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Possible disease-protective roles of fibroblasts in cancer and fibrosis and their therapeutic application

Yukihiro Shiraki, Shinji Mii, Nobutoshi Esaki and Atsushi Enomoto

Department of Pathology, Nagoya University Graduate School of Medicine, Nagoya, Japan

ABSTRACT

Cancer and fibrotic diseases are characterized by continuous inflammation, tissue wounds, and injuries. Cancer is a "wound that does not heal," and the uncontrolled proliferation of cancer cells disrupts normal tissue integrity and induces stromal fibroinflammatory reactions. Fibroblasts proliferate extensively in the stroma, playing a major role in the development of these diseases. There has been considerable evidence that fibroblasts contribute to fibrosis and tissue stiffening and promote disease progression via multiple mechanisms. However, recent emerging findings, mainly derived from single-cell transcriptomic analysis, indicated that fibroblasts are functionally heterogeneous, leading to the hypothesis that both disease-promoting and -restraining fibroblasts exist. We recently showed that a fibroblast population, defined by the expression of the glycosylphosphatidylinositol-anchored membrane protein Meflin may suppress but not promote fibrotic response and disease progression in cancer and fibrotic diseases. Although currently hypothetical, the primary function of Meflin-positive fibroblasts may be tissue repair after injury and cancer initiation occurred. This observation has led to the proposal of a potential therapy that converts the phenotype of fibroblasts from pro-tumor to anti-tumor. In this short review, we summarize our recent findings on the function of Meflin in the context of cancer and fibrotic diseases and discuss how we can utilize this knowledge on fibroblasts in translational medicine. We also discuss several aspects of the interpretation of survival analysis data, such as Kaplan-Meier analysis, to address the function of specific genes expressed in fibroblasts.

Keywords: perivascular fibroblasts, mesenchymal stem cells, Meflin, cancer-associated fibroblasts, Immunoglobulin superfamily containing leucine-rich repeat (ISLR)

Abbreviations:
α-SMA: α-smooth muscle actin
PVF: perivascular fibroblast
MSC: mesenchymal stem cell
CAF: cancer-associated fibroblast
ECM: extracellular matrix
PDAC: pancreatic ductal adenocarcinoma
ISLR: immunoglobulin superfamily containing leucine-rich repeat

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Corresponding Author: Atsushi Enomoto, MD, PhD
Department of Pathology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
TEL: +81-52-744-2093, Fax: +81-52-744-2098, E-mail: enomoto@iar.nagoya-u.ac.jp

INTRODUCTION

Fibroblasts secrete extracellular matrix (ECM) molecules such as collagen and fibronectin that are essential to maintain the normal architecture of tissues and organs.[1] The ECM produced by fibroblasts is also essential for tissue repair after wounds, injuries, and acute inflammation. In diseases such as cancer and chronic inflammatory or fibrotic diseases, fibroblasts become activated and proliferate in the stroma, constituting an essential component of fibroinflammatory reactions.[2,3] Activated fibroblasts, often called myofibroblasts owing to the high expression level of α-smooth muscle actin (α-SMA) in these cells, produce a large amount of ECM molecules and proteases as well as inflammatory cytokines and chemokines evoking inflammation.[4] To date, a multitude of studies have investigated the function of fibroblasts that proliferate under various disease conditions, leading to the accepted dogma that these cells promote fibrosis and tissue stiffening. Therefore, the development of drugs and tools that tactfully inhibit continuous activation and proliferation of fibroblasts has been the focus of a substantial amount of research. Our group has been also interested in the role of fibroblasts in cancer progression.[5-9] However, one major obstacle in these studies is the lack of markers specific to fibroblasts that enable us to distinguish between non-activated and activated fibroblasts.[10]

Previous studies have highlighted the importance of fibroblast heterogeneity by demonstrating that the suppression of fibroblast proliferation or their genetic ablation unexpectedly resulted in the progression of pancreatic ductal adenocarcinoma (PDAC).[2,3,11-14] These studies led to the notion that fibroblasts may both promote cancer (cancer-promoting cancer-associated fibroblasts [pCAFs]) and suppress cancer (cancer-restraining CAFs [rCAFs]).[2,3,14] The heterogeneity of CAFs has been also supported by recently emerging, single-cell transcriptomic analysis, which showed that the extent of CAF heterogeneity and their cells of origin differ across tumor types. This suggests that there is more than one mechanism linking fibroinflammatory reactions to cancer progression and that CAFs are not simply binary in nature ("good" or "bad").[2,3,10,15] There is a plethora of recently published review articles on CAF diversity and heterogeneity that readers can refer to for further understanding.[2,3,10,15-19]

We have previously identified Meflin as a fibroblast marker.[3,20-27] Interestingly, our recent studies on human pathological samples and mouse disease models have suggested that the function of Meflin-positive (Meflin$^+$) fibroblasts is distinct from that of conventional activated fibroblasts (myofibroblasts and CAFs) in fibrotic disease and cancer conditions. In this review, we briefly summarize the findings from our own laboratory and other studies on the functions of Meflin protein and Meflin$^+$ fibroblasts. We also describe a caveat when interpreting the results of the Kaplan-Meier analysis in studying the molecular function of CAF markers.

