（様式 10）

（海外特別研究員事業）

令和　1 年　12 月　01 日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

採 用 年 度　平成 29 年度

受 付 番 号　496

氏　　　名　小川　久貴　　　小川久貴

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。

なお、下記及び別紙記載の内容については相違ありません。

記

1.　用務地（派遣先国名）用務地：ヒューストン　　　　　　　　　（国名：　　アメリカ　）

2.　研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。

膵臓癌に対する温熱キメラ抗原受容体 T 細胞療法の開発

3.　派遣期間：平成　29 年　4 月　1 日～平成　29 年　12 月 30 日（ 274 日間）

4.　受入機関名及び部局名

　　Dept of Pediatrics, UTHealth Science Center at Houston

5.　所期の目的の遂行状況及び成果…書式任意　書式任意（A4 判相当 3 ページ以上、英語で記入も可）

（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）

（注）「6.研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Pancreatic ductal adenocarcinoma (PDA) is the most aggressive GI tract adenocarcinoma with a 5-year survival of less than 10%. Although so many clinicians and researchers have been seriously trying to improve the prognosis over 100 years, we are yet to have a satisfactory treatment strategy. There may be two different reasons. First, it is severely hard to find PDA at an early stage because of the lack of definitive screening test. Most of the patients are diagnosed advanced stage at initial diagnosis, and about 80% of the patients are diagnosed with inoperable status mainly due to the existence of concurrent distant metastasis. Next, to make matters worse, patients are not likely to escape from distant metastasis even after curative surgery and subsequent anti-cancer drug. It is because pancreatic cancer is so aggressive by nature that it has already invisibly infiltrated the surrounding organs such as lymph nodes and systemically spread into the bloodstream and metastasized microscopically to liver and lung. We must consider pancreatic cancer as a systemic disease and develop a new therapy that can eradicate cancer in the body in concert with curative surgery.

In cancer research, cancer stem cell (CSC) theory has emerged as an attractive hypothesis that this unique population is responsible for cancer development and progression (Fig1).

Fig1. CSC theory
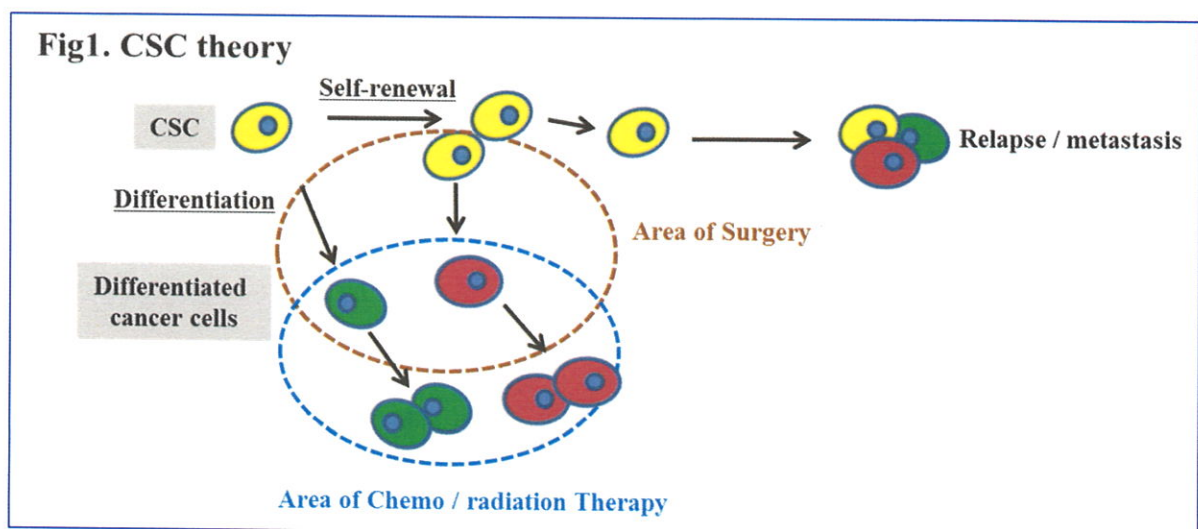
The theory demonstrates that cancer tissue consists of heterogeneous populations, and this small CSC population has the features of self-renewal and differentiation into diverse cancer cells similar to normal stem cells. The theory also suggests that we cannot thoroughly remove cancer without attacking CSC because surviving CSC can lead to regrowth and metastasis. CSC theory perfectly fits the reality in pancreatic cancer patients. This CSC population has been identified by some cell surface antigens in pancreatic cancer by flow cytometry-based analysis. Among them, I aimed surface antigen X that is a critical antigen and closely related to cancer progression in pancreatic cancer. I hypothesized the development of a new treatment system targeting X expressing cells would be anti-CSCs therapy.

