Chapter

KLF4-Mediated Plasticity of Myeloid-Derived Suppressor Cells (MDSCs)

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Abstract

Robustness of tissues refers to their capability to maintain normal functions despite perturbation such as injuries. Recent studies suggest a key role of the immune system in injury repair. In this process, several immune cell lineages exhibit considerable plasticity as they migrate toward the site of damage and contribute to repair. For example, myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature cells and possess phenotypic plasticity in cancer, a pathological status that is considered as "wounds that do not heal." They are characterized by their potent ability to suppress immune responses. In cutaneous wound healing, MDSCs not only execute their immunosuppressive function to inhibit inflammation but also stimulate cell proliferation once they adopt a fate of a totally different cell type. At a molecular level, we found that Krüppel-like factor 4 (KLF4), a transcription factor with multiple roles in homeostasis and disease development plays a critical role in regulating MDSCs. In this review, KLF4-mediated plasticity of MDSCs and the underlying mechanisms are discussed.

Keywords: KLF4, FSP-1, myeloid-derived suppressor cells (MDSCs), plasticity, cancer, wound healing

1. Introduction

KLF4 is a member of the Krüppel-like factor family, a group of zinc finger-containing transcription factors that are highly homologous with the Drosophila Krüppel protein [1–4]. It has important functions in a variety of cellular processes that include cell proliferation, differentiation, development, and maintenance of normal tissue homeostasis [5]. KLF4 has also been shown to act either as a tumor suppressor or an oncoprotein in a context-dependent manner [6–8]. Moreover, KLF4 is critical to barrier function of the skin and promotes physiological and pathological wound healing [9–11].

MDSCs are bone marrow-derived cells present in bone marrow, spleen, and circulation. They are a heterogeneous collection of immature myeloid cells. These immature cells possess typical $CD11b^{+}Ly6G^{+}$ markers in mice with a wider range of markers in humans. The main function of MDSCs is their potent ability to suppress the host immune responses, especially T-cell proliferation and cytokine production [12]. They possess phenotypic plasticity in cancer [13, 14], a pathological status that is considered as "wounds that do not heal." However, while the involvement of MDSCs in wound healing has been shown by their recruitment to the wound sites [15], the

role of their plasticity in wound healing has not been fully examined. On the other hand, two immune cell lineages closely related to MDSCs, namely neutrophils and macrophages, demonstrated their phenotypical and functional plasticity in wound repair [16]. In addition, we showed that in wound healing MDSCs not only execute their immunosuppressive function to inhibit inflammation, but also stimulate cell proliferation once they adopt a fibrocyte fate [11]. Collectively, these observations support a key role of MDSC plasticity in wound healing leading to tissue robustness, though the underlying cellular and molecular mechanisms are not clear.

We recently reported that KLF4 promotes cancer development by regulating the recruitment and function of MDSCs [8, 17, 18]. In addition, we found that KLF4 regulates generation of fibrocytes, emerging effector cells in chronic inflammation [19, 20], from MDSCs in cancer [8], wound healing [11], allergic asthma [21]. Given the importance of plasticity of macrophages, a highly relevant cell type to MDSCs, in tissue repair and regeneration [22], we postulate that KLF4 also regulates myeloid plasticity in wound healing. In this review, the role of KLF4 in regulating plasticity of MDSCs in wound healing and the underlying molecular mechanisms will be discussed.

2. Plasticity of MDSCs in cancer and wound healing

MDSCs represent a group of heterogeneous monocytes during myeloid cell development with a major attribute of immunosuppressive activities. The population of these cells increases in a number of conditions associated with chronic inflammation, autoimmune diseases, and cancer. These heterogeneous cells are now further divided into two major subgroups including polymorphonuclear (PMN) and monocytic (M)-MDSCs [23]. Although non-immunosuppressive MDSCs exist in tumor-bearing hosts or in conditions of chronic inflammation [24], in which MDSCs can be classified as MDSC-like cells (MDSC-LC), demonstration of immunosuppressive activities is required to accurately define MDSCs after the initial phenotypical characterization by cell surface markers. In term of immunosuppressive activities of MDSCs, different mediators were reported, such as arginases, nitric oxide (NO), reactive oxygen species (ROS), indoleamine 2,3-dioxygenase (IDO), transforming growth factor-β1 (TGF-β1), and prostaglandin E2 (PGE2) among others, depending on specific conditions. As MDSCs are heterogeneous and suppress immune functions with different mechanisms, it is not surprising that they possess phenotypical and functional plasticity [25], reflecting their adaptation to varied environmental conditions. Note that immune cell plasticity could be understood from two different and important senses [16]. The first one is *intra-lineage cell plasticity*, that is, changes in cell function within a given cell lineage. This is also known as functional plasticity. The second sense is *trans-lineage cell plasticity*, that is, the switch from one lineage to another. Alternatively, this can be called "transdifferentiation" or "phenotypical plasticity." We will mainly use "phenotypical plasticity" and "functional plasticity" to discuss MDSC functions in this chapter.