MEFLIN IS A MARKER OF MESENCHYMAL STEM CELLS AND PERIVASCULAR FIBROBLASTS

Meflin is a glycosylphosphatidylinositol-anchored membrane protein encoded by the immunoglobulin superfamily containing leucine-rich repeat (*ISLR*) gene.[20] We previously identified Meflin as one of the proteins specifically expressed in contact-inhibited fibroblasts.[20] Further studies showed that Meflin was expressed by rare stromal cells that adhere to the abluminal side of endothelial cells or localize in the perivascular area of micro- or small capillaries in almost all organs in adult mice (Figure 1). In mouse embryos, Meflin expression was found in immature mesenchymal cells that constitute the stroma of all tissues and the cartilage primordia of skeletal tissues. In adult mice, Meflin expression was detected in stromal fibroblasts found in the bone

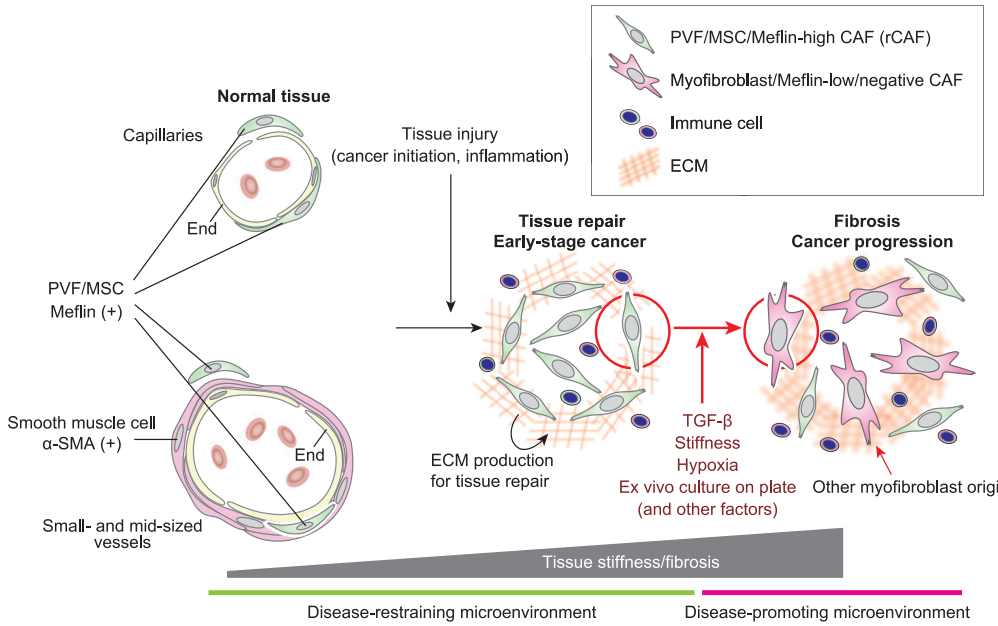

Fig. 1 Localization of Meflin$^+$ cells in normal tissues and their proliferation after tissue injury, cancer initiation or inflammation

Meflin$^+$ cells are PVFs or MSCs found on the abluminal side of endothelial cells, perivascular areas, and adventitia of capillaries and vessels. They are also sprinkled in the media of small- and mid-sized vessels. The findings reported by our group and others suggest that these Meflin$^+$ cells proliferate after tissue injury, including cancer initiation and inflammation. Some Meflin$^+$ cells differentiate into α-SMA$^+$ myofibroblasts or CAFs during disease progression. The factors that induce the differentiation of Meflin$^+$ PVFs/MSCs into myofibroblasts that are negative or weakly positive for Meflin include TGF-β, stiffness of substrate, hypoxia, and ex vivo culture on plastic.
α-SMA: α-smooth muscle actin
CAF: cancer-associated fibroblast
End: endothelial cells
ECM: extracellular cell matrix
MSC: mesenchymal stem cell
PVF: perivascular fibroblast
TGF-β: transforming growth factor beta

marrow, skeletal muscle, periosteum, adipose tissue, and immature chondroblasts.[20,25] Lineage tracing experiments using a mouse line expressing the tamoxifen-sensitive Cre recombinase CreER^{T2} under the Meflin gene promoter (Meflin-CreER^{T2}) showed that Meflin$^+$ cells differentiated into mature white and brown adipocytes, osteocytes, chondrocytes, and skeletal muscles.[25,28] These findings suggest that Meflin is a marker of MSCs that exist in almost every organ. Interestingly, Meflin was not expressed in terminally differentiated cells such as mature adipocytes, chondrocytes, and osteocytes, suggesting that Meflin specifically marked undifferentiated MSCs.[20,25] As described above, most Meflin$^+$ cells were found in the perivascular area of micro- and small capillaries and the adventitia of muscular vessels, some of which appear to be pericytes (Figure 1). These findings are consistent with the previous notion that MSCs exist as pericytes in tissues or that MSCs contain a subpopulation of pericytes.[29,30] Indeed, in situ hybridization (ISH) and the analysis of a Meflin reporter mouse line showed that the number of Meflin$^+$ cells was much lower than that of conventional desmin$^+$ pericytes.[20,25] Although further detailed analysis should

