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

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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 Mefflin may suppress but not promote fibrotic response and disease progression in cancer and fibrotic diseases. Although currently hypothetical, the primary function of Mefflin-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 Mefflin 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, Mefflin, 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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INTRODUCTION

Fibroblasts secrete extracellular matrix (ECM) molecules such as collagen and fibronectin that are essential to maintain the normal architecture of tissues and organs. 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. 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. 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. 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.

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). 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]). 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”). There is a plethora of recently published review articles on CAF diversity and heterogeneity that readers can refer to for further understanding.

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. We previously identified MeFlin as one of the proteins specifically expressed in contact-inhibited fibroblasts. 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. 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...
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marrow, skeletal muscle, periosteum, adipose tissue, and immature chondroblasts.\textsuperscript{20,25} Lineage tracing experiments using a mouse line expressing the tamoxifen-sensitive Cre recombinase CreER\textsuperscript{12} under the Meffin gene promoter (Meffin-CreERT\textsuperscript{2}) showed that Meffin\textsuperscript{+} cells differentiated into mature white and brown adipocytes, osteocytes, chondrocytes, and skeletal muscles.\textsuperscript{25,28} These findings suggest that Meffin is a marker of MSCs that exist in almost every organ. Interestingly, Meffin was not expressed in terminally differentiated cells such as mature adipocytes, chondrocytes, and osteocytes, suggesting that Meffin specifically marked undifferentiated MSCs.\textsuperscript{20,25} As described above, most Meffin\textsuperscript{+} 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.\textsuperscript{20,30} Indeed, in situ hybridization (ISH) and the analysis of a Meffin reporter mouse line showed that the number of Meffin\textsuperscript{+} cells was much lower than that of conventional desmin\textsuperscript{+} pericytes.\textsuperscript{20,25} Although further detailed analysis should

Fig. 1 Localization of Meffin\textsuperscript{+} cells in normal tissues and their proliferation after tissue injury, cancer initiation or inflammation

Meffin\textsuperscript{+} 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 Meffin\textsuperscript{+} cells proliferate after tissue injury, including cancer initiation and inflammation. Some Meffin\textsuperscript{+} cells differentiate into $\alpha$-SMA\textsuperscript{+} myofibroblasts or CAFs during disease progression. The factors that induce the differentiation of Meffin\textsuperscript{+} PVFs/MSCs into myofibroblasts that are negative or weakly positive for Meffin include TGF-\(\beta\), stiffness of substrate, hypoxia, and ex vivo culture on plastic. $\alpha$-SMA: $\alpha$-smooth muscle actin CAF: cancer-associated fibroblast End: endothelial cells ECM: extracellular cell matrix MSC: mesenchymal stem cell PVF: perivascular fibroblast TGF-\(\beta\): transforming growth factor beta

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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). 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. 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. 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. 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. 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. 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). 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
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The (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+ PVFsMSCs 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+ PVFsMSCs gave rise to Meflin+/α-SMA+ myofibroblasts in fibrosis conditions (Figure 1). However, the exact contribution of Meflin+ PVFsMSCs 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+ PVFsMSCs 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

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

Fig. 2 Hypothesis on the role of Meflin+ PVFsMSCs or fibroblasts in fibrotic diseases
Meflin is expressed by quiescent PVFsMSCs 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+ PVFsMSCs, but there are other sources of cells that give rise to α-SMA+ myofibroblasts.

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et al, unpublished observation). Although the data obtained from such lineage trace experiments using the CreER 

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

A search for Meffin ligands identified bone morphogenetic protein 7 (BMP7). Our biochemical assay showed that Meffin bound to BMP7 to augment its signaling in cultured cells. Given that BMP7 has an anti-fibrotic function by counteracting TGF-β signaling, it was hypothesized that the primary function of Meffin may be TGF-β and fibrosis suppression in physiological and disease conditions (Figures 1 and 2). The anti-fibrotic role of Meffin was also shown in cultured fibroblasts isolated from the lungs of wild-type and Meffin KO mice. Lung fibroblasts from Meffin 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 Meffin in cellular senescence remains unknown; however, recent findings have shown that lung fibroblasts isolated from Meffin KO mice acquired an accelerated senescence phenotype compared to those from wild-type mice when they were stimulated with TGF-β. In addition, the quantification of Meffin mRNA in mouse hearts showed that its expression was significantly lower in aged mice than in young mice. Thus, the role of Meffin in aging and age-dependent physiological decline of organ function should be a focus of future research.