2.1 MDSC plasticity in cancer

Immunotherapies against cancer rely on activated T cells or NK cells to recognize and eliminate tumor cells. However, the effector cells in the tumor microenvironment encounter a wide array of factors that limit their activities. MDSC-mediated immune suppression represents one of the major mechanisms by which the functions of immune effector cells are blocked in cancer. In addition, MDSCs are implicated not only in regulating tumor immune response, but also in tumor angiogenesis, tumor cell invasion, and formation of pre-metastatic niches [26].

Phenotypical plasticity of MDSCs in cancer could be first understood from the capacity of myeloid regulatory cells to convert from each other under certain conditions. Such plasticity could explain confusing observations on the role of MDSCs in tumor growth or tumor inhibition [13]. For example, while MDSCs are well known for their tumor promoting function because of their immunosuppressive activities against T cells, they can be converted to dendritic cells (DCs) in the presence of nature killer T (NKT) cells and α-galactosylceramide, leading to an anti-tumor immune response against HER2/CT26 tumor [27]. Mechanistically, it was proposed that NKT cells interact with MDSCs. This interaction leads to the conversion of MDSCs to DCs by increasing gene expression of CD80, CD86 and CD70. Consequently, interactions of CD80 and CD70 on newly converted DCs with CD28 and CD27 on T cells support these T cell responses to the tumor cells resulting in elimination of MDSC-mediated immune suppression [13].

Phenotypical plasticity of MDSCs could also be understood from the existence of MDSC subtypes and their differentiation into macrophages under normal and abnormal conditions. Because PMN-MDSCs are short lived, M-MDSCs have been studied in a more detail. In addition, most studies did not correlate M-MDSCs with monocytes expressing high levels of Ly-6C (Ly-6C^{hi} cells). These Ly-6C^{hi} cells are frequently referred to inflammatory monocytes. Given their elevated function at the tumor site and their potent immunosuppressive activities, Ly-6C^{hi} monocytes in the tumor microenvironment most likely represent *bona fide* M-MDSCs [14]. M-MDSCs have been shown to differentiate into tumor-associated macrophages (TAMS) after they are recruited to the tumor site [28]. It was shown that the CD45-mediated inhibition of STAT3 in MDSCs promotes TAM differentiation [29]. Besides TAMs and DCs as we discussed earlier, MDSCs differentiate into fibrocytes, an emerging group of cells with multiple functions in inflammation and cancer [19, 20, 30, 31].

Functional plasticity of MDSCs could be understood by their intrinsic features especially their immunosuppressive activities. It is known that immunosuppressive activities of MDSCs are mainly detected in tumors, but rarely in other tissues or organs including bone marrow or spleen. However, MDSCs in tumor and other chronic inflammatory conditions may not always be immunosuppressive. For example, in the initiation stage of chronic inflammation or early stage tumors, there are cells with MDSC phenotypical markers but without potent immunosuppressive activities. Moreover, even in advanced stage tumors, not all cells with a MDSC phenotype possess immune suppressive activity. For example, recent studies showed that in chronic inflammation, cells with an MDSC phenotype lacking suppressive activity actually contribute to the early stages of tumor inflammation [32]. However, the exact nature and the mechanism of how MDSCs acquire their immune suppressive activities are not entirely clear.

2.2 Potential role of MDSC plasticity in tissue repair

Though immunologists generally consider the immune system as a system of defense, recent studies suggest a key role of the system in tissue robustness, the capability of an organism to maintain its function and performance despite perturbations [33, 34]. One of the major ways by which the immune system contributes to robustness is through immune cell plasticity. Most studies of tissue repair have focused on the innate immune system, which may reflect the evolutional conservation of the repair-mediated robustness. Although plasticity of γδT cells [35, 36], innate lymphoid cells [37], and regulatory T cells [38] is also involved in tissue repair, we will mainly discuss the role of neutrophils, macrophages, and MDSCs in the process.