be performed, we hypothesize that Meflin marks a subpopulation of pericytes or perivascular fibroblasts (PVFs), some of which are equivalent to tissue-resident MSCs. It is currently unknown, but possible, that differentiation capacity and lineage commitment of Meflin$^+$ PVFs/MSCs differ depending on the organ, developmental stage, and pathological context. Of note, Meflin$^+$ cells were sparsely found in the tunica media of some, but not all, mid-sized muscular vessels (Figure 1). The role and significance of these Meflin$^+$ cells in tunica media remain unknown. Throughout this review, we will describe Meflin$^+$ cells prior to activation and differentiation as Meflin$^+$ PVFs/MSCs; however, it should be noted that strict discrimination between the terms "PVFs/MSCs" and "normal tissue-resident fibroblasts" will not be made.

FUNCTION OF MEFLIN IN TISSUE REPAIR AFTER ACUTE TISSUE INJURY

The response of Meflin$^+$ PVFs/MSCs to acute tissue injuries was first investigated using a mouse model of acute myocardial infarction (AMI).[21] On inducing AMI, Meflin$^+$ PVFs/MSCs proliferated extensively in the border zone between the intact and necrotic myocardial tissues. Notably, the proliferation of Meflin$^+$ PVFs/MSCs preceded that of α-SMA$^+$ myofibroblasts in the border zone after inducing AMI, suggesting that Meflin$^+$ fibroblasts could sense or detect signals from injured tissues or necrotic cells with a higher sensitivity than conventional myofibroblasts (Figure 1). In addition, most Meflin knockout (KO) mice died because of cardiac rupture after AMI, and collagen type I production in fibroblasts was significantly affected in Meflin KO hearts compared with that in wild-type hearts.[21] These data suggest that Meflin KO hearts exhibit increased vulnerability to ischemia or tissue injury.

The role of Meflin in tissue repair after acute tissue injury was also demonstrated by subjecting Meflin KO mice to bleomycin (BLM)-induced lung injury and fibrosis or dextran sulfate sodium (DSS)-mediated colon inflammation.[26,31] The transtracheal administration of BLM induced the proliferation or infiltration of Meflin$^+$ fibroblasts in the lungs. As observed in the AMI model, Meflin KO mice presented severe lung hemorrhage and died more quickly than wild-type mice after BLM administration (Nakahara Y and Hashimoto N, unpublished observation); furthermore, they had severe intestinal damage and impaired regeneration after DSS treatment.[26,31]. These data further support the view that Meflin is essential for tissue repair after acute tissue injury (Figure 1).

The rapid mobilization of Meflin$^+$ PVFs/MSCs to diseased lesions was also observed in a mouse model of pancreatic cancer.[3,22] We found that Meflin$^+$ fibroblasts appeared even in small lesions with acinar-ductal metaplasia, a precancerous lesion in the KPC pancreatic cancer mouse model, where few α-SMA$^+$ myofibroblasts were found.[3] Given that cancer initiation is essentially equivalent to repeated wounding and regeneration, the above finding is consistent with the notion that Meflin$^+$ PVFs/MSCs are involved in acute injury and wound repair (Figure 1). Currently, the mechanisms by which Meflin$^+$ PVFs/MSCs/fibroblasts infiltrate or get recruited to injured tissues have not been elucidated.

FUNCTION OF MEFLIN IN FIBROSIS

The role of Meflin in fibrotic diseases was first investigated in a mouse model of chronic heart failure induced by transverse aortic constriction (TAC).[21] In the normal heart, Meflin is expressed by PVFs in the interstitium of the ventricular walls and the pericardium and endocardium. A lineage-trace experiment using a mouse line expressing Cre recombinase under the Meflin

