

# siRNA DDS Technology (Cancer Tissue)

## Background and Overview

It is generally believed that drug delivery system(DDS) is necessary to deliver nucleic acid drugs to cancer tissue. By complexing siRNA with a folic acid (ligand) -conjugated cationic peptide, it is possible to deliver nucleic acid drugs to cancer tissue, which has been difficult to do in the past. Dr. Takeshi Wada of Tokyo University of Science and Dr. Keisuke Taniuchi of Kochi University reported that siRNA can be efficiently delivered to cancer cells with high expression of folate receptor by using folic acid/cationic peptide conjugate (hereafter referred to as Fol-Dab8) as a delivery enhancer and forming a complex with siRNA<sup>(Ref. 1)</sup>.

Pancreatic cancer has one of the worst prognoses because early detection is difficult, pancreatic cancer cells are highly motile, and even small cancers invade the surrounding blood vessels, gastrointestinal tract, nerves, etc. and easily metastasize to distant sites. Dr. Keisuke Taniuchi at Kochi University discovered that snora22 RNA is involved in the invasion and metastasis of pancreatic cancer<sup>(Ref. 2)</sup>, and found that inhibition of the snora RNA by a complex of Fol-Dab8 and siRNA (Fol-Dab8/siRNA complex) suppresses the invasion and proliferation of pancreatic cancer cells<sup>(Ref. 1)</sup>. These results are expected to lead to the creation of innovative therapeutic agents for pancreatic cancer and the development of new therapeutic agents that can deliver siRNA to various cancer cells with high expression of folate receptor, such as brain tumors and breast cancers.

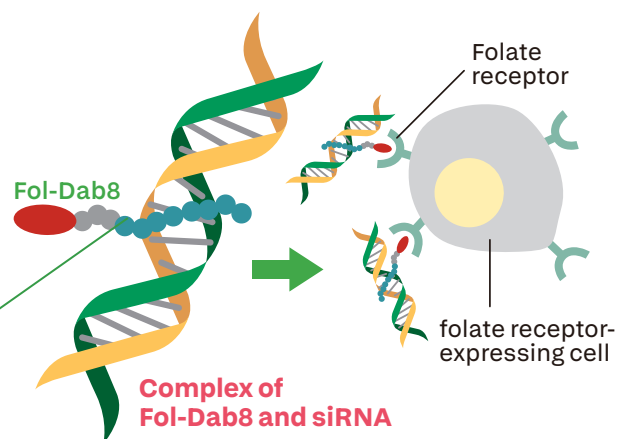
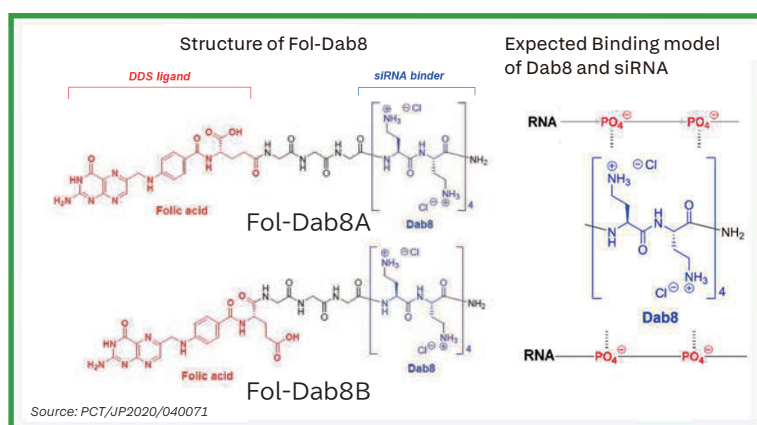
※Reference 1 : PCT/JP2020/040071

※Reference 2 : K. Taniuchi and M. Ogasawara, *Oncotarget*, 2020, 11:131

※Fol-Dab8 is an abbreviation for 2,4-diaminobutyric acid octamer in which folic acid is linked via a linker.

## Patent information

PCT/JP2020/040071: Nucleic acid delivery enhancer



## Features of Fol-Dab8 technology

**siRNA delivery can be achieved by simply mixing Fol-Dab8 and siRNA.**

**Fol-Dab8/siRNA complex acquires resistance to RNase.**

**Fol-Dab8/siRNA complex can be delivered to pancreatic cancer cells and pancreatic cancer tissues via folate receptor.**

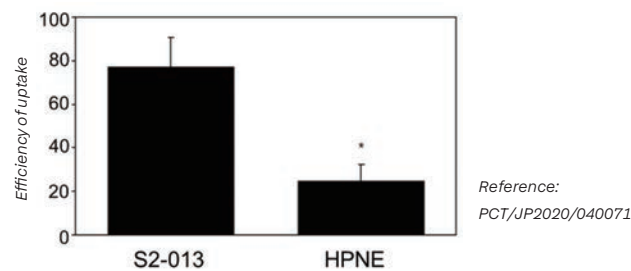
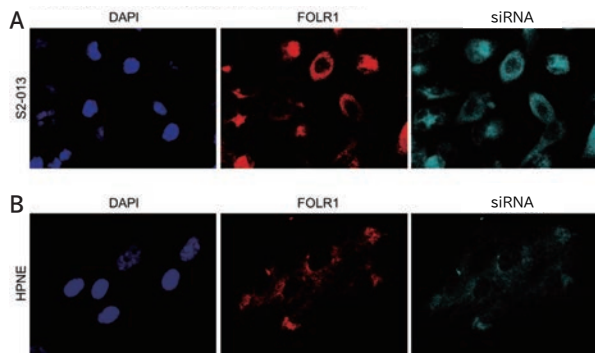
**Fol-Dab8/SNORA22 siRNA complex inhibits pancreatic cancer invasion, metastasis, and tumor growth.**