Neutrophils are the major innate cells recruited to the damage site and are considered as the first line of defense against infection [39]. However, these cells

can switch phenotypes, display distinct subpopulations, and produce a large variety of cytokines and chemokines [40]. In tissue repair, neutrophils can show their intra-lineage or functional plasticity by pro- or anti-inflammation, during the early stage of a typical wound repair. In addition, in an inflammatory and pro-type 2 microenvironment of a lesion, neutrophils transdifferentiate into antigen presenting cells (APCs) [41]. Such transdifferentiation into APCs has also been studied in rheumatism, where it could drive sustained inflammation, thereby preventing normal repair [42]. Besides neutrophils, macrophages fulfill roles that change over the duration of wound healing [43]. Initially they are bactericidal, and voraciously phagocytose cell and matrix debris, particularly red blood cells and any spent neutrophils at the wound site. These early stage macrophages are called M1 macrophages, and they are pro-inflammatory. Later in the repair process, macrophages develop the pro-repair capacity. These macrophages are called M2 macrophages, and they are anti-inflammatory and pro-reparative. The resting macrophages are called M0 macrophages. Not surprisingly, the plasticity of macrophages, namely the changeable cellular phenotypes and the range of differentiation and activation states, helps to explain the pleiotropic nature of these cells and their complex functions in wound repair [22, 44]. Beside their role in the early inflammatory stage of wound healing, macrophages contribute to tissue remodeling in wound healing by transdifferentiation, notably into endothelial cells [45, 46], a phenotypical plasticity.

When compared to those of neutrophils and macrophages, the role of MDSCs and their plasticity in wound healing are less studied [47]. However, there is ample evidence supporting a critical role of MDSC plasticity in repair. For example, as a heterogeneous and immature population of myeloid cells, recruited MDSCs at wound sites can differentiate into macrophages, DCs, and neutrophils [25]. In addition, because of their immunosuppressive function, MDSCs appear to dampen inflammation at the early stage but then promote healing after inflammation wanes by adopting a fate of fibrocytes [11], a cell type that can further differentiate into myofibroblasts that produce extracellular matrix in wound closure [48, 49]. In cancer, a pathological condition considered as "wounds that do not heal," fibrocytes are viewed as a subpopulation of MDSCs [50, 51], further highlighting a dynamic and plastic nature of MDSCs in wound healing.

3. KLF4-mediated plasticity of MDSCs

3.1 KLF4 promotes cancer development through regulating plasticity of M-MDSCs

KLF4 is expressed in many tissues and cells types. Besides in epithelial cells, it is also expressed in bone marrow-derived cells and is key to inflammation [52, 53] and monocyte differentiation [54, 55]. However, it was not clear whether and how immune cell-expressing KLF4 is involved in the development of tumor. It is our hypothesis that the overall function of KLF4 depends on its expression in immune cells and in the resident epithelial cells. In the following discussion, we will focus on the role of MDSC-expressing KLF4 in cancer.

To study the function of KLF4 in MDSCs, we used a 4T1 mammary tumor model. This model is unique due to its similar characteristics with human breast cancer, particularly the ability to spontaneously metastasize to lungs. Based on 4T1 cells, we generated stable KLF4 knockdown cells and control cells using siRNA technology. They were designated as siKLF4 and siCon, respectively. We found that in siCon cell-inoculated BALB/c mice tumors were observed as early as Day 9 and the tumor size reached to 18.2 ± 1.6 mm in diameter. However, in siKLF4 cell-inoculated

mice the primary mammary tumors became visible on Day 14 and the tumor size was only 11.3 ± 1.4 mm in diameter [18]. These data were in agreement with our previous results showing that KLF4 knockdown delayed the onset of mammary tumor development and inhibited lung metastasis in immunocompromised NOD/SCID mice inoculated with MDA-MB-231 human breast cancer cells [56]. We then tested whether MDSCs were involved in KLF4-mediated tumor development. We examined MDSCs in bone marrow, spleen, and tumor by flow cytometry. We found that after implantation of 4 T1 cells, KLF4 knockdown significantly reduced the numbers of MDSCs in bone marrow and spleen when compared to siCon counterparts [18]. As a critical control, we examined the immunosuppressive activities of MDSCs from control cell- and KLF4 knockdown cell-inoculated mice [57, 58]. As expected, MDSCs from siKLF4 cell-inoculated mouse inhibited proliferation of CD4+ and CD8+ T-cell significantly less than their siCon counterparts. The same assay using MDSCs purified from mouse tumors confirmed this observation. Moreover, consistent with higher T cell proliferation upon KLF4 knockdown, the arginase activities in MDSCs from siKLF4 cell-inoculated mice were lower when compared to those in siCon counterparts. Furthermore, we examined the infiltration of T cells into tumor sites by CD3 immunofluorescence staining. We found that there were more T cells accumulated in siKLF4 cell-inoculated mice than in siCon group.