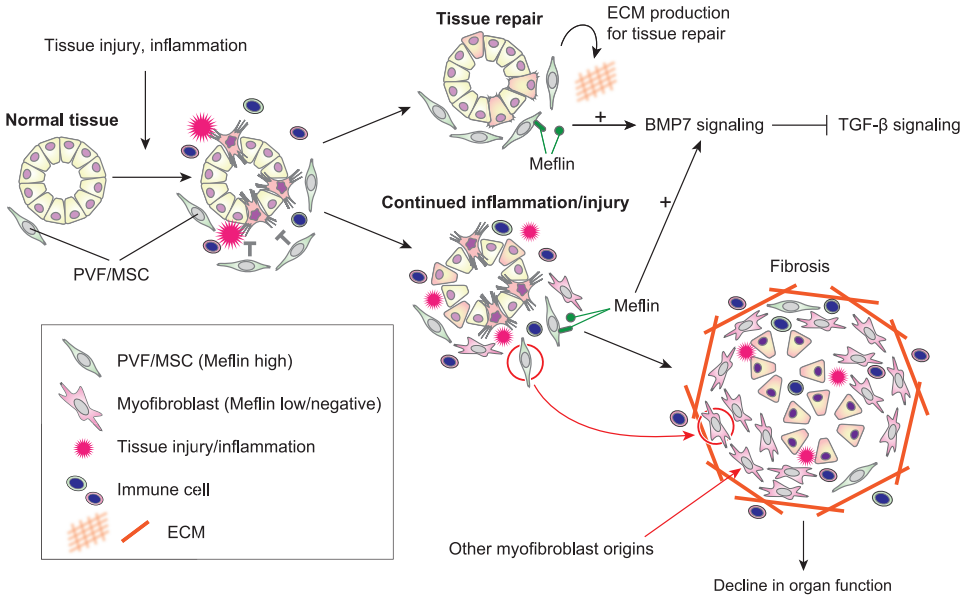

Fig. 2 Hypothesis on the role of Meflin$^+$ PVFs/MSCs or fibroblasts in fibrotic diseases
Meflin is expressed by quiescent PVFs/MSCs in normal tissues, but also in cells that proliferate in the early stages of inflammatory and fibrotic diseases. Tissue injuries or inflammatory lesions are either resolved or become chronic, depending on the underlying etiology and the initial cause of the disease. Our data suggest that Meflin functions include the augmentation of BMP7 signaling and suppression of Lox-mediated crosslinking of the extracellular matrix. Furthermore, some α-SMA$^+$ myofibroblasts derive from Meflin$^+$ PVFs/MSCs, but there are other sources of cells that give rise to α-SMA$^+$ myofibroblasts.
α-SMA: α-smooth muscle actin
BMP: bone morphogenetic factor
CAF: cancer-associated fibroblast
ECM: extracellular cell matrix
MSC: mesenchymal stem cell
PVF: perivascular fibroblast

(*ISLR*) promoter (Meflin-Cre) showed proliferation of Meflin-lineage cells in the fibrotic area. These cells were positive for myofibroblast markers such as α-SMA and periostin, suggesting that Meflin$^+$ cells were one of the origins of myofibroblasts in cardiac fibrosis (Figure 1, 2). We also identified factors that induced the differentiation of Meflin$^+$ PVFs/MSCs into α-SMA$^+$ myofibroblasts using cultured MSCs and cardiac fibroblasts. These factors included transforming growth factor-β (TGF-β), substrate stiffness, hypoxic conditions, and long-term culture or passages on plastic (Figure 1). Notably, significant downregulation of Meflin expression in cultured fibroblasts was observed when α-SMA expression was increased by TGF-β stimulation, leading to the speculation that Meflin$^+$ PVFs/MSCs gave rise to Meflin$^{-/low}$/α-SMA$^+$ myofibroblasts in fibrosis conditions (Figure 1). However, the exact contribution of Meflin$^+$ PVFs/MSCs in generating α-SMA$^+$ myofibroblast populations has not been elucidated, and our recent data suggest that it is less than we had expected and differs among diseases (Figures 1 and 2). Lineage tracing in a mouse model of renal fibrosis showed that Meflin$^+$ PVFs/MSCs gave rise to an approximately 10% of all α-SMA$^+$ myofibroblasts. Additionally, Meflin lineage cells comprised two types of fibroblasts that exhibited (1) high or (2) low/negative α-SMA expression (Minatoguchi and Saito

et al, unpublished observation). Although the data obtained from such lineage trace experiments using the CreER^{T2} system do not generally reflect the entire lineage potential of cells of origin, we entertain the possibility that the above finding is one of the mechanisms that underlie fibroblast heterogeneity in disease conditions.

Interestingly, Meflin KO hearts exhibited accelerated fibrosis and severe diastolic dysfunction after TAC induction compared to wild-type hearts.[21] This was accompanied by increased stiffness of the cardiac tissue and poor prognosis in Meflin KO mice. Notably, ejection fraction (EF), a marker of global systolic function, was comparable between wild-type and Meflin KO hearts after TAC induction, indicating that Meflin KO mouse hearts after TAC induction may be a model of human heart failure with preserved EF (HFpEF).[21] These data suggest that Meflin plays a role in suppressing cardiac fibrosis.

A search for Meflin ligands identified bone morphogenetic protein 7 (BMP7).[21] Our biochemical assay showed that Meflin bound to BMP7 to augment its signaling in cultured cells.[21,24] Given that BMP7 has an anti-fibrotic function by counteracting TGF-β signaling,[32] it was hypothesized that the primary function of Meflin may be TGF-β and fibrosis suppression in physiological and disease conditions (Figures 1 and 2). The anti-fibrotic role of Meflin was also shown in cultured fibroblasts isolated from the lungs of wild-type and Meflin KO mice.[26] Lung fibroblasts from Meflin KO mice exhibited a significant increase in α-SMA and collagen expression compared to wild-type fibroblasts when stimulated with TGF-β.

