主論文の要旨

# Silencing of STRN4 suppresses the malignant characteristics of cancer cells

（STRN4 の抑制はがん細胞の悪性形質を抑える）

名古屋大学大学院医学系研究科　分子総合医学専攻
病態内科学講座　消化器内科学分野
（指導：後藤 秀実　教授）
王 美鴻

<Background>

STRN4 is a member of striatin family of proteins that harbor multiple protein-binding domains. Accumulating evidence has shown that striatin family proteins act as a regulatory subunit of protein phosphatase 2A (PP2A). PP2A is a family of mammalian serine/ threonine phosphatase and is involved in many cellular functions, such as metabolism, cell cycle, protein synthesis, apoptosis, and cellular signaling. STRN4 has been reported to associate with members of germinal center kinase (GCK), including MINK1, TNIK and MAP4K4. Several lines of evidence revealed that these protein kinases exert tumor-promoting or tumor-suppressing activity. However, whether STRN4 is associated with tumor progression remains unknown. Therefore, we examine the role that STRN4 plays in cancer progression using several cancer cell lines.

<Materials and methods>

STRN4 expression was depleted in multiple cancer cell lines using siRNA and shRNA. Proliferation, migration, invasion, anchorage-independent growth and sensitivity to gemcitabine were examined to evaluate the malignant characteristics of the cancer cells. The cells were then subcutaneously injected into mice, at both sides of the femoral area and via intravenous injection, to evaluate tumor proliferation and metastasis.

<Results>

The expression of STRN4 in multiple cell lines was confirmed by western blot (Fig. 1A). We used two different siRNAs to deplete STRN4 expression in these cell lines and we observed the proliferation of KP4, PK9 and HCT116 was clearly reduced by STRN4 knockdown, data showed KP4 cells (Fig 1B). To determine whether the reduction in proliferation of these cells was mediated by the inhibition of cell cycle progression or by the induction of apoptosis, we performed an EdU incorporation assay and a TUNEL assay. As shown in Fig. 1C, depletion of STRN4 significantly reduced the ratio of EdU-positive cells. The TUNEL assay demonstrated an induction of apoptosis by STRN4 depletion in KP4, PK9 and HCT116 cells (Fig. 1D).

To assess changes in cell migration, we first performed a wound healing assay. The migration of STRN4-depleted cells was clearly delayed compared with that of the control siRNA-transfected cells, data showed KP4 cells (Fig. 2A). To further confirm this result, we used a modified Boyden chamber assay. The migration of STRN4-depleted cells was suppressed compared with that of the control siRNA-transfected cells (Fig. 2B). A Matrigel-coated Boyden chamber was used to investigate the effect that STRN4 suppression had on cell invasion. The invasion of cancer cells was significantly reduced by STRN4 knockdown (Fig. 2C).

We next assessed the anchorage-independent growth of the STRN4-depleted cells. The

STRN4 siRNA-transfected cells formed significantly fewer colonies than the control siRNA-transfected cells (Fig. 3A). Anoikis is a form of cell apoptosis induced by the detachment of cells from the extracellular matrix. We hypothesized that the suppression of colony formation by STRN4 knockdown was associated with the promotion of anoikis. Cells transfected with siRNAs were cultured in suspension for 48 h, and apoptotic cells were then detected via TUNEL assay. As shown in figure 3B, STRN4 depletion clearly increased the ratio of apoptotic cells after 48 h of suspension culture.

Gemcitabine is a nucleoside analog used for pancreatic cancer treatment. To avoid the toxicity of the transfection reagent, we established KP4 cells that constitutively expressed two different shRNAs targeting STRN4 (shSTRN4-1 and shSTRN4-2) as well as control shRNA-expressing cells (shCtrl). The expression of STRN4 was reduced in both the shSTRN4-1 and shSTRN4-2 cells (Fig. 4A). These cells were cultured in the presence of various concentrations of gemcitabine, and cell growth was assessed. As shown in figure 4B, the STRN4-depleted KP4 cells were more sensitive to gemcitabine treatment than the control KP4 cells. To further confirm the increased sensitivity to gemcitabine, we performed a TUNEL assay and we observed a significant increase in apoptotic cells depleted of STRN4 (Fig. 4C).

We next investigated the growth of STRN4 knockdown cells in mice. shCtrl and shSTRN4-1 KP4 cells were subcutaneously injected into the femoral area of nude mice, and tumor formation was examined. Both cell lines formed five subcutaneous tumors out of a total of five injected sites. The tumor formation of the shSTRN4-1 cells was suppressed compared with the tumor formation of the shCtrl cells (Fig. 5A). The average weight of the shSTRN4-1 cell derived tumors was significantly reduced compared with that of the shCtrl cells (Fig. 5B). We also examined the effect of STRN4 depletion on the metastasis of KP4 cells. Both shCtrl and shSTRN4-1 cells were injected in the lateral tail vein of mice, and metastasis in the lung and liver was examined. Three out of the five mice injected with shCtrl cells formed metastatic foci in the lung or liver; however, we did not observe any metastatic foci in the lung and liver of the mice injected with shSTRN4-1 (Fig. 5C). We maintained the mice for 8 weeks after tumor injection. Three shCtrl-injected mice died within 8 weeks, but all the mice injected with shSTRN4-1 cells survived for over 8 weeks (Fig. 5D).

## <Discussion>

In this report, we show that STRN4 is expressed in multiple cancer cell lines and that depletion of STRN4 in some cancer cell lines inhibits cell cycle progression and induces apoptosis. Depletion of STRN4 reduced migration, invasion and survival in suspension conditions. Additionally, STRN4 knockdown increased the sensitivity of KP4 cells to gemcitabine. Striatins have been shown to associate with multiple protein kinases that

play a role in tumor progression. Connector of kinase to AP-1 (CKA) is a homolog of striatin family proteins in *Drosophila melanogaster*. CKA promotes AP-1 activation and associates with HEP and BSK, which are human homologs of JNKK (Jun kinase kinase) and JNK (Jun kinase), respectively [27]. AP-1, which is a heterodimeric transcription factor comprising c-Fos, c-Jun, ATF and JDP family proteins, plays a pivotal role in the progression of numerous tumor types. We have previously reported that STRN4 directly associates with MINK1, TNIK, and MAP4K4 [13]. MINK1 is activated by Ras activation and mediates p38 activation during growth arrest and senescence [24]. Both TNIK and MAP4K4 exert tumor-promoting activity. TNIK phosphorylates T-cell factor 4 (TCF4) to activate Wnt signaling and stimulate colorectal cancer cell proliferation [26]. MAP4K4 is overexpressed in many types of human cancer, and silencing of MAP4K4 inhibits tumor cell migration and proliferation [25, 28]. STRN4 has been reported to regulate the activity of associating kinases [13]; thus, depletion of STRN4 may affect the activity of these kinases and suppress signaling pathways critical for the invasion and survival of cancer cells.