Consistently, in a mouse B16-F10 implantation melanoma model, we showed that KLF4 deficiency in bone marrow drastically reduced lung metastasis accompanied by decreased recruitment of monocytic CCR2+ MDSCs (M-MDSCs) in the lungs. Interestingly, bone marrow KLF4 deficiency was linked with significantly reduced numbers of fibrocytes and myofibroblasts in metastatic lungs [8]. We further performed a cause-effect study to exclude the effect of KLF4-mediated development of MDSCs and to test the direct effect of KLF4-regulated fibrocyte generation from M-MDSCs on tumor metastasis. We sorted M-MDSC subset from the lungs of mice bearing B16-F10 melanoma. They were mixed with B16-F10 tumor cells and then injected wild-type mice with the mixture intravenously. We then induced KLF4 knockout in these mice by tamoxifen injection. In the control mice, they only received B16-F10 tumor cells, but were still injected with tamoxifen or sunflower seed oil as controls. Mice were sacrificed at Day 7 after tumor cell inoculation. We found that no difference was observed in the incidence of lung metastasis between the mice administrated with tamoxifen or sunflower seed oil. However, in the $KLF4^{-/-}$ and control groups, metastatic nodules in the pulmonary were drastically fewer than those in the $KLF4^{+/+}$ group. The results strongly suggest that KLF4 controls the process in which M-MDSCs facilitate the seeding and growth of pulmonary metastatic nodules. We also took advantage of the EGFP marker in the transplanted M-MDSCs. We examined MDSC differentiation in the lung by immunofluorescence using COL1A1 and α-SMA antibodies. We found that although there was no difference in the total number of $EGFP^+$ cells between the $KLF4^{+/+}$ and $KLF4^{-/-}$ group, in KLF4 deficient mice the number of $COL1A1^+EGFP^+$ cells decreased significantly when compared to that in the $KLF4^{+/+}$ mice. Similarly, $\alpha\text{-}SMA^+EGFP^+$ cells also decreased in $KLF4^{-/-}$ mice, further supporting our speculation that KLF4 regulates the differentiation of M-MDSCs into fibrocytes and myofibroblasts after they are recruited to the lungs *in vivo*.

3.2 KLF4 deficiency compromised cutaneous wound healing depending on functional MDSCs

A pressure ulcer (PU) is defined as an injury caused by unrelieved pressure that results in damage to the skin and underlying tissue [59, 60]. They are thought to be caused by local tissue ischemia, interstitial and lymphatic blockage, reperfusion injury,

and mechanical deformation of cells by compressive forces [61]. PUs are detrimental to the patients by prolonging their hospital stay, affecting social life-styles, and contributing to negative psychological consequences [62, 63]. Generally, wound healing includes the early inflammatory phase and the later proliferative and remodeling phases [64–66]. However, this process in PU is frequently stalled in the inflammatory stage [67]. This is the reason why PU has been considered a chronic wound [68].