The possible role of Meflin in cellular senescence remains unknown; however, recent findings have shown that lung fibroblasts isolated from Meflin KO mice acquired an accelerated senescence phenotype compared to those from wild-type mice when they were stimulated with TGF-β.[26] In addition, the quantification of Meflin mRNA in mouse hearts showed that its expression was significantly lower in aged mice than in young mice.[21] Thus, the role of Meflin in aging and age-dependent physiological decline of organ function should be a focus of future research.

FUNCTION OF MEFLIN IN CANCER PROGRESSION

Our recent study showed that Meflin was a marker of pancreatic stellate cells (PSCs), which are resident fibroblasts of the pancreas with a high capacity to restore retinol and retinyl esters in their cytoplasm.[22,33] Consistent with the notion that PSCs are a cell of origin of CAFs in PDAC,[33] Meflin$^+$ PSCs proliferate in the stroma of both human PDAC and tumors developed in the KPC PDAC mouse model.[22] A lineage tracing study using Meflin-CreER^{T2} mice bearing a subcutaneous mT5 pancreatic cancer xenograft showed the possibility that Meflin$^+$ PSCs gave rise to CAFs that were highly positive for α-SMA. The contribution of Meflin lineage cells to CAFs has also been shown in an autochthonous mouse model of colorectal cancer.[34] Multiplex ISH assay showed that there were at least two subsets of CAFs in the stroma of human PDAC: Meflin-high and α-SMA-low CAFs and Meflin-low and α-SMA-high CAFs[22] (Figure 3). We further found that the numbers of Meflin$^+$ CAFs differed across patients with PDAC, suggesting intertumoral heterogeneity and diversity of CAFs. Interestingly, patients with a high number of Meflin$^+$ CAFs showed better prognosis than those with a low number of Meflin$^+$ CAFs, and Meflin KO mice developed more advanced PDAC than wild-type mice when they were crossed with the KPC PDAC model.[22] Moreover, exogenous expression of Meflin in CAFs retarded tumor progression in a subcutaneous tumor model.[22] Thus, our findings suggest that Meflin$^+$ PSCs and CAFs play a role in suppressing tumor progression but give rise to Meflin$^{low/-}$ and α-SMA$^+$ CAFs. Another recent study from our group suggested that Meflin was biochemically bound to lysyl oxidase (Lox), which crosslinked collagen or elastin to form stiff tissues with increased

tensile strength and integrity, thereby inhibiting its enzymatic activity.[35] Given the role of Lox in cancer progression,[36] we hypothesized that Meflin suppressed cancer progression by inhibiting Lox activity and augmenting BMP7 signaling, as described earlier (Figure 3). The tumor-suppressive role of Meflin$^+$ CAFs was also demonstrated in our recent study, which showed that Meflin competed with another CAF marker protein, Gremlin 1.[24] Gremlin 1 is a negative regulator of BMP signaling in the tumor microenvironment of colorectal cancer.[24] The study also showed the therapeutic potential of the anti-Gremlin 1 antibody that neutralized the activity of Gremlin 1 and adeno-associated virus-mediated transduction of the Meflin gene to modulate CAF function in treating patients with metastatic colorectal cancer.

