**News Release**

**Title**

**Development of a therapeutical strategy to convert the phenotype of cancer-associated fibroblasts and improve the efficacy of anti-cancer drugs in pancreatic cancer**

**Key Points**

1. Recalcitrant cancers including pancreatic cancer are characterized by extensive proliferation of cancer-associated fibroblasts (CAFs) in the tumor stroma. Previous studies have shown that there exist cancer-promoting CAFs ("friends of cancer cells") and cancer-restraining CAFs ("foes of cancer cells").
2. Nagoya University researchers and colleagues previously identified Meflin as a functional marker of cancer-restraining CAFs in pancreatic cancer.
3. In the present study, the research team has identified the synthetic unnatural retinoid AM80 as a compound that effectively induces the expression of Meflin in CAFs and convert cancer-promoting CAFs to cancer-restraining CAFs.
4. AM80 administration induced a decrease in tumor stiffness in a pancreatic cancer mouse model, which was accompanied by an increase in tumor vessel area and improvement of the delivery of the anti-cancer drug Gemcitabine. The AM80 and Gemcitabine combination therapy significantly improved the outcome of pancreatic cancer mouse models compared with Gemcitabine monotherapy.
5. Based on the findings of the present study, an investigator-initiated clinical trial to test the safety and efficacy of the combination therapy of AM80 and Gemcitabine/nab-Paclitaxel has started in Nagoya University and the University of Tokyo (principal investigator: Mitsuhiro Fujishiro, ClinicalTrials.gov Identifier: NCT05064618).

**Summary**

It is well known that intractable cancers such as pancreatic cancer exhibit extensive fibroinflammatory reaction in the stroma. Cancer-associated fibroblasts (CAFs) are one of the major components of the tumor stroma and promote cancer progression through various mechanisms including the secretion of cytokines, chemokines and extracellular matrix (ECM) proteins. Therefore, CAFs have been considered a target for the development of anti-cancer therapeutics. However, previous therapeutic attempts to deplete CAFs or inhibit their proliferation in pancreatic cancer were not successful in mice or patients. Thus, researchers have argued that CAFs may be tumor suppressive or heterogeneous, with distinct cancer-restraining and -promoting CAFs (rCAFs and pCAFs, respectively). In the present study, Nagoya University researchers and colleagues showed that induced expression of the glycosylphosphatidylinositol-anchored protein Meflin, a rCAF-specific marker, in CAFs by genetic and pharmacological approaches improved the

chemosensitivity of mouse models of pancreatic cancer. The research team performed a chemical library screen and identified AM80 (tamibarotene), a synthetic non-natural retinoid, as a reagent that effectively induced Meflin expression in CAFs. They found that AM80 administration improved the sensitivity of pancreatic cancer to chemotherapeutics, accompanied by increases in tumor vessel area and drug delivery. The team also provided an insight into the function of Meflin, where they found that Meflin was involved in the suppression of tissue stiffening by interacting with lysyl oxidase (Lox) to inhibit its collagen crosslinking activity. These data suggested that the modulation of CAF heterogeneity may represent a strategy for pancreatic cancer treatment.

The study, "Pharmacologic conversion of cancer-associated fibroblasts from a protumor phenotype to an antitumor phenotype improves the sensitivity of pancreatic cancer to chemotherapeutics" was published online in the journal *Oncogene* on April 13, 2022 at DOI 10.1038/s41388-022-02288-9.

**Research Background**

A well-known feature of pancreatic cancer is the proliferation of CAFs, excessive deposition of the ECM proteins produced by CAFs, and ECM remodelling in the stroma (**Figure 1**). CAFs secrete various soluble factors, including growth factors, chemokines and cytokines, insoluble ECM, proteases, and extracellular vesicles, which may promote the proliferation, invasion, and metastasis of cancer cells and contribute to drug resistance and suppression of antitumor immunity (ref. 1). These findings have facilitated recent efforts to develop therapeutics that deplete CAFs or inhibit their proliferation and functions in academic and pharmaceutical sectors.