FUNCTION OF MEFFIN IN CANCER PROGRESSION

Our recent study showed that Meffin 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. Consistent with the notion that PSCs are a cell of origin of CAFs in PDAC, Meffin+ PSCs proliferate in the stroma of both human PDAC and tumors developed in the KPC PDAC mouse model. A lineage tracing study using Meffin-CreER 

mice bearing a subcutaneous mT5 pancreatic cancer xenograft showed the possibility that Meffin+ PSCs gave rise to CAFs that were highly positive for α-SMA. The contribution of Meffin lineage cells to CAFs has also been shown in an autochthonous mouse model of colorectal cancer. Multiplex ISH assay showed that there were at least two subsets of CAFs in the stroma of human PDAC: Meffin-high and α-SMA-low CAFs and Meffin-low and α-SMA-high CAFs (Figure 3). We further found that the numbers of Meffin+ CAFs differed across patients with PDAC, suggesting intertumoral heterogeneity and diversity of CAFs. Interestingly, patients with a high number of Meffin+ CAFs showed better prognosis than those with a low number of Meffin+ CAFs, and Meffin KO mice developed more advanced PDAC than wild-type mice when they were crossed with the KPC PDAC model. Moreover, exogenous expression of Meffin in CAFs retarded tumor progression in a subcutaneous tumor model. Thus, our findings suggest that Meffin+ PSCs and CAFs play a role in suppressing tumor progression but give rise to Meffinlow/high and α-SMA+ CAFs. Another recent study from our group suggested that Meffin 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.\textsuperscript{35} Given the role of Lox in cancer progression,\textsuperscript{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\textsuperscript{+} CAFs was also demonstrated in our recent study, which showed that Meflin competed with another CAF marker protein, Gremlin 1.\textsuperscript{24} Gremlin 1 is a negative regulator of BMP signaling in the tumor microenvironment of colorectal cancer.\textsuperscript{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\textsuperscript{+} PVF/MSCs or fibroblasts in cancer
As in fibrotic and inflammatory diseases, Meflin\textsuperscript{+} PVFs/MSCs are the source of CAFs in cancer. We hypothesize that Meflin\textsuperscript{+} PVFs/MSCs proliferate and constitute rCAFs in the early stages of cancer but give rise to \(\alpha\)-SMA\textsuperscript{+} CAFs during cancer progression. Ami80 administration increases the number of Meflin\textsuperscript{+} rCAFs, which leads to a decrease in tumor stiffness and increase in tumor vessel area and tumor sensitivity to chemotherapeutics.\textsuperscript{23}

\(\alpha\)-SMA: \(\alpha\)-smooth muscle actin  
BMP: bone morphogenetic factor  
CAF: cancer-associated fibroblast  
ECM: extracellular cell matrix  
MSC: mesenchymal stem cell  
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The role of Meflin\textsuperscript{low/–} and \(\alpha\)-SMA\textsuperscript{+} CAFs remains controversial. A previous study showed that the genetic depletion of \(\alpha\)-SMA\textsuperscript{+} CAFs resulted in the acceleration of PDAC progression in mice,\textsuperscript{13} whereas a number of studies demonstrated a significant correlation between the number of \(\alpha\)-SMA\textsuperscript{+} CAFs and poor outcomes in many types of human cancers.\textsuperscript{3} Our data showed that \(\alpha\)-SMA was weakly expressed in Meflin\textsuperscript{+} CAFs\textsuperscript{21} and that the depletion of \(\alpha\)-SMA\textsuperscript{+} CAFs resulted in the depletion of Meflin\textsuperscript{+} 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 \(\alpha\)-SMA protein and the roles of \(\alpha\)-SMA\textsuperscript{+} 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

![Diagram](image)

**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.

\(\alpha\)-SMA: \(\alpha\)-smooth muscle actin
CAF: cancer-associated fibroblast

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progression, such as fibroblast activation protein-α. 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. 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 Me3lin was a protein with a tumor-suppressive role, whereas the role of Me3lin+ 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.