We have reported that KLF4 ablation delayed cutaneous wound healing in KLF4-CreER/KLF4(flox) [69] and RosaCreER/KLF4(flox) double transgenic mice [11], in which KLF4 was knocked out upon tamoxifen induction. To further test the possibility that KLF4 deficiency-induced delay of cutaneous wound healing may be attributed to bone marrow cells, we transplanted bone marrow cells from RosaCreER/KLF4(flox)/β-actin-EGFP triple transgenic mice into wild type C57BL/6 mice and used these chimeric mice to perform full-thickness wound healing experiments. The wound-closure kinetics showed that wound healing was significantly delayed upon KLF4 knockout in bone marrow. In addition, M-MDSCs but not total MDSCs in the skin wounding bed significantly decreased in the $KLF4^{-/-}$ group compared to those in the $KLF4^{+/+}$ group. By flow cytometric analysis, after we gated EGFP+ cells and analyzed $COL1A1^{+}CD45^{+}CD11b^{+}$ populations to examine bone marrow-derived fibrocytes in the skin wounding bed, we showed that fibrocytes decreased in $KLF4^{-/-}$ group compared to those in $KLF4^{+/+}$ group. This finding was further confirmed by immunofluorescent staining of the wounding bed, as demonstrated by significantly reduced numbers of COL1A1/EGFP and α-SMA/EGFP co-expressing cells in $KLF4^{-/-}$ group. Moreover, we transplanted bone marrow cells from KLF4/EGFP transgenic mice, in which KLF4-expressing cells are labeled with EGFP [69], to the wild type mice and performed full thickness wound healing experiments. Four days after the wound placement, the wound healing tissues were collected and slides prepared, followed by immunofluorescent staining. We found that KLF4 expressing EGFP cells in the wound bed adapted elongated morphology and were co-localized with those expressing α-SMA, a marker of myofibroblasts that play a critical role in wound healing [70, 71].

KLF4 was highly expressed in M-MDSCs, and we postulated that KLF4 in M-MDSCs may directly regulate the cutaneous wound healing. Because of the highest expression level of FSP-1 in M-MDSCs among all MDSC subpopulations, to test our hypothesis, we used FSP-1-Cre/KLF4(flox) mice to produce PUs [72]. The dorsal skin of WT and FSP-1-Cre/KLF4(flox) (KLF4 null) mice were shaved, gently pulled up and placed between two cylinders of magnets (12 mm in diameter and 5 mm in thickness), producing a compressive pressure of 50 mmHg between the two magnets according to the established PU model [72–74]. A single ischemia-reperfusion cycle (I/R) consisted of a period of magnet placement for 16 h followed by a release or rest of 8 h. Three I/R cycles were used in each animal to initiate decubitus ulcer formation. Ulcers were typically formed at Day 3 (at the end of third I/R cycle) accompanied by full-thickness loss of skin. To assess the wound healing of PU, the detached full-thickness skin (ulcered skin) was removed at Day 3 right after the third I/R cycle, and the closure of open ulcer area in each mouse was monitored and photographed consecutively for 10 days. We found that 1 day after the ulcered skin was removed, the opening areas were increased in both WT and KLF4 null mice, probably because of the acute responses. From Day 2 to Day 10, wounds were gradually healed in WT mice, but the healing was delayed in KLF4 null mice as also indicated by an unclosed wound at Day 10. H&E staining showed an increased suprabasal layer of the skin and decreased hair follicle densities. The infiltrated lymphocytes were almost doubled in granule tissue of the skin in KLF4 null mice. These results suggest an elevated inflammatory status in KLF4 null mice. In agreement with reduced numbers of M-MDSCs and fibrocytes upon KLF4 knockout in

bone marrow in our full-thickness wound healing model, these populations were also decreased in FSP-1-Cre/KLF4(flox) mice in the PU model. Interestingly, we found that the populations of CD11b+Ly6C++ cells, which may represent inflammatory monocytes [75], in both blood and skin wounding beds were increased when compared to those in wild type mice. This observation is consistent with the increased inflammation in KLF4 null mice.