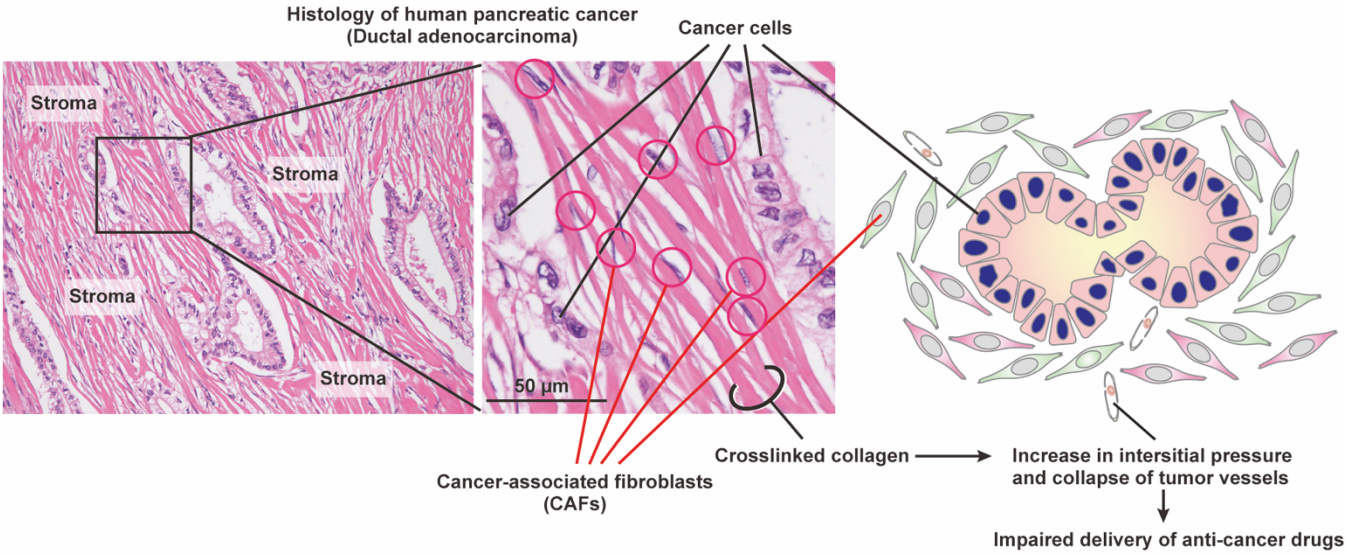


**Figure 1.** Representative histology of human pancreatic cancer.

CAFs were previously thought to be a uniform population of cells with cancer-promoting functions. However, several preclinical and clinical attempts to target CAFs in pancreatic cancer mouse models and patients were not therapeutically successful, and some intervention strategies resulted in disease progression (ref. 1). Thus, certain subtype(s) of CAFs may suppress rather than support the

progression of pancreatic cancer (**Figure 2**). The heterogeneity of CAFs has also been shown by recent single-cell transcriptomic analyses that have shown that CAFs can be classified into many subsets. The most relevant and characterised CAF subsets in pancreatic cancer are myofibroblastic CAFs (myCAFs), which are positive for α-smooth muscle actin (α-SMA), and inflammatory CAFs (iCAFs) that have a high capacity to secrete inflammatory cytokines (ref. 1). Clinicopathological and pharmacological studies have shown that these CAFs exert protumor effects through diverse mechanisms. However, the existence of cancer-restraining CAFs (rCAFs) and their marker proteins has not been investigated.

