti-PD-1 Ab. To evaluate efficacy and safety of GPC1 specific CAR-T

cells therapy, human and mouse GPC1 specific CAR vectors derived from anti-GPC1 mAb

which cross-react with human and mouse GPC1 was developed (**chapter 1**). GPC1 specific

human CAR-T cells were developed and evaluated the antitumor efficacy against human GPC1

endogenously overexpressing human tumors *in vitro* and *in vivo* using xenogeneic mouse

models (**chapter 2**). Furthermore, GPC1 specific murine CAR-T cells were developed and their

adverse effects and antitumor efficacy *in vivo* using syngeneic mouse models were evaluated.

Finally, the feasibility of novel combination immunotherapy combined with CAR-T cells and

anti-PD-1 Ab was evaluated (**chapter 3**).

[A] https://www.who.int/cancer/PRGlobocanFinal.pdf

[B] https://ganjoho.jp/reg_stat/statistics/stat/summary.html

[C]

https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProduct
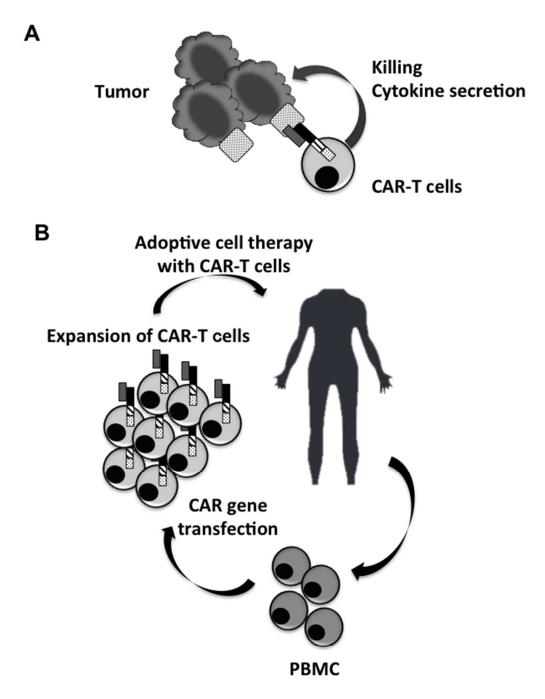
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A

Tumor

Killing
Cytokine secretion

CAR-T cells

B

Adoptive cell therapy
with CAR-T cells

Expansion of CAR-T cells

CAR gene
transfection

PBMC

Fig. Concepts of CAR-T cell therapy

Fig. Concepts of CAR-T cell therapy

A) CAR-T cells specifically secreted cytokines and killed tumor cells in the target antigen-dependent manner. B) Therapy procedure of CAR-T cell therapy. After CAR genes were transduced into patients derived PBMC, expanded CAR-T cells were intravenously injected in the patients.

Chapter 1

Generation of GPC1 specific chimeric antigen receptors (CAR)

Introduction

Adoptive transfer of CAR-T cells specific for a tumor cell surface antigen has emerged as a promising new approach for the cancer immunotherapy. Success in patients with advanced B cell malignancies treated with CD19-specific CAR-T cells has been leading to optimism that this approach will be useful for treating common solid tumors[15]. However, CAR-T cells for patients with solid tumors have faced some problems and CAR-T cells have not demonstrated expected clinical responses[14]. One of the major problems is the lethal on-target/off-tumor adverse effects caused by attacking normal tissues by CAR-T cells, although it is rare case[17].

Overexpression of glypican-1 (GPC1), a cell-surface heparansulphate proteoglycan, have been reported in various solid cancers including glioma, mesothlioma, several squamous cell carcinoma such as esophagus[29] and cervical cancers[30], and several adenocarcinoma such as breast[27] and pancreatic cancer[23]. It has been demonstrated that GPC1 was overexpressed in 98.8 % of patients with esophageal squamous cell carcinoma (n= 175) and 48 % of patients with cervical cancer (n=110)[22]. The major function of GPC1 is reported to be the development of nervous systems in the fetal phase. GPC1 knockout mice showed 10 % reduction of brain volume in adults, however the slight brain hypoplasia does not cause physiological problems. In

addition, the adult GPC1 knockout mice show no abnormalities in morphology, behavior, or life

span[21]. Thus, GPC1 would not have a critical function in the healthy adult body. Therefore,

GPC1 is one of an attractive target antigen for the development of CAR-T cell therapy in

patients with GPC1 positive solid tumors.

Although primate model would have more homogenous protein expression in human,

primate cannot be inoculated syngeneic tumor due to the ethical problem. To evaluate the

antitumor efficacy and adverse effects of CAR-T cells simultaneously in preclinical models,

usage of a syngeneic mouse model is thought to be a promising alternative[31]. To evaluate

antitumor efficacy and adverse effects of GPC1 specific CAR-T cells in both a human tumor

xenografted immunodeficient mouse model and an immunocompetent syngeneic mouse model,

it is necessary to generate GPC1 specific human and murine CAR-T cells from anti-GPC1 mAb

which cross-react with human and mouse GPC1. It is difficult to generate Ab recognizing an

evolutionally conserved epitope among mammals by using mammal hosts because of

immunotolerance against self-antigens. Recently, chicken is used as a host for generation of

monoclonal Ab instead of mammal hosts[32]. Specific epitope immunization in chicken can make

high-affinity Ab against the evolutionally conserved epitope among mammals to evaluate the

feasibility of GPC1 specific CAR-T cells for human patients using xenogeneic and syngeneic

mouse models.

The purpose of this chapter is to generate anti-GPC1 mAb which cross-react human and

mouse GPC1 by chicken immunization and generate GPC1-specific CAR vectors.

本章の以降の内容は、学術論文として出版する計画があるため公表できない。 5 年

以内に公表予定。

Chapter 2

Evaluation of antitumor efficacy of GPC1 specific

human CAR-T cells in a xenogeneic mouse model

本章の以降の内容は、学術論文として出版する計画があるため公表できない。 5 年

以内に公表予定。

Chapter 3

Evaluation of the safety and antitumor effect of GPC1 specific

murine CAR-T cells and the combination therapy

with anti-PD-1 Ab in a syngeneic mouse model

本章の以降の内容は、学術論文として出版する計画があるため公表できない。 5 年

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Summary and conclusion

本章の以降の内容は、学術論文として出版する予定があるため公表できない。 5 年以内に公表予定。

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