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 Me3lin expression levels sometimes shows that...
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the Meffin-high group exhibits poorer outcomes than the Meffin-low group? (Figure 5). We believe that to conclude that Meffin 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 Meffin+ 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 Meffin+ CAFs had worse prognosis than those with lower number of Meffin+ 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 Meffin+ CAFs than in those with low Meffin+ CAFs (Figure 5b). Furthermore, the number of Meffin+ 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. Meffin expression could be a marker that predicts worse outcomes in patients with cancer, but the functions of Meffin and Meffin+ 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 Meffin 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 Meffin expression in CAFs35 (Figure 3). Oral administration of Am80 resulted in a significant increase in Meffin expression but suppressed α-SMA expression in CAFs in a mouse model of pancreatic cancer. Consistent with the finding that Meffin 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-
administration 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\textsuperscript{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).\textsuperscript{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.\textsuperscript{44,45}

As described earlier, the function of the Meflin protein and that of Meflin\textsuperscript{+} 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.
REFERENCES

1. LeBleu VS, Neilson EG. Origin and functional heterogeneity of fibroblasts. *FASEB J.* 2020;34(3):3519-3536. doi:10.1096/fj.201903188R.
2. Kobayashi H, Enomoto A, Woods SL, Burt AD, Takahashi M, Worthley DL. Cancer-associated fibroblasts in gastrointestinal cancer. *Nat Rev Gastroenterol Hepatol.* 2019;16(5):282–295. doi:10.1038/s41575-019-0115-0.
3. Miyai Y, Esaki N, Takahashi M, Enomoto A. Cancer-associated fibroblasts that restrain cancer progression: Hypotheses and perspectives. *Cancer Sci.* 2020;111(4):1047–1057. doi:10.1111/cas.14346.
4. Pakshir P, Noskovicova N, Lodyga M, et al. The myofibroblast at a glance. *J Cell Sci.* 2020;133(13):jcs227900. doi:10.1242/jcs.227900.
5. Yamamura Y, Asai N, Enomoto A, et al. Akt-Girdin signaling in cancer-associated fibroblasts contributes to tumor progression. *Cancer Res.* 2015;75(5):813–823. doi:10.1158/0008-5472.CAN-14-1317.
6. An J, Enomoto A, Weng L, et al. Significance of cancer-associated fibroblasts in the regulation of gene expression in the leading cells of invasive lung cancer. *J Cancer Res Clin Oncol.* 2013;139(3):379–388. doi:10.1007/s00432-012-1328-6.
7. Kato T, Enomoto A, Watanabe T, et al. TRIM27/MRTF-B-dependent integrin β1 expression defines leading cells in cancer cell collectives. *Cell Rep.* 2014;7(4):1156–1167. doi:10.1016/j.celrep.2014.03.068.
8. Weng L, Enomoto A, Ishida-Takagishi M, Asai N, Takahashi M. Girding for migratory cues: roles of the Akt substrate Girdin in cancer progression and angiogenesis. *Cancer Sci.* 2010;101(4):836–842. doi:10.1111/j.1349-7006.2009.01487.x.
9. Ichihara R, Shiraki Y, Mizutani Y, et al. Matrix remodeling-associated protein 8 is a marker of a subset of cancer-associated fibroblasts in pancreatic cancer. *Pathol Int.* 2022;72(3):161–175. doi:10.1111/pin.13198.
10. Sahai E, Astsaturov I, Cukierman E, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer.* 2020;20(3):174–186. doi:10.1038/s41568-019-0238-1.
11. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell.* 2014;25(6):735–747. doi:10.1016/j.ccr.2014.04.021.
12. Özdemir BC, Pentcheva-Hoang T, Carstens JL, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell.* 2014;25(6):719–734. doi:10.1016/j.ccr.2014.04.005.
13. Lee JJ, Perera RM, Wang H, et al. Stromal response to Hedgehog signaling restrains pancreatic cancer progression. *Proc Natl Acad Sci USA.* 2014;111(30): E3091-E3100. doi:10.1073/pnas.1411679111.
14. Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer.* 2016;16(9):582–598. doi:10.1038/nrc.2016.73.
15. Chen Y, McAndrews KM, Kalluri R. Clinical and therapeutic relevance of cancer-associated fibroblasts. *Nat Rev Clin Oncol.* 2021;18(12):792–804. doi:10.1038/s41571-021-00546-5.
16. Ishii G, Ochiai A, Neri S. Phenotypic and functional heterogeneity of cancer-associated fibroblast within the tumor microenvironment. *Adv Drug Deliv Rev.* 2016;99(PT B):186–196. doi:10.1016/j.addr.2015.07.007.
17. Mezawa Y, Orimo A. The roles of tumor-and metastasis-promoting carcinoma-associated fibroblasts in human carcinomas. *Cell Tissue Res.* 2016;365(3):675–689. doi:10.1007/s00441-016-2471-1.
18. Gieniec KA, Butler LM, Worthley DL, Woods SL. Cancer-associated fibroblasts—heroes or villains? *Br J Cancer.* 2019;121(4):293–302. doi:10.1038/s41416-019-0509-3.
19. Neesse A, Bauer CA, Öhlund D, et al. Stromal biology and therapy in pancreatic cancer: ready for clinical translation? *Gut.* 2019;68(1):159–171. doi:10.1136/gutjnl-2018-316451.
20. Maeda K, Enomoto A, Hara A, et al. Identification of Meflin as a potential marker for mesenchymal stromal cells. *Sci Rep.* 2016;6:22288. doi:10.1038/srep22288.
21. Hara A, Kobayashi H, Asai N, et al. Roles of the mesenchymal stromal/stem cell marker Meflin in cardiac tissue repair and the development of diastolic dysfunction. *Circ Res.* 2019;125(4):414–430. doi:10.1161/CIRCRESAHA.119.314806.
22. Mizutani Y, Kobayashi H, Iida T, et al. Meflin-positive cancer-associated fibroblasts inhibit pancreatic carcinogenesis. *Cancer Res.* 2019;79(20):5367–5381. doi:10.1158/0008-5472.CAN-19-0454.
23. Ohara Y, Enomoto A, Tsuyuki Y, et al. Connective tissue growth factor produced by cancer-associated fibroblasts correlates with poor prognosis in epithelioid malignant pleural mesothelioma. *Oncol Rep.* 2020;44(3):838–848. doi:10.3892/or.2020.7669.
24. Kobayashi H, Gieniec KA, Wright JA, et al. The balance of stromal BMP signaling mediated by GREM1 and ISLR drives colorectal carcinogenesis. *Gastroenterology.* 2021;160(4):1224–1239.e30. doi:10.1053/j.gastro.2020.11.011.
25. Hara A, Kato K, Ishihara T, et al. Meflin defines mesenchymal stem cells and/or their early progenitors.
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with multilineage differentiation capacity. *Genes Cells.* 2021;26(7):495–512. doi:10.1111/gtc.12855.

26 Nakahara Y, Hashimoto N, Sakamoto K, et al. Fibroblasts positive for meflin have anti-fibrotic property in pulmonary fibrosis. *Eur Respir J.* 2021;58(6):2003397. doi:10.1183/13993003.03397-2020.

27 Takahashi M, Kobayashi H, Mizutani Y, et al. Roles of the mesenchymal stromal/stem cell marker Meflin/Islr in cancer fibrosis. *Front Cell Dev Biol.* 2021;9:749924. doi: 10.3389/fcell.2021.749924.

28 Kuwano T, Izumi H, Aslam MR, et al. Generation and characterization of a Meflin-CreERT2 transgenic line for lineage tracing in white adipose tissue. *PLoS One.* 2021;16(3):e0248267. doi:10.1371/journal.pone.0248267.

29 Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell.* 2011;21(2):193–215. doi:10.1016/j.devcel.2011.07.001.