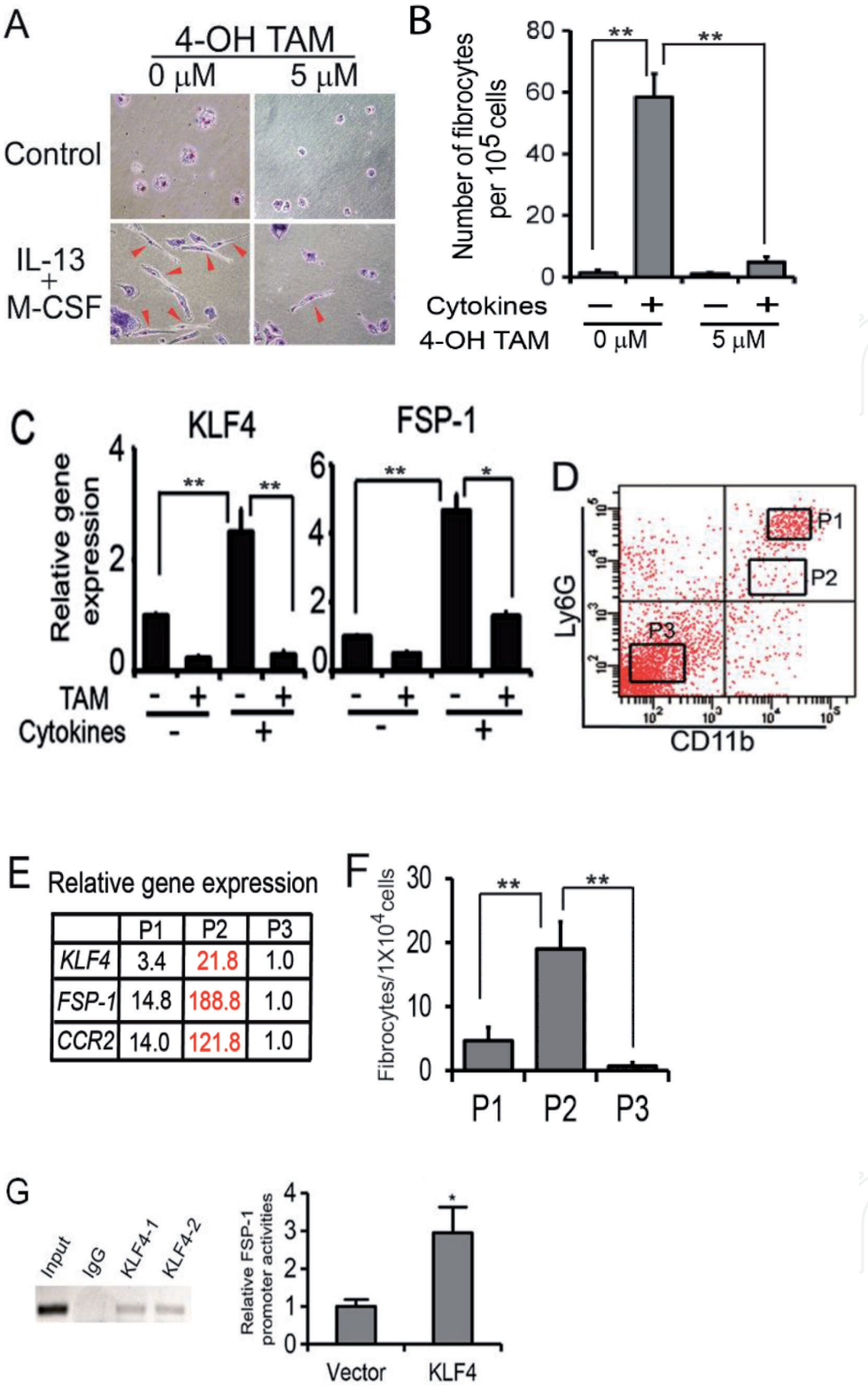
3.3 Mechanisms of KLF4-mediated MDSC plasticity

MDSC plasticity, and in general, myeloid plasticity, is regulated by the local microenvironment. These cells are environmental sensors and adapters [25]. In tumor, myeloid cells are the most abundant immune cells, and signals within the tumor microenvironment instruct these cells to change their dynamics and plasticity. There are many potential factors/mechanisms in these processes, including hypoxia, tumor ER stress, exosomes, and tumor-derived soluble factors [76]. In the following discussion, we will focus on KLF4-mediated plasticity of MDSCs in cancer and wound healing based on our recent studies.

3.3.1 KLF4 regulates FSP-1 in fibrocyte generation from MDSCs

FSP-1, also known as S100A4, is widely accepted as a fibroblast-specific marker [77, 78]. Given the fact that FSP-1 is expressed in more than 90% of monocytes of the host immune system [79] and that it has a "specific" expression in fibroblasts, it is challenging to reconcile the function of FSP-1 at the cellular level between these two very different cell types. On the other hand, fibrocytes are bone marrow-derived progenitor cells that can differentiate into myofibroblasts and promote cutaneous would healing and cancer development [20, 51, 80, 81]. Therefore, fibrocytes are very good candidates for carrying the expression/function of FSP-1 from the host immune cells such as MDSCs to fibroblasts.