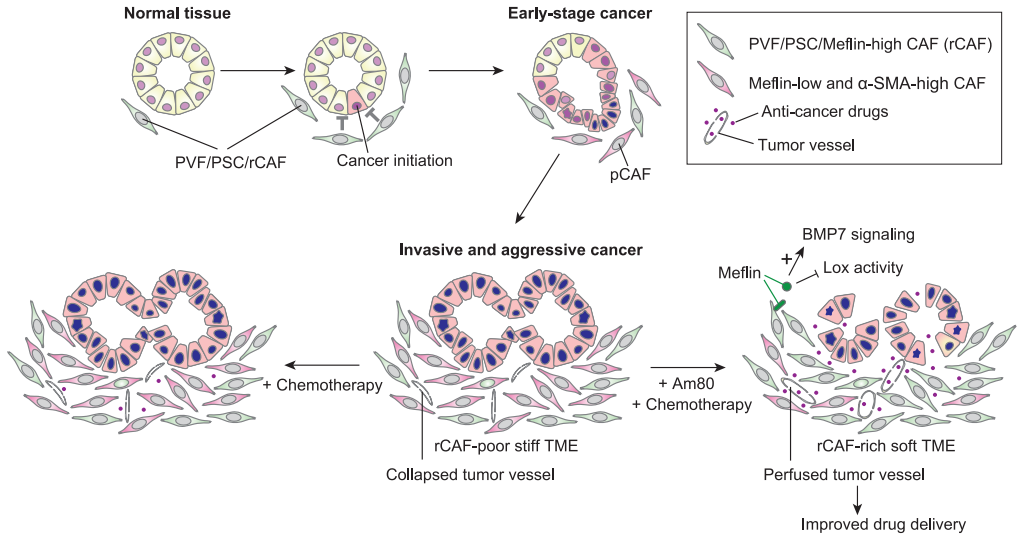

Fig. 3 Hypothesis on the role of Meflin$^+$ PVF/MSCs or fibroblasts in cancer
As in fibrotic and inflammatory diseases, Meflin$^+$ PVFs/MSCs are the source of CAFs in cancer. We hypothesize that Meflin$^+$ PVFs/MSCs proliferate and constitute rCAFs in the early stages of cancer but give rise to α-SMA$^+$ CAFs during cancer progression. Am80 administration increases the number of Meflin$^+$ rCAFs, which leads to a decrease in tumor stiffness and increase in tumor vessel area and tumor sensitivity to chemotherapeutics.
α-SMA: α-smooth muscle actin
BMP: bone morphogenetic factor
CAF: cancer-associated fibroblast
ECM: extracellular cell matrix
MSC: mesenchymal stem cell
PVF: perivascular fibroblast

The role of Meflin$^{low/-}$ and α-SMA$^+$ CAFs remains controversial. A previous study showed that the genetic depletion of α-SMA$^+$ CAFs resulted in the acceleration of PDAC progression in mice,[12] whereas a number of studies demonstrated a significant correlation between the number of α-SMA$^+$ CAFs and poor outcomes in many types of human cancers.[3] Our data showed that α-SMA was weakly expressed in Meflin$^+$ CAFs[21] and that the depletion of α-SMA$^+$ CAFs resulted in the depletion of Meflin$^+$ CAFs. Thus, it would be inaccurate to neatly divide CAFs into pCAFs and rCAFs, which may confound the interpretation of experimental results in preclinical models and clinical samples. Most importantly, one should distinguish between the functions of α-SMA protein and the roles of α-SMA$^+$ CAFs, which are often confounded by researchers.

CAF DIVERSITY: A DIFFERENT PHENOTYPE OF THE SAME CELLS?

Recent advances in single-cell transcriptomic analysis have enabled a deeper understanding of CAF heterogeneity across many tumor types, which led to the identification of a number of genes expressed in CAFs defining different potential CAF subsets.[15] Readers can refer to several review articles describing in details the proposed CAF classification, which are not included in this review.[2,10,14,15,37] One of the major problems in the current CAF classification is that it is based on arbitrary descriptions that are primarily rooted in existing knowledge on CAF marker functions and their correlation with the outcome in animal models and patients. Furthermore, the biological significance of each CAF subset has not necessarily been experimentally proven. We and other authors have proposed a simple CAF classification model, in which CAFs were classified according to their functions in cancer progression.[2,3,14,18] Based on our previous data that showed the forced expression of exogenous Meflin in CAFs retarded their pro-tumor function,[22,24,35] we proposed a model in which each CAF expressed both proteins with anti-tumor functions and those with pro-tumor functions, and the role of each CAF was determined by the balance between the levels of these proteins (Figure 4). For example, analysis of publicly available data from single-cell analysis of CAFs and immunohistochemical staining for various CAF markers showed that Meflin was co-expressed with CAF marker proteins that promoted cancer

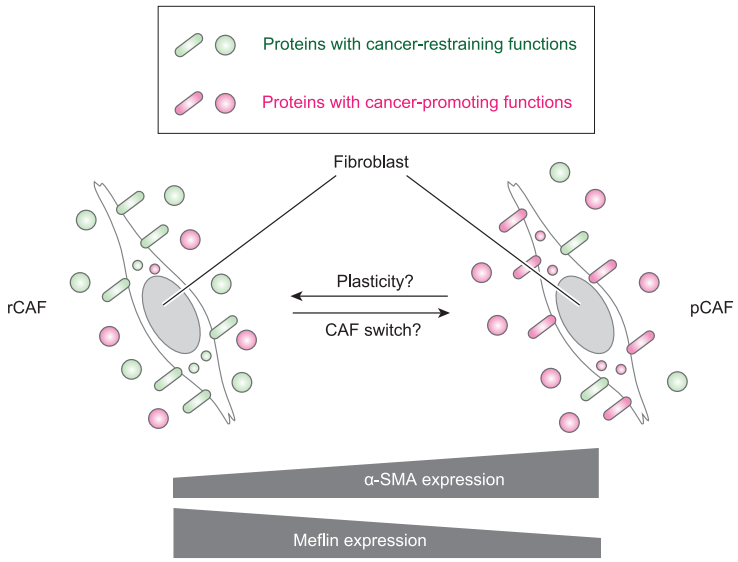

Fig. 4 Hypothesis on the mechanism of fibroblast heterogeneity
The most plausible hypothesis based on a number of previous studies on the diversity and heterogeneity of fibroblasts is that they have different origin or derived from various lineages during disease development. However, it is possible that the function of fibroblasts could be determined by the relative amounts and balance of proteins with cancer-restraining functions and those with cancer-promoting functions. The involvement of liquid factors derived from other cells, including cancer and inflammatory cells, and epigenetic mechanisms should also be considered as regulators of the conversion and plasticity between rCAFs and pCAFs. Most importantly, one should discriminate the function of a CAF subset expressing a particular protein and the function of that protein in terms of whether they are pro-tumor or anti-tumor.
α-SMA: α-smooth muscle actin
CAF: cancer-associated fibroblast