\*SNORA22 is a non-coding RNA (small nucleolar RNA) present in the nucleolus. It binds to RNA binding protein KHSRP and is localized in cell protrusion (lamellipodia) of pancreatic cancer cells. It is involved in the motility and invasion of pancreatic cancer cells by regulating the translation of mRNA bound to IGF2BP3 transferred intracellularly to lamellipodia.

## 1. Uptake into pancreatic cancer cells

**[Method]** SNORA22-siRNA labeled with fluorescent dye was mixed with Fol-Dab8B (2 equivalents), and then added into the culture medium of S2-013 human pancreatic cancer cells or HPNE normal human pancreatic duct epithelial cells in 4-well chambers and incubated at 37°C overnight, then the nuclear (DAPI), folate receptor (FOLR1) and siRNA (Alexa488) signals were observed.

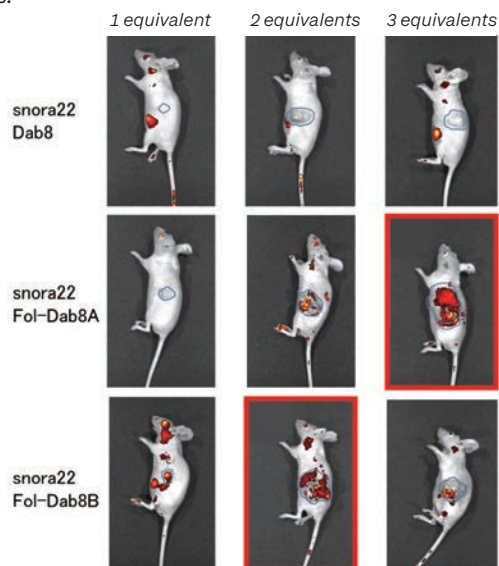
**[Result]** In S2-013 pancreatic cancer cells, the staining intensity of folate receptors was higher than in HPNE normal pancreatic duct epithelial cells, and at the same time, the fluorescence intensity of SNORA22-siRNA incorporated into the cells was stronger.



## 2. Delivery to pancreatic cancer tissue

**[Method]** Six weeks after subcutaneous implantation of human pancreatic cancer organoids in nude mice, fluorescence-labeled SNORA22 siRNA (5 µg) with Dab8, Fol-Dab8A, or Fol-Dab8B was administered by tail vein injection. 24 h later, the images were taken using “in vivo imager”.

**[Result]** Despite differences in equivalence both Fol-Dab8A and Fol-Dab8B were effective in enhancing delivery to pancreatic cancer tissues.

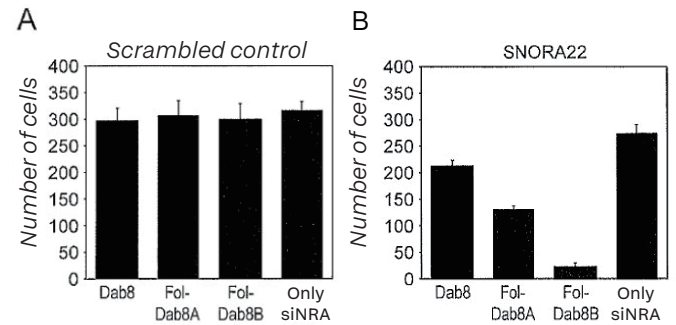


Reference: PCT/JP2020/040071

## 3. Inhibition of pancreatic cancer cell invasion

**[Method]** Scrambled control siRNA and SNORA22 siRNA with Fol-Dab8A and Fol-Dab8B were added to the culture medium of S2-013 pancreatic cancer cells, and Matrigel invasion assays were performed 48 hours later.

**[Result]** The addition of SNORA22 siRNA with Fol-Dab8A or Fol-Dab8B significantly inhibited the invasion of S2-013 pancreatic cancer cells compared to the scrambled control siRNA with Fol-Dab8A or Fol-Dab8B and the siRNA alone.



## 4. Inhibition of tumor growth

**[Method]** To study the effects of SNORA22 siRNA on tumor growth in vivo, a mouse model subcutaneously transplanted with human pancreatic cancer organoids (S2-013-organoid model) was used.

1 week after subcutaneous implantation of human pancreatic cancer organoids in nude mice, SNORA22 siRNA (5 µg) with Dab8 (2 equivalents) or Fol-Dab8 (2 equivalents) was administered weekly by tail vein injection, and tumors were measured weekly using a caliper (n = 8 per group). As controls, scrambled control siRNA with Fol-Dab8 was administered in the same manner.

**[Result]** Compared to the non-treated control group (Control), the group treated with scrambled control-siRNA (Scr-siRNA/Fol-Dab8), and SNORA22 siRNA with Dab8 (SNORA22 siRNA/Dab8), SNORA22 siRNA with Fol-Dab8 (SNORA22 siRNA/Fol-Dab8) significantly inhibited tumor growth after week 8.