It has been reported that fibrocytes can be generated from bone marrow-derived cells such as MDSCs [82]. We postulated that KLF4 controls MDSC-mediated generation of fibrocytes. To test this hypothesis and to examine the underlying mechanisms, we isolated spleen cells from KLF4 inducible knockout Rosa26CreER/KLF4(flox) mice and examined fibrocyte differentiation using an *ex vivo* assay with murine IL-13 and M-CSF [83]. We found that the application of IL-13 and M-CSF resulted in 58 ± 7 fibrocytes per 1×10^5 cells (**Figure 1A**) in the control group. However, the same treatment decreased the number of fibrocytes to 5 ± 2 cells per 1×10^5 splenocytes when KLF4 was knocked out by induction of 5 μM 4-OH tamoxifen (**Figure 1B**). Furthermore, we examined KLF4 and FSP-1 expression in the process of fibrocyte generation by quantitative RT-PCR analysis. As shown in **Figure 1C**, both KLF4 and FSP-1 mRNA levels were significantly elevated after the application of IL-13 and M-CSF, which was consistent with *ex vivo* generation of fibrocytes. The induction of KLF4 deficiency by 4-OH tamoxifen correlates with a significant decrease in FSP-1 expression, suggesting a KLF4-mediated regulation of FSP-1 in the process. Since splenocytes are a mixed group of cells, we proceeded to examine KLF4 and FSP-1 expression in different subsets of MDSCs from the wild type mouse splenic tissues (**Figure 1D**). Highest levels of KLF4, FSP-1, and CCR2 expression were found in the $CD11b^{+}Ly6G^{Int}$ subpopulation of MDSCs (P2 in **Figure 1D** and **E**), known as M-MDSCs [84, 85]. Note that these M-MDSCs had the highest potential for fibrocyte generation (**Figure 1F**), thus supporting the observation that KLF4 deficiency led to significant decrease in FSP-1 expression and fibrocyte generation (**Figure 1A–C**) in the MDSC pool. To test whether KLF4 directly regulates FSP-1 gene expression, we first using two different KLF4 antibodies to perform a chromatin



E	P1	P2	P3
KLF4	3.4	21.8	1.0
FSP-1	14.8	188.8	1.0
CCR2	14.0	121.8	1.0

Figure 1.
*KLF4 regulates FSP-1 gene expression in fibrocyte generation. (A) Representative photographs of morphological fibrocyte generation from splenocytes in the absence and presence are indicated by red arrows. KLF4 deficiency was induced by 4-OH tamoxifen (TAM). (B) Quantification of the data from (A). (C) Relative levels of KLF4 and FSP-1 mRNA in fibrocyte generation as assessed by qRT-PCR. (D) Different MDSC subsets in mouse splenocytes measured by flow cytometry. (E) Relative levels of KLF4, FSP-1 and CCR2 mRNA in different MDSC subsets by qRT-PCR. (F) Potential of fibrocyte generation from MDSC subsets in mouse spleen. (G) Left—binding of KLF4 to the FSP-1 promoter as assessed by chromatin immunoprecipitation assay using two KLF4 antibodies (KLF4-1 and KLF4-2). IgG was used as a negative control. Right—the effect of KLF4 overexpression on FSP-1 promoter activities, as examined by transient transfection and dual luciferase assays, *P < 0.05, **P < 0.01.*

immunoprecipitation (CHIP) assay. We found that KLF4 directly bound to the FSP-1 proximal promoter region (**Figure 1G** left). Then we constructed a FSP-1 promoter luciferase reporter containing ~2.3 kb of the FSP-1 promoter region. By transient transfection and dual luciferase assays, we found that KLF4 overexpression resulted in three fold increase of the FSP1 promoter activity (**Figure 1G** right), suggesting a direct regulation of FSP-1 by KLF4 at the transcriptional level.

3.3.2 Epigenetic control of MDSC plasticity

The studies of epigenetics, heritable changes to gene expression without changes to DNA, are significantly advancing our knowledge of the inflammatory conditions [86]. They include DNA modifications mainly methylation, histone tail modifications, and non-coding RNA-mediated gene regulation. Recent data revealed that epigenetic mechanisms could provide novel strategies for modulating wound healing [87–89].