progression, such as fibroblast activation protein-α.[22] This notion was further corroborated by recent studies that showed that CAF expression of collagen type I alpha 1, which was reported to be expressed in some pCAFs, played a role in suppressing cancer progression by affecting the immune response to tumors and mechanistically restraining tumor spread.[38,39] This was proven by an induction of tumor development in mouse lines in which the gene for collagen type I alpha 1 was conditionally deleted in α-SMA$^+$ cells or hepatic stellate cells. Those experiments showed that collagen type I alpha 1 protein was tumor suppressive. However, the role of collagen type I alpha 1$^+$ CAFs remains unknown and should be carefully determined by the net balance of the expression of cancer-promoting and -restraining proteins. Likewise, our data showed that Meflin was a protein with a tumor-suppressive role, whereas the role of Meflin$^+$ CAFs was not necessarily tumor suppressive. Our previous study showed that the genetic depletion of Meflin$^+$ CAFs resulted in the development of poorly differentiated tumors and a decrease in α-SMA$^+$ CAFs but did not change tumor volume in a subcutaneous tumor transplantation model.[22]

A CAVEAT IN EVALUATING THE FUNCTION OF GENES EXPRESSED IN CAFS USING KAPLAN-MEIER ANALYSIS

One question that we are frequently asked is: Why is it that a simple comparison of the outcomes in cancer patients stratified based on Meflin expression levels sometimes shows that

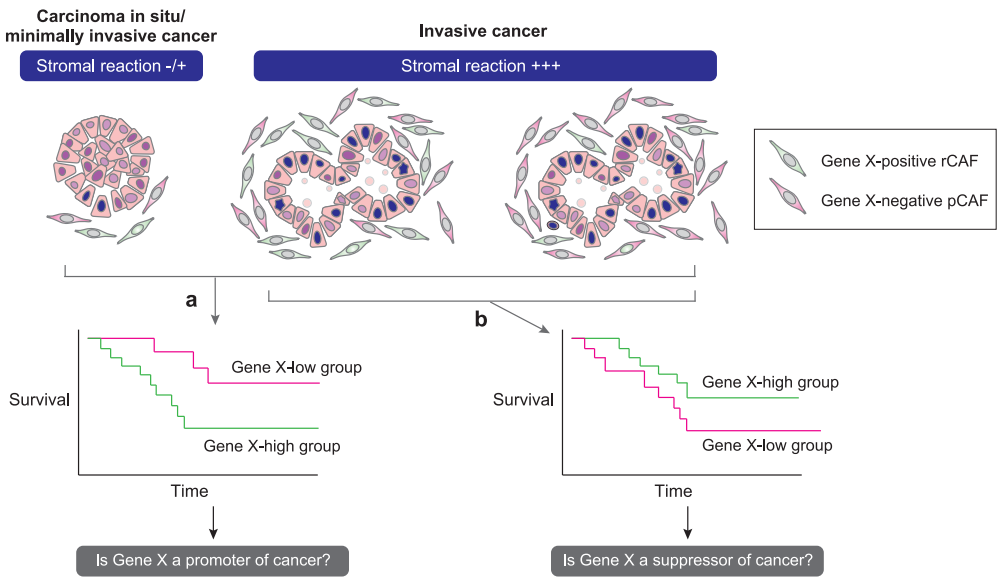

Fig. 5 A trap in interpretation of the data obtained from Kaplan-Meier analysis
Tumors are diverse in terms of the extent of fibroinflammatory and fibrotic reactions in the stroma, even in the same type of cancer. Less invasive tumors, which exhibit favorable prognosis, tend to have fewer stromal reactions with limited numbers of rCAFs and pCAFs, whereas more invasive tumors are accompanied by greater stromal reactions with large numbers of rCAFs and pCAFs. Therefore, simple interpretation of Kaplan-Meier analysis based on the expression level of proteins or genes expressed in rCAFs does not accurately depict their roles in cancer development (**a**). Correct conclusions may be made when tumors with the same extent of stromal reactions are analyzed for the expression of genes or proteins specifically in rCAFs (**b**).
CAF: cancer-associated fibroblast