On the other hand, genetic engineering technology moved cancer immunotherapy to the next stage. Chimeric antigen receptor (CAR) T cell therapy is an impressive choice of treatment that genetic manipulation technology endowed in immunotherapy. CAR construct consists of an extra-cellular targeting domain that can bind to a specific antigen on the surface of target cells, trans-membrane, and endo-cellular domain. The extra-cellular domain is derived from a single chain fragment of variance (scFv) consists of the variable heavy and light chains of the monoclonal body. The binding to the specific antigen on the surface of cancer cells was major histocompatibility complex -unrestricted interaction and processed between scFv and antigen. Once stimulated by binding, CAR T cells start both executing cytolysis and proliferating to spread and patrol surviving target cells. CAR T cell therapy can be called "living drugs" and also the ultimate way of molecularly targeted therapy. From these ideas, I developed Sleeping Beauty transposon-based

CAR T therapy targeting X (Fig2).

Fig2. CAR T cell
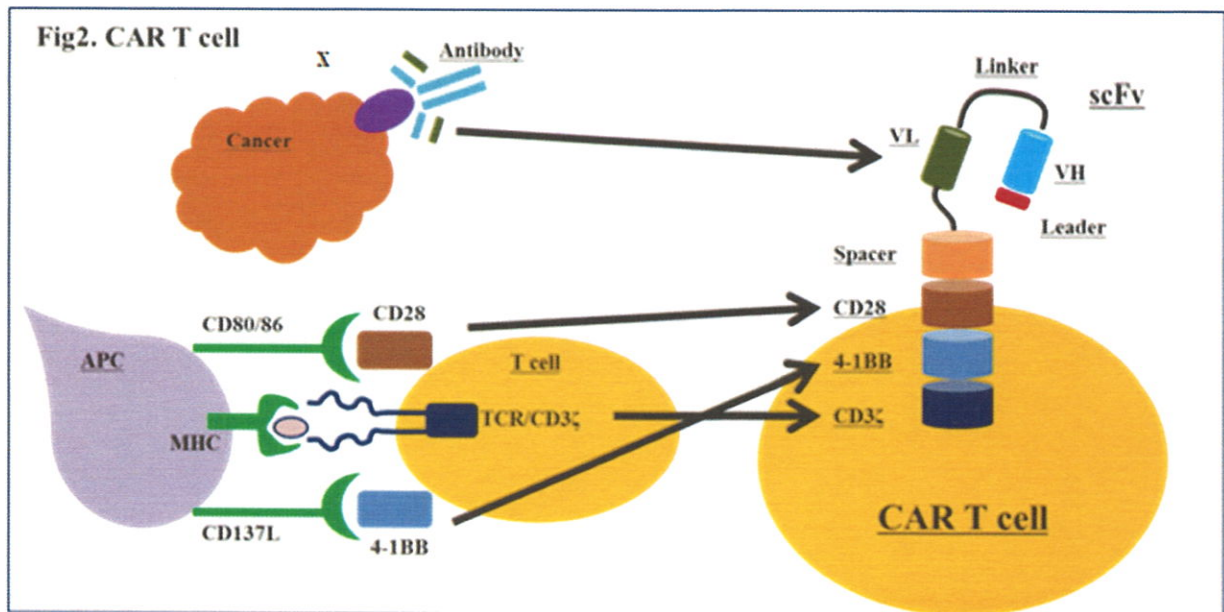
Sleeping Beauty transposon system can stably integrate vectors into chromosomal and last for the long term. Furthermore, it seems safer with lower immunogenicity and insertional mutagenesis compared with a viral vector for clinical use. First, I transfected the CAR vector into the isolated human PBMC together with transposase (SB100) by electroporation method and, next day stimulated them with OKT-3 loaded KT86/64 cells under human IL-2 for expansion. On day3, I started Puromycin selection due to the Puromycin resistant gene incorporated in the CAR vector.