Critical functions of KLF4 have been shown in the generation of induced pluripotent stem cells and in cancer development through epigenetic mechanisms [90, 91]. In addition, there are numerous reports showing that microRNAs regulate KLF4 [92–94] or KLF4 regulate microRNAs [95, 96] in varied pathological conditions. KLF4-mediated DNA methylation have also been reported in hTert promoter [97] and methylation of KLF4 promoter is associated with urothelial cancer progression and early recurrence [98]. Moreover, the correlation of KLF4 and histone modifications has also been reported. For example, histone methyltransferase KMT2D, a frequently aberrant epigenetic modifier in various cancer, sustains prostate carcinogenesis and metastasis via epigenetically activating KLF4 [99]. From the perspective of MDSCs, epigenetic regulation of their differentiation and function is not completely understood. However, there is evidence to indicate the importance of epigenetic regulation. Shang et al. showed that long non-coding RNA retinal non-coding RNA3 (RNCR3) promotes C/EBP homologous protein (Chop) expression by sponging microRNA 185-5p during MDSC differentiation [100]. In addition, although histone modifications related to myeloid differentiation have been extensively studied [101], currently there is no clear indication about epigenetic markers that can discriminate specific MDSC subsets. Given the role of KLF4 in epigenetic regulation and the importance of MDSC plasticity in cancer and wound healing, it will be very interesting to examine how KLF4 is involved in epigenetic control of MDSC subsets or plasticity.

3.3.3 Is there potential molecular plasticity of KLF4 in cancer and wound healing?

KLF4 is a transcription factor with multiple functions in different physiological and pathological conditions, notably in cancer development. For example, KLF4 is well known for its tumor suppressive effect on tumor development in the gastrointestinal tract [102]. However, high expression of KLF4 is associated with skin cancer and breast cancer development [56, 103, 104], suggesting a tumor promoting function of KLF4 in these tissues. Recently, a tumor suppressive function of KLF4 was also reported in breast cancer [105]. These contradictory reports suggest context-dependent functions of KLF4 in cancer development [106]. At a molecular level, different KLF4 transcripts were found in testis [107], and alternative splicing of KLF4 has been proposed to explain context-dependent functions of KLF4 [108]. Consistently, an oncogenic KLF4 isoform, named KLF4α, has been found in both pancreatic cancer [109] and breast cancer [110]. In line with these observations, there is dynamic expression of KLF4 isoforms in mouse embryogenesis [111].

Interestingly, another human KLF4 isoform with an additional 34 amino acid-fragment in the C-terminal region has been reported in leukemia patients [112] and in myeloid cells [113], which further supports the importance of differential expression of KLF4 in different conditions.

We speculate that the existence of different isoforms of KLF4 and possibly relative ratios of these isoforms may explain different functions of KLF4 in cancer development and even in wound healing. Because KLF4 is a transcription factor that regulates gene expression, different isoforms of KLF4 will have different patterns of gene regulation of the downstream targets. In analogy to MDSC dynamics and plasticity, we propose a concept of KLF4 plasticity, which reflects the dynamic nature of KLF4 expression under different conditions. It is likely that under one condition, a major isoform of KLF4 regulates a group of genes that are responsible for one signaling transduction pathway. This pathway may be linked to one functional or phenotypical MDSC group. Under a different condition, another KLF4 isoform dominates and regulates a different group of genes and a different signaling pathway. This kind of differential regulation may cause the plastic change of MDSCs in cancer or wound healing. To confirm our hypothesis, future experiments will be needed to characterize the different KLF4 isoforms during the dynamic change of MDSCs. Validation of our hypothesis will not only reveal novel molecular mechanisms whereby KLF4 regulates MDSC plasticity, but also help design KLF4-based therapeutic strategies to manipulate MDSC plasticity in the treatment of cancer and wound healing.

4. Conclusion remarks

Studies of immune cell plasticity have recently gained momentum due to their novel functions in tissue repair and robustness beside their well-known functions in system defense. MDSCs, as a myeloid population with unique functions in tumor and tissue repair, are less studied regarding their phenotypical and functional plasticity, compared to macrophages and neutrophils. Given the ample evidence showing MDSC plasticity in cancer and wound healing, it is essential to elucidate the underlying molecular mechanisms in order to harness MDSCs in tissue repair and cancer treatment. In the meantime, we have shown KLF4 as a key molecule to regulate MDSC plasticity in cancer, wound healing, and allergic asthma. KLF4-controlled FSP-1 expression and possible epigenetic alterations are two possible mechanisms underlying MDSC plasticity. In addition, the existence of different KLF4 isoforms prompts us to hypothesize that KLF4 isoforms control gene expression of different signaling pathways that may contribute to MDSC dynamics and plasticity in both cancer and wound healing. In this regard, future studies to characterize different KLF4 isoforms during MDSC plastic changes and the relevant signaling pathways will pave the way to harness MDSC plasticity in the treatment of cancer and wound healing.

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Conflict of interest

The authors declare no conflict of interest.

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