the Meflin-high group exhibits poorer outcomes than the Meflin-low group? (Figure 5). We believe that to conclude that Meflin is a bad gene that promotes tumor progression from such an analysis is an oversimplification. Because tumors with more desmoplastic and fibroinflammatory reactions, which are accompanied by increased infiltration of Meflin$^+$ CAFs, are more invasive and show worse outcomes than those with less desmoplastic reactions, the comparison of all the patients with minimally invasive tumors, including carcinoma in situ, to those with highly invasive tumors, will lead to misunderstanding the function of genes expressed in CAFs (Figure 5a). For example, the analysis of all non-small cell lung cancer patients deposited in The Cancer Genome Atlas database showed that patients with an increased number of Meflin$^+$ CAFs had worse prognosis than those with lower number of Meflin$^+$ CAFs.[40] However, analysis of patients with stage III non-small cell lung cancer who presumably exhibited comparable stromal reactions tended to show more favorable outcomes in patients with high Meflin$^+$ CAFs than in those with low Meflin$^+$ CAFs[40] (Figure 5b). Furthermore, the number of Meflin$^+$ CAFs correlates well with the favorable response of patients with non-small cell lung cancer to immune checkpoint inhibitors.[41] Thus, it is suggested that simple interpretation of Kaplan-Meier analysis based on the expression level of CAF marker genes in whole tumor tissue samples does not necessarily lead to a correct understanding of the functions of these genes. We believe that the same holds true when interpreting the function of genes expressed in cancer and immune cells. Thus, it is important to remember that Kaplan-Meier analyses are suitable to identify markers that predict patient outcome and compare different arms of treatment or cohorts in clinical studies but do not necessarily reveal the function of genes of interest in terms of whether they are pro-tumor or anti-tumor. Meflin expression could be a marker that predicts worse outcomes in patients with cancer, but the functions of Meflin and Meflin$^+$ cells should be examined by multifaceted approaches, including studies on animal models and cultured cells.

MODULATION (REPROGRAMMING) OF FIBROBLAST FUNCTION IN CANCER

The failure of preclinical and clinical studies to target pathways that regulate the proliferation of CAFs or deplete them have led to the idea that CAFs have a role in restraining cancer progression as described above.[2,3,11-13,19] Our recent line of studies showed that Meflin may be one of the proteins that conferred anti-tumor functions to CAFs.[3,22,24] Another approach targeting CAFs that recently emerged was to reprogram or engineer pro-tumor CAFs such that they become anti-tumor. A pioneering study by Sherman et al showed that the administration of the vitamin D analog calcipotriol increased the sensitivity of pancreatic cancer to chemotherapy in an autochthonous pancreatic cancer mouse model.[42] This effect of calcipotriol was mediated by transcriptomic changes, such as alterations in the expression of genes involved in the regulation of inflammation and the extracellular matrix, in CAFs or activated PSCs that proliferate in the stroma of pancreatic cancer. These observations led to several clinical trials that investigated the effect of combinations of calcipotriol and other conventional chemotherapies or immune checkpoint inhibitors in patients with PDAC (https://clinicaltrials.gov).

Our group recently screened a chemical library of nuclear receptor ligands, which led to the discovery of Am80 as a compound that significantly upregulated Meflin expression in CAFs[35] (Figure 3). Oral administration of Am80 resulted in a significant increase in Meflin expression but suppressed α-SMA expression in CAFs in a mouse model of pancreatic cancer. Consistent with the finding that Meflin suppresses fibrosis by augmenting BMP7 signaling and inhibiting the activity of Lox, tumors administered Am80 were softer than control tumors, which was accompanied by an increase in vessel area in the developed tumors. Furthermore, Am80 ad-

ministration resulted in increased intratumor concentrations of gemcitabine and its efficacy in a pancreatic cancer mouse model. These effects of Am80 were not observed in Meflin KO mice, suggesting that Am80 exerted its anti-tumor effect by upregulating Meflin in CAFs[34] (Figure 3). Based on these observations, our colleagues (Dr Mitsuhiro Fujishiro, Tokyo University and Dr Hiroki Kawashima, Nagoya University) have started an investigator-led clinical trial to investigate the safety and tolerability of Am80 (generic name: tamibarotene; developmental code: MIKE-1) in combination with the conventional tumoricidal drugs gemcitabine and nab-paclitaxel in patients with unresectable PDAC and determine the recommended dose and explore its efficacy (ClinicalTrials.gov identifier: NCT05064618).[43]

CONCLUSION AND PERSPECTIVES

In this short review, we briefly described the pathological roles of Meflin in PVFs, MSCs, and CAFs in various disease conditions. The precise molecular function of Meflin remains to be understood, but we hypothesize that Meflin plays a role in inhibiting fibrotic reactions by augmenting BMP7 signaling and suppressing Lox activity. The involvement of Meflin in the regeneration of skeletal tissues and myoblast differentiation has also been evaluated in other studies but it was not covered in this review.[44,45]

As described earlier, the function of the Meflin protein and that of Meflin$^+$ cells should be determined separately to better understand their specific roles in disease progression. It is also important to assess whether diverse fibroblasts are derived from different lineages or represent different faces of the same fibroblasts with plasticity. Furthermore, identification of transcription factors and epigenetic mechanisms that regulate fibroblast heterogeneity and plasticity is vital to this field of research. We believe that the accumulation of studies on the functions of fibroblast markers will lead to a deeper understanding of fibroblast heterogeneity and the development of therapies for cancer and fibrotic diseases.

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DISCLOSURE STATEMENT

The authors declare no competing interests.

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