From our protocol, I could obtain a sufficient number of CAR T cells just within two weeks with about 100% purity from 100 ml blood. Then, I screened the expression of surface antigen X by flow cytometry-based analysis to choose the target cells. Of note, I figured out the expression of surface antigen X in many kinds of cancers, including pancreatic cancer, gastric cancer, colon cancer, breast cancer, lung cancer, thyroid cancer, uterine cancer and skin squamous cell cancer. That suggested our CAR T cell could be universally useful regardless of cancer types. Next, I examined the cytolysis and cytokine

secretion ability against pancreatic cell lines in vitro. Our CAR T cell tremendously eradicated a variety of pancreatic cell lines only for 24 hours and, consistently, highly secreted cytotoxic-related cytokine secretion depending on antigen strength. From the analysis of differentiation status, I found central memory and effector memory subsets account for the majority, possibly leading to better engraftment in vivo.

In an established subcutaneous pancreatic cancer xenograft NSG mouse model, I experienced the sudden death of mice just after the injection of our CAR T cells via the tail vein. Then, I tweaked our the hinge portion in our CAR vector so that I never experienced the mice's death with our new CAR vector. Our CAR T cell abundantly accumulated in the xenograft, whereas T cell without CAR construct did not, and much showed reduced tumor burden. From these results, I believed our new CAR T cell therapy might be promising next cancer immunotherapy for pancreatic cancer. Meanwhile, I had to face the unique characteristics of pancreatic cancer to proceed into clinical trials without a doubt.

We realized there must be two main features of pancreatic cancer to attenuate CAR T cell therapy. First, pancreatic cancer is a quite solid cancer with abundant fibrosis and scarce tumor vessels in the stroma. Abundant stroma impedes drug delivery to the cancer cells. No matter what an effective drug we will create, it cannot work without proper delivery to the tumor. Then, to solve the first problem, we employed and evaluated physiological fever-range hyperthermia to improve access to subcutaneous xenograft by enhancing tumor perfusion. As expected, hyperthermia efficiently increased tumor perfusion and enabled many more CAR T cells to get access to the xenografts leading to a more significant anti-tumor effect. Unexpectedly and

surprisingly, hyperthermia-induced the enhanced surface antigen X expression on the fever-treated cancer cells.

So far, there is no clinical CAR T trial with hyperthermia conducted, but physiological fever-range hyperthermia has been well used in concert with chemotherapy in a clinical setting, and the feasibility of this mild heating was confirmed well. From these results, I get to assure that hyperthermia synergizes our CAR T cell therapy due to better delivery to the tumor and better surface antigen X exposure on the surface of cancer cells. While I was in Houston, I accomplished my initial aim to develop a new CAR T cell therapy against PDAC. From there, I successfully proposed and validated a new treatment strategy 'mild heating CAR T cell therapy,' but we are yet to solve another, but more critical, problem. Reviewing the outcomes from the completed unsuccessful CART trials, a promising result in an experimental mouse model is not reproducible in clinical trials. It is because all previous CAR T research used the immunodeficient mouse to validate the CART function and have omitted the process of finding out if we have to take some more measures in the patients with a healthy immune system. Next, we have to deal with the more critical problem about the immune escape induced by regulatory T cell (Treg), the unique population of helper T cells, have an influential role in attenuating CAR T cell therapy. Treg accounts for only approximately 5-10% of CD4+T cells but plays a central role in the maintenance of self-tolerance and homeostasis. Recent research has shown another critical function of Treg in cancer immunity that Treg accelerates tumor progression by their suppressive function in a variety of immune cells. It is well also reported that there are abundant infiltrating Treg cells in pancreatic cancer and the ratio of cytotoxic T cells to Tregs in cancer is closely

correlated with poor prognosis in pancreatic cancer patients. Treg cell transient depletion, in combination with our current treatment has been shown to enhance cancer-specific T cell activation in preclinical studies. We realize the importance of Treg in cancer immunity, and the understanding of the mechanisms underlying the immunosuppressive effects of Treg in cancer is essential to overcome for the treatment of PDA. Although a variety of the mechanism for Treg mediated suppression for T cell therapy in crosstalk with the antigen-presenting cells and other immune cells have been suggested, there has never been such a useful experimental method to analyze how Treg can directly interact with cancer cells and cytotoxic T cells before CAR technology. Taking advantage of my precious and hardened expertise in CAR technology, I am currently challenging this problem at my facility.