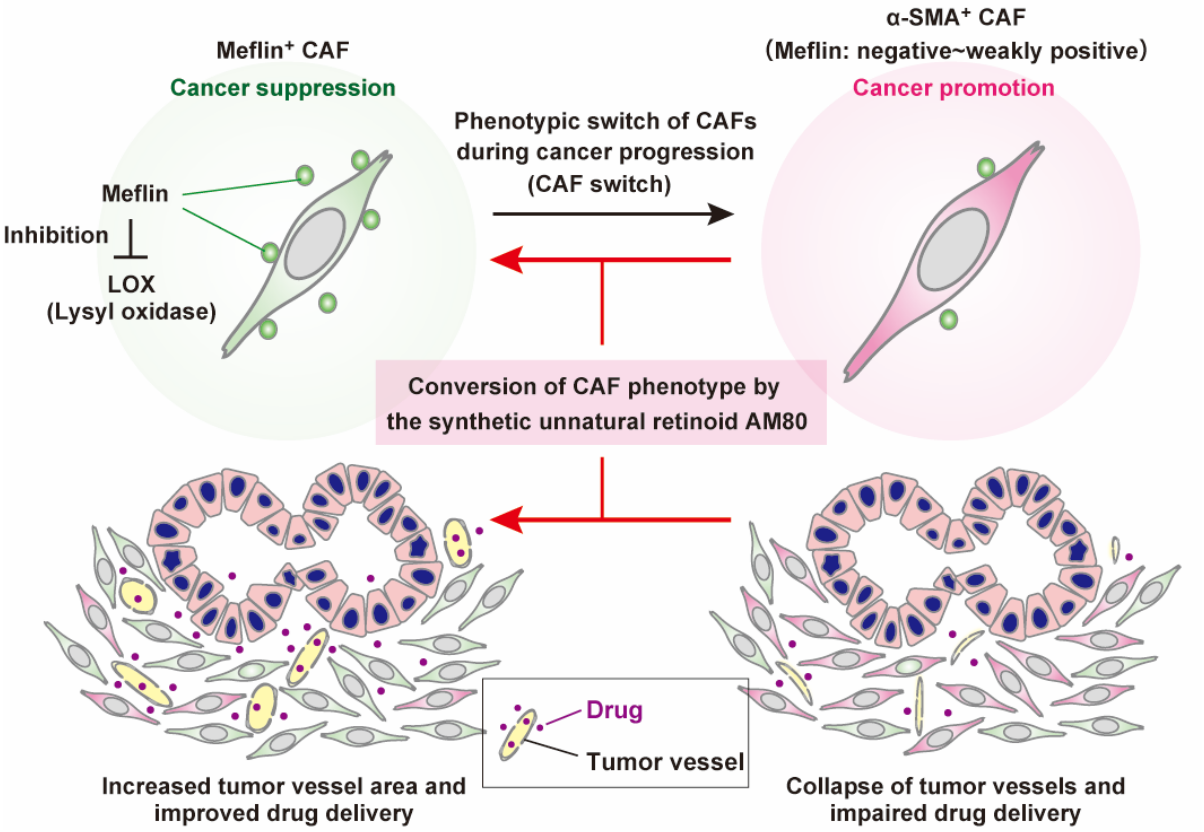


**Figure 2.** Conversion of CAF phenotype by AM80 administration and its effect on drug delivery.

The research team previously identified the new CAF marker Meflin, a glycosylphosphatidylinositol (GPI)-anchored membrane protein, in both mouse and human pancreatic and colorectal cancer (ref. 2-5). The analyses of human pancreatic cancer samples and mouse models showed that Meflin suppresses cancer progression, suggesting that Meflin is a marker of rCAFs. Interestingly, a lineage tracing experiment showed that Meflin$^+$ rCAFs give rise to α-SMA$^+$ CAFs that are negative or weakly positive for Meflin during cancer progression (ref. 3), suggesting that Meflin$^+$ rCAFs differentiate into other CAF types, including myCAFs and iCAFs. Another recent study showed that the number of Meflin$^+$ CAFs well correlated with the efficacy of immune checkpoint inhibitors in patients with non-small cell lung cancer (ref. 6). However, any therapeutics that convert the protumor CAFs (myCAFs and iCAFs) into antitumor rCAFs or induce Meflin expression in CAFs have not been developed so far.