30 Caplan AI. New MSC: MSCs as sentinels and gatekeepers. *J Orthop Res.* 2017;35(6):1151–1159. doi:10.1002/jor.23560.

31 Xu J, Tang Y, Sheng X, et al. Secreted stromal protein ISLR promotes intestinal regeneration by suppressing epithelial Hippo signaling. *EMBO J.* 2020;39(7):e103255. doi:10.15252/embj.2019103255.

32 Tsujimura T, Idei M, Yoshikawa M, Takase O, Hishikawa K. Roles and regulation of bone morphogenetic protein-7 in kidney development and diseases. *World J Stem Cells.* 2016;8(9):288–296. doi:10.4252/wjsc.v8.i9.288.

33 Apte MV, Park S, Phillips PA, et al. Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells. *Pancreas.* 2004;29(3):179–187. doi:10.1097/00006676-200410000-00002.

34 Kobayashi H, Gieniec KA, Lannagan TRM, et al. The origin and contribution of cancer-associated fibroblasts in colorectal carcinogenesis. *Gastroenterology.* 2022;162(3):890–906. doi:10.1053/j.gastro.2021.11.037.

35 Iida T, Mizutani Y, Esaki N, et al. Pharmaceutical conversion of cancer-associated fibroblasts from a protumor phenotype to an antitumor phenotype improves the sensitivity of pancreatic cancer to chemotherapeutics. *Oncogene.* 2022;41(19):2764–2777. doi:10.1038/s41388-022-02288-9.

36 Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. *Nat Rev Cancer.* 2012;12(8):540–552. doi:10.1038/nrc3319.

37 Helms E, Onate MK, Sherman MH. Fibroblast heterogeneity in the pancreatic tumor microenvironment. *Cancer Discov.* 2020;10(5):648–656. doi:10.1158/2159-8290.CD-19-1353.

38 Chen Y, Kim Jiha, Yang S, et al. Type I collagen deletion in αSMA⁺ myofibroblasts augments immune suppression and accelerates progression of pancreatic cancer. *Cancer Cell.* 2021;39(4):548–565.e6. doi:10.1016/j.ccell.2021.02.007.

39 Bhattacharjee S, Hamberger F, Ravichandra A, et al. Tumor restriction by type I collagen opposes tumor-promoting effects of cancer-associated fibroblasts. *J Clin Invest.* 2021;131(11):e146987. doi:10.1172/JCI146987.

40 Miyai Y, Sugiyama D, Hase T, et al. Meflin-positive cancer-associated fibroblasts enhance tumour response to immune checkpoint blockade therapy. *Research Square.* 2021. doi:10.21203/rs.3.rs-258152/v1.

41 Miyai Y, Sugiyama D, Hase T, et al. Meflin-positive cancer-associated fibroblasts enhance tumour response to immune checkpoint blockade. *Life Sci Alliance.* 2022;5(6):e202101230. doi:10.26508/lsa.202101230.

42 Sherman MH, Yu RT, Engle DD, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreaticitis and enhances pancreatic cancer therapy. *Cell.* 2014;159(1):80–93. doi:10.1016/j.cell.2014.08.007.

43 Mizutani Y, Iida T, Ohno E, et al. Safety and efficacy of MIKE-1 in patients with advanced pancreatic cancer: a study protocol for an open-label phase I/II investigator-initiated clinical trial based on a drug repositioning approach that reprograms the tumour stroma. *BMC Cancer.* 2022;22(1):205. doi:10.1186/s12885-022-09272-2.

44 Zhang K, Zhang Y, Gu L, et al. Islr regulates canonical Wnt signaling-mediated skeletal muscle regeneration by stabilizing Dishevelled-2 and preventing autophagy. *Nat Commun.* 2018;9(1):5129. doi:10.1038/s41467-018-07638-4.

45 Cui C, Han S, Shen X, et al. ISLR regulates skeletal muscle atrophy via IGF1-Pi3K/Akt-Foxo signaling pathway. *Cell Tissue Res.* 2020;381(3):479–492. doi:10.1007/s00441-020-03251-4.