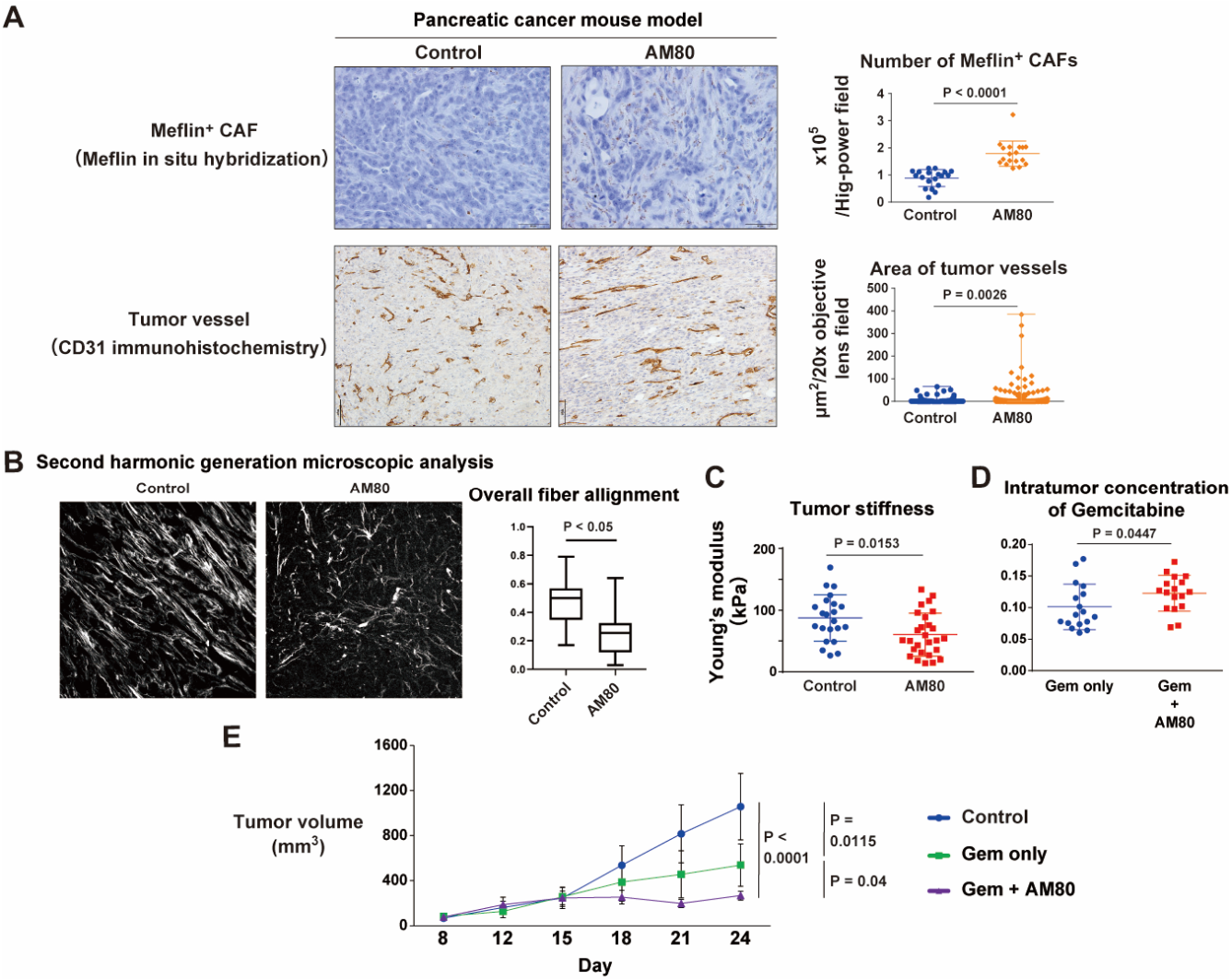


**Figure 3.** AM80 administration changes CAF phenotype and improves the delivery of the anti-cancer drug Gemcitabine.

**Research Results**

The research team performed a chemical library screen for compounds that convert protumor CAFs into rCAFs by inducing Meflin expression. As a result, they identified the synthetic retinoid AM80 as a candidate drug that achieves the CAF conversion (**Figure 2**). Experiments on pancreatic cancer mouse models showed that AM80 itself exhibited no effect on tumor growth, whereas the combination of AM80 and Gemcitabine significantly increased the tumoricidal effects of Gemcitabine, which was accompanied by increases in the number of Meflin-positive CAFs and tumor vessel area, changes in the alignment and architecture of collagen fibers, and a decrease in the stiffness (elastic modulus) of tumor tissues (**Figure 3A-C**). Interestingly, these changes in tumor stroma resulted in an increase in the intratumor concentration of Gemcitabine, suggesting that AM80-medicated changes in CAFs and stroma improved the efficacy of drug delivery (**Figure 2, 3D**). Accordingly, the AM80 and Gemcitabine combination therapy resulted in better outcomes than the Gemcitabine alone group in the pancreatic cancer mouse model (**Figure 3E**). Notably, this AM80 effect was not observed in Meflin knock out mice. The research team also demonstrated that

the transduction of the Meflin gene (*Islr gene*) into CAFs through the Sendai virus system augmented the efficacy of Gemcitabine in pancreatic cancer mouse models.

The research team finally showed that Meflin directly bound Lox and functioned to suppress its collagen crosslinking activity, which may be the mechanism of low stiffness of tumors administered AM80 or transduced with the Meflin gene through the Sendai virus system.

**Research Summary and Future Perspective**

These data provide a rationale for therapeutic strategies that increase the number of Meflin-positive rCAFs in the treatment of patients with pancreatic cancer. The research team has just started an investigator-initiated clinical trial to examine the safety and efficacy of the combination of AM80 and conventional anti-cancer drugs Gemcitabine and nab-Paclitaxel in patients with unresectable pancreatic cancer in Nagoya University and the University of Tokyo (principal investigator: Mitsuhiro Fujishiro, ClinicalTrials.gov Identifier: NCT05064618) (ref. 7). Furthermore, the present study clearly demonstrated the importance of CAF heterogeneity in understanding the biology of cancer and development of new anti-cancer therapeutics.

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